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IN THE CIRCUIT COURT OF THE FIRST CIRCUIT

STATE OF HAWAI‘I

HAWAI‘I UNITES, a 501(c)(3) nonprofit corporation; TINA LIA, an individual, Plaintiffs, v. BOARD OF LAND AND NATURAL RESOURCES, STATE OF HAWAI‘I, and DEPARTMENT OF LAND AND NATURAL RESOURCES, STATE OF HAWAI‘I, Defendants.	Civil No. 1CCV-23-0000594 (JMT) (Environmental Court) **PLAINTIFFS’ MOTION FOR TEMPORARY RESTRAINING ORDER AND PRELIMINARY INJUNCTION; MEMORANDUM IN SUPPORT OF MOTION; DECLARATION OF COUNSEL; DECLARATION OF TINA LIA; DECLARATION OF DR. LORRIN W. PANG; EXHIBITS 1-11; NOTICE OF HEARING; CERTIFICATE OF SERVICE** HEARING MOTION Judge: Hon. John M. Tonaki Hearing Date: July 21, 2023 Hearing Time: 9:00 a.m.

PLAINTIFFS' MOTION FOR TEMPORARY RESTRAINING ORDER
AND PRELIMINARY INJUCTION

COMES NOW Plaintiffs Hawai'i Unites, a 501(c)(3) nonprofit corporation ("Hawai'i Unites") and Tina Lia ("Lia") (collectively, "Plaintiffs"), by and through their attorneys Margaret Wille and Timothy Vandeveer of MARGARET WILLE AND ASSOCIATES LLLC and move this Honorable Court to issue a temporary restraining order ("TRO") and preliminary injunction against the above-captioned Defendants Board of Land and Natural Resources, State of Hawai'i and Department of Land and Natural Resources, State of Hawai'i ("Defendants").

Due to current, ongoing harm to the environment as a result of actions that Defendants and their agents and other representatives or employees, including any of those agencies in its multi-agency partnership *Birds, Not Mosquitoes* ("BNM") have taken via the "mark release recapture" segment of the project known as "Suppression of Non-Native Wild Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui", Plaintiffs are here asking that the Court immediately issue an order that Defendants and their agents and other representatives, employees, or agencies in its BNM partnership, refrain from:

- Releasing incompatible male mosquitoes using Incompatible Insect Technique ("IIT") method on any of the fragile ecosystems of East Maui's Haleakalā National Park, Ko'olau Forest Reserve, Hāna Forest Reserve, Hanawī Natural Area Reserve, Kīpahulu Forest Reserve, Makawao Forest Reserve, and Waikamoi Preserve (The Nature Conservancy), as well as in the privately managed lands of East Maui Irrigation Company, LLC, Mahi Pono, and Haleakalā Ranch; and

- Releasing any biopesticide lab-reared *Wolbachia*-bacteria-infected mosquitoes on any of the fragile ecosystems of East Maui's Haleakalā National Park, Ko'olau Forest Reserve, Hāna Forest Reserve, Hanawī Natural Area Reserve, Kīpahulu Forest Reserve, Makawao Forest Reserve, and Waikamoi Preserve (The Nature Conservancy), as well as in the privately managed lands of East Maui Irrigation Company, LLC, Mahi Pono, and Haleakalā Ranch.

This Motion is based on Hawai‘i Rules of Civil Procedure (“HRCP”) Rule 65 “Injunctions” section (b) “Temporary restraining order; notice; hearing; duration”, the Memorandum in Support of the Motion, the declarations and exhibits attached hereto, the records and files of the case, and such other and further matters that may arise at the time of the hearing on this Motion. Consistent with HRCP Rule 65, section (a) “Preliminary injunction” in addition to the TRO, Plaintiffs request a preliminary injunction.

To the extent to that the Court decides that a preliminary injunction is appropriate at this time, Plaintiffs request that the Court immediately order Defendants and their agents and other representatives or employees including any of those agencies in its multi-agency partnership *Birds, Not Mosquitoes* (“BNM”) to refrain from the stated releases to allow time for a proper Environmental Impact Statement to first be conducted. Plaintiffs fear that without an appropriate thorough review of the proposed biopesticide project, native birds as well the affected ecosystem generally are at risk of being subject to far greater harm than currently exists.

DATED: Honolulu, Hawai‘i, June 20, 2023.

/s/ Timothy Vandeveer
Margaret (Dunham) Wille
Timothy Vandeveer

Attorneys for Plaintiffs
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HAWAI‘I UNITES, a 501(c)(3) nonprofit corporation; TINA LIA, an individual, Plaintiffs, v. BOARD OF LAND AND NATURAL RESOURCES, STATE OF HAWAI‘I, and DEPARTMENT OF LAND AND NATURAL RESOURCES, STATE OF HAWAI‘I, Defendants.	Civil No. 1CCV-23-0000594 (JMT) (Environmental Court) **MEMORANDUM IN SUPPORT OF MOTION FOR TEMPORARY RESTRAINING ORDER AND PRELIMINARY INJUNCTION** HEARING MOTION Judge: Hon. John M. Tonaki Hearing Date: July 21, 2023 Hearing Time: 9:00 a.m.

MEMORANDUM IN SUPPORT OF MOTION FOR TEMPORARY RESTRAINING ORDER AND FOR PRELIMINARY INJUNCTIVE RELIEF

I. SUMMARY OF RATIONALE FOR REQUESTING TRO AND PRELIMINARY INJUNCTIVE RELIEF

Plaintiffs Hawaiʻi Unites, a 501(c)(3) nonprofit corporation ("Hawaiʻi Unites") and Tina Lia ("Lia") (collectively, "Plaintiffs") seek this TRO/preliminary injunction enjoining Defendants Board of Land and Natural Resources, State of Hawaiʻi ("BLNR") and Department of Land and Natural Resources, State of Hawaiʻi ("DLNR") (collectively, "Defendants") and their agents and employees, and all persons acting under, in concert with, or for them, including any of those agencies in its multi-agency partnership *Birds, Not Mosquitoes* ("BNM") from any conduct in furtherance of their action on the basis of the "Environmental Assessment and Finding of No Significant Impact for Suppression of Non-Native Wild Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui" ("FEA/FONSI")[1] until such time as the Hawaiʻi Constitution and Hawaiʻi Revised Statutes ("HRS") chapter 343 have been adequately and properly complied with.

Defendants failed to comply with the Hawaiʻi Environmental Policy Act ("HEPA") and violated Plaintiffs' rights under the Hawaiʻi Constitution, including the right to a clean and healthful environment. What is at issue here is a massive mosquito biopesticide project ("experiment") with no meaningful mitigation measures in place if things don't go according to plan. Based on information and belief, it appears that the "mark release recapture" pilot project phase of the action has already started with plans to implement future phases of the experiment in the immediate future. Plaintiffs request injunctive relief at this time due to the current,

[1] A copy of the BNM FEA/FONSI dated March for the is attached hereto as **Exhibit ("Ex.") 1**.

ongoing, and imminent harm to the environment as a result of actions that Defendants and their agents and partners in BNM have taken.

Plaintiffs' right to a clean and healthful environment, right to conduct native Hawaiian traditional and customary practices, and right to due process have and will continue to be violated by Defendants' conduct. By failing to comply with HRS chapter 343 and the Hawaiʻi Constitution, Defendants have not taken the required precautionary steps to prevent harm to the fragile ecosystems and the health and safety of the communities of Maui. There is no adequate remedy at law for the violations of Plaintiffs' due process rights, and public policy strongly supports the protection of the environment and protection of Plaintiffs' constitutional rights.

II. PARTIES

Plaintiff Hawaiʻi Unites is a 501(c)(3) nonprofit organization dedicated to the conservation and protection of Hawaiʻi's environment and natural resources. The mission of Hawaiʻi Unites is honoring and protecting our sacred connection to the natural world. The organization has conducted extensive research into the science, data, and documentation of Defendant's biopesticide mosquito project ("experiment") that is being proposed to save endangered native birds from avian malaria using the Incompatible Insect Technique ("IIT") for mosquito population control.

Plaintiff Tina Lia is the founder of Hawaiʻi Unites and current Board President. She resides on Maui, the island where the proposed biopesticide mosquito experiment area is located. Lia has participated throughout the review process for the proposed action via public comment and attendance at hearings.[2]

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[2]A copy of Lia's written testimony to the Board of Land and Natural Resources and comment in regard to the proposed action entitled "Suppression of Non-Native Wild Mosquito Populations to

Defendant BLNR is the "agency that issues an approval prior to implementation of an applicant action" for the use of state lands for the project, including a Conservation District Use Permit and management plan. BLNR was the "approving agency" for the proposed biopesticide mosquito project under HEPA. *See* Hawaiʻi Administrative Rules ("HAR") § 11-200.1-2. As the "approving agency," BLNR was responsible for determining "whether the anticipated effects constitute a significant effect" and "the need for an EIS." *Id.* Defendant BLNR was also the agency with the authority to conduct a proceeding in which the "legal rights, duties, or privileges of specific parties are required by law to be determined after an opportunity for agency hearing." *See* HRS § 91-1(5).

Defendant DLNR is responsible for managing, administering, and exercising control over the State's public lands, the water resources, ocean waters, navigable streams, coastal areas (excluding commercial harbor areas), and minerals and all other interests therein. DLNR was the submitting agency for the FEA/FONSI and co-applicant (as part of BNM) in the proposed action.

III. RELEVANT BACKGROUND FACTS

According to the FEA/FONSI, the proposed action is the release of up to 775,992,000 biopesticide lab-reared *Wolbachia*-bacteria-infected mosquitoes per week over a period of "likely at least 20 years," potentially resulting (at highest estimated frequency) in over 807 billion mosquitoes released in the fragile ecosystems of East Maui's Haleakalā National Park, Koʻolau Forest Reserve, Hāna Forest Reserve, Hanawī Natural Area Reserve, Kīpahulu Forest Reserve, Makawao Forest Reserve, and Waikamoi Preserve (The Nature Conservancy); as well

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Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui" is attached hereto as **Exhibits 3-5**.

as in the privately managed lands of East Maui Irrigation Company, LLC; Mahi Pono; and Haleakalā Ranch.

The FEA/FONSI studied two alternatives: 1.) the "Proposed Action" (release the mosquitoes) and 2.) "No-Action" (not release the mosquitoes). *See* Ex. 1.

On March 24, 2023, the FEA/FONSI submitted by Defendant DLNR for the proposed multi-agency action entitled "Suppression of Non-Native Wild Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui" was accepted by Defendant BLNR. Declaration ("Dec.") of Lia at ¶ 5.

On March 24, 2023, the BLNR denied Plaintiff Tina Lia's request (on behalf of Plaintiff Hawai'i Unites) for a contested case hearing. This denial came *before* the BLNR voted on whether to accept the FEA/FONSI submitted by Defendant DLNR. The reason provided for the denial for a contested case on "the board's action to either accept or deny the Environmental Assessment" was that "your remedy…" is "you can sue under Chapter 343." Dec. of Lia at ¶ 6.

On April 8, 2023, the FEA/FONSI submitted by Defendant DLNR for the proposed experiment was published in *The Environmental Notice*. Dec. of Lia at ¶ 7.

On May 8, 2023, Plaintiffs timely filed their Complaint for Declaratory and Injunctive Relief ("Complaint") pursuant to HRS § 343-7 "Limitation of actions." Docket ("Dkt.") No. 1.

On May 11, 2023, a BNM agency partner representative was quoted in a Hawai'i Public Radio news story that stated "[t]he project to suppress the mosquito population and reduce avian malaria will move forward despite the lawsuit. Infertile mosquitoes could be released as early as this month."[3]

[3] A copy of a Hawai'i Public Radio article entitled "Hawai'i Unites sues DLNR over mosquito suppression plan to save native birds" dated May 11, 2023 is attached hereto as **Exhibit 6**.

On May 12, 2023, the BLNR again denied Plaintiff Lia's request (on behalf of Plaintiff Hawaiʻi Unites) for a contested case hearing. The reasons given for the second denial were that "there is already a piece of litigation going on by the same group before the courts, and I think this would be a duplication of it" and "because in the write-up, it explains that the petitioner did not assert a property interest that would trigger the due process analysis, and also that the recommendation has been to seek relief in the environmental court, which the applicant said she has." Dec. of Lia at ¶ 8.

On May 17, 2023, BNM posted the following message on its Facebook page: "The Environmental Assessment for East Maui was approved…now what?? On Maui, Birds, Not Mosquitos is starting to pilot small-scale releases of Wolbachia-incompatible male mosquitoes as part of a phased approach to landscape-scale mosquito control (Incompatible Insect Technique). The partnership is excited to take this momentous step toward mosquito control on Maui. This next phase will be used to fine-tune the release parameters–learning where and how far the incompatible male mosquitoes travel after they are released. These pilot releases alone are not expected to affect the overall population size of the southern house mosquito."[4]

On June 2, 2023 BNM wrote in an online article that they had, in fact, started "small-scale pilot releases of *Wolbachia*-incompatible male mosquitoes … in mid-May after the Finding of No Significant Impact for the East Maui Environmental Assessment." They also stated that "These pilot releases will also inform the larger landscape use of this mosquito control technique

[4] A copy of the *Birds, Not Mosquitoes* Facebook post entitled "The Final Environmental Assessment for East Maui was approved…now what??" dated May 17, 2023 is attached hereto as **Exhibit 7**.

on Maui."[5] The article did not specify exactly what date or location the "small-scale pilot releases of *Wolbachia*-incompatible male mosquitoes" had occurred.

IV. SCOPE OF TRO AND PRELIMINARY INJUNCTIVE RELIEF REQUESTED

Plaintiffs seek the issuance of a TRO, immediately enjoining Defendant DLNR and its agents and other representatives or employees including any of those agencies in its multi-agency partnership *Birds, Not Mosquitoes* from:

- Releasing incompatible male mosquitoes using Incompatible Insect Technique ("IIT") method on any of the fragile ecosystems of East Maui's Haleakalā National Park, Ko'olau Forest Reserve, Hāna Forest Reserve, Hanawī Natural Area Reserve, Kīpahulu Forest Reserve, Makawao Forest Reserve, and Waikamoi Preserve (The Nature Conservancy) as well as in the privately managed lands of East Maui Irrigation Company, LLC, Mahi Pono, and Haleakalā Ranch; and

- Releasing any biopesticide lab-reared *Wolbachia*-bacteria-infected mosquitoes on any of the fragile ecosystems of East Maui's Haleakalā National Park, Ko'olau Forest Reserve, Hāna Forest Reserve, Hanawī Natural Area Reserve, Kīpahulu Forest Reserve, Makawao Forest Reserve, and Waikamoi Preserve (The Nature Conservancy), as well as in the privately managed lands of East Maui Irrigation Company, LLC, Mahi Pono; and Haleakalā Ranch.

V. PLAINTIFFS HAVE A SUBSTANTIAL LIKELIHOOD OF SUCCESS ON THE MERITS

Plaintiffs have a substantial likelihood of success on the merits of its action for a preliminary and permanent injunction as well as for other requested relief. In the instant action, Plaintiffs seek declaratory judgment that the biopesticide mosquito project may have a significant impact on the environment, that Defendants have violated and are violating HRS

[5] A copy of the *Birds, Not Mosquitoes* online article entitled "Giving Thirst Traps a Whole New Meaning" dated June 2, 2023 is attached hereto as **Exhibit 8**.

Chapter 343 by failing to require an EIS; that the BLNR's acceptance of the final EA and FONSI fails to comply with HEPA and its implementing rules and is otherwise legally improper and invalid and that Defendant DLNR (and its BNM partner applicants) be required to prepare an EIS for the proposed biopesticide mosquito experiment due to the significant impact the proposed experiment will have on the environment. Plaintiffs also seek relief enjoining Defendants from issuing approvals for the proposed project or otherwise allowing it to proceed until compliance with HEPA and the fundamental requirements of administrative procedure under the Hawai'i Administrative Procedures Act, HRS chapter 91, and due process under article I, section 5 and article XI, sections 1 and 9 of the Hawai'i Constitution has occurred.

A. SUBSTANTIVE FAILURE TO COMPLY WITH HRS CHAPTER 343

HEPA requires the preparation of an EIS for any action that "***may*** have a significant effect on the environment." HRS § 343-5(c) (emphasis added). The Hawai'i Supreme Court has made clear that under the "may have a significant effect" standard, "plaintiffs need not show that significant effects <u>will in fact occur</u> but instead need only raise substantial questions whether a project may have a significant effect." *Unite Here! Local 5 v. City & Cnty. of Honolulu*, 123 Hawai'i 150, 178, 231 P.3d 423, 451 (2010) (internal citations omitted) (emphasis in original)

In determining whether an action may have a significant impact on the environment, "the agency shall consider every phase of a proposed action, the expected impacts, and the proposed mitigation measures." HAR § 11-200.1-13(b). The agency must consider certain "significance criteria" outlined in HAR § 11-200.1-13:

"[A]n action ***shall*** *be determined to have a significant effect on the environment if it* ***may***," among other factors:

(1) Irrevocably commit a natural, cultural, or historic resource;

(2) Curtail the range of beneficial uses of the environment;

(3) Conflict with the State's environmental policies or long-term environmental goals established by law;

(4) Have a substantial adverse effect on the economic welfare, social welfare, or cultural practices of the community and State;

(5) Have a substantial adverse effect on public health;

(6) Involve adverse secondary impacts, such as population changes or effects on public facilities;

(7) Involve a substantial degradation of environmental quality;

(8) Be individually limited but cumulatively have substantial adverse effect upon the environment or involves a commitment for larger actions;

(9) Have a substantial adverse effect on a rare, threatened, or endangered species, or its habitat;

(10) Have a substantial adverse effect on air or water quality or ambient noise levels;

(11) Have a substantial adverse effect on or be likely to suffer damage by being located in an environmentally sensitive area such as a flood plain, tsunami zone, sea level rise exposure area, beach, erosion-prone area, geologically hazardous land, estuary, fresh water, or coastal waters.

(12) Have a substantial adverse effect on scenic vistas and viewplanes, during day or night, identified in county or state plans or studies; or

. . .

HAR § 11-200.1-13(b)(emphasis added).

The criteria are expressly listed in the disjunctive. Thus, the existence of a single factor is sufficient to require preparation of an EIS. *See id.*

In the instant case, Defendant BLNR simply took Defendant DLNR's word that the BNM project would not have any significant impact and approved the FEA/FONSI instead of taking a "hard look" at the information presented and giving serious consideration to the impacts as required by chapter 343.

1. Improper Segmentation

The pilot phase of the project known as "mark release recapture" that BNM has announced is *currently underway* is a part of the experiment that was referenced without detail in the EA and was expressly exempted from the assessment. Ex. 2; *See* Ex. 8. On June 17, 2022, BLNR Chairperson Suzanne D. Case signed an exemption notice for "Mosquito Control Research Using *Wolbachia*-based Incompatible Insect Technique."[6] The final EA states that the DLNR filed the exemption notice "to conduct limited import of male mosquitoes for preliminary transport trials and mark release recapture studies."

However, Plaintiffs argue that this is an important part of the project and one that should not have been segmented and exempted from review. Plaintiffs contend that all actions of the mosquito project – including trial imports, mark release recapture studies, and field releases – should be addressed in one EIS. HAR § 11-200.1-10; *Hawaiʻi Environmental Policy Act Citizen's Guide* (2014). Per HEPA, "a proposed action must be described in its entirety and cannot be broken up into component parts, which if each is taken separately, may have minimal

[6] A copy of the DLNR "Exemption Notice Regarding Preparation of an environmental assessment" dated June 17, 2023 is attached hereto as **Exhibit 2**.

impact on the environment. Segmenting a project generally is forbidden." HAR § 11-200.1-10 – "Multiple or phased actions," provides:

> A group of actions shall be treated as a single action when:
>
> (1) The component actions are phases or increments of a larger total program;
>
> (2) An individual action is a necessary precedent to a larger action;
>
> (3) An individual action represents a commitment to a larger action; or
>
> (4) The actions in question are essentially identical and a single EA or EIS will adequately address the impacts of each individual action and those of the group of actions as a whole.

Because the project has been improperly segmented, there have been no details or analysis of the preliminary trials or the "mark release recapture" studies. For example, there has been no disclosure as to what type of mosquito is being transported, where the mosquitoes are being transported from, and whether or not the mosquitoes are being tested for pathogens prior to transport. Dec. of Lia at ¶ 9. As detailed below, this lack of detail is crucial given the potential for horizontal transmission of the introduced *Wolbachia* strains between the introduced biopesticide mosquitoes and the existing wild mosquitoes. Importantly, this is the segment of the proposed action *that is currently underway*, thus making Plaintiffs' request for issuance of a TRO both timely and appropriate.

2. Experimental Nature of the Project

Defendants don't have enough information about the experiment to conclusively state if any of the above criteria for environmental impact are being met or what those impacts might be. Though Defendant DLNR and its BNM agency partners contend that the proposed action is "neither an experiment nor a novel technique being tested in Hawai'i" they admit that it "has not been employed in Hawai'i nor for wildlife conservation" thus making it novel (as a new or unusual technique for Hawai'i and for wildlife conservation) and experimental (based on untested ideas or techniques and not yet established or finalized) by definition. *See* Ex. 1 at Appendix H, pgs. H-8, H-9. Federal documentation connected to this project also confirms that "although used world-wide for human health, *Wolbachia* IIT is a novel tool for conservation purposes and its degree of efficacy in remote forest landscapes is unknown." *U.S. Department of the Interior Strategy for Preventing the Extinction of Hawaiian Forest Birds* (2022).

Defendants also admit that because the proposed action has never been employed in Hawai'i nor for wildlife conservation that "protocols will need to be developed for its use in Maui's native forest and other local conditions." *See* FEA at Appendix H, pgs. H-8, H-9. However, such "protocols" are never listed or detailed in the FEA/FONSI, including important protocols detailing mitigation measures in the event that things don't go according to plan. The species planned for use in this project, *Culex quinquefasciatus* ("*Culex q.*"), has never been used for a stand-alone Incompatible Insect Technique (IIT) biopesticide mosquito field release. The *Culex q.* mosquito has never been lab-bred and *Wolbachia*-bacteria-infected and then released for mosquito suppression or population replacement. Although *Culex q.* was lab-bred and infected with *Wolbachia* in a 2020 study by Ant et al., the mosquitoes were not released for the purpose of mosquito suppression or population replacement. Ant et al. were studying the ability to make the mosquitoes incompatible, but they did not release any *Culex q.* mosquitoes. *Wolbachia transinfections in Culex quinquefasciatus generate cytoplasmic incompatibility* (2020).

Landscape level control of *Culex quinquefasciatus* mosquitoes using the Incompatible Insect Technique (IIT) has never been done before. Even with *Aedes* mosquitoes, the largest project area was 724 acres. The East Maui project area is 64,666 acres. This means that the East Maui project area would be the largest area ever to be used for any IIT - over 89 times larger than the current 724-acre maximum. The largest release area to date globally for a mosquito suppression project was the "Fresno DeBug" project which released *Aedes aegypti* mosquitoes in a 724-acre area. The only known time that the southern house mosquito was released for mosquito suppression was a 1982 study in India (by Curtis et al.) that used *Wolbachia* with a translocation that induced sterility. Because of the translocation, this was not a "stand-alone" project. The closest study to using *Culex q.* with *Wolbachia* to suppress mosquitoes was the 1967 Laven study in Okpo ("Okpho"), Burma ("Myanmar"), which was done with a *Culex pipiens fatigans* hybrid species closely related to *Culex quinquefasciatus*. *Crawford et al.* (2020); *Curtis et al.* (1982); *Eradication of Culex pipiens fatigans through Cytoplasmic Incompatibility* (Laven, 1967).

Without further study, the development of protocols or mitigation measures is difficult or impossible and the risks unknown.

3. Inadequacy of the Final Environmental Assessment

Plaintiffs contend that Defendants failed to take a "hard look" in both assessing impacts to the environment and accepting the FEA. This includes the failure to examine the broader impacts of the project, especially in light of the total lack of detail regarding mitigation plans and biosecurity protocols in the FEA and the failure to adequately address public comments and concerns (e.g., contradictory, misleading, or incomplete responses).

In the instant case, Defendant's own documents raise the possibility that the experiment may have a significant effect on the environment.

a. Horizontal Transmission

Tropical disease and vector expert Dr. Lorrin Pang[7] ("Dr. Pang"), speaking as a private citizen, has expressed concerns about horizontal transmission ("horizontal spread" or "horizontal transfer") of the introduced *Wolbachia* bacteria strain to wild mosquitoes and other insects, including other insect vectors of disease[8]. *See* Dec. of Dr. Pang at ¶ 4. Horizontal transmission is defined as the spread of an infectious agent from one group or individual to another, directly or indirectly.

Peer-reviewed studies document horizontal transmission of *Wolbachia* bacteria. The evidence of horizontal spread of *Wolbachia* shows that the bacteria go not only to sexual cells, but also to somatic cells (non-sexual cells of the body). *Wolbachia* can also live outside of intra-cellular systems for several months. Horizontal transmission of the *Wolbachia* bacteria can occur through mating, shared feeding sites, and serial predation of adult insects and of larva in standing water breeding sites. Studies that downplay the possibility of horizontal transmission based on *aedes aegypti* mosquitoes are flawed references because *aedes aegypti* are resistant to *Wolbachia*. *See* Dec. of Dr. Pang at ¶ 5; *see also Wolbachia infection in wild mosquitoes (Diptera: Culicidae): implications for transmission modes and host-endosymbiont associations in Singapore* (2020); *Wolbachia Horizontal Transmission Events in Ants: What Do We Know and What Can We Learn?* (2019); *The Intracellular Bacterium Wolbachia Uses Parasitoid Wasps as Phoretic Vectors for Efficient Horizontal Transmission* (2015).

b. Increased Pathogen Infection and Disease-Spreading Capability

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[7] A copy of Dr. Lorrin W. Pang's current Curriculum Vitae is attached as **Exhibit 9**.
[8] A copy of Dr. Pang's statement further detailing his concerns regarding the action called "Suppression of Invasive Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui" is attached as **Exhibit 10**.

Peer-reviewed studies have shown *Wolbachia* bacteria in mosquitoes to cause increased pathogen infection and to cause mosquitoes to become more capable of spreading diseases such as avian malaria and West Nile virus. West Nile virus can infect birds and humans. This project has the potential to cause the extinction of endangered native birds, and it could impact human health. *Wolbachia Can Enhance Plasmodium Infection in Mosquitoes: Implications for Malaria Control?* (2014); *Wolbachia Enhances West Nile Virus (WNV) Infection in the Mosquito Culex tarsalis* (2014). Dec. of Dr. Pang at ¶ 6.

The final EA also fails to adequately address the accidental release of lab-bred *Wolbachia*-infected females who bite, breed, and spread disease. Further, male mosquitoes transmit bacteria and pathogens to females. Infected females can spread disease to birds, other animals, and humans. Further, the final EA's assertion that released mosquitoes pose no risk to human health is based on unsound science. An example of this includes a 2010 article (by Popovici et al.) cited in the FEA that has been discredited by the EPA. *See Assessing key safety concerns of a Wolbachia-based strategy to control dengue transmission by Aedes mosquitoes* (2010); *April 24-26, 2018, Meeting of the Human Studies Review Board; April 24-26, 2018, EPA Human Studies Review Board Meeting Report.*

c. Biopesticide Wind Drift

The final EA fails to address biopesticide wind drift – the movement of biopesticide mosquitoes through wind to unintended areas. Mosquitoes carried on the wind into and out of the release sites of the project area have not been factored into the math model or the overall plan. *See* Dec. of Dr. Pang at ¶ 7.

d. Unexpected Evolutionary Events and Population Replacement

The final EA fails to adequately address the potential for the release of biopesticide mosquitoes to cause unexpected evolutionary events and population replacement. *Wolbachia infection in wild mosquitoes (Diptera: Culicidae): implications for transmission modes and host-endosymbiont associations in Singapore* (2020); *Wolbachia-mediated sterility suppresses Aedes aegypti populations in the urban tropics* (2021). *See* Dec. of Dr. Pang at ¶ 8.

4. Scope of the Project

The scope, risks, and experimental nature of the project require detailed, comprehensive studies and documentation of the impacts to our native birds, wildlife, environment, and public health. In the instant case, this impact is more likely than not to happen, given the sheer scope of the project. Due to the limited information provided in the FEA/FONSI, it is hard to determine exactly what the number of mosquitoes released would actually be if all phases of the project are implemented. However, Plaintiffs estimate that the experiment could result in over 807 billion mosquitoes released in one of the most unique and fragile ecosystems in the world. The sheer size of the experiment makes it more likely than not that there will be significant environmental impact.

B. FAILURE TO COMPLY WITH HAWAII ADMINISTRATIVE RULES AND DENIAL OF DUE PROCESS

1. Defendant DLNR Did not Comply with HAR §§ 11-200.1-20

In the "Statement of Compliance" in their FEA dated March 24, 2023, Defendant DLNR inaccurately states that "[t]he document attached here serves as the DLNR's final EA and has been revised and adjusted as needed to meet Hawai'i Revised Statutes (HAR) Chapter 343 compliance." Ex. 2 at p. vii.

HAR § 11-200-1-20 "Public review and response requirements for draft environmental assessments" provides, in relevant part:

> (c) **For agency actions, the proposing agency shall,** and for applicant actions, **the applicant shall: respond in the final EA in the manner prescribed in this section to all substantive comments received or postmarked during the statutorily mandated review period, incorporate comments into the final EA as appropriate, and include the comments and responses in the final EA. In deciding whether a written comment is substantive, the proposing agency or applicant shall give careful consideration to the validity, significance, and relevance of the comment to the scope, analysis, or process of the EA, bearing in mind the purpose of this chapter and chapter 343, HRS**. Written comments deemed by the proposing agency or applicant as non-substantive and to which no response was provided shall be clearly indicated.
>
> (d) Proposing agencies and applicants shall respond in the final EA to all substantive comments in one of two ways, or a combination of both, so long as each substantive comment has clearly received a response:
>
> > (1) By grouping comment responses under topic headings and addressing each substantive comment raised by an individual commenter under that topic heading by issue. **When grouping comments by topic and issue, the names of commenters who raised an issue under a topic heading shall be clearly identified in a distinctly labeled section with that topic heading.** All substantive comments within a single comment letter must be addressed, but may be addressed throughout the applicable topic areas with the commenter identified in each applicable topic area. **All comments, except those described in subsection (e), must be appended in full to the final EA**; or
> >
> > (2) By providing a separate and distinct response to each comment **clearly identifying the commenter and the comment receiving a response for each comment letter submitted. All comments, except those described in subsection (e), must either be included with the response or appended in full to the final EA.**

Haw. Code R. 11-200.1-20 (Weil)(**emphasis added**)

In their FEA, Defendant DLNR appears to have chosen the first response method listed above, however they did not include the comments that they received as a part of each response and did not append the comments in full to their FEA. *See* Ex. 1 – Appendix H, pp. H-1 – H-18. Defendants also did not clearly identify the names of commenters who raised the issue addressed in a distinctly labeled section with the topic heading. *Id*. Bearing in mind one of the main two purposes of chapter 343 is public participation during the review process, this failure to comply with the provision of the HAR further bolsters Plaintiffs chances of success on the merits in showing that Defendant BLNR's acceptance of the final EA and FONSI for the proposed biopesticide mosquito project violated the letter and purpose of HEPA. *See* HRS §343-1.

2. Due Process

The BLNR's approval of the final EA and FONSI immediately following the Board's improper addition to the March 24, 2023 agenda of Plaintiff Lia's verbal request for a contested case hearing on behalf of Plaintiff Hawaiʻi Unites, and the Board's subsequent vote to deny Plaintiffs' request without having received or reviewed Plaintiffs' petition for a contested case hearing, violate the letter and purpose of HEPA as well as fundamental requirements of administrative procedure and due process. Defendants' violations in this instance again worked to nullify HEPA's fundamental purpose to "ensure that environmental concerns are given appropriate consideration in decision making" so that "environmental consciousness is enhanced, cooperation and coordination are encouraged, and public participation during the review process benefits all parties involved and society as a whole." *Id*.

Plaintiffs argue that their procedural standing is grounded in the Hawaiʻi constitutional provision, article XI, section 9, which guarantees a "clean and healthful environment" which creates a private right of action.

Appropriate consideration and public participation have both been lacking or denied in the instant case, where the proposal involves a massive experiment with no meaningful mitigation plan in place if things don't go according to plan. It is therefore essential to have a high level of trust and confidence that the planned action has been thoroughly assessed and evaluated. Based on the foregoing facts and authority, Plaintiffs, as shown herein, have satisfied the first test that they are likely to prevail on the merits. The second test is also satisfied.

VI. PLAINTIFFS HAVE AND WILL CONTINUE TO SUFFER IRREPERABLE INJURY WITHOUT THE TRO

With every release it is unknown whether the experiment will result in unintended consequences, any of which could potentially threaten public health and the environment. What is known is that the science shows that as long as infected mosquitoes are being released, the project is a current and ongoing threat of causing possible irreparable injury to the fragile ecosystem of Maui. The precautionary principle calls for further study of the potential for collateral damage resulting from the experiment.

Plaintiff here seeks injunctive relief not only due to the likelihood of significant impact on the environment, but the lack of biosecurity protocols if project plans go awry or there is an unintended transfer of *Wolbachia* bacteria. In other words, there is no comprehensive mitigation plan in place and no "putting the genie back in the bottle" if something goes wrong. The only mention of mitigation in the FEA in reference to the experimental biopesticide mosquito releases involves "develop[ing] mitigation measures" - essentially a "plan to come up with a plan." The oversight of such potentially catastrophic, irreversible unintended consequences and lack of any serious plans for dealing with possibilities such as horizontal transfer underscores not only the threat to the fragile ecosystem of Maui, but the need for further study - namely a full environmental impact statement.

A. The Requested Relief Sought By Plaintiffs Cannot Be Replaced With Monetary Damages As To Deprive Plaintiffs Of Fair And Reasonable Redress

Under the balance of irreparable harm analysis, a TRO should be issued to preserve the status quo. *See Wahba, LLC v. USRP (Don), LLC*, 106 Haw. 466, 472, 106 P.3d 1109, 1115 (2005). The term "status quo" has been defined as ". . . the last actual, peaceable, noncontested condition which preceded the controversy . . ." *Porter v. K.S. Partnership*, 627 P.2d 836 (Montana 1981)(other citations omitted).

It is recognized that, "[I]njury is irreparable where it is such a character that a fair and reasonable redress may not be had in a court of law." *Penn v. Transportation Lease Hawai'i, Ltd.*, 2 Haw.App. 272, 276, fn.1, 630 P.2d 646 (Haw.App. 1981). It is further recognized that where monetary damages would be inadequate to compensate the Plaintiffs, an injunction should issue. *Klausmeyer v. Makaha V.F. Ltd.*, 41 Haw. 287, 340 (Haw. 1956). In *Klaumeyer*, the Hawai'i Supreme Court held that:

> . . . an injury is irreparable, within the law of injunctions, where it is of such a character that a fair and reasonable redress may not be had in a court of law, so that to refuse the injunction would be a denial of justice; where, in other words, from the nature of the act, or from the circumstances surrounding the person injured, or from the financial condition of the person committing it, it cannot be readily, adequately, and completely compensated with money. *** the term 'irreparable damage' does not have reference to the amount of damage caused, but rather to the difficulty of measuring the amount of damages inflicted . . . .
> Id. at 340.

Plaintiffs are seeking injunctive relief in their Complaint filed herein. Under the facts of this case, Plaintiffs have raised substantial questions as to why the proposed experiment *is likely to have a significant effect* on the environment and as such, the status quo should be preserved until such time as an environmental impact statement, studying the feasibility of the experiment, including consideration of all of the critical aspects of the proposed project in order to determine possible long-term detrimental consequences to island ecologies, to native birds, and public health, can be completed.

VII. THE REQUESTED INJUNCTIVE RELIEF WILL NOT SUBSTANTIALLY INJURE OTHERS

"In essence, a TRO is a prohibitory form of injunction. In a prohibitory injunction, the matter complained of is a consequence of present conduct and the injunction simply orders a defendant to refrain from engaging in the designated acts." *Wahba, LLC v. USRP (Don), LLC*, 106 Haw. 466, 472, 106 P.3d 1109, 1115 (2005)(internal citations omitted). Further, "the more the balance of irreparable damage favors issuance of the injunction, the less the party seeking the injunction has to show the likelihood of success on the merits." *Nuuanu Valley Ass'n v. City & Cnty. of Honolulu*, 119 Haw. 90, 106, 194 P.3d 531, 547 (2008), as corrected (Nov. 25, 2008).

The scope of the requested TRO and preliminary injunction is intended to avoid harm to others or the environment. The status quo in the instant case means that the release of incompatible mosquitoes would not occur, that is, no biopesticide experimentation, in essence the "no-action" alternative as detailed in Defendants' FEA/FONSI and the condition which preceded the proposed action. Defendant DLNR's assertion that "the mosquitoes that carry this (avian malaria) disease would remain uncontrolled" as a result of the "no-action" alternative fails to include the important caveat that the biopesticide mosquito release is an experiment. The TRO and preliminary injunction relief requested is simply aimed to halt Defendant's biopesticide experiment before a comprehensive study can be conducted and proper mitigation and biosecurity protocols developed and put in place.

This Honorable Court is authorized to issue a temporary restraining order based on the cited facts and authorities presented herein. Plaintiffs argue that the final test for the issuance of an injunction is also satisfied.

VIII. THE REQUESTED INJUNCTIVE RELIEF FURTHERS AND DOES NOT DISSERVE THE PUBLIC INTEREST

This requested TRO and preliminary injunction furthers the public interest by ensuring accountability and preserving the safety and health of the natural environment and the people of Maui. It also furthers the public interest in ensuring that experiments are properly studied and implemented, including with appropriate mitigation measures. Hawai‘i Unites is dedicated to the conservation and protection of Hawai‘i’s environment and natural resources, including endangered birds, and it is precisely for this reason that they are requesting injunctive relief until such time as adequate study of this experiment can be conducted.

Our Hawai‘i State Constitution provides in Article IX, Section 8, entitled, “Preservation of a Healthful Environment,” that, “[t]he State shall have the power to promote and maintain a healthful environment, including the prevention of any excessive demands upon the environment and the State's resources.” Furthermore, Article IX, Section 1, entitled “Public Health” states, “[t]he State shall provide for the protection and promotion of the public health.” In addition to the constitutional protections stated above, the Plaintiffs in this case have a constitutional right to seek injunctive relief to protect environmental rights, as recreational users of the subject area. Article XI, Section 9, entitled, “Environmental Rights” states:

> **Each person has the right to a clean and healthful environment, as defined by laws relating to environmental quality, including control of pollution and conservation, protection and enhancement of natural resources.** Any person may enforce this right against any party, public or private, through appropriate legal proceedings, subject to reasonable limitations and regulation as provided by law. (**Emphasis added**).

IX. CONCLUSION

For the aforementioned reasons, Plaintiffs ask the Court to grant the above-requested injunctive relief.

DATED: Honolulu, Hawai‘i, June 20, 2023.

/s/ Timothy Vandeveer

Margaret (Dunham) Wille
Timothy Vandeveer

Attorneys for Plaintiffs
Hawai‘i Unites and Tina Lia

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IN THE CIRCUIT COURT OF THE FIRST CIRCUIT

STATE OF HAWAI‘I

HAWAI‘I UNITES, a 501(c)(3) nonprofit corporation; TINA LIA, an individual, Plaintiffs, v. BOARD OF LAND AND NATURAL RESOURCES, STATE OF HAWAI‘I, and DEPARTMENT OF LAND AND NATURAL RESOURCES, STATE OF HAWAI‘I, Defendants.	Civil No. 1CCV-23-0000594 (JMT) (Environmental Court) **DECLARATION OF COUNSEL**

DECLARATION OF TIMOTHY VANDEVEER

I, TIMOTHY VANDEVEER, under pain of perjury of law, do hereby state and declare as follows:

1. I am an adult resident of the State of Hawaiʻi.

2. I am licensed to practice law in the State of Hawaiʻi.

3. I am a member of the law firm of Margaret Wille & Associates LLLC, and one of the attorneys for Plaintiffs Hawaiʻi Unites and Tina Lia in the above-captioned case.

4. On June 5, 2023 the Complaint was served on the Department of the Attorney General as pursuant to Hawaiʻi Rules of Civil Procedure ("HRCP") Rule 4(d)(4).

5. As of this filing, a responsive pleading has not been filed by Defendants.

AUTHENTICATION

1. Attached hereto as **Exhibit 1** is a true and correct copy of the Environmental Assessment and Finding of no Significant Impact for the action entitled "Suppression of Non-Native Wild Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui" dated March 24, 2023.

2. Attached hereto as **Exhibit 2** is a true and correct copy of the DLNR "Exemption Notice Regarding Preparation of an environmental assessment" dated June 17, 2023.

FURTHER DECLARANT SAYETH NAUGHT

This Declaration is based upon my personal knowledge or as otherwise indicated, and I am competent to testify as to the truth of the statements contained herein.

DATED: Honolulu, Hawaiʻi, June 20, 2023.

Signed: ***/s/ Timothy Vandeveer***
Timothy Vandeveer

MARGARET WILLE & ASSOCIATES LLLC
Margaret Wille #8522
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IN THE CIRCUIT COURT OF THE FIRST CIRCUIT

STATE OF HAWAI‘I

HAWAI‘I UNITES, a 501(c)(3) nonprofit corporation; TINA LIA, an individual, Plaintiffs, v. BOARD OF LAND AND NATURAL RESOURCES, STATE OF HAWAI‘I, and DEPARTMENT OF LAND AND NATURAL RESOURCES, STATE OF HAWAI‘I, Defendants.	Civil No. 1CCV-23-0000594 (JMT) (Environmental Court) **DECLARATION OF TINA LIA**

DECLARATION OF TINA LIA

I, TINA LIA, under pain of perjury and law, do hereby state and declare as follows:

1. I am a resident of the County of Maui in the State of Hawai‘i.

2. I am over the age of eighteen (18).

3. I am the founder and current Board President of Plaintiff Hawai‘i Unites and also an individual Plaintiff in the above-captioned case.

4. I commented on the proposed action entitled “Suppression of Non-Native Wild Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui” throughout the Draft and Final Environmental review process.

5. On March 24, 2023, the Final Environmental Assessment/Finding of No Significant Impact (“FEA/FONSI”) submitted by Defendant DLNR for the proposed multi-agency action entitled “Suppression of Non-Native Wild Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui” was accepted by Defendant BLNR.

6. On March 24, 2023, the BLNR denied Plaintiff Tina Lia’s request (on behalf of Plaintiff Hawai‘i Unites) for a contested case hearing. This denial came before the BLNR voted on whether to accept the FEA/FONSI submitted by Defendant DLNR. The reason provided for the denial for a contested case on “the board’s action to either accept or deny the Environmental Assessment” was that “your remedy…” is “you can sue under Chapter 343.”

7. On April 8, 2023, the FEA/FONSI submitted by Defendant DLNR for the proposed experiment was published in The Environmental Notice.

8. On May 12, 2023, the BLNR again denied Plaintiff Tina Lia’s request (on behalf of Plaintiff Hawai‘i Unites) for a contested case hearing. The reasons given for the second denial were that “there is already a piece of litigation going on by the same group before the courts, and I think this would be a duplication of it” and “because in the write-up, it explains that the petitioner did not assert a property interest that would trigger the due process analysis, and also that the recommendation has been to seek relief in the environmental court, which the applicant said she has.”

9. There has been no disclosure as to what type of mosquito is being transported, where the mosquitoes are being transported from, and whether or not the mosquitoes are being tested for pathogens prior to transport for the “mark release recapture” studies.

AUTHENTICATION

1. Attached hereto as **Exhibit 3** is a true and correct copy of my written testimony to the Board of Land and Natural Resources dated August 24, 2022 in regard to the proposed action entitled "Suppression of Non-Native Wild Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui."

2. Attached hereto as **Exhibit 4** is a true and correct copy of my written comment in opposition to the Draft Environmental Assessment for the "Mosquito Suppression Environmental Assessment - December 2022" for the island of Maui dated January 21, 2023.

3. Attached hereto as **Exhibit 5** is a true and correct copy of my written testimony opposing the request for approval of the Final Environmental Assessment for the planned biopesticide mosquito releases on Maui dated March 22, 2023.

4. Attached hereto as **Exhibit 6** is a true and correct copy of an article from Hawaiʻi Public Radio entitled "Hawaiʻi Unites sues DLNR over mosquito suppression plan to save native birds" dated May 11, 2023.

5. Attached hereto as **Exhibit 7** is a true and correct copy of a Facebook post from *Birds, Not Mosquitoes* entitled "The Final Environmental Assessment for East Maui was approved…now what??" dated May 17, 2023.

6. Attached hereto as **Exhibit 8** is a true and correct copy of an online article from *Birds, Not Mosquitoes* entitled "Giving Thirst Traps a Whole New Meaning" dated June 2, 2023.

FURTHER DECLARANT SAYETH NAUGHT

This Declaration is based upon my personal knowledge or as otherwise indicated, and I am competent to testify as to the truth of the statements contained herein.

DATED: Kīhei, Hawaiʻi, June 20, 2023.

Signed: ______________
Tina Lia

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IN THE CIRCUIT COURT OF THE FIRST CIRCUIT

STATE OF HAWAIʻI

HAWAIʻI UNITES, a 501(c)(3) nonprofit corporation; TINA LIA, an individual, Plaintiffs, v. BOARD OF LAND AND NATURAL RESOURCES, STATE OF HAWAIʻI, and DEPARTMENT OF LAND AND NATURAL RESOURCES, STATE OF HAWAIʻI, Defendants.	Civil No. 1CCV-23-0000594 (JMT) (Environmental Court) **DECLARATION OF** **DR. LORRIN W. PANG**

DECLARATION OF DR. LORRIN W. PANG

I, DR. LORRIN W. PANG, under pain of perjury and law, do hereby state and declare as follows:

1. I am a resident of the County of Maui in the State of Hawaiʻi.

2. I am over the age of eighteen (18).

3. I believe that the intent to save rare birds is sound and if the action called "Suppression of Invasive Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui" ("the Action") goes as planned, this would be a valuable tool for future interventions. However, with new life forms coming to the islands, there is too much potential for unexpected, dangerous, irreversible "evolutionary" events. This is especially true when the new organisms cannot be contained to their target ecosystem.

4. I have been compiling studies documenting horizontal *Wolbachia* bacterial spread, and I'm concerned about the potential for significant adverse outcomes of the Action.

5. The evidence of horizontal spread of *Wolbachia* bacteria (documented in peer-reviewed studies) shows that the bacteria go not only to sexual cells, but also to somatic cells (non-sexual cells of the body). *Wolbachia* can also live outside of intra-cellular systems for several months. Horizontal transmission of the *Wolbachia* bacteria can occur through mating, shared feeding sites, and serial predation of larva in standing water breeding sites. Studies that downplay the possibility of horizontal transmission based on aedes aegypti mosquitoes are flawed references because aedes aegypti are resistant to *Wolbachia*.

6. Peer-reviewed studies have shown *Wolbachia* bacteria in mosquitoes to cause increased pathogen infection and to cause mosquitoes to become more capable of spreading diseases such as avian malaria and West Nile virus. West Nile virus can infect birds and humans. This project has the potential to cause the extinction of endangered native birds, and it could impact human health.

7. The final EA for the Action failed to address biopesticide wind drift – the movement of biopesticide mosquitoes through wind to unintended areas. Mosquitoes carried on the wind into and out of the release sites of the project area have not been factored into the math model or the overall plan.

8. The final EA for the Action failed to adequately address the potential for the release of biopesticide mosquitoes to cause unexpected evolutionary events and population replacement.

AUTHENTICATION

1. Attached hereto as **Exhibit 9** is a true and correct copy of my CV/Resume.

2. Attached hereto as **Exhibit 10** is a true and correct copy of a statement further detailing my concerns regarding the action called "Suppression of Invasive Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui".

3. Attached hereto as **Exhibit 11** is a true and correct copy of an undated draft research article that I co-authored with other scientists entitled "Barriers with valve mechanisms are predicted to protect crops from Rat Lungworm disease transmitted by slug hosts" highlighting how population changes are often determined by pathways set up in parallel, not just sequentially; that models must be set up by the initial assumptions with the math derivations of the formula to follow; that the models must predict intuitively the changes in populations when extreme limits are reached (steady state and non-steady state); that tracking units of the parameters of the math expression is a very useful practice in complicated models. Because this draft article is awaiting publication and the copyright does not belong to me, I asked that it be filed under seal.

FURTHER DECLARANT SAYETH NAUGHT

This Declaration is based upon my personal knowledge or as otherwise indicated, and I am competent to testify as to the truth of the statements contained herein.

DATED: Wailuku, Hawai'i, June 18, 2023.

Signed: ______________________
Dr. Lorrin W. Pang

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JOSH GREEN, M.D.
GOVERNOR | KE KIAʻĀINA

SYLVIA LUKE
LIEUTENANT GOVERNOR | KA HOPE KIAʻĀINA

STATE OF HAWAIʻI | KA MOKUʻĀINA ʻO HAWAIʻI
DEPARTMENT OF LAND AND NATURAL RESOURCES
KA ʻOIHANA KUMUWAIWAI ʻĀINA

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DAWN N.S. CHANG
CHAIRPERSON
BOARD OF LAND AND NATURAL RESOURCES
COMMISSION ON WATER RESOURCE MANAGEMENT

LAURA H.E. KAAKUA
FIRST DEPUTY

M. KALEO MANUEL
DEPUTY DIRECTOR - WATER

AQUATIC RESOURCES
BOATING AND OCEAN RECREATION
BUREAU OF CONVEYANCES
COMMISSION ON WATER RESOURCE MANAGEMENT
CONSERVATION AND COASTAL LANDS
CONSERVATION AND RESOURCES ENFORCEMENT
ENGINEERING
FORESTRY AND WILDLIFE
HISTORIC PRESERVATION
KAHOOLAWE ISLAND RESERVE COMMISSION
LAND
STATE PARKS

March 28, 2023

Director
State of Hawai'i
Office of Planning and Sustainable Development
Environmental Review Program
235 South Beretania Street, Room 702
Honolulu, HI 96813

RE: ENVIRONMENTAL ASSESSMENT AND FINDING OF NO SIGNIFICANT IMPACT FOR SUPPRESSION OF NON-NATIVE WILD MOSQUITO POPULATIONS TO REDUCE TRANSMISSION OF AVIAN MALARIA TO THREATENED AND ENDANGERED FOREST BIRDS ON EAST MAUI

Dear Director:

With this letter, the State of Hawai'i Department of Land and Natural Resources hereby transmits the final Environmental Assessment and Finding of No Significant Impact (EA-FONSI) for "Suppression of Non-native Wild Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui" for publication in the next available edition of The Environmental Notice.

In addition to this letter, the online Environmental Review Program (ERP) Publication Form has been submitted through the ERP website.

Should you have any questions, please contact Cynthia King of the Division of Forestry and Wildlife at (808) 587-0019 or cynthia.b.king@hawaii.gov.

Sincerely,

DGS

Dawn N. S. Chang
Chairperson

Signature:

Email: david.g.smith@hawaii.gov

EXHIBIT 1

From: webmaster@hawaii.gov
To: DBEDT OPSD Environmental Review Program
Subject: New online submission for The Environmental Notice
Date: Wednesday, March 29, 2023 11:16:53 AM

Action Name

Suppression of Invasive Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui

Type of Document/Determination

Final environmental assessment and finding of no significant impact (FEA-FONSI)

HRS §343-5(a) Trigger(s)

- (1) Propose the use of state or county lands or the use of state or county funds
- (2) Propose any use within any land classified as a conservation district

Judicial district

Maui - multiple districts

Tax Map Key(s) (TMK(s))

2-1-6-999:999; 2-2-4-016:999; 2-2-4-013:999; 2-2-4-016:004; 2-1-3-001:002; 2-1-4-001:003; 2-1-5-001:001; 2-1-5-001:002; 2-1-5-011-002; 2-1-5-011:007; 2-1-5-011:008; 2-1-6-001:001; 2-1-6-001:002; 2-1-6-001:003; 2-1-6-001:004; 2-1-6-001:005; 2-1-6-001:007; 2-1-6-001:009; 2-1-6-002:001; 2-1-6-002:002; 2-1-6-002:003; 2-1-6-002-004; 2-1-6-002:005; 2-1-6-002:006; 2-1-6-002:007; 2-1-6-002:008; 2-1-6-002:009; 2-1-6-002:010; 2-1-6-002:011; 2-1-6-002:012; 2-1-6-003:001; 2-1-6-003:002; 2-1-6-003:003; 2-1-6-003:007; 2-1-6-003:008; 2-1-6-003:009; 2-1-6-003:010; 2-1-6-003:013; 2-1-6-003:015; 2-1-6-003:016; 2-1-6-003:017; 2-1-6-003:021; 2-1-6-003:022; 2-1-6-003:023; 2-1-6-003:025; 2-1-6-003:026; 2-1-6-003:027; 2-1-6-003:028; 2-1-6-003:029; 2-1-6-004:001; 2-1-6-004:002; 2-1-6-004:006; 2-1-6-004:007; 2-1-6-004:008; 2-1-6-004:020; 2-1-6-005:013; 2-1-6-005:016; 2-1-6-005:022; 2-1-6-005:024; 2-1-6-005:028; 2-1-6-005:035; 2-1-6-006:001; 2-1-6-006:002; 2-1-6-006:003; 2-1-6-006:004; 2-1-6-006:005; 2-1-6-006:006; 2-1-6-006:007; 2-1-6-006:012; 2-1-6-006:014; 2-1-6-006:015; 2-1-6-006:016; 2-1-6-006:020; 2-1-6-006:021; 2-1-6-006-023; 2-1-6-010:004; 2-1-6-010:006; 2-1-6-010:007; 2-1-7-001:001; 2-1-7-001:002; 2-1-7-001:033; 2-1-7-001:034; 2-1-7-004:016; 2-1-3-001:001; 2-1-7-001:003; 2-1-7-004:004; 2-1-6-005:007; 2-2-4-013:073; 2-2-4-013:185; 2-2-4-016:001; 2-2-4-016:003; 2-2-4-033:010; 2-2-4-033:013; 2-2-7-015:001; 2-2-8-008:001; 2-2-8-008:008; 2-2-8-008:009; 2-2-9-014:001; 2-1-2-004-013; 2-1-2-004:005; 2-1-1-002:002; 2-1-2-004:007; 2-1-3-001:003; 2-1-8-001:007; 2-2-3-005:001; 2-1-7-004:006; 2-1-7-002:073; 2-1-4-001:001; 2-1-1-001:050; 2-1-1-001:044; 2-2-3-005:004; 2-2-3-005:003; 2-1-6-001:006; 2-1-6-010:002; 2-2-8-008:007; 2-2-7-015:003; 2-1-6-010:001; 2-2-4-016:002; 2-1-6-010:008; 2-2-3-005:999;

Action type

Agency

Proposing/determining agency

Department of Land and Natural Resources

Agency contact name

Cynthia King

Agency contact email (for info about the action)

cynthia.b.king@hawaii.gov

Agency contact phone

(808) 587-0019

Agency address

1151 Punchbowl Street
Room 325
Honolulu, Hawaii 96813
United States
Map It

Was this submittal prepared by a consultant?

Yes

Consultant

Tetra Tech

Consultant contact name

Keith Pohs

Consultant contact email

Keith.Pohs@tetratech.com

Consultant contact phone

(928) 600-2958

Consultant address

350 Indiana Street
Golden, Colorado 80401
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Map It

Action summary

Populations of endangered Hawaiian forest birds have decreased substantially over the last 20 years, and it is predicted that at least two more species could be extinct in the next 2 to 10 years. The primary cause of declines in native forest birds is avian malaria, which is spread by non-native mosquitoes. The purpose of the project is to substantially suppress or eliminate non-native mosquitoes in threated and endangered forest bird habitat on East Maui, thereby reducing the risk of their extinction and contributing to the recovery of these species. The approved action consists of repeatedly releasing incompatible male mosquitoes, which will reduce the reproductive potential of wild mosquitoes. When conducted repeatedly over time, releases of incompatible mosquitoes will suppress the wild mosquito population by at least 90%. The primary tool used to release mosquitoes on the landscape will be drones and, to a lesser extent, helicopters and ground-based methods.

Reasons supporting determination

See Appendix G – DLNR HEPA Significance Criteria Analysis

Attached documents (signed agency letter & EA/EIS)

- ERP-letter_East-Maui-EA-FONSI_03282023-part-1-signed.pdf
- DLNR-Mosquito-Suppression_EA_Main-Body_Final_Complete_03242023.pdf

Shapefile

- The location map for this Final EA is the same as the location map for the associated Draft EA.

Action location map

- East_Maui_MosquitoEA_Project_Area.zip

Authorized individual

Cynthia King

Authorization

- The above named authorized individual hereby certifies that he/she has the authority to make this submission.

Suppression of Invasive Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui

Final Environmental Assessment

Hawaiʻi Department of Land and Natural Resources

March 24, 2023

STATEMENT OF COMPLIANCE

This Environmental Assessment (EA), titled "Suppression of Invasive Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui", was produced through a cooperative effort between the National Park Service (NPS) and the Hawaiʻi Department of Land and Natural Resources (DLNR). The NPS served as lead for drafting this EA for National Environmental Policy Act (NEPA) compliance, and DLNR served as the cooperating agency. The document attached here serves as the DLNR's final EA and has been revised and adjusted as needed to meet Hawaiʻi Revised Statutes (HAR) Chapter 343 compliance.

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ACRONYMS AND ABBREVIATIONS

AGL	above ground level
CEQ	Council on Environmental Quality
dBA	A-weighted decibel
DLNR	Hawai‘i Department of Land and Natural Resources
DOFAW	Hawai‘i Division of Forestry and Wildlife
EA	Environmental Assessment
EPA	Environmental Protection Agency
ESA	Endangered Species Act
FAA	Federal Aviation Administration
HEPA	Hawai‘i Environmental Policy Act
IIT	Incompatible Insect Technique
IVUMC	Interagency Visitor Use Management Council
LOS	line of sight
LZ	landing zone
MFBRP	Maui Forest Bird Recovery Project
NEPA	National Environmental Policy Act
NPS	National Park Service
OGG	Helibase - Kahului Airport
OSHA	Occupational Safety and Health Administration
PEPC	Planning, Environment, and Public Comment
TNC	The Nature Conservancy
USFWS	U.S. Fish and Wildlife Service
USGS	U.S. Geological Survey

CHAPTER 1: PURPOSE OF AND NEED FOR ACTION

INTRODUCTION

This environmental assessment (EA) analyzes the impacts of the proposed action to suppress invasive mosquito populations with the goal of addressing the effects of avian malaria on threatened and endangered forest birds on Maui, Hawaiʻi versus a no-action alternative. The project area primarily consists of Haleakalā National Park and adjacent properties managed by the State of Hawaiʻi, The Nature Conservancy (TNC), and other private conservation lands. This EA has been prepared consistent with the National Environmental Policy Act (NEPA) and the Hawaiʻi Environmental Policy Act (HEPA) and provides compliance for project implementation on both federal and state lands.

Haleakalā National Park (the park) was established in 1916 and manages over 33,000 acres of federal land on the island of Maui. There are two districts in the park: the Summit District and the Kīpahulu District. The Summit District includes a portion of Haleakalā Highway (known as Crater Road within the park), Haleakalā Crater, Kaupō Gap, and Nuʻu. The Kīpahulu District includes ʻOheʻo Gulch, Kīpahulu Valley, Manawainui, and Kaʻāpahu. Recognized as an International Biosphere Reserve, the park's stated purpose is: *"For the inspiration of current and future generations, [the park] protects a wild volcanic landscape with a wild array of fragile and diverse native ecosystems, including plant and animal species found nowhere else on Earth. Our stewardship perpetuates the unique and continuing connections between Hawaiian culture and this sacred and evolving land"* (NPS 2015; see Appendix A: References). The National Park Service (NPS) is the lead agency for this EA.

The State of Hawaiʻi Department of Land and Natural Resources (DLNR) Division of Forestry and Wildlife (DOFAW) manages forest and wildlife resources, including plant and wildlife habitats and native ecosystems, lands designated as forest reserves, natural area reserves, wildlife sanctuaries, and game management areas, and partners broadly for the protection and management of natural resources on agency and private land throughout the state. DOFAW reserves on East Maui include the Koʻolau Forest Reserve, Hāna Forest Reserve, Hanawī Natural Area Reserve, Kīpahulu Forest Reserve, and Makawao Forest Reserve. DLNR is serving as a cooperating agency for this project.

TNC manages lands in the Waikamoi Preserve, while several private partners, including East Maui Irrigation, Mahi Pono, LLC., and Haleakala Ranch, manage adjacent properties on East Maui to protect native ecosystems and watersheds. TNC, NPS, DLNR, and partners would work together to implement this mosquito suppression project on these lands. These cooperative actions do not alter the jurisdiction of each agency, organization, or private landowner; rather, this collaboration is the most efficient way to achieve the goals of the project.

PROJECT BACKGROUND

More than 30 species of forest birds known as Hawaiian honeycreepers have gone extinct over the last 20–200 years (Banko and Banko 2009, Elphick et al. 2010, USFWS 2021). Many of the remaining 17 species are considered at risk, with some populations exhibiting rapid and recent declines (Paxton et al. 2016, Judge et al. 2021). The primary cause of these declines is avian malaria, a non-native disease that is caused by a parasite (*Plasmodium relictum*) spread by the invasive southern house mosquito (*Culex quinquefasciatus*). Hawaiian honeycreepers have little resistance to avian malaria, and most cannot survive infection (Atkinson et al. 1995, LaPointe and Atkinson 2009). Until recently, honeycreepers were able to persist in high elevation forests where it is too cold for mosquitoes and the avian malaria parasite to reproduce. Recent climate changes have allowed mosquitoes and associated avian malaria to start invading these upper elevation forests on Maui, killing native forest birds in their last remaining locations. At least two endangered bird species on East Maui, kiwikiu (Maui Parrotbill, *Pseudonestor xanthophrys*) and ʻākohekohe (*Palmeria dolei*), are expected to become extinct within two to fifteen years if avian malaria is left unchecked (Mounce et al. 2018, Paxton et al. 2022). There are currently fewer than 200 kiwikiu and fewer than 2,000 ʻākohekohe persisting in the wild, all of which are located within the project area of this EA on East Maui (Judge et al. 2021). Both species have declined by more than 70 percent over the last two decades. Four additional Hawaiian honeycreepers also reside on

East Maui: the threatened ʻiʻiwi (*Drepanis coccinea*), Maui ʻalauahio (only lives on Maui; *Paroreomyza montana*), Hawaiʻi ʻamakihi (*Chlorodrepanis virens*), and ʻapapane (*Himatione sanguinea*). These species are also affected by avian malaria and addressed in this EA.

The NPS and DLNR propose to reduce native forest bird mortality from avian malaria by suppressing southern house mosquito populations on East Maui. These non-native invasive mosquitoes are the only insect that transmits avian malaria in this area. The proposed action consists of repeatedly releasing incompatible male southern house mosquitoes (hereafter "incompatible mosquitoes"), which would prevent mosquitoes within the project area from being able to reproduce. This approach employs the incompatible insect technique (IIT), which uses a naturally occurring bacteria called *Wolbachia* that is present in many insect species on Maui. When male mosquitoes with an incompatible strain of *Wolbachia* are introduced to a population of female mosquitoes, mating is unproductive, thereby suppressing mosquito populations (Atyame et al 2015). When releases are done repeatedly over time, they further suppress the mosquito population and, in turn, would suppress transmission of avian malaria.

In response to comments received during public scoping, the following are key points regarding mosquitoes and the proposed action:

1. Male mosquitoes do not bite animals or humans. This project would only release male mosquitoes.
2. *Wolbachia* is already present in many insects in Hawaiʻi, including the southern house mosquito populations present on Maui. This project would release only male mosquitoes with a different strain of *Wolbachia* bacteria to that occurring in southern house mosquitoes in East Maui.
3. *Wolbachia* bacteria cannot transfer between animal species or to humans. Similarly, it cannot transfer between male mosquitoes and female mosquitoes; mosquitoes can only inherit *Wolbachia* from their mother.
4. The southern house mosquito, like all mosquitoes in Hawaiʻi, is an invasive species on Maui. It occupies higher elevations and cooler environments than other species of mosquitoes found on Maui. Other mosquito species would not expand their ranges in response to elimination of southern house mosquitoes.
5. Southern house mosquitoes are not an important source of food for native bats, birds, or other insects in Hawaiʻi.
6. Neither southern house mosquitoes nor *Wolbachia* bacteria are new organisms to Maui; this project would not result in introduction of any new species to the island.
7. The proposed use of IIT does not include genetic engineering techniques that result in genetically modified organisms (GMOs).

PURPOSE OF AND NEED FOR ACTION

The purpose of the project is to substantially suppress or eliminate southern house mosquitoes and, thus, avian malaria in threatened and endangered forest bird populations on East Maui, thereby reducing extinction risks and contributing to the recovery of these species. To prevent the extinction of threatened and endangered forest birds on East Maui, timely management action needs to be taken to control avian malaria. The populations of two endangered Hawaiian forest birds, kiwikiu and ʻākohekohe, have decreased by more than 70 percent over the last 20 years, and population projections predict their extinction in the next two to ten years (Mounce et al. 2018, Paxton et al. 2022). The avian malaria parasite and the mosquitoes that spread avian malaria are unable to successfully reproduce in cold environments, thus these two honeycreepers have been able to persist in high elevation native forest habitat on East Maui. Recently, increasing temperatures associated with climate change are allowing mosquito populations and avian malaria to expand into these high elevation native forests where some of the last populations of these forest birds remain. This expansion is the primary cause of these endangered species' rapid decline and threat of extinction (Fortini et al. 2015, Mounce et al. 2018, Judge et al. 2021, Paxton et al. 2022).

PROJECT AREA

The NPS and DLNR identified the project area through a collaborative process, during which all public lands within much of the current and historic ranges of threatened and endangered forest birds on East Maui were evaluated for inclusion. The project area (**Figure 1**) includes areas downslope from many birds' current ranges that may serve as high-density mosquito breeding grounds from which mosquitoes may move upward in elevation into native forest bird habitat. The upper elevation limit of the project area was defined by the boundary of the park along the north slope and Palikū Ridge between Pōhaku Pālaha and Kuiki, separating native forest from Haleakalā Crater. The lower limit of the project area, 1,969 feet above sea level, is the low elevation range of vulnerable native forest birds, such as the ʻapapane and ʻiʻiwi (Judge et al. 2019) except within the boundaries of the park in the lower Kīpahulu Valley and Kaʻapahu where the project area extends to sea level.The project area includes approximately 64,666 acres, including NPS land (12,042 acres), DLNR lands in forest reserves and natural area reserves (37,989 acres), adjacent lands privately managed in a conservation easement by TNC (8,606 acres), East Maui Irrigation Company, LLC (4,409 acres), Haleakala Ranch (393 acres), and Mahi Pono (1,227 acres) lands managed for conservation (**Table 1** and **Figure 1**).

TABLE 1: PROJECT AREA ACREAGE AND MANAGEMENT

Name	Management	Acres
Haleakalā National Park	NPS	12,042
Koʻolau Forest Reserve	DLNR/DOFAW	15,179
Hāna Forest Reserve	DLNR/DOFAW	10,679
Hanawī Natural Area Reserve	DLNR/DOFAW	7,713
Kīpahulu Forest Reserve	DLNR/DOFAW	2,318
Makawao Forest Reserve	DLNR/DOFAW	2,100
Waikamoi Preserve (TNC)	TNC	8,606
East Maui Irrigation Company, LLC	Private	4,409
Mahi Pono	Private	1,227
Haleakala Ranch	Private	393
TOTAL		**64,666**

ISSUES AND IMPACT TOPICS ANALYZED IN THIS ENVIRONMENTAL ASSESSMENT

This EA analyzes environmental consequences associated with the implementation of the proposed action or the no-action alternative. Issues and impact topics address the following resources and values: threatened and endangered wildlife species and wildlife species of concern, threatened and endangered plant species and state plant species at risk, wilderness character, acoustic environment, and visitor use and experience. Numerous other issues and impact topics were considered but dismissed from further analysis for reasons specified in "Appendix B: Issues, Impact Topics, and Alternatives Dismissed from Detailed Analysis."

The interdisciplinary team consulted with scientific experts and environmental planners from NPS, DLNR, U.S. Fish and Wildlife Service (USFWS), and U.S. Geological Survey (USGS) familiar with the native forest bird species and ecosystems of East Maui to determine which environmental issues would be carried forward for detailed analysis in the EA. The team also reviewed public scoping comments for additional insight on issues and impact topics relevant to this project. Details of the civic engagement and public scoping processes are available in Chapter 4 of this EA.

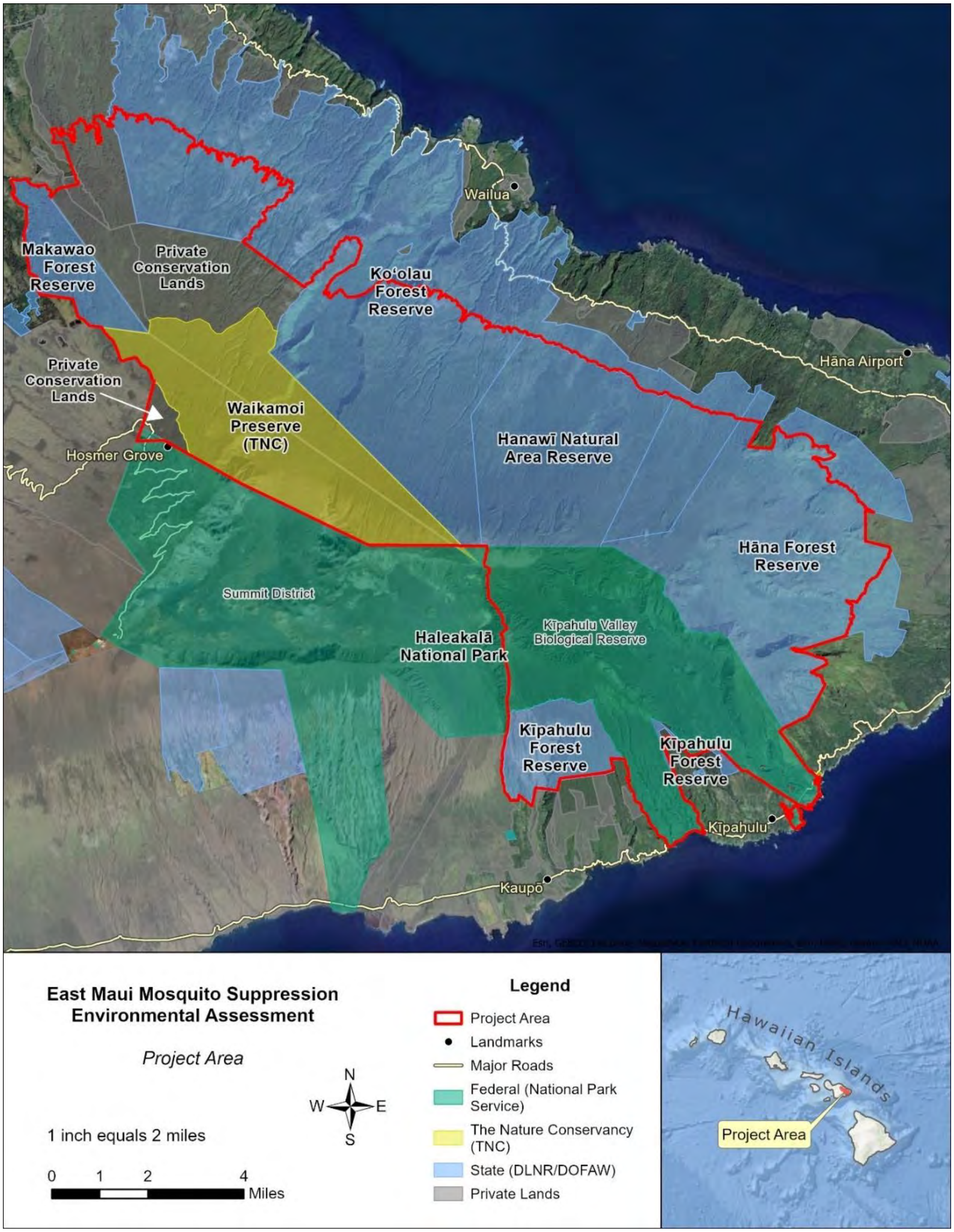

FIGURE 1: PROJECT AREA FOR RELEASE OF INCOMPATIBLE MOSQUITOES

CHAPTER 2: ALTERNATIVES

INTRODUCTION

This chapter describes alternatives for reducing mosquito populations and, thus, avian malaria transmission to threatened and endangered forest birds on East Maui, consistent with the purpose and need for action. Two alternatives are presented: the no-action alternative and the proposed action. Mitigation measures are included in the proposed action. Several other potential alternatives were considered and discussed during internal and public scoping but were dismissed from detailed analysis in this EA as described in "Appendix B: Issues, Impact Topics, and Alternatives Dismissed from Detailed Analysis."

NO-ACTION

Under the no-action alternative, release of incompatible mosquitoes would not occur. Although ongoing conservation and other management activities would continue on East Maui (e.g., fencing, removal of non-native ungulates and predators, invasive plant control), native forest birds would continue to be adversely affected by their primary threat, avian malaria, because the mosquitoes that carry this disease would remain uncontrolled.

PROPOSED ACTION

The NPS and DLNR propose to reduce threatened and endangered forest bird mortality from avian malaria by suppressing mosquito populations on East Maui. The proposed action was developed in consideration of park and state statutory missions and responsibilities; environmental factors; preliminary impact analysis; Native Hawaiian consultation and public input; existing infrastructure (such as helibases, landing zones [LZs], camps, trails, fence lines, and roads); and input from agency personnel, technical experts, and the public. The proposed action consists of repeatedly releasing incompatible male mosquitoes to reduce the reproductive potential of mosquitoes in the project area. This approach employs IIT, which as described in Chapter 1, uses a naturally occurring bacteria called *Wolbachia* that is present in the eggs and sperm of many insect species, including the southern house mosquito (Hilgenboecker et al. 2008, Bennett et al. 2012). When male mosquitoes with an incompatible strain of *Wolbachia* are introduced to a population of female mosquitoes, mating is unproductive, thereby suppressing mosquito populations (Atyame et al 2015). Releases under the proposed action must be conducted repeatedly over time to achieve and maintain significant suppression of the mosquito population, and like other similar mosquito suppression projects, this project has the potential to suppress the mosquito population by 90 percent or more (Beebe et al. 2021, Crawford et al. 2020, and Zheng et al. 2019). Monitoring mosquito populations would guide the frequency, number, and location of releases, and would need to continue for as long as the proposed action is implemented. The park would oversee implementation on federal lands and DLNR on state and private conservation lands or those managed by TNC.

Effective implementation of the proposed action would be dependent on the numbers and availability of lab-reared southern house mosquitoes that carry incompatible strains of *Wolbachia*. The proposed action would start with small scale on-the-ground or aerial releases of incompatible mosquitoes within the project area, where field teams would be able to monitor effectiveness of IIT implementation. The majority of the project area is inaccessible by ground, and thus would require uncrewed aircraft systems (i.e., drones) to implement large-scale mosquito releases throughout the project area. Releases via helicopter may be required as a short-term (up to two months), temporary release method if drones are not available. Mosquito release technologies would resemble those established for IIT (or related techniques) suppression projects of the yellow fever mosquito (*Aedes aegypti*), which have been successfully implemented in the United States and other parts of the world (Mains et al. 2016, Bouyer et al. 2020, Crawford et al. 2020, Moreira et al. 2009, Hoffman et al. 2011, Ritchie et al. 2014, Dutra et al. 2016). Releases would be expected to continue until southern house mosquito populations are significantly reduced and the status of threatened and endangered forest birds stabilizes, or until new mosquito population suppression techniques are developed. Releases may be conducted in a piecemeal fashion over the project area because of limitations in resources (e.g., availability of drones, personnel, or incompatible mosquitoes). The details of the proposed action are described below and include descriptions of the project area, frequency, timing, mosquito release methodology, and monitoring techniques.

Mosquito Transport and Storage

Under the proposed action, incompatible mosquitoes would be reared under sterile conditions in a laboratory environment to ensure that they are free from invasive organisms, parasites, and diseases. The lab-reared incompatible mosquitoes would be derived from southern house mosquito eggs initially collected in Hawai‘i. The *Wolbachia* strain transinfected into the southern house mosquitoes is also found in Hawai‘i, including on Maui. As such, no foreign organisms would be introduced to Maui via the proposed action. The lab would likely be located at a U.S. mainland facility, at least at the outset of this project, and incompatible mosquitoes would be transported to Maui from the rearing facility in containers designed for transport and/or field release. After arriving on Maui and following agricultural inspection, the incompatible mosquitoes would be held by a permitted importer in a climate-controlled environment, then promptly distributed by NPS or DLNR staff and designated agents. The timing of release following shipment is critical for success as the survivorship, and thus time to find a mate, of the incompatible mosquitoes is influenced by the length of time held in transport containers. During implementation, mosquitoes may be released directly from drones or handheld containers, or from small biodegradable packages that could be dispersed by drones or helicopters (as discussed in the following sections).

Number of Mosquitoes to be Released

As previously mentioned, the goal of the proposed action is to dramatically reduce the distribution and abundance of the mosquito population within the project area. Many previous successful IIT projects resulted in mosquito population declines of 90 percent or more (Beebe et al. 2021, Crawford et al. 2020, and Zheng et al. 2019). A similar decline would ensure that there would be very few remaining mosquitoes capable of biting and infecting threatened or endangered forest birds with avian malaria. The number of incompatible mosquitoes per release would be based on the local population densities of wild mosquitoes. Population densities of mosquitoes are dependent on precipitation patterns, habitat availability, and temperature. Adults, eggs, and larvae develop faster and in higher densities within warmer low-elevation areas (Ahumada et al. 2004). Estimates range from an abundance of approximately 600 mosquitoes per acre near sea level on Hawai‘i Island where monthly temperatures average 70–75° F, to an abundance of five mosquitoes per acre at an elevation of approximately 4,000 feet where temperatures average 55–60° F (Samuel et al. 2011, Giambelluca et al. 2014). Estimates assume an equal sex ratio of males to females; therefore, the number of prescribed incompatible mosquitoes released would be based on approximately one-half of the estimated mosquito population. Incompatible males would need to outcompete wild males; thus, it is desirable to release males in such numbers as to “overflood” the wild males. Statistical models suggest that 10 to 20 incompatible males for every wild male mosquito in the population may be required to achieve population suppression (McClure 2020). Based on current estimates, we expect to release between 50 and 6,000 incompatible mosquitoes per acre per treatment (which would occur up to twice per week) depending on elevation and local temperature and capture data gathered during monitoring. The quantity of incompatible mosquitoes released for this project would likely be less than other IIT mosquito projects that have occurred in urban areas throughout the world (involving yellow fever mosquitoes) because the southern house mosquito population density in East Maui is believed to be lower than yellow fever mosquito population densities in these urban areas. In addition, the uppermost elevations in the project area may have even fewer mosquitoes than estimated by Samuel et al. (2011) and population suppression in these areas may only require infrequent releases of incompatible mosquitoes. Alternatively, suppression at lower elevations may be sufficient to reduce or eliminate the threat of disease at the higher elevations by eliminating the individuals that could disperse uphill.

Release Locations and Spacing

The project team used all available data to estimate the distribution of mosquitoes within the project area. The current range of kiwikiu and ‘ākohekohe (Judge et al. 2021) and mosquito movements were applied to identify areas where mosquitoes might occur and spread disease. This information was also used to determine the locations to release mosquitoes. Based on past research, southern house mosquitoes are estimated to travel (disperse) approximately 650 feet in a 24-hour period (LaPointe 2008); thus, incompatible males would have the highest probability of finding a female and mating during the first day of release when locations are spaced 1,300 feet apart. Based on the estimated dispersal of mosquitoes into the range of threatened and endangered birds, a total of 1,389 proposed release locations were identified within the center of the project area (**Figure 2**). The area encompassing these 1,389 release locations is hereafter referred to as the "core area." The number of release locations, based on 1300-foot spacing within the core area, within each land management area are included in **Table 2**. The core area may expand, contract, or shift within the project area. Release spacing would be determined through a series of trials within the core area and may differ from those estimated here. This spacing would dictate the total number of release locations. Releases would be conducted systematically within each management area (the park, state forest and natural area reserves, TNC's Waikamoi Preserve, and private conservation lands), potentially by a variety of tools simultaneously (release methods are described in the following sections).

TABLE 2: MOSQUITO RELEASE LOCATIONS PER MANAGEMENT UNIT IN THE PROPOSED PROJECT AREA.

Land Manager and Reserve	Area (acres)	% Of Project Area	Release Locations
Hawai'i Dept. of Land and Natural Resources			
Hāna Forest Reserve	9,117	14.1%	262
Hanawī Natural Area Reserve	6,072	9.4%	174
Kīpahulu Forest Reserve	1,953	3%	51
Ko'olau Forest Reserve	11,668	18%	340
Makawao Forest Reserve	1,986	3.1%	59
National Park Service			
Haleakalā National Park	7,099	11%	211
Private			
East Maui Irrigation, LLC	3,927	6%	112
Haleakala Ranch Company	15	<0.1%	0
Mahi Pono	1,226	1.9%	36
The Nature Conservancy			
Waikamoi Preserve	5,101	7.8%	144
Grand Total	**48,164**	**74.5% ***	**1,389**

Note: Release locations are spaced 1,300 feet apart. The core area is smaller than the project area because the distribution of mosquitoes and range of native forest birds do not overlap (in all months of the year) in some high-elevation areas.
* At this time, 74.5% of the project area represents the core area between 2,200 and 4,300 feet where incompatible mosquito releases would be most important (as described in earlier sections).

Frequency and Timing of Release

Incompatible mosquito releases could occur throughout the project area during all seasons. However, releases would likely occur across the largest portion of the project area in the summer and fall months when mosquito populations in Hawai'i peak (LaPointe 2000; Gaudioso-Levita et al. 2005; Warren et al. 2020). These are months when the temperatures are suitable for avian malaria transmission within the greatest elevation extent, including areas above 4,300 feet in elevation (where most threatened and endangered birds currently live and breed). Incompatible mosquito releases may be reduced during the cooler spring and winter months when the abundance of mosquitoes at high elevations is thought to be reduced. The breeding season of most native forest birds peaks during the colder months from December through April (Berlin and Vangelder 2020, Fancy and Ralph 2020a,b, Simon et al. 2020), when incompatible mosquito releases may be curtailed at higher elevations due to temperature (and low mosquito density). Limited disturbance from release efforts to breeding forest birds is expected during this time. Concurrent monitoring would help identify seasonal fluctuations in mosquito populations and help guide the release strategy. Implementation may also be limited by inclement weather conditions and availability of mosquitoes.

To achieve the greatest possible reduction in the mosquito population, incompatible mosquitoes would be released at a maximum of twice per week per release location and potentially less frequently as wild mosquito population suppression is achieved over time. Release frequency would be determined by initial trials to determine longevity and dispersal of the incompatible males. The rate of release would be determined by the length of time the incompatible males survive at sufficient densities after release. The frequency of releases may also be reduced if there are advances in technologies for transporting mosquitoes (including lab-rearing in Hawai‘i) or releases, both of which could reduce mortality and improve longevity and competitiveness (the ability of incompatible male mosquitoes to compete with wild male mosquitoes for breeding). The release locations shown in **Figure 2**, each spaced 1,300 feet apart, have distinct temperature and precipitation characteristics because of elevation, topography, and aspect. Low elevation areas (red release locations) would require releases throughout the year, while high elevation areas (blue release locations) may require less frequent releases primarily during summer months. Higher frequency release locations (red) are in areas with temperatures that are conducive to year-round reproduction of mosquitoes and the avian malaria parasite (in infected mosquitoes). Medium frequency (orange) and low frequency (blue) release locations correspond to areas with lower average monthly temperatures and reduced distribution of mosquitoes and avian malaria during cooler months, typically from December through April. The frequency and number of incompatible mosquitoes released could decrease over time depending on the project’s success in suppressing the mosquito population.

Release Methods

Mosquito releases would be primarily conducted via drones. If there are obstacles to using drones for aerial releases in the core area, NPS and DLNR would release incompatible mosquitoes from helicopters over the short term (up to two months), either from a release device attached to the belly of a helicopter or from a long cable affixed with a device that could allow release of mosquitoes closer to the forest canopy or floor (described below). It is expected that limited pedestrian releases and monitoring would be conducted simultaneously with broadscale aerial releases.

Drone Release

Drones would allow for efficient incompatible mosquito releases throughout the core area and are considerably safer, less expensive, and quieter than helicopters. This method has been successfully used elsewhere for other mosquito control projects (Virginio et al. 2018, Bouyer et al. 2020). Although the specific mosquito release mechanism is still under development, it is expected that it will be available by the time the project is ready for implementation. It has also been assumed that drones would be flown from “front country” locations accessible by major roads and that no helicopter use would be required to transport drone operation crews into the remote or ground-inaccessible “backcountry” areas.

Drones would operate somewhat automatically (monitored by an operator), flying a prescribed route and releasing incompatible mosquitoes at the pre-determined release locations in the core area (**Figure 2**). It is estimated that drones would fly approximately 50–100 feet above the tree canopy during mosquito releases but no higher than 500 feet above ground level (AGL) when ferrying between release locations and the operator. Larger areas would require multiple days to conduct releases (e.g., Ko‘olau Forest Reserve), while smaller areas (e.g., Kīpahulu Forest Reserve) may only require a few hours for each aerial release. The drone operator would ensure that the drone and release mechanism are operating correctly and safely during each flight. Incompatible mosquitoes would likely be released in small biodegradable packages designed to open on contact with the canopy or forest floor.

The drone model(s) to be used has yet to be determined and would depend on a host of factors including environmental conditions and agency approvals. The choice of drone model affects the release rate as different models have varying flight speed capabilities and battery capacities. Available convertible fixed wing/multirotor drone models that could be used for this project can fly approximately 15 minutes in multirotor mode or 90 minutes during fixed wing mode before battery life is expended with a maximum payload (carrying weight). The flight speeds possible during releases of incompatible mosquitoes are also dependent on drone model and weather conditions (e.g., wind speed) as well as optimal speeds for the release mechanism, which are still to be determined. Estimates provided are based on a flight speed of 22 mph (following Bouyer et al. 2020) during mosquito releases and 62 miles per hour while in fixed wing mode when ferrying to and from release locations and the drone operator.

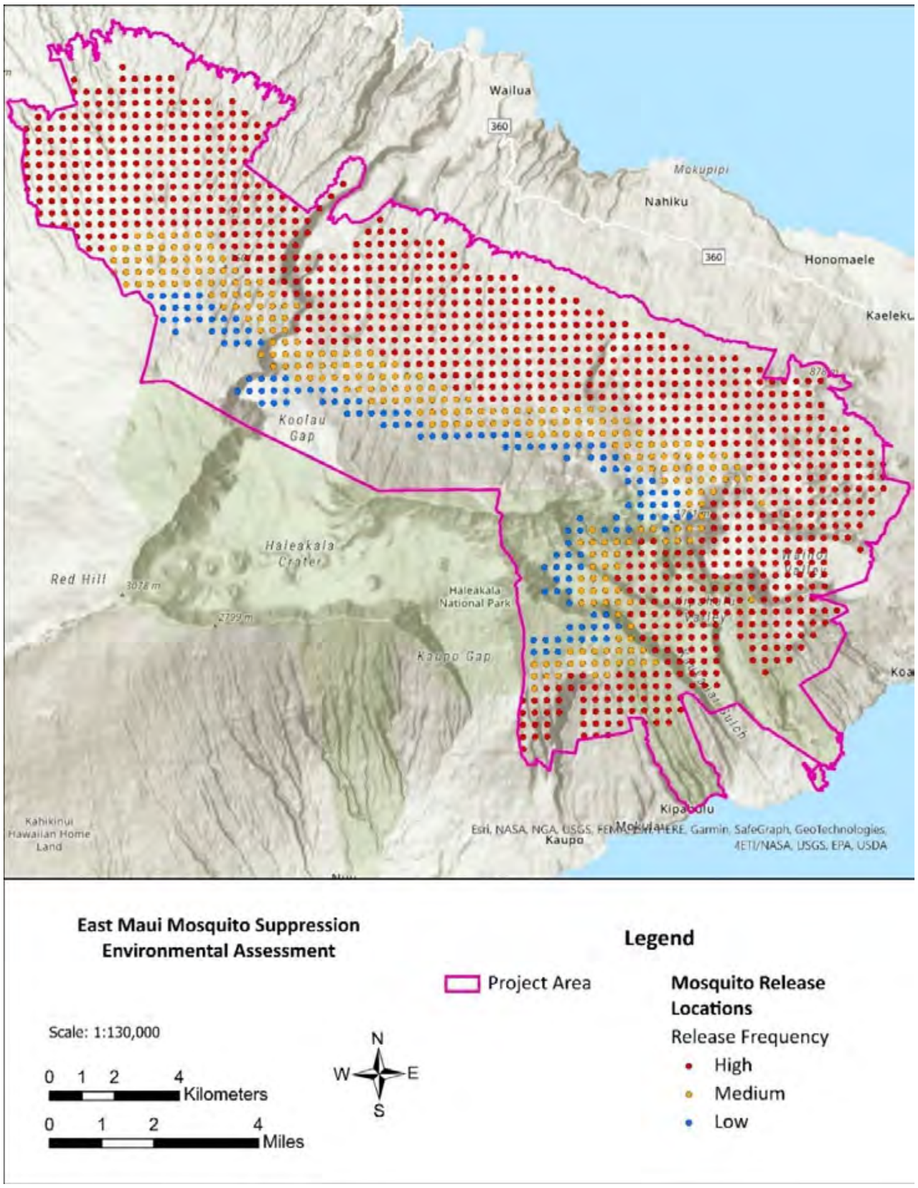

FIGURE 2: RELEASE LOCATIONS[1], SPACED 1,300 FEET APART, WITHIN THE PROJECT AREA BETWEEN 2,300 AND 6,000 FEET IN ELEVATION.

[1] Release frequency was determined by seasonal temperature patterns where warmer low elevation areas (red) may require releases throughout the year, while high elevation areas (blue) would only require releases during warmer summer months.

Proposed release locations would be spaced 1,300 feet apart, so a drone flying at 22 mph would be able to release incompatible mosquitoes at 24 release locations in a 15-minute period. At 62 miles per hour, the ferry times for the various parts of the core area vary widely. For example, a drone would only need to travel for approximately 1.5 minutes to reach some release locations in Makawao Forest Reserve but would need more than 5 minutes to reach certain areas of Hanawī Natural Area Reserve and Hāna Forest Reserve from a drone operator located in the front country. **Figure 3** provides a depiction of the drone launch locations (temporary helibases) and the general directions that the drones could fly into the core area to reach release locations. With an estimated maximum of 6 hours of release time possible per day, 576 release locations could be reached per day by one drone, based on the flight assumptions (e.g., speed, battery life) described above. The drone would likely spend 15 seconds or less hovering over each mosquito release location; it may be possible that drones would be able to release without pausing. A “treatment” is defined as releasing incompatible mosquitoes at all release locations within the entire core area. At least two drones would need to be working simultaneously each week to achieve two complete treatments per week in the core area. As described in the previous sections, however, the number of release locations could vary based on release location spacing and/or because of seasonal temperature trends, which can be simplified into “warm months” and “cold months.” Additionally, the number of release locations planned for a given treatment may be less than what is estimated for the entire core area based on limited available resources.

Table 3 illustrates the expected number of drone flight hours and total flights, both per week and for the entire core area, in both cold (December–April) and warm months (May–November). Note that the number of release locations during cold months is a minimum estimate and numbers of locations could increase incrementally over the course of each year to include the entire core area under unusually warm climatic conditions. The estimates presented in **Table 3** are for the entire core area. Multiple drones operating simultaneously would greatly decrease the number of total flying days. The estimates in **Table 3** are based on a convertible fixed wing/multicopter drone type and other drone models may be available that have increased speed, payload, and battery capacity that would alter flight estimates provided in the table.

TABLE 3. ESTIMATED NUMBER OF DRONE FLIGHT HOURS AND ROUND-TRIP FLIGHTS PER TREATMENT (RELEASING MOSQUITOES AT EACH LOCATION) AND PER WEEK (ASSUMING 2 TREATMENTS PER WEEK) PER LAND MANAGER.

Land Manager	Per Treatment				Per Week			
	warm months		cold months		warm months		cold months	
	hrs	flights	hrs	flights	hrs	flights	hrs	flights
Hawai‘i Dept. of Land and Natural Resources	23.2	43	18.2	35	46.5	87	36.4	70
National Park Service	5.5	10	2.9	6	11.0	21	5.9	11
Private	3.4	7	3.0	6	6.7	14	6.1	12
The Nature Conservancy	3.6	7	0.3	1	7.3	14	0.6	1
TOTAL	**36**	**67**	**24**	**48**	**72**	**134**	**49**	**94**

Note: Presented in this table are estimated flight information for lower elevations only (2000–4300 ft) during colder months (December–April) when releases at higher elevations are not expected to be needed (“cold months”) and all elevations (2000–5600 ft) within the core area where releases are expected to be needed during warmer months. These elevations are based on thermal limits of the malaria parasite (>55° F) below which transmission from mosquitoes is limited (Ahumada et al. 2004).

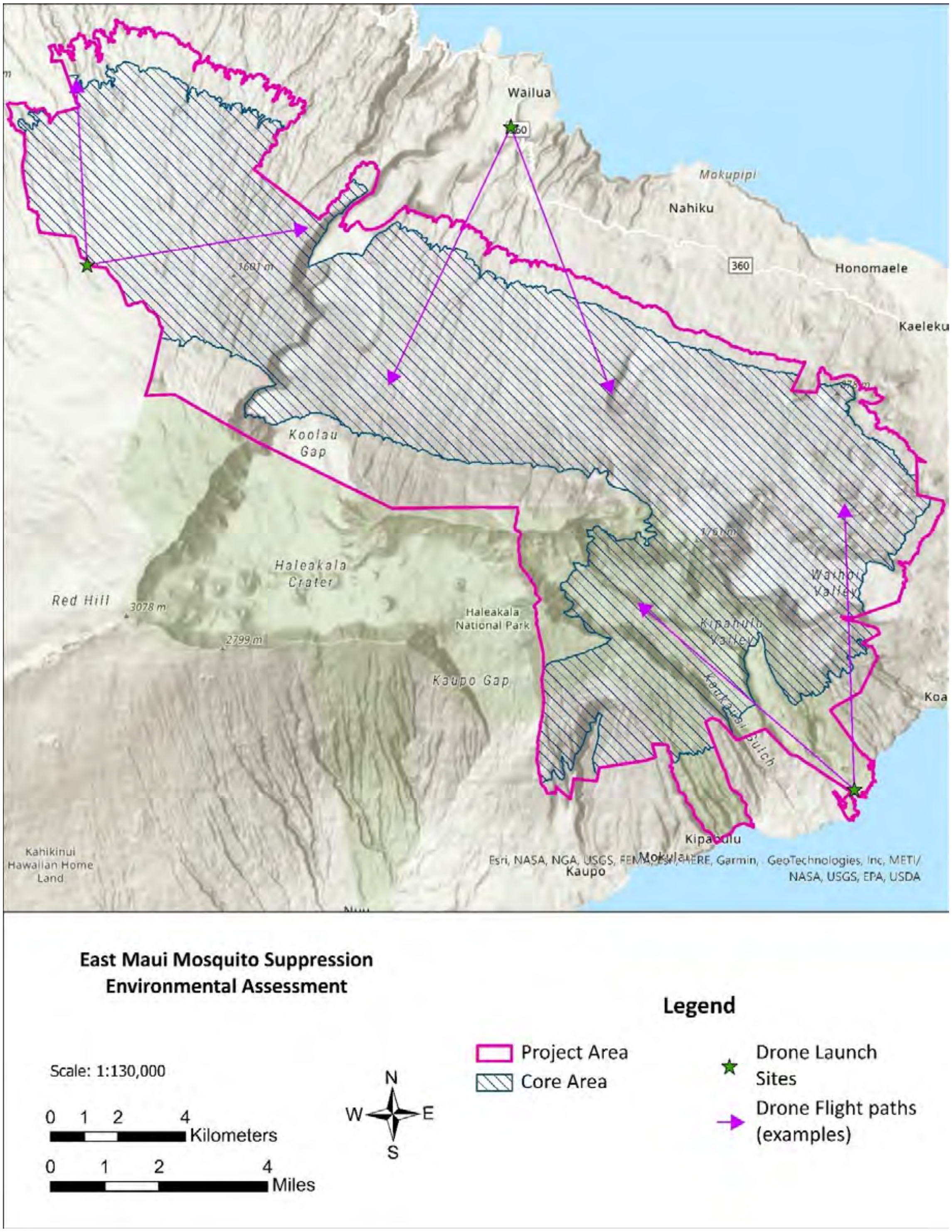

FIGURE 3: EXAMPLE DRONE FLIGHT PATHS FROM POSSIBLE LAUNCH LOCATIONS INTO THE CORE AREA.

Helicopter Longline Release

Helicopters are an essential tool for natural resource management on East Maui. Because of the steep topography and dense vegetation in the project area, helicopters are invaluable for transporting personnel and equipment to remote areas. Given the noise and visual impacts, logistics, and financial requirements of helicopters, the use of helicopters for releasing incompatible mosquitoes is proposed as a short-term (up to two months), temporary release method if drone releases are unavailable. In that event, helicopters could release incompatible mosquitoes for up to two months in management units where population suppression can be sustained.

Several projects worldwide have used helicopters to release sterile insects to control or eradicate agricultural pests (Dyck et al. 2021), and several projects on Maui have had success controlling pests and weeds from devices attached to longlines (Tuttle et al. 2008). The helicopter, operated by a pilot and carrying one spotter (unless the load calculation precludes the weight of a passenger), would be equipped with an approximately 50–100-foot longline attached to the belly hook of the helicopter. Longlines are heavy-duty steel cables that can be attached to the underside of a helicopter. This type of cable allows the helicopter to place loads in areas where the helicopter could not safely land or distribute a load while hovering above the surface.

With an approximately 50-foot tree canopy, a 50–100-foot longline, and a 50-foot buffer for safety, the helicopter would fly approximately 150–200 feet AGL while releasing mosquitoes above the tree canopy. A release mechanism would be attached to the end of the longline, and mosquito releases may be triggered remotely by the pilot or spotter. While the detailed design for longline release of mosquitoes is not yet known, the method is considered feasible based on current longline operations on federal and state lands in Hawai‘i. On East Maui, the NPS and DLNR regularly conduct helicopter herbicide applications using longlines to control high priority invasive plants and animals. These methods would be adapted to mosquito releases within the core area, and have been used to estimate flight speed, flight times, and specific logistics for suppression of mosquito populations on East Maui.

During a typical operation, it is expected that the helicopter would fly at a speed of 69 miles per hour and approximately 500–2000 feet AGL from the main heliport (Kahului Airport, OGG) to a designated temporary helibase (20–90 miles; 10–25 minutes) where the longline and release mechanism would be attached by ground teams. The helicopter would then fly at a slower speed with the longline to the core area (approximately 22 miles per hour) for releases. The helicopter could complete 68–74 release locations per hour and 137–148 release locations per flight before refueling (based on the spacing assumptions previously described for drones). The helicopter could complete three flights per day. Thus, one day of helicopter flights could consist of six hours of flying covering 412–443 release locations. The helicopter would likely spend 15 seconds or less hovering over each mosquito release location. Here we assume repeat visits to any given area would not likely occur more than twice per week, based on logistic constraints, but would be refined over time based on monitoring of mosquito populations.

Table 4 describes the estimated number of helicopter flights (including round trip to and from the main heliport [OGG] and round trip from a front country launch location and release area) and number of flight hours required to release mosquitoes via helicopter longline throughout the entire core area. These estimates are further broken down into warm and cold months.

Pedestrian Mosquito Release

Pedestrian release is not expected to be a primarily release method as it is much less efficient than aerial release methods and it is only possible in limited areas within the project area. Under this method, pedestrian teams would receive helicopter deliveries and then distribute mosquitoes to the release locations and conduct concurrent mosquito monitoring. Pedestrian releases would involve field teams walking the terrain on foot, using existing management trails and fence lines, as well as camping at established remote camps or helicopter LZs if necessary. Teams may spend several days hiking and releasing mosquitoes at designated release locations every 1,300 feet along existing management trails. The number of release locations that can be accessed would be determined by the terrain and availability of management trails at each location. Some non-mechanized trail clearing and re-flagging would be required by NPS or DLNR staff in some areas, with generally more effort required at the lower elevation locations

TABLE 4. ESTIMATED NUMBER OF HELICOPTER FLIGHT HOURS AND ROUND-TRIP FLIGHTS PER TREATMENT (RELEASING MOSQUITOES AT EACH LOCATION) AND PER MONTH (ASSUMING 2 TREATMENTS PER MONTH) PER LAND MANAGER AS A SHORT-TERM, TEMPORARY MEASURE.

Land Manager	Per Treatment				Per Month			
	warm months		cold months		warm months		cold months	
	hrs	flights	hrs	flights	hrs	flights	hrs	flights
Hawai'i Dept. of Land and Natural Resources	12.4	6.2	10.1	5.0	24.9	12.4	20.2	10.1
National Park Service	3.1	1.5	1.7	0.9	6.1	3.1	3.4	1.7
Private	2.0	1.0	1.8	0.9	4.0	2.0	3.6	1.8
The Nature Conservancy	2.0	1.0	0.2	0.1	3.9	2.0	0.4	0.2
TOTAL	**20**	**10**	**14**	**7**	**39**	**20**	**28**	**14**

Note: Presented in the above table are estimated flight information for lower elevations only (2000–4300 ft) during colder months (December–April) when releases at higher elevations are not expected to be needed ("cold months") and all elevations (2000–5600 ft) within the core where releases are expected to be needed during warmer months. These elevations are based on thermal limits of the malaria parasite (>55° F) below which transmission from mosquitoes is limited.

where brushy vegetation is thicker and encroaches on trails and fence lines more frequently. As such, trail maintenance may take more effort per release location at the lower elevation locations. Protocols would be followed to prevent invasive weed dispersal, particularly from lower elevation areas to higher-elevation areas, including sanitation procedures and limiting all movement between camps (either hiking or successive trips) from only higher to lower elevations.

Pedestrian mosquito release, especially at remote sites, would likely be primarily for necessary field trials because it can be implemented immediately and would allow for simultaneous monitoring. Consistent pedestrian release is only possible in portions of Makawao Forest Reserve and Waikamoi Preserve. Although pedestrian releases could occur throughout the year in Makawao Forest Reserve and Waikamoi Preserve, pedestrian releases may only be possible within Haleakalā National Park, Hanawī Natural Area Reserve, and other remote sites on a quarterly basis simultaneous with ground-based mosquito monitoring. A helicopter would be required to transport crews into the field to reach LZs near monitoring and release locations in Haleakalā National Park and Hanawī Natural Area Reserve, and the frequency and duration of these helicopter flights is described in the following section, "Mosquito Monitoring."

Mosquito Monitoring

DLNR will work with State and Federal partners to prepare a detailed monitoring plan. Field teams would conduct a variety of monitoring activities to measure the effectiveness of the proposed action. Field teams would trap mosquitoes in release areas to determine relative abundance of the mosquito population, dispersal distance of incompatible mosquitoes, and estimated hatch success. Field teams would place traps along existing trails and fence lines, collect mosquitoes from traps, and preserve the captured mosquitoes for additional testing, e.g., for absence or presence of avian malaria. As a result of monitoring, the NPS and DLNR would be able to prioritize future releases, optimize the number and location of incompatible mosquitoes, improve mosquito release methods, and minimize costs for project implementation. Sustained and regular mosquito trapping would be necessary to understand the proposed action's effectiveness and track seasonal fluctuations in population densities.

Monitoring would likely occur quarterly (four times/year). Baseline monitoring data are available from areas of Kīpahulu Valley, TNC's Waikamoi Preserve, and Hanawī Natural Area Reserve (Aruch et al. 2007, MFBRP unpublished), and monitoring would be continued at these locations. Monitoring would be more frequent at the start of the project and would vary depending on the availability of incompatible mosquitoes and personnel. It is assumed that four locations would be selected on state lands (e.g., two within Hanawī Natural Area Reserve and two within Forest Reserves), two locations within the park (within the Kīpahulu Valley Biological Reserve), and two locations within TNC's Waikamoi Preserve.

_A total of five sites within Haleakalā National Park, TNC's Waikamoi Preserve, and Hanawī Natural Area Reserve are helicopter access only, where mosquito monitoring field teams would camp at established remote shelters or helicopter LZs. Crews would conduct monitoring activities remotely for approximately one week at a time and would need to use portable generators to charge mosquito trap batteries, GPS units, and field radios. **Table 5** estimates helicopter flight hours required to transport teams in and out of the field for necessary mosquito population monitoring. **Figure 4** shows existing helicopter infrastructure that includes the main heliport at Kahului Airport (OGG) and several LZs throughout the project area. Three other sites within the analysis area are accessible by vehicle, where field teams could commute from management offices daily for monitoring activities.

TABLE 5. ESTIMATED HELICOPTER FLIGHT HOURS TO TRANSPORT MONITORING TEAMS

Land Manager	Helicopter Flight Hours	
	per quarter	per year
	hrs	hrs
Hawai'i Dept. of Land and Natural Resources	7	28
National Park Service	7	28
The Nature Conservancy	3.5	14
TOTAL	**17.5**	**70**

Note: The flight estimates in this table are based on the need to reach 2 monitoring locations within DLNR, 2 within the park, and 1 within TNC Waikamoi Preserve with a helicopter. Additional monitoring may be conducted but helicopter assistance may not be required. Flights hours are estimated for one visit per location quarterly (4 × per year).

Vehicle Support

Where access roads exist (shown in **Figure 5**), motorized vehicles (trucks or SUVs) would be used to transport field teams and equipment for ground-based monitoring and pedestrian releases. Vehicles would be used in the project area on a quarterly basis to support monitoring and likely more frequently to support pedestrian mosquito releases. Vehicles would be used on existing roads that are currently used and maintained by their respective landowners for maintenance, management, and public recreation. None of these existing roads are within designated wilderness. During monitoring, vehicles would drive along the Flume Road shown (in brown) on **Figure 5** for up to 4 hours per day for 7 consecutive days on a quarterly basis to reach three monitoring locations in Makawao Forest Reserve and TNC's Waikamoi Preserve. Vehicles would drive along the same road once or twice weekly for up to 2 hours per day when or if pedestrian mosquito releases are occurring (for perhaps 50-100 locations in Makawao Forest Reserve and TNC's Waikamoi Preserve). This road crosses Makawao Forest Reserve and private conservation lands but provides pedestrian access to TNC's Waikamoi Preserve.

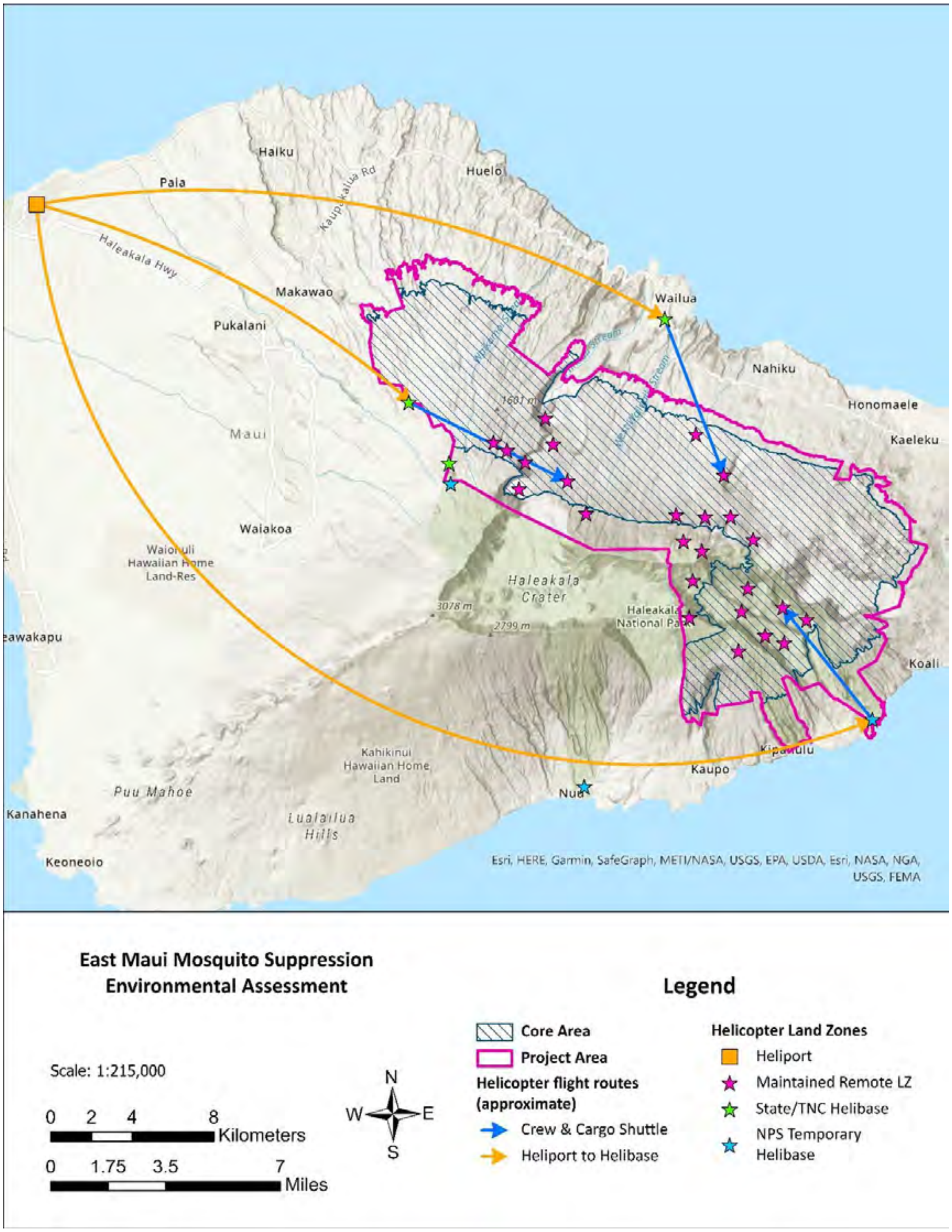

FIGURE 4: EXAMPLE FLIGHT PATHS[1] FROM THE HELIPORT TO HELIBASES (ORANGE) AND THEN ON TO REMOTE LANDING ZONES (BLUE).

[1] Many of the landing zones without arrows would also be used during project implementation but this map has been provided to show several example scenarios for various flight paths from the heliport to helibases and then to remote landing zones.

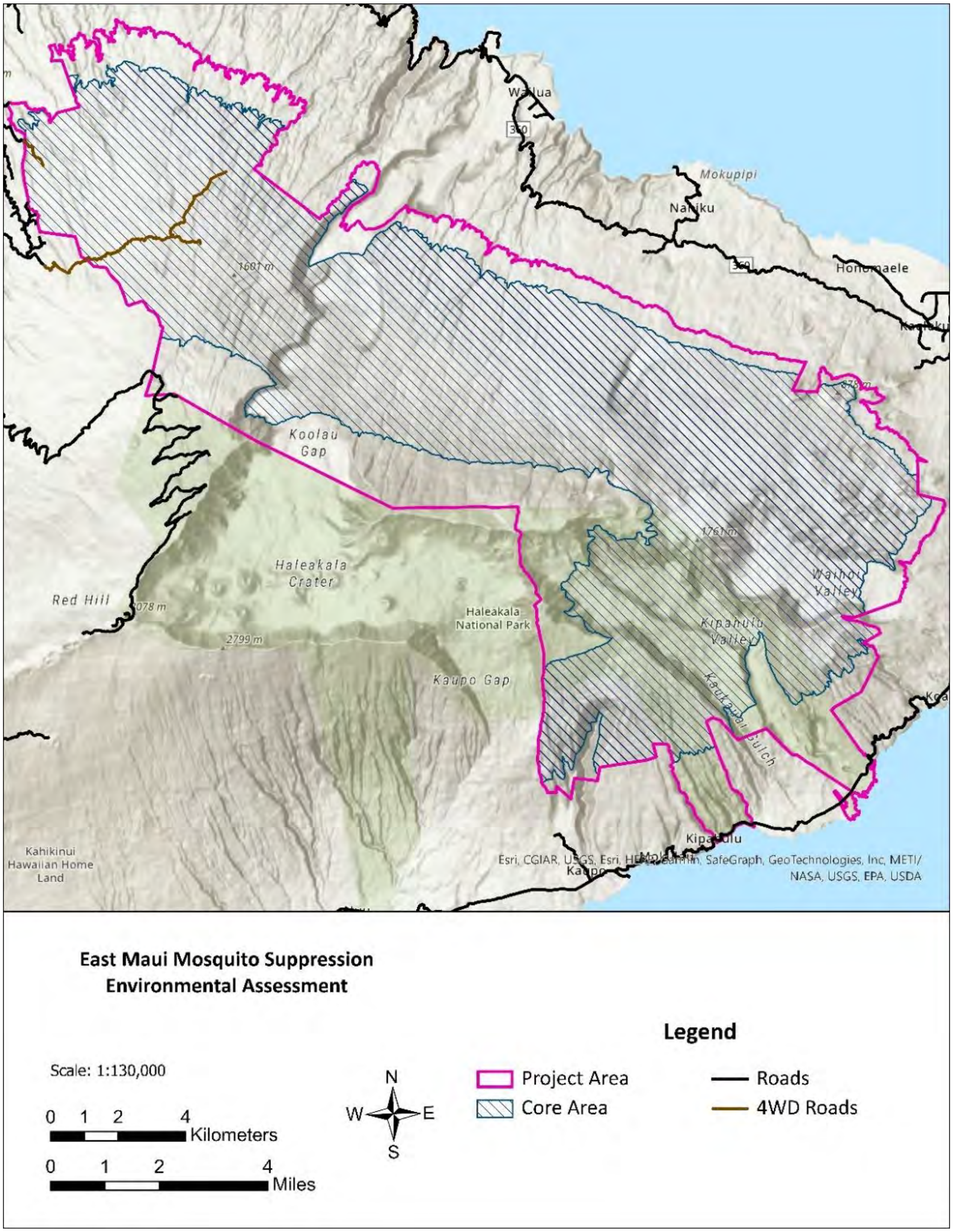

FIGURE 5: ROAD ACCESS TO THE PROJECT AREA

Required Permits and Approvals

To implement the proposed action as described, the NPS expects to obtain approval from the Department of the Interior to operate drones in the park. Currently, the state can use drones on state Forest and Natural Area Reserves and TNC within Waikamoi Preserve if compliant with the Federal Aviation Administration (FAA) regulations. Certification by the FAA would be required for drone use in all areas, and all drone and helicopter flights would comply with all FAA rules and regulations.

In June 2022, the State of Hawaiʻi Board of Agriculture approved the addition of the southern house mosquito to the Chapter 4-71, Hawaiʻi Administrative Rules (HAR) "Non-Domestic Animal Import Rules" list of restricted animals (Part A) and set permit conditions to allow the importation and field release of male southern house mosquitoes inoculated with incompatible strains of *Wolbachia* bacteria. In October 2022, the Hawaiʻi Department of Agriculture (HDOA), Plant Quarantine Branch issued a permit to DLNR to allow for the import of southern house mosquitoes for mosquito control projects; however, the permit would need to be amended for broad-scale implementation of releases as part of this project. The Environmental Protection Agency (EPA) regulates incompatible mosquitoes as biopesticide products. An EPA Section 18 application has been prepared for submittal by the HDOA, in collaboration with USFWS and DLNR, to request an emergency exemption from Section 3 pesticide registration, given the imminent extinction risks to threatened and endangered forest bird species. If approved, the Section 18 process would result in temporary product registration and a label that identifies appropriate product use, application rates, restrictions, safety, and quality control requirements. If control projects are initiated for the southern house mosquito, HDOA, DLNR and USFWS would then collect and share post-application monitoring data with the EPA to contribute towards a formal Section 3 pesticide registration package.

The release of incompatible mosquitoes for landscape scale control of the southern house mosquito on state lands is contingent on the results of the impact analysis in this EA. However, in June 2022, DLNR filed an exemption notice regarding the preparation of an environmental assessment under the authority of Chapter 343, Hawaiʻi Revised Statutes (HRS) and Section 11-200.1-17, HAR, to conduct limited import of male mosquitoes for preliminary transport trials and mark release recapture studies. The Chairperson of the DLNR has the authority to declare exempt from the preparation of an environmental assessment those department actions that are included in the DLNR exemption list when the Board of Land and Natural Resources has delegated authority to conduct those actions. The exemption notice cited General Exemption Type 5 "Basic data collection, research, experimental management and resource and infrastructure testing and evaluation activities that do not result in a serious or major disturbance to an environmental resource" (DLNR exemption list November 10, 2020).

Mitigation Measures and Best Management Practices

Table 6 summarizes general best management practices that would be implemented for this project to avoid and minimize potential impacts.

TABLE 6: GENERAL BEST MANAGEMENT PRACTICES INCLUDED IN THE PROPOSED ACTION

Resource Mitigation Measures	
Wildland Fire	Especially in dry areas, personnel would take all precautions to avoid igniting wildland fires. Vehicles would not be left to idle, especially in tall grass. Vegetation within LZs would be maintained to avoid possible ignition by helicopters. Personnel would follow all applicable DLNR and NPS regulations in the project area that includes but is not limited to no open fires and closed cooking devices.
	Helicopters would use appropriate mufflers to minimize fire potential.

Resource	Mitigation Measures
	NPS and DLNR staff regularly conduct on-site measurements of temperature, humidity, and wind to determine fire risk. If the level is moderate-high, fire teams would warn staff and restrict or eliminate activity in high-fire risk areas. Water tanks would be maintained and could provide a water source for suppression if needed.
	Although not anticipated, the local fire department, in coordination with NPS and DLNR, would respond to and extinguish potential fires ignited by project activities as soon as possible.
	All uncrewed aircraft systems (UAS) will be closely monitored by the operator and field teams while adhering to guidance developed by the NPS Natural Resource Stewardship and Science Directorate and policies established by Federal Aviation Administration. The DLNR Division of Forestry and Wildlife (DOFAW) is mandated under the Land Fire Protection Law, Chapter 185, Hawaiʻi Revised Statute to take measures for the prevention, control, and extinguishment of wildland fires within all forest reserves and natural area reserves on East Maui (DLNR, DOFAW 2018). DOFAW is statutorily required to cooperate with county and federal government fire control agencies to develop plans for wildfire prevention. UAS operators under NPS or DOFAW operational control will be required to have an up-to-date FAA 14 CFR Part 107 Remote Pilot Certificate and FAA Certificate of Waiver or Authorization. UAS operations will follow best practice protocols established by the National Wildfire Coordinating Group, which provides guidance detailed in the Interagency Helicopter Operation Guide. NPS law enforcement will monitor UAS operations and approve flight plans and thus will be able to respond immediately to UAS mishaps. The Maui Fire Department, in coordination with NPS Fire Management officers and the DOFAW Fire Management Program, will respond to any on-site emergency, including downed UAS vehicles to assure that there is no risk of wildfire.
Vegetation	Transport of weeds by equipment, including helicopters, would be mitigated by strictly following NPS and DLNR sanitation protocols. Specifically, concerns regarding the spread of invasive weeds would dictate the order of which LZs are accessed, who is sent to each LZ and when. Project personnel would implement and follow the USFWS "*Avoidance, Minimization, and Conservation Measures for listed plants in the Pacific Islands*" (revised September 2020; Appendix D), the USFWS January 20, 2022, letter addressed to the park regarding this project (Appendix D), and the [*Pacific Islands Fish and Wildlife Office] PIFWO Invasive Species Biosecurity Protocol* (USFWS 2022a; Appendix D). Personnel would follow DLNR and NPS Rapid ʻŌhiʻa Death sanitation protocols.
Wildlife	NPS and DLNR staff would observe native wildlife while conducting mosquito suppression and monitoring activities. If noise-producing activities appear to be adversely affecting native wildlife, the park or DLNR wildlife biologists would be consulted as to what, if any, restrictions would be implemented. Restrictions could include re-routing, delaying, or modifying flight times or motor vehicle use. No flights (either drone or helicopter) would occur between "civil sunset" and "civil sunrise."

Resource Mitigation Measures	
Special Status Plant Species	NPS and DLNR personnel and contractors working in the area would be required to demonstrate the ability to identify special status plants (i.e. federally- and state-listed plants and plant species at risk) and would be trained on how to avoid adverse impacts to them. Project personnel would implement and follow the USFWS "*Avoidance, Minimization, and Conservation Measures for listed plants in the Pacific Islands*" (revised September 2020; Appendix D), the *PIFWO Invasive Species Biosecurity Protocol* (USFWS 2022a; Appendix D), and the mitigation measures provided in the USFWS January 20, 2022, letter addressed to the park regarding this project (Appendix D). The boundary of the area occupied by listed plants and plant species at risk would be marked with flagging by a surveyor and these areas would be avoided. All project personnel would be provided with maps showing the locations of designated critical habitat areas and trained on how to avoid unnecessary adverse impacts within designated critical habitat, including disturbance to native and special status plant species and activities that could accelerate erosion. This sensitive information (i.e. localities of listed plants) would be protected and not shared outside of the personnel assigned to this project.
Special Status Wildlife Species	All team members working on the project would be trained in special status wildlife species identification and ways to minimize impacts to listed species. This information would include maps showing locations of all known nesting or roosting sites. This sensitive information would be protected and not shared outside of the personnel assigned to this project. Project personnel would implement and follow the USFWS "*Animal Avoidance and Minimization Measures"* for listed wildlife in the Pacific Islands (February 2022; summarized in **Table 7**; Appendix D), the "*PIFWO Invasive Species Biosecurity Protocol*" (Appendix D), and the mitigation measures provided in the USFWS January 20, 2022, letter addressed to the park regarding this project (Appendix D). Additionally, the park does not fly out of ʻOheʻo/Kīpahulu temporary helibase until after 8 am to prevent early morning noise disturbance, which would double as a mitigation for birds that are active at dawn. No flights (either drone or helicopter) would occur between "civil sunset" and "civil sunrise."
Special Status Species Habitat	Personnel tasked with working in or traversing across designated critical habitat would be trained and evaluated in plant identification (especially listed plant identification). Disturbance to special status species would be avoided. Avoidance measures would include confining pedestrian travel to existing trails and camps and restricting project activities for a certain period of time or in a certain area. If deemed necessary by park or DLNR wildlife biologists, noise-producing activities may be prohibited near breeding or nesting habitat of endangered or threatened wildlife. All project personnel would be provided with maps showing the locations of critical habitat areas and trained in biosecurity (see Invasive Species below) and on how to avoid adverse impacts within critical habitat.
Invasive Species	All vehicles, equipment, clothes, and footwear would be inspected and cleaned to prevent transport and establishment of introduced species including weeds and diseases/pathogens before and after field deployments.
Cultural, Historic, and Ethnographic Resources	Archaeological features would be avoided during all ground-based activities. Staff would be provided with maps depicting the locations of cultural and historic resources and buffer zones and trained in best practices for avoiding adverse impacts. This sensitive information would be protected and not shared outside of the personnel assigned to this project.

	Resource Mitigation Measures
	Project-related helicopter and drone flights would be avoided on park's six (6) designated commercial free days (calendar dates vary slightly from year to year) to avoid disturbance of traditional cultural practices (see Appendix C for more information): • End of Makahiki (January) • Zenith Noon (May) • Summer Solstice (June) • Zenith Noon (July) • Start of Makahiki (October) • Winter Solstice (December)
Human Health and Safety	All appropriate precautions and safety measures would be taken when operating helicopters and drones and conducting release activities to avoid threats to human health and safety. Specifically, regulations for safe operation of helicopters/drones, camping, and hiking during release activities would be strictly enforced.
Acoustic Environment	LZs, camps, helibases, flight paths, timing of flights, and height above ground level would be selected to minimize noise impacts on visitors, nearby landowners or communities, wilderness, and sensitive environmental resources. Helicopter flights out of the ʻOheʻo/Kīpahulu temporary helibase would not occur until after 8 am to prevent early morning noise disturbance. A communication plan would be developed to include coordination with interpretation staff to avoid conducting flights when an interpretive program is scheduled or when Native Hawaiian ceremonies, plant collecting, or other traditional activities would be conducted.
Visitor Experience	There would be no flights or operations conducted after dark, before civil sunrise, or on weekends. When flights are conducted near areas open to public access, flight path and timing would be selected to minimize noise and viewscape impacts on visitor experience.
Wilderness Preservation	All actions taken that involve a prohibited use pursuant to Section 4(c) of the Wilderness Act would be subject to a Minimum Requirements Analysis and would strive to minimize the impacts to wilderness character.

Table 7 summarizes USFWS-recommended mitigation measures (Appendix D) that would be implemented to avoid and minimize potential impacts on federally listed wildlife species.

TABLE 7. USFWS-RECOMMENDED MITIGATION MEASURES

Resource	Mitigation Measures
Nēnē (Hawaiian Goose)	• Personnel would not approach, feed, or disturb nēnē. • If nēnē are observed resting or foraging within a particular release location or helicopter/drone launch location during the breeding season (October through May), a biologist familiar with nēnē nesting behavior would survey for nests in and around the launch site prior to the resumption of work. Repeat surveys after any subsequent delay of work of three or more days (during which the birds may attempt to nest). • If a nest is discovered within 150 feet of a proposed worksite, all work within 150 feet would cease and USFWS would be contacted for guidance before resuming work within this area proximate to the nest. • In areas where nēnē are known to be present, personnel would post and implement reduced speed limits, and inform project personnel and contractors about the presence of endangered species on-site.
Hawaiian Forest Birds	• Personnel would avoid activities that may increase the wildfire threat to montane forest habitats. • Personnel would avoid removing tree cover during the typical breeding season between November 1 and June 30. • Personnel would prevent the spread of invasive species. • Personnel would avoid increasing stagnant water habitat. • To the extent possible, personnel would conduct mosquito suppression in threatened and endangered forest bird habitat outside the peak of the breeding season (January-March). Where breeding seasons cannot be avoided, drone operations would occur only above tree height level, and hovering in one place would be minimized to limit the risk that breeding birds would flush from active nests. Helicopters would avoid flying low near forest bird habitats to avoid rotor wash and disturbing nesting forest birds.
Hawaiian Seabirds	• During the seabird breeding season (February 1 to November 15), NPS and DLNR would avoid flights between dusk and dawn to protect night-flying seabirds.
Hawaiian Waterbirds	• Endangered waterbirds do not occupy, or breed within the project area. If waterbirds were to be detected, personnel would post and implement reduced speed limits and inform project personnel and contractors about the presence of endangered species on-site.
ʻŌpeʻapeʻa (Hawaiian Hoary Bat)	• Personnel would not disturb, remove, or trim woody plants greater than 15 feet tall during the bat birthing and pup rearing season (June 1 through September 15). • NPS and DLNR would avoid drone and helicopter flights between dusk and dawn to protect flying bats. • During the breeding season, drone operations would occur only 50-150 feet above tree height level, and hovering in one place would be minimized to limit the risk of disturbing pup rearing. Helicopters would avoid flying low near ʻŌpeʻapeʻa habitats to avoid rotor wash and disturbing day roosting bats.

CHAPTER 3: AFFECTED ENVIRONMENT AND ENVIRONMENTAL CONSEQUENCES

INTRODUCTION

This chapter describes both the affected environment (the existing conditions of resources, including trends and ongoing and planned actions) and environmental consequences (impacts) of the proposed action on each resource. The affected environment and environmental consequences if no action is taken are described in each "Current and Expected Future Condition of the [Resource] if No Action is Taken" section. This is consistent with direction from the Council on Environmental Quality (CEQ), which states that agencies "may contrast the impacts of the proposed action and alternatives with the current and expected future conditions of the affected environment in the absence of the action, which constitutes consideration of a no-action alternative" (85 FR 43323). The environmental consequences of the proposed action are described in the "Effects of the Proposed Action on [Resource]" section for each resource. For the purposes of describing the affected environment and resource trends, past, present, and reasonably foreseeable future actions on NPS, DLNR, and TNC lands were assessed and are further described in Appendix E.

Methods and Assumptions

The following analysis evaluates direct, indirect, and cumulative impacts that would result from the implementation of the alternatives. A factual description of the direct and indirect impacts provides the reader with an understanding of how the current condition of a resource would likely change as a result of implementing the alternatives. The approach includes the following elements:

- The analysis is focused, to the greatest extent possible, on management changes and associated issues that could have meaningful impacts on the resources being evaluated.
- The description of the affected environment and analysis of impacts follow the CEQ NEPA regulations, as amended in May of 2022, the Department of the Interior NEPA regulations, and the 2015 NPS NEPA Handbook.
- As the proposed action is a joint NPS/DLNR project, the impact analysis in this EA is also in accordance with the Hawai‘i Environmental Policy Act (HEPA). According to HAR Chapter 11-200.1, Environmental Impact Statement Rules, "(a) In considering the significance of potential environmental effects, agencies shall consider the sum of effects on the quality of the environment and shall evaluate the overall and cumulative effects of an action. (b) In determining whether an action may have a significant effect on the environment, the agency shall consider every phase of a proposed action, the expected consequences, both primary and secondary, and the cumulative as well as the short-term and long-term effects of the action." HEPA Significance criteria are evaluated in Appendix G.
    - One of the specific considerations under HEPA is that the effects of a proposed action on the cultural practices of the community be analyzed. Impacts to cultural resources were considered and dismissed from detailed analysis, as described in Appendix B. However, a Cultural Impact Assessment was prepared for the project as required by HEPA and is included in Appendix C.

The NPS and DLNR interdisciplinary planning team reviewed a substantial body of scientific literature and studies applicable to the proposed mosquito release methods, project area, and associated resource issues and impact topics. This information augmented previous site-specific observations and documentation gathered by team personnel to support the qualitative and quantitative statements presented for each analyzed resource.

The following basic guiding assumptions were used to provide context for this analysis:

- *Mitigation.* All mitigations/best management practices included in Chapter 2 would be implemented for the proposed action.

- *Analysis Period.* The proposed action provides objectives and specific implementation actions needed to manage mosquito populations into the future. To understand the potential long-term impacts associated with mosquito population management, this document considers actions and effects over a 20-year period.
- *Overall Analysis Area.* The overall analysis area includes 64,666 acres of NPS, DLNR, and private lands managed for conservation. Haleakalā National Park lands within the analysis area include the Kīpahulu District and small portions of the northern edge of the Summit District. State lands within the analysis area include the Koʻolau, Hāna, Kīpahulu, and Makawao Forest Reserves and Hanawī Natural Area Reserve. Private lands managed by TNC, East Maui Irrigation, and Mahi Pono are also within the analysis area. Based on proposed ground activities under the proposed action or a more limited extent of a resource within the analysis area, a smaller area was analyzed (such as for threatened and endangered plants, which would only potentially be impacted by pedestrian teams, and wilderness character, which is only applicable to the designated Haleakalā Wilderness within Haleakalā National Park).

Cumulative Impacts

The CEQ regulations for implementing NEPA require the assessment of cumulative impacts in the decision-making process for federal projects. Cumulative impacts are defined as "effects on the environment that result from the incremental impact of the action when added to other past, present, and reasonably foreseeable future actions regardless of what agency (federal or nonfederal) or person undertakes such other actions" (40 CFR 1508.1(g)(3)).

Cumulative impacts were determined for each impact topic by combining the impacts of other past, present, and reasonably foreseeable future actions that also would result in beneficial or adverse impacts. Therefore, it was necessary to identify other ongoing or reasonably foreseeable future projects and plans at the park, on adjacent DLNR and TNC-managed lands in the project area, and, if applicable, the surrounding region. Past projects or plans with ongoing effects and reasonably foreseeable future projects or plans on NPS, DLNR, and TNC-managed lands are identified in Appendix E). Cumulative impacts of past, present, and reasonably foreseeable actions are included in the "Current and Expected Future Condition of the [Resource] if No Action is Taken" section of each resource, and the cumulative impacts of the proposed action are included under the "Effects of the Proposed Action on [Resource]" section of each resource.

ACOUSTIC ENVIRONMENT

The acoustic environment is the combination of all the acoustic resources and sounds within a given area as modified by the environment (such as meteorological conditions, absorption, reverberation, reflection, and diffraction). Acoustic resources are the individual types of sounds, including both natural sounds (for example, wind, water, wildlife, weather) and cultural sounds (for example, Native Hawaiian ceremonies). The natural soundscape of a park, according to the NPS soundscape management policy (Section 4.9 in NPS 2006), refers to the combination of all the natural sounds occurring in the park, absent the human-induced sounds, as well as the physical capacity for transmitting those natural sounds that can be perceived and comprehended by humans. Natural sounds include those within and beyond the range that humans can perceive and can be transmitted through air, water, or solid materials (NPS 2006b). The character and quality of the acoustic environment influence human perceptions of an area, providing a sense of place that differentiates it from other regions. In addition, the acoustic environment is a critical component of wilderness character and plays an important role in wildlife communication, behavior, and other ecological processes (Wood 2015).

Noise generally refers to sounds that are unwanted or intrusive, either because of its effects on humans and wildlife, or its interference with the perception or detection of other sounds (Section 4.9 in NPS 2006; Lee et al. 2016). Primary sources of human-caused noise can include cars, aircraft, buses, and other motorized vehicles and equipment. Sound levels can vary greatly, depending on location, topography, vegetation, biological activity, weather conditions, and other factors. The magnitude of sound levels is usually described by its sound pressure. The A-weighted decibel (dBA) scale is commonly used to describe sound levels because it reflects the frequency range to which the human ear is most sensitive.

Current and Expected Future Condition of the Acoustic Environment if No Action is Taken

The current condition of the acoustic environment is described below. A detailed discussion of past, present, and reasonably foreseeable future projects within the park contributing to the existing conditions and current trends of the acoustic environment are described in more detail in Appendix E. The description below includes an overview of how these ongoing and future actions would affect the acoustic environment. Details regarding impacts of noise from the no-action alternative on wildlife, visitors, and wilderness are discussed further in the "Federally Listed Wildlife Species and Wildlife Species of Concern," "Visitor Use and Experience," and "Wilderness" sections of this chapter.

Under the no-action alternative, the acoustic environment would remain the same or similar to existing conditions, including trends and impacts from past, present, and foreseeable planned actions. Therefore, the affected environment and impacts of no-action are the same and discussed only once here.

Haleakalā National Park

NPS *Management Policies 2006* and Director's Order 47 require the agency to manage, preserve, and restore park acoustical environments and soundscapes. These policies require the NPS to protect and restore the natural soundscapes of parks, including those that have been affected by unnatural and unacceptable noise. In addition to these policies, the park's Foundation Document (NPS 2015b) identifies natural sounds as one of the fundamental resources and values of the park. As discussed in the Foundation Document, natural soundscapes are vital components of a healthy, intact, biological community, that play an important role in wildlife communication and behavior and are critical to effective wilderness management. In addition, natural soundscapes are highly desired by park visitors. As a fundamental resource and value, natural soundscapes are "*warranted primary consideration during planning and management processes"* (NPS 2015b).

The natural acoustic environment of the park is a key fundamental resource and value (NPS 2015b), and is important for wildlife, visitors, and native Hawaiian ceremonies. Because of this importance, the park has invested in over three decades of extensive acoustic monitoring, scientifically documenting the acoustic environment and where human caused noise may impact key resources. Overall, the findings of these studies revealed that across the park, the acoustic environment is generally in good condition, while aircraft are documented as the most prevalent noise source affecting the soundscape (Wood 2015, Lee et al. 2016). Helicopters are most common during the daytime and high-altitude jets are most common at night (Wood 2015). Further, the crater of Haleakalā National Park boasts intensely quiet sound pressure levels, around 10 dBA (Wood 2015). It is necessary to note that the intent of these acoustic monitoring reports is to identify the general acoustic conditions of the park. Sampling locations are generally chosen to represent larger areas of the park based on considerations such as vegetation cover and topography. The acoustic monitoring in these reports was not intended to measure any specific noise, including aircraft or air tour noise. Further, what is mostly reported below are median sound pressure levels during the day from 6am to 6pm ($L_{A50,\ 12hr,\ daytime}$). Like any median measure, this metric does not drastically change if only a few loud events per day occur. Additionally for reference, because decibels are measured on a logarithmic scale, an increase in 3 dB represents a doubling of sound pressure level.

Most of the project area within park lands is in the Kīpahulu District. Common natural sounds in that portion of the project area include weather-related sounds (wind in the forest canopy, thunder, and rain), water flowing, waterfalls rushing, bird calls, insects buzzing, and other animal calls or communications (Lynch 2012, Lee et al. 2016, Job et al. 2018). **Table 8** presents the results of acoustical monitoring conducted for the park within or near the project area and **Figure 6** depicts noise monitoring locations identified in the table. The project area includes the entire Kīpahulu District and a small portion of the Summit District of the park.

The baseline acoustic measurement of natural ambient sound levels for upper Kīpahulu Valley (ST9) is approximately 30 dB ($L_{A50,\ 12hr,\ daytime}$) and the measurement of existing ambient sound levels is approximately 35 dB ($L_{A50,\ 12hr,\ daytime}$) (Lee et al. 2016). Natural ambient in upper Kīpahulu Valley is 30 dB ($L_{A50,\ 12hr,\ daytime}$) due to more vegetation, rain and streams, birds, and insects. The existing ambient of 35 dB ($L_{A50,\ 12hr,\ daytime}$) includes the noise of aviation, which is the dominant (and possibly the only) non-natural sound that could be heard in the area. Visitors are not allowed in Kīpahulu Valley Biological Reserve (see **Figure 6**).

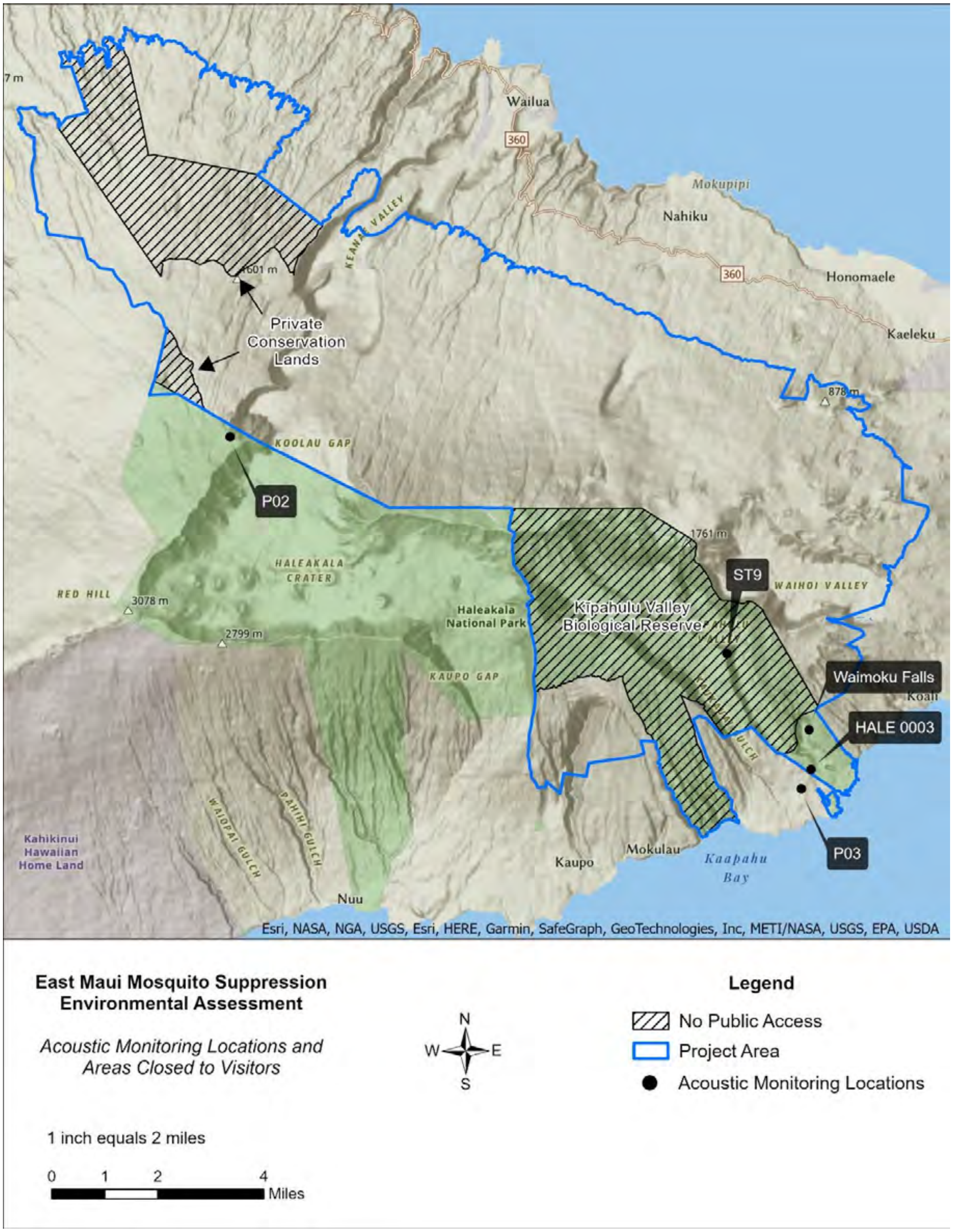

FIGURE 6: AREAS CLOSED TO PUBLIC ENTRY AND ACOUSTIC MONITORING LOCATIONS WITHIN HALEAKALĀ NATIONAL PARK

Commercial air tours, commercial flights, private aviation, and other administrative flights contribute noise to this area. The difference between the natural and existing ambient, as measured in 2003 for upper Kīpahulu Valley, represents a meaningful change. In other words, the natural ambient is noticeably quieter than the existing ambient due to the factors described earlier in this section.

The lower-elevation portion of the Kīpahulu District acoustic measures are represented by Kīpahulu Coastal (P03) measured in 2003 and Kīpahulu (HALE003) measured in 2008 (Lee et al. 2016, Lynch 2012). Natural ambient sound levels for these areas were 45.3 dB ($L_{A50,\ 12hr,\ daytime}$) for P03 and 38.0 dB ($L_{A50,\ 12hr,\ daytime}$) for HALE003. The existing ambient sounds levels were 43.5 dB ($L_{A50,\ 12hr,\ daytime}$) for P03 and 38.9 dB ($L_{A50,\ 12hr,\ daytime}$). These sound pressure levels (both natural and existing) are much higher due to the proximity to the coast and more natural sound activity; however, the small differences between natural and existing ambient here suggest lower levels of noise than the upper portion of the valley. The higher natural and existing ambient sound pressure levels do allow for masking of anthropogenic or unwanted noise in these areas.

A small portion of the project area occurs within the Summit District of the park. **Table 8** includes the results of acoustical monitoring conducted along the Supply Trail within the Summit District (P02) with the natural ambient at approximately 27.2 dB ($L_{A50,\ 12hr,\ daytime}$) and the measurement of existing ambient sound levels is approximately 27.7 dB ($L_{A50,\ 12hr,\ daytime}$) (Lee et al. 2016). These measures indicate a relatively quiet acoustic environment, dominant in natural sounds such as the sound of wind, rain, and the occasional animal noise (Lee et al. 2016). In addition to sounds of hikers, day-use visitors, campers, and human-generated noises that are part of the soundscape on a regular to intermittent basis, there is also noise generated by park management activities, vehicles along the small portion of Crater Road within the project area, and administrative and commercial aircraft flying overhead (primarily helicopter flights).

TABLE 8: SUMMARY OF SOUNDSCAPE DATA COLLECTED WITHIN THE PARK PORTIONS OF THE PROJECT AREA

Site Name (Site Number)	Vegetation Type	Year Data Collected[1]	$L_{Anat,12hr,\ daytime}$[2]	$L_{A50,\ 12hr,\ daytime}$[3]	$L_{A90,\ 12hr,\ daytime}$[3]
Kīpahulu (HALE003) *	Grassland; coastal	2008	38.0	38.9	35.1
Upper Kīpahulu Valley / Kīpahulu Scientific Reserve (ST9) *	Evergreen forest	2003	30.7	34.9	30.0
Kīpahulu Coastal (P03) *	Forested upland	2003	45.3	43.5	38.2
West Rim Crater / Supply Trail (P02)	Shrubland	2003	27.7	27.2	21.5

Sources: Lee et al. 2016 Lynch 2012.
[1] Different techniques were used to calculate natural ambient sound in 2003 versus 2008. See Lee et al. 2016 for data collection protocol in 2003 and see Lynch 2012 for data collection protocol for data collected in 2008
[2] L_{nat} = natural ambient sound level and is the natural sound conditions in national parks, which exist in the absence of any human-produced noise.
[3] L_{50} and L_{90} = metric used to describe existing sound pressure level (L) in decibels, exceeded 50 and 90 percent of the time respectively; in other words, half the time the measured levels of sound are greater than the L_{50} value, while 90 percent of the time the measured levels are higher than the L_{90} value.
* Located in lower Kīpahulu Valley.

Helicopters are used for transporting park personnel to various park locations for resource monitoring, rescue actions, and maintenance activities. These flights contribute noise to the park's acoustic environment. Park staff conduct management and resource monitoring activities in remote areas of the park and fieldwork may last a few hours to a week at a time. Ongoing activities that use mechanized tools include fencing to exclude ungulates and facilities maintenance for existing cabins within wilderness enclaves. Helicopter use for these administrative activities averaged approximately 200 hours/year (approximately 100 operations) between 2011 and 2022 (T. Bailey, *pers. comm. 5/26/2022*) and would likely continue at current levels into the future. Approximately 30 percent of current administrative flights travel within the Summit District and 70 percent (140 hours/year or 12 hours/month) travel within the Kīpahulu District including many areas where incompatible mosquito releases would occur under this project. The park would continue current management actions and respond to future needs and conditions without major changes in the present course.

Unlike administrative flights, commercial air tours in the park occur seven days a week year-round except during inclement weather and on the following commercial-free days (end of Makahiki [January], Zenith Noon [May], Summer Solstice [June], Zenith Noon [July], start of Makahiki [October], and Winter Solstice [December]). Between 2013 and 2019, the number of commercial air tours in the park ranged between 4,543 and 4,932 per year (Lignell 2020). From 2013 through 2018, the number of commercial air tours averaged approximately 13 air tours per day (an estimated 2.05 hours per day or 750 hours per year). In 2019, a study identified a total of 321 helicopter air tours between March 15–April 15, with an average of 10 flights per day over this period (Beeco et al. 2020). **Figure 7** displays the travel patterns and helicopter model of these flights (figure from Beeco et al. 2020). These flights intersect the project area in the southernmost reaches, primarily around Kīpahulu Valley, Ka‘āpahu, and Kaupō Gap. Based on acoustical monitoring in 2003, commercial aircraft were audible 10.2 percent of the time at the Supply Trail (P02) monitoring station (in the Summit District) and 27.8 percent of the time at the monitoring station located in the highest monitoring station in upper Kīpahulu Valley (ST9), Kīpahulu District (Lee et al. 2016). The park is developing an Air Tour Management Plan (ATMP) with the FAA to mitigate or prevent substantial adverse impacts of commercial air tour operations on the park's natural and cultural landscapes and resources, areas of historic and spiritual significance to Native Hawaiians, wilderness character, and visitor experience. A decision is expected in 2023.

The impacts of these ongoing and future actions (Appendix E) have been considered. Under the no-action alternative, the acoustic environment would remain the same or similar to existing conditions, including trends and impacts from past, present, and foreseeable planned actions. Because these actions are part of the existing acoustic environment conditions, the no-action alternative would not result in any indirect or direct impacts to the acoustic environment on NPS lands. In turn, because there are no direct or indirect effects of the no-action alternative, there would be no cumulative effects associated with the no-action alternative.

State Lands

The State of Hawai‘i regulates noise through the HAR, Title 11, Chapter 46 (HAR 11-46), "Community Noise Control." The purpose of these rules is to *"provide for the prevention, control, and abatement of noise pollution in the State from the following noise sources: stationary noise sources; and equipment related to agricultural, construction, and industrial activities"* (HAR 11-46). Community Noise Control Regulations are not applicable to most moving sources, i.e., transportation and vehicular movements.

State lands within the project area are depicted in **Figure 1**. Other than administrative and commercial helicopter flights and the occasional noise from hunters and management activities on state forest reserves (e.g., invasive animal and plant control, habitat restoration, resource monitoring, rare species protection and research, fire management, and infrastructure maintenance), the state forest and natural area reserves are extremely quiet (based on anecdotal experience of state staff working in the project area). Although the state has no acoustic monitoring data in the project area, the soundscape likely consists primarily of natural sounds coming from wind, rain, animal noises, and waterfalls, based on NPS acoustic monitoring results as the baseline for the entire project area. Most state forest reserves within the project area are open to the public, however, visitor use is very low due to the difficult terrain and limited roads and trails. State natural area reserves within the project area (such as Hanawī Natural Area Reserve) are open to the public, but access is extremely difficult, and permits may be required for access and certain activities. Therefore, visitor use in these areas is extremely limited.

On state lands, DOFAW oversees fence construction and maintenance, control of ungulates, control of invasive plants, and predator control to preserve native ecosystems and species. The Maui Forest Bird Recovery Project (MFBRP) conducts mosquito and avian malaria monitoring. Mechanized equipment and ground teams would generate noise during fencing activities and regular planned maintenance of trails and LZs. Approximately 165 helicopter operations are conducted per year for management activities within the reserves. These flights are typically quick trips to drop off field staff and supplies. Over the past 12 years, DOFAW used helicopters for approximately 208 hours/year (4 hours/week) to conduct natural resource management activities (Safecom 2022). It is unknown how many commercial or tour flights fly over the state forest reserves within the project area, and site-specific acoustic data have

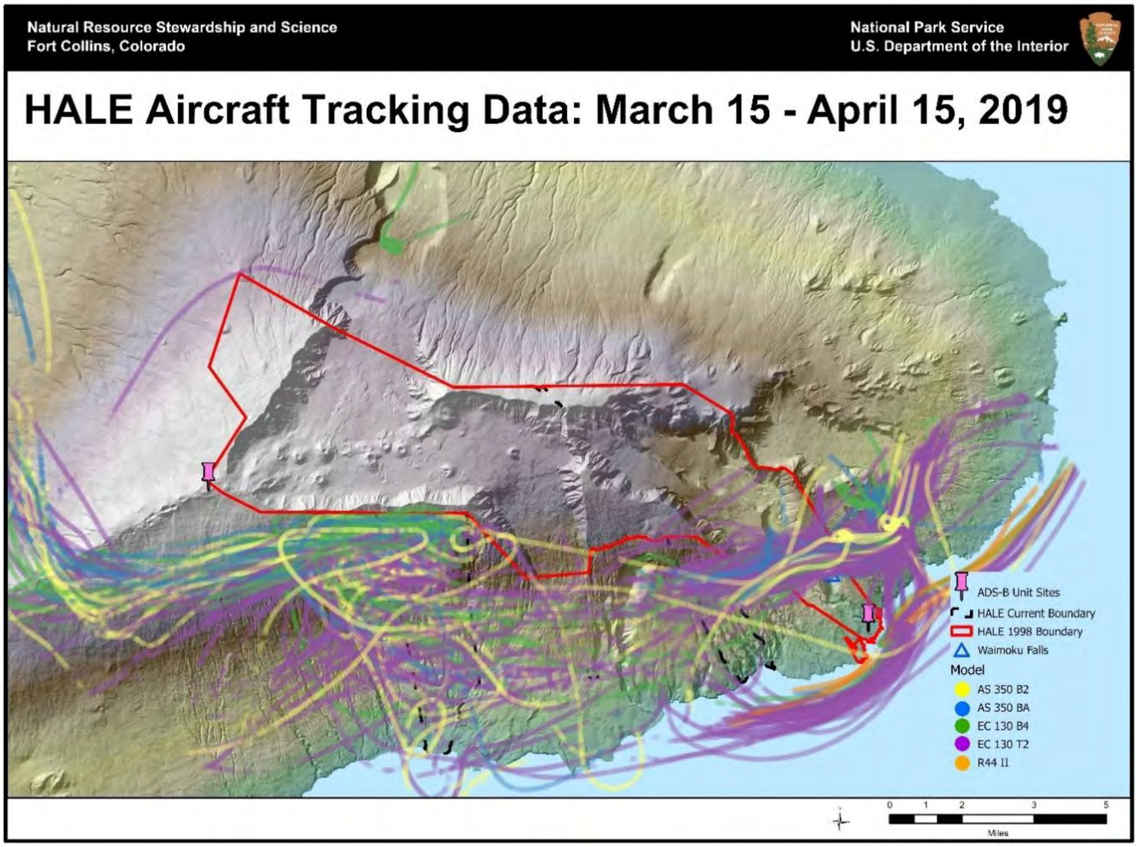

FIGURE 7: COMMERCIAL HELICOPTER FLIGHTS OVER THE PROJECT AREA (BEECO ET AL. 2020).

not been collected within the state forest reserves. However, for analysis purposes and because the majority of state forest reserves occur within the project area, it has been assumed that approximately 200 hours/year of administrative helicopter flights occur within or immediately near the project area. As in the park, the number of state administrative helicopter flights and associated noise levels would likely continue at current levels within the project area. There are no anticipated changes to public access within the project area, so ongoing noise impacts to visitors would remain unchanged in the foreseeable future.

The impacts of these ongoing and future actions (as listed in Appendix E) have been considered as part of the affected environment as described above. Under the no-action alternative, the acoustic environment would remain the same or similar to existing conditions, including trends and impacts from past, present, and foreseeable planned actions. Because these actions are part of the existing acoustic environment conditions, the no-action alternative would not result in any indirect or direct impacts to the acoustic environment on state lands. In turn, because there are no direct or indirect effects of the no-action alternative, there would be no cumulative effects associated with the no-action alternative.

The Nature Conservancy and Other Private Conservation Lands

Other than the noise associated with occasional commercial air tours and helicopter administrative flights, as well as the sounds of human visitors and employees, TNC's Waikamoi Preserve is extremely quiet, with natural sounds coming from wind, rain, vegetation, and animals (assuming conditions are similar to those within the park). Because acoustic data has not been collected within the preserve (other than bird recordings), the NPS acoustic monitoring results were used as a baseline for the entire project area. However, within the other private conservation lands adjacent to the park and Waikamoi Preserve, human-caused noise is likely lower than in the park, state, and TNC-managed lands because there is no public access allowed within these areas. Noises in the private conservation lands primarily consist of occasional commercial overflights, and vehicle use for management activities and landowner/manager/employee recreational hunting.

Public access to the Waikamoi Preserve is limited to guided hikes, educational and service trips, and scientific research. TNC staff typically lead public hikes into the preserve three times per month with a maximum of 15 participants per hike. In addition, approximately one volunteer work trip is conducted per month and TNC typically provides trips into the preserve twice a month (once for local groups, and once for donors or other special guests). Research projects typically occur for a period of one week a couple of times a year. In total, visitation to the preserve is approximately 1,000 people per year (A. Cohan, *pers. comm. 9/30/21*).

In addition to the sound of walking and talking by visitors, noises are generated by management activities including fence maintenance, ungulate control, treatment of non-native plant species, and resource monitoring within the preserve. Because much of Waikamoi Preserve is remote and relatively inaccessible by foot, many management activities are conducted by helicopter. Approximately 60 helicopter operations are conducted per year (estimated 75 flight hours/year) into and out of the preserve (A. Cohan, *pers. comm. 9/30/21*). These flights typically drop off employees and supplies. Management activities are expected to continue as described in this section on the private conservation and TNC-managed lands. In addition to helicopter flights to and from the preserve, commercial flights over the preserve also create intermittent noise.

The impacts of these ongoing and future actions (see Appendix E) have been considered. Under the no-action alternative, the acoustic environment would remain the same or similar to existing conditions, including trends and impacts from past, present, and foreseeable planned actions. Because these actions are part of the existing acoustic environment conditions, the no-action alternative would not result in any indirect or direct impacts to the acoustic environment of TNC and other private conservation lands. In turn, because there are no direct or indirect effects of the no-action alternative, there would be no cumulative effects associated with the no-action alternative.

Effects of the Proposed Action on the Acoustic Environment

Activities associated with the proposed action would result in noise that could impact the acoustic environment, visitor experience, sensitive wildlife, and wilderness character. Noise impacts would be mitigated through careful planning of flight paths and timing of mosquito releases (see mitigation measures in Chapter 2). Details regarding impacts of noise on wildlife, visitors, and wilderness are discussed further in the "Federally Listed Wildlife Species and Wildlife Species of Concern," "Visitor Use and Experience," and "Wilderness" sections of this chapter.

Methods and Assumptions

The baseline for evaluating potential impacts to the acoustic environment was developed using the available existing ambient sound measurements in the park (Lynch 2012, Lee et al. 2016). No baseline sound metrics are available for state, TNC, or privately managed conservation lands; however, given the similarity of conditions on state, TNC, and privately managed lands to park lands, existing sound levels in these areas are assumed to be similar to those within the park. The existing ambient sound measurements were then compared to the expected noise levels that would occur during incompatible mosquito releases, specifically the use of drones, and occasionally other mechanized equipment such as ground vehicles, generators, and helicopters, relative to the existing ambient sound levels. Impacts were evaluated based on the potential for mosquito release activities to create noise impacts over sustained periods of time that would surpass ambient existing sound levels and indicators for human and wildlife impacts. Notably, the attenuation (reduction) of noise depends on site-specific conditions such as the terrain conditions between the noise source and receiver (i.e. visitors and/or wildlife), vegetation, and meteorological conditions. A detailed analysis of mechanized noise (from drone, helicopter, ground vehicle, or other mechanized equipment) in specific locations that take these factors into account would be impracticable because impacts to the acoustic environment would be dispersed over the entire core area; however, a general understanding of how the acoustic environment may be impacted is presented.

The acoustic environment analysis area includes not only the core area where most releases would occur, but also the area surrounding it where project-related noise could impact the acoustic environment. Specific locations included in the acoustic environment analysis area that lie outside of the project area include temporary helibases outside of the core area and drone or helicopter flight paths to and from the core area.

Sound pressure levels are often measured with the logarithmic decibel (dB) scale relative to a reference value. The relative loudness of sounds as perceived by the human ear is expressed in dBA (OSHA 2013). The following values illustrate some key sound level indicators and the effects that they have on humans:

- 35 dBA – This value is designed to address health effects of sleep interruption; noises at this loudness can have effects on blood pressure while sleeping (Harabaldis et al. 2008).
- 45 dBA – This value represents the recommendation from the World Health Organization that noise levels inside bedrooms remain below 45 dBA (Berglund, Lindvall, and Schwela 1999).
- 52 dBA – This value is based on the U.S. Environmental Protection Agency's level for speaking in a raised voice to an audience at 33 feet (EPA 1974). This represents the sound level at which an interpretive program would be affected.
- 60 dBA – This value is the sound level where normal communications with individuals standing 3.3 feet apart would be interrupted. This represents the sound level at which recreational visitors conversing would be affected, including hikers (EPA 1974).

Although noise levels are usually measured and expressed in dBA, which is based on the sensitivity of the human ear to different frequencies, this measurement may not reflect the noise sensitivity of birds or other wildlife (NPS 1995). For additional information regarding noise impacts to wildlife from the proposed action, refer to the "Noise Impacts on Wildlife" section within the Threatened and Endangered Wildlife Species and Wildlife Species of Concern impact analysis.

An increase of the existing ambient sound level affects the ability of humans and animals to perceive other sounds within a certain distance or area. In general, the higher the existing ambient sound level, the shorter the distance from which other sounds (for example, those of a forest bird) can be heard. This concept is expressed in terms of listening area (the area in which humans and wildlife can perceive sounds) and alerting distance (distance at which alerting communications can be heard). Reduction in listening area and altering distance is a way of quantifying degradation of hearing performance in humans and animals as a result of an increase in ambient noise level. **Table 9** shows the relationship between increases in ambient sound levels and percent reduction in listening area and alerting distance. The impact criteria are based on the distance at which project impacts would result in a 3 dBA increase over ambient conditions (EPA 1974). A 3 dBA increase above the existing ambient sound level is considered an important indicator of potential noise impact because it results in a 50 percent reduction in listening area for humans and animals and a 30 percent reduction in alerting distance, as shown in **Table 9** (NPS 2010).

TABLE 9: REDUCTION IN LISTENING AREA AND ALERTING DISTANCE DUE TO INCREASES IN AMBIENT SOUND LEVELS

50% dBA Ambient Increase	Percent Reduction in Listening Area	Percent Reduction in Alerting Distance
3	50%	30%
6	75%	50%
10	90%	70%
20	99%	90%

Drone Noise Levels

The primary method of incompatible mosquito release within the project area would be through the use of drones. The sound produced by a consumer-grade battery-powered rotary or fixed-wing drone at ground level is similar to loud highway noise (Schaffer et al. 2021). Most consumer-grade drones are far quieter than helicopters with some being up to 40 dBA quieter than a manned helicopter at roughly 328 feet AGL (Airborne Drones 2020). For this project, drones would fly at approximately 50–100 feet above the tree canopy (likely approximately 100–200 feet AGL) during mosquito releases. When multiple drones are in use, they would likely be releasing in different areas (such as one on state lands and one in the park) rather than releasing in close proximity. Therefore, it is not anticipated that noise impacts would be compounded by the use of multiple drones. When ferrying to and from release locations, drones would fly no higher than 500 feet AGL. Drone noise levels for various heights above ground are presented in **Table 10** and are based on a decrease of 6 dB for every doubling of distance from a sound perceiver. Along the same lines, the noise produced by a drone would likely blend in with the existing ambient noise levels of the project area at a lateral distance of approximately 0.25–0.5 mile depending on the height of flight (Airborne Drones 2020, Schaffer et al. 2021). Notably, the noise levels presented in this section are not actual measured noise levels; actual noise levels during mosquito releases would vary during specific operations depending on altitudes, topography, vegetation, speed, and drone power settings.

TABLE 10: DRONE NOISE LEVELS AT VARIOUS HEIGHTS

Drone Type	Height Above Ground Level (AGL) from Source (feet)			
	25 feet AGL	100 feet AGL	200 feet AGL	500 feet AGL
Consumer Multirotor	~ 68–75 dBA	~ 58–65 dBA	~ 52–59 dBA	~ 44–52 dBA
Small, fixed wing drone	~ 63–70 dBA	~ 53–60 dBA	~ 47–54 dBA	~ 40–47 dBA
Quiet Commercial Multirotor	~ 57–68 dBA	~ 47–58 dBA	~ 41–52 dBA	< 44 dBA

Source: Airborne Drones (2020) and Schaffer et al. (2021)

Helicopter Noise Levels

Helicopter noise levels were estimated using the sliding scale approach presented in the Interagency Visitor Use Management Council Framework (IVUMC 2016) and the NPS Natural Sounds and Night Skies Division (NSNSD) developed Attenuation Calculator. The Attenuation Calculator maps and provides noise metric statistics for the attenuation (i.e. spread and reduction) of noise using the ISO 9613-2 (*Attenuation of sound during propagation outdoors — Part 2: General method of calculation*) standard. The main limitation of this tool is that terrain effects are not incorporated into the calculation; it is strictly the attenuation loss due to the atmosphere and distance. Practically, this means that the distances with associated noise metrics identified are a worst-case scenario. Further, the Attenuation Calculator only calculates a single operational mode (hover in ground effect) and cannot incorporate multiple operational parameters such as aircraft performance, thrust settings, directivity, and other operational modes. Despite these limitations, the tool provides valuable information regarding noise attenuation and is a means of comparison between different release methods for this project. For the purposes of this analysis, two primary approaches were taken. First, noise was calculated for the helicopter in transit and the other for the helicopter hovering.

For the transit analysis, it was assumed that:

- the park and state would be using a Hughes 500D helicopter for all flight operations, which is a typical aircraft used for park and state administrative flights;
- the analysis used the Hughes 500D in the ‘hover in ground effect’ operational mode;
- the speed was set to 57 mph, which is similar to the anticipated transiting flight speed (62 mph)
- altitude was set at 500 feet above the receiver (person on the ground).
- natural ambient and existing ambient sounds levels were set to 30 dB (A-weight) and 35 dB, respectively, which are consistent with the baseline acoustic measures of natural ambient for the upper Kīpahulu Valley (Lee et al. 2016).

For the hovering analysis, the assumptions were the same as for transit, except:

- the speed was set to stationary.
- altitude was set at 150 feet above the receiver (person on the ground).

As summarized in **Table 11**, results of the Attenuation Calculator under the worst-case scenario suggest that helicopter noise could be audible[1] up to 3.5 miles from a given flight path at 500 feet AGL, and noise could be above existing ambient levels (35 dB) up to1.8 miles from the flight path. Modeled flight paths were chosen as representative flight paths into and out of the Kīpahulu Valley portion of the project area for mosquito releases that would commonly be used for dropping off teams for monitoring or conducting helicopter longline releases. Speech or interpretive program interference (levels above 52 dB) could begin to occur at 0.47 miles from a flight path. Speech or interpretive program interference is based on the EPA's level for when speaking in a raised voice to an audience at 33 feet would begin to be affected (EPA 1974). Finally, when hovering within 50 lateral feet of a given location at 150 feet AGL, helicopter sound levels could reach a maximum of 82 dB (L_{AMax}) at ground level. The maximum sound pressure levels directly under the helicopter at 50 feet AGL are estimated to be 93 dB (L_{AMax}).

TABLE 11: ATTENUATION CALCULATOR HELICOPTER SOUND EXPOSURE LEVELS AT DIFFERENT DISTANCES

		Lateral Distance from Source (feet or miles)				
Aircraft Name	**Operational Mode**	**0 feet**	**50 feet**	**0.47 miles**	**1.8 miles**	**3.5 miles**
Hughes 500D	Hover in ground effect	93.1 dB (L_{AMax}) at 50 feet AGL	82.1 dB (L_{AMax}) at 150 feet AGL	Speech Interference (> 52 dB) at 500 feet AGL	Above Existing Ambient (> 35 dB) at 500 feet AGL	Audible (~27 dB) at 500 feet AGL

Source: NSNSD attenuation calculation for this project

Generators and Vehicle Noise Levels

The estimated noise levels of generators and vehicles proposed for use under the proposed action are included in **Table 12**. Trucks or SUVs would be used on existing roads (see **Figure 5**) to reach monitoring and pedestrian release sites in Makawao Forest Reserve, TNC's Waikamoi Preserve, Mahi Pono, and East Maui Irrigation lands and to reach drone launch sites at road-accessible helibases. Generators would only be used at up to four monitoring locations on state lands, two locations within the park, and two locations within TNC's Waikamoi Preserve. Notably, these noises would occur at ground level and would be substantially muffled by the surrounding dense vegetation.

TABLE 12: GROUND MECHANIZED EQUIPMENT PROPOSED FOR USE IN THE ANALYSIS AREA.

Type of Equipment	Estimated Maximum Noise Level (dBA)
Quiet Honda Generator	~ 52–58 dBA* at 23 feet from source
Truck or SUV	~75 dBA** at 50 feet from source

* Source: Honda (2022)
** Source: FHWA (2006)

Analysis

Drone Release

The park and state would use drones as the primary mosquito release method in the core area. Drone operators would be positioned at temporary helibases accessible by ground vehicles. Noise from vehicles would only occur when drone operators drive (outside the project area) to or from helibases at the beginning and end of each drone operation day

[1] Audibility was defined to be sounds levels that are 8 dB below existing ambient levels

(operation days could include five workdays per week) or if resupply trips are needed. As described in Chapter 2, it is conservatively estimated that this project would require up to 72 hours of drone flight time per week during warm months and up to 49 hours during cold months to achieve the desired mosquito release rate (**Table 3**). Of those hours, 37–47 flight hours per week would occur over state lands (approximately 64 percent of the core area or 30,796 acres), 6–11 hours per week would occur over NPS lands (approximately 15 percent of the core area or 7,099 acres), 6–7 hours per week would occur over private conservation lands (approximately 11 percent of the core area or 5,168 acres), and 1–7 hours per week would occur over TNC-managed lands (approximately 10 percent of the core area or 5,101 acres). Two or more drones would fly for up to five days per week between civil sunrise and civil sunset on weekdays. Drone flight paths would vary substantially depending on the release locations being treated each day, and drones would likely only pass over a specific location twice per week. Depending on the drone model in use, noise levels experienced by wildlife or a person on the ground during releases where the drone is flying at 100 feet AGL could range from 47 to 65 dBA and from 41 to 59 dBA at 200 feet AGL for less than 15 seconds because the drones would be moving swiftly through the core area during releases (up to 22 mph; see **Table 10**) (Airborne Drones 2020; Schaffer et al. 2021). For birds or other wildlife near the top of the tree canopy, drone noise levels could range from 47 to 71 dBA for less than 15 seconds again depending on the type of drone in use and the height above canopy. For reference, a Hughes 500D helicopter at 150 feet AGL would produce a maximum noise level of 82 dBA (**Table 11**). During ferrying flights at approximately 500 feet AGL, drone sound levels would range between less than existing ambient (~35 dBA in upper Kīpahulu Valley) to 53 dBA (see **Table 10**) for wildlife or a person on the ground or wildlife in the tree canopy. These noise levels are estimated to only last for less than 5 seconds at a time because the drones can travel up to 62 mph while ferrying.

Under the worst-case scenario, drone noise could potentially be heard (above approximately 27 dBA) up to 0.5 mile of the drone. Notably, the extensive tree canopy cover and rugged terrain can have a dampening effect on sound and may reduce the distance (likely by half or more based on anecdotal experience of park and state staff working in the project area) where sound is heard. The nearest recreational areas where people could experience drone noise are in Makawao Forest Reserve and lower Kīpahulu District. People in these areas could very briefly experience drone noise if drones pass within 0.5 mile of recreational trails or other public use areas, but these impacts could be reduced, for example, by conducting regular pedestrian releases in Makawao Forest Reserve, should that be deemed necessary. Most of the areas where drones would be conducting releases would be out of earshot for hikers along the Pīpīwai Trail to Waimoku Falls. Drone flight paths, timing of flights, and flight heights would be selected to reduce noise impacts on visitors, nearby landowners or communities, wilderness, and sensitive environmental resources. Mosquito releases would occur outside the breeding season of Hawaiian forest birds, to the extent possible. Where breeding seasons cannot be avoided, drone operations would occur above the tree canopy to limit the noise impact to nesting forest birds. It should also be noted that there would be no mosquito releases at night or on the weekends, so noise impacts from drones would only occur during daylight hours on weekdays.

Overall, people would not likely notice a noise difference at popular visitor use areas near the park and other publicly accessible areas during the anticipated 49–72 hours of drone flights per week. The anticipated drone use under the proposed action would require two or more drones flying simultaneously in different areas of the core area for a total of approximately 220–325 hours per month throughout the core area. The perceived drone noise levels (approximately 47–59 dBA at 100–200 feet AGL) experienced by wildlife or people on the ground in the core area would fluctuate rapidly because drones would be moving swiftly, and noises would be muffled by the tree canopy and rugged terrain. At the upper limit of the estimated decibel levels, drone noise could possibly be loud enough to disrupt conversations, but this disruption would be brief, due to the minimal time that a drone would be overhead in one location. With the exception of the Kīpahulu District of Haleakalā National Park and Makawao Forest Reserve, very little recreation or hunting occurs in the project area, so noise impacts to visitors or other users would be minimal. Noise from drones would be present in the project area during release operations until sufficient mosquito population suppression is achieved but would largely go unnoticed by humans and may only briefly cause annoyance to wildlife.

Helicopter Longline Release

The helicopter longline release method would only be used as a short-term (up to two months), temporary release method for intermittent time periods if drones are unavailable. Decibel levels directly under flight paths are expected to be substantially higher than existing ambient levels based on the maximum sound levels produced by a helicopter. Notably, higher sound level estimates were used for a more conservative analysis. Under this worst-case scenario, helicopter noise could be audible up to 3.5 miles from a given flight path at 500 feet AGL, and noise could be above existing ambient levels up to 1.8 miles from the flight path. Speech interference could occur at 0.47 miles (the area within a 0.47 mile-radius is 448 acres; see **Table 11**). When a helicopter is hovering within 50 feet laterally of a given location at approximately 150 feet AGL (at which most helicopter longline releases would occur), helicopter sound levels at the ground could reach a maximum of 82 dBA for 15 seconds or less at any given location in the core area. Sound levels would decrease as the distance from a given flight path increases. Notably, actual distances and sound levels would likely be far lower than the modeled results provided in **Table 11** due to the rugged terrain and extensive tree canopy cover in the project area, which would block and absorb some of the helicopter noise. Additionally, the noise levels presented in this section are not actual measured noise levels; actual noise levels would vary during specific operations depending on the altitudes, types of maneuvers, speed, and power settings during helicopter flight. These factors also affect the intensity, duration, and spatial distribution of noise.

For purposes of estimating helicopter noise impacts during mosquito releases, it has been assumed that an average of two treatments of the entire core area could occur per month for up to two months per year. The average anticipated helicopter flight time would occur for up to 6 hours per day, 5–7 days per month for a total of 39 hours per month during warm months and 28 hours per month during cold months. Flight time would not exceed 56–78 flight hours/year. However, as stated in Chapter 2, this estimate is a maximum and the occasional helicopter longline releases would only be needed as a short-term (up to two months), temporary release method should drones become unavailable. For reference, this maximum estimate of helicopter flight hours for helicopter longline release (56–78 flight hours/year) is far less than the current estimate of annual administrative flight hours (approximately 415 hour/year) for park, state, and TNC-managed lands within the project area.

Helicopters would avoid flying low near forest bird breeding habitats to avoid rotor wash and excessive noise disturbance to nesting forest birds. There would be no mosquito releases at night or on the weekends, so noise impacts would only occur during daylight hours (between civil sunrise and civil sunset) on weekdays. During helicopter longline releases, adverse impacts on the acoustic environment would primarily occur along flight paths, at helibases, and when hovering over mosquito release locations. Helicopters would hover for less than 15 seconds over each mosquito release location. At any given location in the core area, the perceived noise levels from helicopter operations would fluctuate for humans or wildlife because helicopters would be moving through the area quickly (22 mph during releases and up to 115 mph during transit). Impacts from helicopter longline releases could occur anywhere within the core area but would be targeted depending on the need at the time. The core area contains many places where there is little to no public use. The most well-used areas with established public trails include Makawao Forest Reserve and the lower Kīpahulu District area where many people use the Pīpīwai Trail to access Waimoku Falls. **Table 13** provides estimates for the duration that helicopter noise along several example flight paths would be audible under the worst-case scenario provided by the Attenuation Calculator (above existing ambient) for a visitor at Waimoku Falls. As shown in the table, most flights to the Kīpahulu Valley and Manawainui locations of the core area would produce audible noise for less than 4 consecutive minutes. The flight paths included in **Table 13** are intended to provide a representative of potential flight paths and the times they would be audible in visitor use areas. Actual flight paths would vary and be determined by weather, and targeted release locations.

TABLE 13: COMMON FLIGHT PATHS IN KĪPAHULU VALLEY AREA

Flight Path	Path Distance (round trip)	Potential Speed of Travel	Travel Time (round trip)	Closest Point along Flight Path to Waimoku Falls	Time Noise above 35dB at Waimoku Falls
‘Ohe‘o to Wing	13.0 miles	115 mph	6.8 min	0.7 mile	3.0 min
‘Ohe‘o to Palikea	11.2 miles	115 mph	5.9 min	0.3 mile	3.5 min
‘Ohe‘o to Charlie	10.6 miles	115 mph	5.6 min	0.4 mile	3.5 min
Nu‘u to Wing	12.0 miles	115 mph	6.3 min	~ 5.0 miles	0 min

Overall, adverse impacts on the acoustic environment from helicopter longline releases could occur anywhere in the core area (up to 48,164 total acres) but would be targeted depending on the need at the time. However, it should be noted that this method would only be used as a short-term (up to two months), temporary release method when drones are not available. Up to 28–39 hours of helicopter flight time could occur per month (up to 6 hours per day, 5–7 days per month) for up to two months per year. Because the helicopters would be flying or hovering well above the canopy, noise levels on the ground would not exceed 82 dBA for a person or wildlife on the ground. While noise levels immediately beneath flight paths would exceed levels that would be expected to disrupt human communication and potentially cause annoyance to wildlife, these noise levels would not be sustained at that level for more than 15 seconds at any given point. Impacts could potentially extend over thousands of acres at a given time, impacting wildlife habitat and visitor use areas within that range. However, little public use occurs in the very remote sections of the core area, and visitors would only experience intermittent noise if flights paths were near their location. Further, this short-term release method would only temporarily occur in necessary situations for less than 2 months per year and would therefore largely be unnoticed by people and would rarely cause annoyance to wildlife.

Pedestrian Release

As stated in Chapter 2, pedestrian release of mosquitoes may occur within an area of up to 5,000 acres in the western portion of the project area including portions of Makawao Forest Reserve (State land), TNC's Waikamoi Preserve, and other private lands. These locations are accessible for pedestrian release due to existing four-wheel-drive roads (shown in brown on **Figure 5**) and established trails with drive-up trailheads. Pedestrian releases may also occur within approximately 400 acres of the park and approximately 400 acres of Hanawī Natural Area Reserve but only on a quarterly basis simultaneous with ground-based mosquito monitoring (see analysis of impacts from pedestrian releases at these monitoring sites in the "Mosquito Monitoring" section below).

Motorized vehicles (SUVs or trucks) would assist in the transportation of field teams and gear for treatments in Makawao Forest Reserve, TNC's Waikamoi Preserve, and other private lands. Noise from vehicles is estimated to not exceed 2 hours per day up to 2 days per week along the Flume Road shown (in brown) on **Figure 5** during pedestrian releases. As previously mentioned, ground vehicles can reach 75 dBA at 50 feet from the source but would be muffled by the surrounding canopy and would not be expected to exceed 60 dBA at 50 feet from the source of noise. The noise produced by crews releasing mosquitoes would be similar to that produced by any other recreational visitor on the trails in Makawao Forest Reserve. Trails within TNC's Waikamoi Preserve are private and not regularly travelled by the public except during guided trips.

Overall, noise from this release method would be minimal and would include noise of up to 75 dBA at 50 feet from vehicles approaching and leaving trailheads up to 2 hours per day, 2 days per week. Noise impacts from vehicles would blend into the vehicle traffic/noise already occurring at trailheads and would largely be unnoticeable to wildlife and humans along the Flume Road.

Mosquito Monitoring

As described in Chapter 2, monitoring activities would consist of intermittent ground-based monitoring to trap and evaluate mosquito populations and would be conducted concurrently with ground-based pedestrian or aerial releases, on a quarterly basis (four times/year). Monitoring activities would continue indefinitely over the life of the project. Four monitoring locations would be selected on state lands, two locations within the park, and two locations within Waikamoi Preserve. It is anticipated that three of the locations (two on state lands and one in Waikamoi Preserve) would be accessible by ground vehicles and the other five locations (two on state lands, two within the park, and one within Waikamoi Preserve) would require helicopter access. Pedestrian releases may occur concurrently with monitoring and could potentially cover up to 1,000 acres within the core area (400 acres in the park, 400 acres on state lands, and 200 acres in Waikamoi Preserve), and potential impacts are discussed in the preceding section.

The estimated total required helicopter flight time for mosquito monitoring is 70 hours/year (approximately 17.5 hours per week for one week each quarter) and would include the time required to land and drop off or pick up crews and supplies at the LZs. For reference, the current estimate of park, state, and TNC administrative flights is 415 hours/year. Helicopters would fly 2–6 hours per day for pick-ups and drop offs at LZs during these quarterly trips. As listed in

Table 11, in the worst-case scenario, helicopter noise could be audible up to 3.5 miles from a given flight path at 500 feet AGL, and noise could be above existing ambient levels up to 1.8 miles from the flight path. Speech interference could occur when helicopters are operating 0.47 miles away. Finally, when hovering within 50 lateral feet of a given location at 150 feet AGL, helicopter sound levels could reach 82 dBA and grow louder (up to 93 dBA) as the helicopter descends below the canopy to land at LZs. As described previously, actual distances and sound levels would likely be far lower than the modeled results provided in **Table 11** due to the rugged terrain and extensive tree canopy cover in the project area, which would block and absorb some of the sound generated by helicopters. Additionally, the noise levels presented in this section are not actual measured noise levels; actual noise levels vary during specific operations depending on the altitudes, types of maneuvers, speed, and power settings during helicopter flight. These factors also affect the intensity, duration, and spatial distribution of noise.

Generators would be needed for monitoring trips and could produce intermittent noise at the five backcountry camps four times per year at each camp, for up to 3 hours per day for up to 7 consecutive days. As listed in **Table 11**, a quiet Honda generator can produce noise levels of up to 58 dBA at 23 feet from the source. However, due to the density of vegetation where generators would be used, this noise is expected to be lower, and generators would only be running in the evening when crews return to camp. While there would be no impact to public visitors from generator noise due to the remote location of these camps, there could be some mild annoyance to wildlife.

Motorized vehicles (SUVs or trucks) would assist in the transportation of field teams and gear to reach three ground-accessible monitoring sites in Makawao Forest Reserve and TNC’s Waikamoi Preserve. Noise from vehicles used during monitoring would primarily occur along the Flume Road shown (in brown) on **Figure 5** and is not expected to exceed 4 hours per day for up to 7 days on a quarterly basis. It should be noted that vehicles would not be running constantly during that 4-hour time period because crews would be stopping periodically to check mosquito traps. As previously mentioned, ground vehicles can reach 75 dBA at 50 feet from the source but would be muffled by the surrounding canopy and would not be expected to exceed 60 dBA at 50 feet from the source of noise.

Overall, the noise from helicopters and generators would be primarily focused at the five helicopter-only accessible monitoring camps and LZs. Noise from approaching or departing vehicles would occur at trailheads in Makawao Forest Reserve and TNC’s Waikamoi Preserve but would be minimal throughout the duration of the project and would blend into the vehicle traffic/noise already occurring at trailheads. Impacts from noise would be affected by topography, vegetation, distance to source, and in the case of helicopters, speed of travel. The duration and frequency of helicopter flights required for monitoring (2–6 hours per day for a total of approximately 17.5 hours per week for one week each quarter) and, therefore, the amount of time visitors or wildlife could experience helicopter noise impacts, would vary by distance from the source. Noise levels along helicopter flight paths would reach less than 72 dBA at 500 feet AGL during overflights at the beginning and end of each monitoring session. Adverse noise impacts from helicopter drop-offs and pick-ups would only occur at five helicopter-accessible only monitoring sites and could reach 82–93 dBA during pick-ups and drop-offs (less than 10 minutes each). During the 7-day quarterly monitoring sessions, adverse noise impacts from generators would be limited to less than 58 dBA at 23 feet for up to 3 hours per day at five monitoring camps, and noise from vehicles would be limited to 4 hours per day in Makawao Forest Reserve and TNC’s Waikamoi Preserve to reach the three ground-accessible monitoring sites. Therefore, adverse impacts on the acoustic environment during monitoring activities from helicopters, generators, and vehicles would be highly variable and not sustained (would only occur every three months). In addition, it is unlikely that any visitors or recreationists would be aware of the helicopter landings or generator noise due to the remoteness of the LZs used during monitoring and the infrequency of trips required for quarterly monitoring.

Cumulative Impacts

The impacts of past, present, and reasonably foreseeable future actions (see Appendix E) and the no-action alternative are as described in the section titled “Current and Expected Future Condition of the Acoustic Environment if No Action is Taken”. As past, present, and reasonably foreseeable future actions are part of the existing acoustic environmental conditions, and because the no-action alternative would result in no indirect or direct impacts to the acoustic environment, there would be no cumulative effects associated with the no-action alternative. When compared to the no-action alternative, mosquito release activities under the proposed action would contribute periodic adverse impacts on the acoustic environment near LZs, helibases, flight paths, trails, trailheads, and roads from the use of drones, mechanized equipment, and helicopters.

Under the proposed action, noise from drones could occur throughout the 48,164-acre core area for 49–72 hours per week. Noise levels from drones could reach a maximum of 47–59 dBA at 100–200 feet AGL (the altitude where most releases would occur) for less than 15 seconds as the drone passes over any given location in the core area one to two times per week. Helicopter noise would only occur for 2–6 hours per day potentially spread over the course of 7 days for a total of approximately 17.5 hours per week for quarterly monitoring trips. Most helicopter flight noise would be highly variable depending on the flight altitude and lateral distance to a person or wildlife but could reach a maximum of 82–93 dBA during pick-ups and drop offs at LZs. Short-term, temporary helicopter longline releases (up to 6 hours of flight time per day, 5–7 days per month for up to two months per year) could produce a maximum of 82 dBA at 150 feet AGL for less than 15 seconds at any given release location in the core area. Generator noise (maximum of 52–58 dBA at 23 lateral feet) could occur for up to 3 hours per day for up to 7 consecutive days on a quarterly basis at the five backcountry monitoring locations. Noise from vehicles (maximum of 75 dBA at 50 feet from the source) would occur intermittently in Makawao Forest Reserve and TNC's Waikamoi Preserve for up to 4 hours per day for up to 7 days during quarterly monitoring and up to 2 hours per day, up to 2 times per week for pedestrian releases that are scheduled to occur in those areas.

As described above, the proposed action would contribute a measurable but largely unnoticeable adverse impact to the acoustic environment. Humans and animals would experience slight increases in perceptible sound/noise compared to the no-action alternative in certain areas at certain times, but in many cases, the project-related noises would be imperceptible due to remoteness of the project area. The locations affected by the proposed action are where most past, present, and reasonably foreseeable management actions are already occurring on park, state, and private conservation lands. When the impacts of the proposed action are added to the impacts of present and reasonably foreseeable actions, an overall adverse cumulative impact on the acoustic environment spread over the entire core area would last until sufficient mosquito population suppression is achieved.

Conclusion

Under the no-action alternative, conditions and trends would remain the same or similar as existing conditions. Compared to the no-action alternative, mosquito release activities under the proposed action would contribute periodic adverse impacts on the acoustic environment near LZs, helibases, flight paths, trails, trailheads, and roads from the use of drones, vehicles, mechanized equipment, and helicopters.

Noise from drones (the primary method for mosquito releases) could occur throughout the 48,164-acre core area (30,796 acres of state land, 7,099 acres of NPS land, 5,168 of private conservation land, and 5,101 acres of TNC-managed land) for 49–72 hours per week. Specifically, 37–47 flight hours per week would occur over state lands, 6–11 hours per week would occur of NPS lands, 6–7 hours per week would occur over private conservation lands, and 1–7 hours per week would occur over TNC-managed lands. Noise levels from drones could reach 47–59 dBA at 100–200 feet AGL (the altitude where most releases would occur) for less than 15 seconds as the drone passes over any given location in the core area one to two times per week.

Helicopter noise would only occur if a short-term (up to two months), temporary release method is needed for releases and when monitoring needs to occur in the backcountry (on a quarterly basis). Helicopter noise impacts would occur primarily at LZs, helibases, and along selected flight paths. To reach the five helicopter-only accessible monitoring sites, helicopter flights could occur for 2–6 hours per day potentially spread over the course of 7 days for a total of approximately 17.5 hours per week. Because monitoring would occur quarterly, the estimate of total annual helicopter flight time is 70 hours. Most helicopter flight noise would be highly variable depending on the flight altitude and lateral distance to a person or wildlife but could reach 82–93 dBA during pick-ups and drop offs at LZs. For short-term temporary helicopter longline releases, it is anticipated that up to 6 hours per day, 5–7 days per month for up to two months could occur and result in a total of up to 56–78 hours of flight time per year. Noise levels could reach a maximum 82 dBA at 150 AGL for up to 15 seconds while the helicopter hovers over release locations within targeted portions of the core area.

Noise from generators (maximum of 52–58 dBA at 23 lateral feet) would be highly variable and would be limited to the five helicopter-only accessible monitoring areas and camps for up 3 hours per day for up to 7 consecutive days on a quarterly basis during monitoring trips. Noise from vehicles (maximum of 75 dBA at 50 feet from the source) would occur intermittently in Makawao Forest Reserve and TNC's Waikamoi Preserve for up to 4 hours per day for up to 7 days during quarterly monitoring and up to 2 hours per day, up to 2 times per week for pedestrian releases that are scheduled to occur in those areas.

Noise from the drone and helicopter longline release methods and monitoring would be the most intense acoustic impacts to result from this project. However, the adverse impacts from the drone and helicopter longline release methods and monitoring would be confined largely to backcountry areas and would largely go unnoticed by humans and would only briefly disturb wildlife. Humans and animals would experience slight perceptible increases in sound/noise compared to the no-action alternative in certain areas at certain times resulting in fleeting disruption or annoyance. Though considerable analysis is presented here, the proposed action would contribute a measurable but largely unnoticeable adverse impact to the acoustic environment during mosquito release and monitoring activities.

WILDERNESS

The Wilderness Act of 1964 established the National Wilderness Preservation System, which is currently comprised of over 800 congressionally designated wilderness areas and over 111 million acres. Congress passed the Act in order to preserve and protect certain lands "in their natural condition" and "to secure for the present and future generations the benefits of wilderness." The Wilderness Act and NPS policy mandate preservation of wilderness character, which includes five tangible qualities (untrammeled, natural, undeveloped, outstanding opportunities for solitude or primitive and unconfined recreation, and other features of value). The Haleakalā Wilderness is designated by federal statue and there is no wilderness on state or private lands.

Analysis Area

The area of analysis for impacts on wilderness character includes the eastern portion of the Haleakalā Wilderness within the park focused on Kīpahulu Valley and Manawainui, where IIT mosquito releases would occur under the proposed action. The area of analysis for wilderness additionally includes locations outside of the mosquito release area where helicopters would travel from helibases outside of wilderness including the lower Kīpahulu Valley and the portion of the designated Haleakalā Wilderness in the park's Summit District.

Current and Expected Future Condition of Wilderness if No Action is Taken

The current condition of these wilderness character qualities is described below. A detailed discussion of past, present, and reasonably foreseeable future actions within the park contributing to the existing conditions and current trends within designated wilderness are located in Appendix E. The description below provides an overview of how these ongoing and future actions would affect wilderness character. Under the no-action alternative, the qualities of wilderness would remain the same or similar to existing conditions, including trends and impacts from past, present, and foreseeable planned actions. The affected environment and impacts of no--action are therefore the same and discussed here only once.

Qualities of Wilderness Character

Formal definitions of wilderness character were developed in 2006 by an interagency monitoring team, including NPS, using the five qualities of wilderness set forth in the Wilderness Act. These qualities are used nationwide to monitor the status and trends in wilderness (preservation or degradation) over time by accounting for stewardship actions as well as impacts from modernization, visitation, and changes occurring outside of the wilderness itself (NPS 2015a). All five qualities occur within the congressionally designated wilderness in Haleakalā National Park and are analyzed in detail: untrammeled, natural, undeveloped, solitude or primitive and unconfined recreation, and other features of value.

Haleakalā Wilderness

Approximately 24,719 acres, or 74 percent, of Haleakalā National Park is congressionally designated wilderness (**Figure 8**). Two distinct areas comprise the Haleakalā Wilderness: the Haleakalā Crater and Kīpahulu Valley above 2,000 feet in elevation, the adjacent Manawainui and Hāna Rainforest areas. Kīpahulu Valley and adjacent areas are a designated Biological Reserve and are closed to visitors. Approximately 14 percent of the project area is in wilderness.

Untrammeled

An untrammeled wilderness is one that is unhindered and free from the intentional actions of modern human control or manipulation. The untrammeled quality is preserved or sustained when actions to intentionally control or manipulate the components or processes of ecological systems inside wilderness (e.g., suppressing fire, stocking lakes with fish, installing water catchments, or removing predators) are not taken. Actions that intentionally manipulate the biophysical environment, such as the removal of nonnative species, intervention in the behavior or lives of native plants and animals, projects to restore the natural conditions of wilderness, and interference in natural processes and energy flows, degrade the untrammeled quality.

Several threats to Haleakalā National Park's unique natural environment have spurred management action to preserve the rare ecological communities and individual species of the park. The ongoing extreme degradation of wilderness ecosystems caused by invasion of non-native species has led the park to take management actions (trammeling) to slow down and address these threats. These include non-native wildlife removal, activities to restore and protect native wildlife, and re-establishment of unique native plant communities. Because of the severe threats to native species, Haleakala's Wilderness is a setting where manipulation of the biophysical environment is required to maintain, protect, and revive the native environment. Because these actions are necessary to preserve the natural environment, it is important to carefully consider restraint before taking actions that impact the untrammeled quality.

The park is currently implementing predator and ungulate control and ground and aerial herbicide spray operations for invasive plant control. Additional ongoing or planned activities include fencing to exclude ungulates, manual removal of invasive plants, and native plant outplantings, all of which adversely affect the untrammeled quality of wilderness. The park would continue current management actions and respond to future needs and conditions to improve the natural quality of the wilderness, while designing these activities to minimize adverse impacts on the untrammeled quality.

Natural

A natural wilderness is one where ecological systems are substantially free from the effects of modern civilization. When indigenous species and ecological conditions are protected and managed to preserve natural conditions, the natural quality is preserved. The natural quality may be improved by controlling or removing non-native species or by restoring ecological conditions. The natural quality is degraded by human-caused changes to the natural environment (i.e. human-caused effects on plants, animals, air, water, ecological processes, etc.).

Kīpahulu Valley and adjacent Manawainui and Hāna Rainforest areas provide refuge for some of Hawaiʻi's most unique native plant communities. East of Palikū Ridge, forests of koa and ʻōhiʻa inhabit Kīpahulu Valley, providing the necessary matrix to sustain intact native watersheds and provide canopy over a wealth of rare species. The diverse plant communities of the Haleakalā Wilderness support several endemic animal species, many of which are now threatened or endangered. Birds are the primary wildlife species here and, like Haleakalā's native plants, native bird species have evolved to occupy a range of specialized niches. For threatened and endangered birds, such as the ʻuaʻu, nēnē, ʻākohekohe, and kiwikiu, the wilderness provides integral habitat and refuge from predators. Important pollinators, such as Hawaiian yellow-faced bees and nocturnal residents such as the ʻōpeʻapeʻa (Hawaiian hoary bat), benefit from and contribute to this diversity (NPS 2015a).

The natural quality of the Haleakalā Wilderness has been severely impacted by non-native species introductions, which have led to the extinction or severe decline of many native species. Invasive plants grow quickly and outcompete native vegetation. Prior to rigorous management, feral ungulates overgrazed, trampled, and severely disturbed the crater and wet forest landscapes, damaging and altering vegetative communities, and significantly impacting ground-nesting birds. Invasive mammalian predators negatively impact the natural quality of wilderness, particularly populations of native bird species that have not evolved with this type of pressure. Avian diseases, such as avian malaria spread by introduced insects, have also taken a toll on native bird distribution and survival (NPS 2015a), thus substantially impacting the natural character of wilderness in the park.

The park is currently implementing predator and ungulate control, forest bird monitoring, and ground and aerial herbicide spray operations for invasive plant control that benefit the natural quality of wilderness. Mosquito surveys and monitoring of avian malaria prevalence have been conducted within the park in the past and recently by USGS and NPS and allow the NPS to evaluate the success of these programs. Additional ongoing or planned activities include fencing to exclude ungulates, manual removal of invasive plants, and native plant outplantings, which also improve the natural quality of wilderness. The park would continue current management actions (see Appendix E) and respond to future needs and conditions to improve the natural quality of the wilderness, while minimizing adverse impacts on the untrammeled and undeveloped qualities of the wilderness. If no new actions are taken, however, avian malaria would continue to devastate native forest bird populations and would likely result in a permanent adverse impact on the natural quality of wilderness character, (i.e., the extirpation and extinction of native forest bird species). This degradation of the natural quality of wilderness would not be a natural phenomenon (the species loss would be caused by invasive mosquitoes and the diseases they transmit).

Undeveloped

An undeveloped wilderness retains its primeval character and influence and is essentially without permanent improvements or modern human occupation. The undeveloped quality is preserved or sustained when it remains free from modern structures, installations, human habitation, motor vehicles, motorized equipment, mechanical transport, and landing of aircraft. It is improved when these prohibited uses are removed or reduced.

Any evidence of human presence, whether large or small, detracts from the undeveloped quality of wilderness. Due to the remote location and difficult access of Kīpahulu Valley and adjacent areas, protection and restoration of this vulnerable environment may sometimes require non-recreational wilderness developments and installations. The developments present within Haleakalā Wilderness include fencing and fence supply caches, snares, monitoring transects, research plots, stream and weather monitoring stations, research shelters, traps and bait stations, trail and tool caches, and administrative trails (NPS 2015a). Developments are intermittent throughout Kīpahulu Valley and are located at maximum distances to achieve management goals. Research shelters exist near adjacent LZs and monitoring transects or administrative trails may be used to strategically travel to both a management site (i.e. invasive plant removal site) and another shelter within an 8-hour hike. These developments would remain in the wilderness in the future and continue to detract from the undeveloped quality of wilderness. A high demand for research permits and research installations also risk impact to the undeveloped nature of this naturally wild area of wilderness, as does the potential need to access these remote areas by helicopter (NPS 2015a).

The 2002 addition of congressionally designated wilderness to the park noted, “construction of fences to exclude feral animals and access into the wilderness via helicopter for fence maintenance, to control destructive invasive alien plants and non-native animals may be necessary to preserve wilderness resources and ecosystem processes” (NPS 2002).

Solitude or Primitive and Unconfined Recreation

Wilderness provides outstanding opportunities for recreation in an environment that is relatively free from the hindrance of modern society. The ability to experience solitude is an integral component of wilderness, while opportunities for primitive and unconfined recreation make the wilderness experience unique. In preserving this wilderness quality, it is important to consider the value of maintaining these places where present and future generations have the opportunity to feel free, at peace, and self-reliant. The solitude or primitive and unconfined recreation quality is preserved or improved when visitors experience minimal encounters, observe landscapes without modern human effects, and experience self-reliance, discovery, self-discovery, traditional skills, and mental and physical challenge. The solitude or primitive and unconfined recreation quality is degraded by sights and sounds of human activity, and by facilities that decrease self-reliant recreation and management restrictions on visitor behavior.

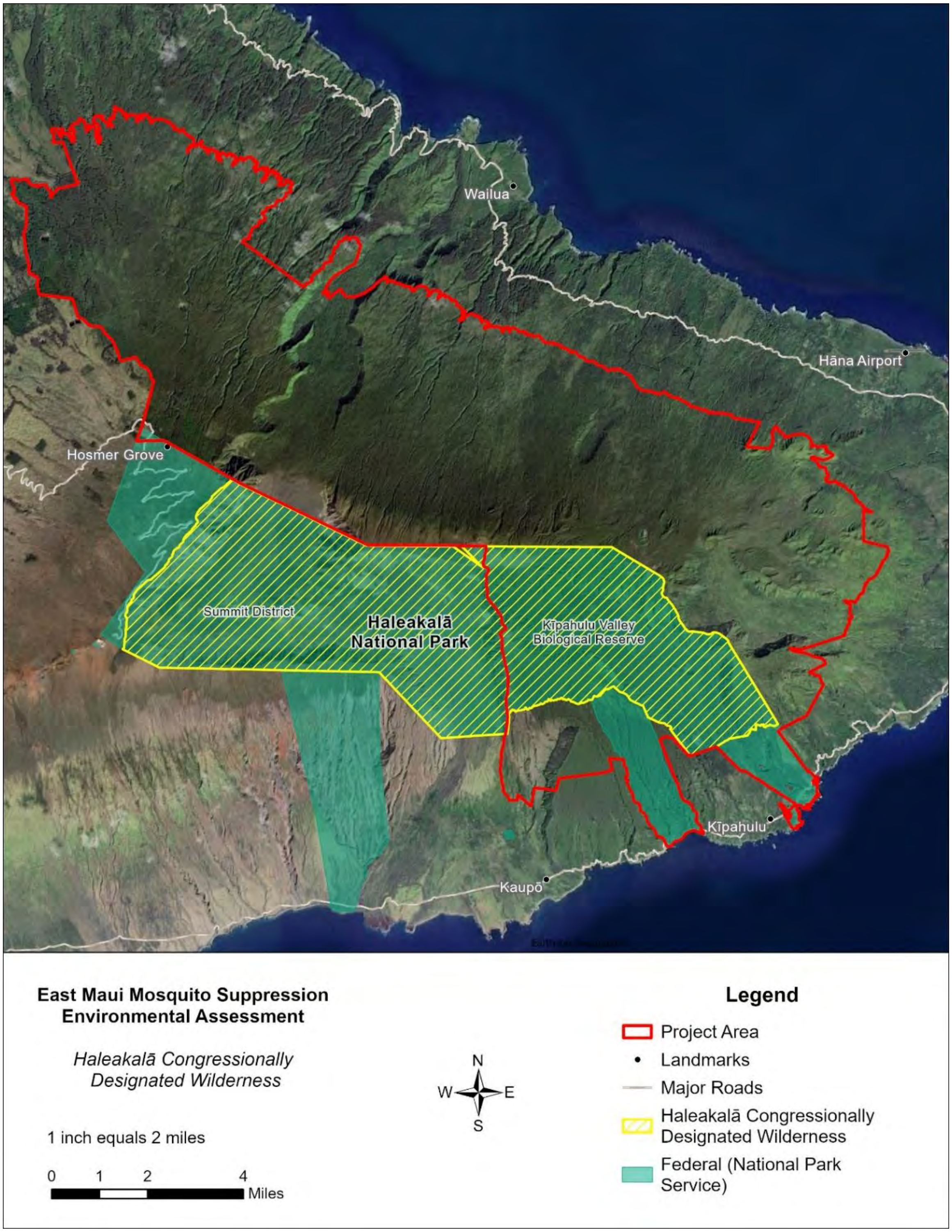

FIGURE 8: DESIGNATED HALEAKALĀ WILDERNESS WITHIN THE PROJECT AREA

Solitude in Haleakalā Wilderness is impacted by administrative flights, commercial helicopter air tours, hikers, campers, and day-use visitors, and administrative use of motorized equipment that audibly and visibly affect the primitive wilderness experience. Administrative flights are more frequent in the Kīpahulu District but are intermittent and do not occur on weekends or after dark (see **Figure 4** for existing flight infrastructure). Alternatively, commercial air tours occur constantly throughout the day and flights that occur just outside of the park can have impacts within Haleakalā Wilderness (see **Figure 7** for flight paths). Haleakalā Wilderness includes enclaves with both visitor and management cabins, and horse pastures to support visitor activities. Recreational infrastructure like cabins that are still visible to visitors may degrade the solitude or primitive and unconfined recreation quality. Sights and sounds of other visitors, along with restrictions for off-trail travel may impact this quality when visiting the Haleakalā Crater area of wilderness. Entry restrictions into areas of the Kīpahulu District of wilderness may also degrade opportunities for unconfined recreation. These impacts to solitude or primitive and unconfined recreation are expected to continue into the future.

Other Features of Value

This quality captures important elements or "features" of a particular wilderness that are not covered by the other four qualities and are truly unique and essential to the character of that wilderness. The Wilderness Act states that wilderness "may also contain ecological, geological, or other features of scientific, educational, scenic, or historical value." Typically, other features of value occur in a specific wilderness location, such as archeological, historical, or paleontological features; some, however, may occur over a broad area such as an extensive geological or paleontological area, or a cultural landscape. This quality is preserved when these "other features of value" are preserved. The other features of value quality are degraded by deterioration or loss of integral site-specific features of value.

Haleakalā, a major geographical and cultural landmark of East Maui, remains intrinsically tied to contemporary Native Hawaiian culture by tangible and intangible cultural resources and values, place names, landscape features, and oral traditions and history. Additionally, the summit of Haleakalā, Kīpahulu Valley, and Kaupō Gap are eligible for the National Register of Historic Places as Traditional Cultural Properties for their association with the cultural landscape of Maui, primarily due to the known uses, oral history, mele (Hawaiian songs and chants), and legends associated with these areas. Potential threats to wilderness cultural sites originate from both environmental and human sources. In a wilderness context, the presence of cultural sites in their natural condition and the continued use of wilderness for traditional practices contribute value to the visitor's sense of human history, provide for the continuation of cultural practices associated with wilderness, and speak to the larger role of humans as an important element of their environment. The rare forest birds within the Haleakalā Wilderness not only have ecological value as captured under the natural quality but also contribute to the cultural resources of the wilderness given their importance to Native Hawaiians. These birds continue to be subject to mortality due to avian malaria, degrading the wilderness character. The proposed action will not adversely affect cultural resources and thus they were dismissed from detailed analysis (see Appendix B); however, a Cultural Impact Assessment has been prepared as required by HEPA (see Appendix C). Cultural resources are briefly considered here as a feature of value of Haleakalā Wilderness that may benefit from the proposed action.

Effects of the Proposed Action on Wilderness

Methods and Assumptions

Potential impacts on designated wilderness were evaluated based on four of the five qualities of wilderness character as described earlier in this section. Impacts on the untrammeled, natural, undeveloped, and solitude or primitive and unconfined recreation qualities are analyzed for the no-action alternative and the proposed action alternative. The analyses only apply to the actions taken within or near the designated Haleakalā Wilderness within the park under each alternative as there is no designated wilderness outside of federal lands.

To ensure an enduring resource of wilderness, the Wilderness Act (section 4(c)) prohibits certain uses within wilderness: "there shall be no temporary road, no use of motor vehicles, motorized equipment, or motorboats, no landing of aircraft, no other form of mechanical transport, and no structure or installation within such area." The exception for utilizing these prohibited uses is only if they are "necessary to meet minimum requirements for the administration of the area."

Analysis

Under the proposed action, incompatible mosquitoes would be released within the project area using aerial methods, primarily drones. Monitoring activities associated with the proposed action would also include helicopter use and landings within two sites in wilderness in addition to the use of portable generators at two sites in wilderness. The untrammeled, natural, undeveloped, and opportunities for solitude or primitive and unconfined recreation qualities of wilderness would be impacted by the proposed action.

Untrammeled

All three mosquito release deployment methods under the proposed action would have the same impact on the untrammeled quality of wilderness. The broad intervention of wildlife through the release of mosquitoes using any of the three methods would result in an adverse impact on the untrammeled quality of wilderness for the life of the plan, likely at least 20 years, as the methods described in the proposed action are used to suppress mosquito populations to reduce avian malaria mortality in native forest birds.

Natural

Minimal clearing of vegetation at LZs, trails, and fence lines would be required at the onset of the project to accommodate mosquito monitoring, but impacts would be limited to areas that have already been cleared for administrative use and mechanized equipment would not be used. Noise from drone flights (maximum of 47–59 dBA at 100–200 feet AGL) once or twice per week would briefly disturb wildlife from 15 seconds to a few minutes. More pronounced noise would occur from short-term (up to two months), temporary helicopter longline releases (maximum of 82 dBA at 150 feet AGL), but from 15 seconds to a few minutes at any given location. Quarterly pedestrian monitoring and release efforts would include helicopter landings, human activity, and generator use resulting in slightly longer and louder noise impacts. The noise from helicopters, however, would only occur for minutes at a time during take-off and landing and just once every three months. Generator noise (maximum of 52–58 dBA at 23 lateral feet) could occur for up to 3 hours per day for up to 7 consecutive days on a quarterly basis at two monitoring locations within wilderness. The presence of and noise from these motorized and mechanized uses would result in adverse impacts on the natural quality of wilderness during mosquito release and monitoring activities. The reduction in the mosquito population under the proposed action, and the subsequent reduction in native forest bird mortality from the transmission of avian malaria, would result in substantial beneficial impacts to the natural quality of wilderness character because of the resultant stabilization or increase in native forest bird populations over time. The planned incompatible mosquito releases would be a long-term action aimed at restoring natural ecosystem processes that have been degraded by invasive mosquitoes spreading avian malaria. Over the long term, the proposed action would substantially benefit the natural quality of wilderness compared to the existing conditions.

Undeveloped

The use of motorized equipment, such as drones, helicopters, and generators (during monitoring) would result in intermittent, direct, adverse impacts on the undeveloped quality of wilderness character given the presence of this technologically advanced equipment in a wilderness setting. Pedestrian releases may occur within designated wilderness in the Kīpahulu Valley Biological Reserve but only on a quarterly basis simultaneous with ground-based mosquito monitoring. Helicopters would land briefly in wilderness during each incompatible mosquito monitoring and release operation, to pick up and drop off teams and supplies. Generators would likely be used for up to 3 hours per day for up to seven consecutive days during the monitoring trips. The presence of helicopters and generators within wilderness would briefly adversely impact the undeveloped quality given the presence of this technologically advanced equipment in a wilderness setting. Incompatible mosquitoes may be released in small biodegradable packages designed to open on contact with the canopy or forest floor. These mosquito packages (dropped via aerial means) would result in an impact to the undeveloped quality of wilderness for as long as they remain in the environment (until they biodegrade).

Solitude or Primitive and Unconfined Recreation

Of lands within the designated Haleakalā Wilderness, only the Kīpahulu Valley Biological Reserve portion is within the project area and is closed to all recreation. However, drone and helicopter flights to and from the project area over the Summit District portion of designated wilderness would occur on an intermittent basis (approximately once or twice per week), very briefly (perhaps 15 seconds to a few minutes) audibly and visibly impacting the primitive wilderness experience. As described in Chapter 2, it is conservatively estimated that this project would require up to 72 hours of drone flight time per week during warm months and 49 hours during cold months to achieve the desired mosquito release rate; flights over or near designated wilderness within the Kīpahulu Valley Biological Reserve (2,318 acres of the 64,666 project area), however, would likely require only approximately 2–3 hours of flight time and at any given location these drones would be perceptible either visually or aurally for less than 30 seconds (hovering would last 15 seconds or less over a particular location). The helicopter longline method could result in a maximum estimate of 28–39 hours of flight time per month, but this method would only be used as a short-term (up to two months), temporary release method if or when drones are unavailable and the time to cover areas near or within designated wilderness would likely be approximately 1–2 hours. When helicopters fly or hover above the canopy, noise levels on the ground would not exceed 82 dBA and would only approach that level for less than 15 seconds in any given location. Although helicopter noise would be short lived in any particular area, it would adversely impact the ability of wilderness users to enjoy a sense of solitude or primitive recreation. Pedestrian releases may also occur within designated wilderness in the Kīpahulu Valley Biological Reserve but only on a quarterly basis simultaneous with ground-based mosquito monitoring. Helicopters would land briefly in wilderness during each incompatible mosquito release operation, to pick up and drop off teams and supplies. Direct adverse impacts on the primitive wilderness experience would result, though these would be rarely and intermittently perceptible to visitors in accessible wilderness areas. Project noise created within the Kīpahulu Valley Biological Reserve portion of designated wilderness that does not travel beyond that boundary would not affect opportunities for solitude and primitive experiences in wilderness areas open to public access.

Other Features of Value

As stated previously, the proposed action would not impact physical historical resources within designated wilderness. The proposed action would likely support a considerable recovery of native forest birds that are of cultural importance to Native Hawaiians, thus benefiting the cultural landscape and the other features of value quality of wilderness.

Cumulative Impacts

When the impacts of the proposed action are added to impacts from past, present, and reasonably foreseeable future projects within the park described in Appendix E, the overall cumulative impact on wilderness character would be beneficial. The proposed action would adversely impact some wilderness character qualities due to the noise and presence of drones, helicopters, and generators. These impacts, however, would not permanently affect wilderness and the overall result of reduced mosquito populations would be a long-term benefit to the natural quality of wilderness and other features of value (native forest bird populations). Natural conditions in wilderness would dramatically improve as a result of the suppression of mosquito populations, which would reduce avian malaria mortality in native forest birds.

Conclusion

The no-action alternative is likely to result in fewer impacts to the untrammeled, undeveloped, opportunity for solitude and other features of value in wilderness compared to the proposed action. Uunder the no-action alternative, however, the natural quality of wilderness would continue to severely degrade with the irreparable harm to native forest bird species. The proposed action would affect additional wilderness character qualities including the untrammeled quality, undeveloped quality, and opportunity for solitude from the use of mechanized equipment for incompatible mosquito releases. This alternative, however, would likely support a considerable recovery to natural conditions previously present on the island, thus benefiting the natural and other features of value qualities of wilderness. Both alternatives therefore detract from wilderness character qualities, but under the proposed action the small adverse impacts to the undeveloped quality, untrammeled quality, and opportunity for solitude from mosquito releases provide a substantial benefit to the natural and other features of value qualities through the protection of native forest birds. Though considerable analysis is presented here, overall adverse impacts to wilderness would be brief and minimal.

VISITOR USE AND EXPERIENCE

Current and Expected Future Condition of Visitor Use and Experience if No Action is Taken

The character and quality of the visitor experience influences perception of natural areas, providing a unique encounter with a place that differentiates it from other regions. Public enjoyment of resources is a fundamental purpose of all national parks (NPS 2006). DLNR manages forest reserves for multiple uses, including visitation. TNC also allows visitors by appointment within the Waikamoi Preserve. The project area lands managed by the park, state, TNC, and private entities are largely inaccessible and remote. Less than 5 percent of the total project area is open to visitors without a permit or readily accessible to visitors, while nearly 40 percent is completely closed to visitation (without a permit) to protect ecologically sensitive resources, including the Kīpahulu Biological Reserve and Hanawī Natural Area Reserve (**Figure 9**).

The current condition of visitor use and experience is described below. A detailed discussion of past, present, and reasonably foreseeable future projects within the park and adjacent lands contributing to the existing conditions and current trends for visitor use and experience are described in more detail in Appendix E. The description below provides an overview of how these ongoing and future actions would affect visitor use and experience.

Under the no-action alternative, visitor use and experience would remain the same or similar to existing conditions, including trends and impacts from past, present and foreseeable planned actions. Therefore, the affected environment and impacts of no-action are the same and discussed only once here.

Haleakalā National Park

The fundamental purpose of the park is to offer opportunities for public education and enjoyment. Visitors come to the park to participate in a range of recreational activities, including viewing sunrise and sunset, hiking, swimming, bicycling, attending ranger programs, scenic flights or driving, stargazing and astronomy, birdwatching, and camping. The enabling legislation that created the park—H.R. 9525, Public, No. 171, Chapter 264—states that, "…the tracts of land on the island of Hawaiʻi and on the island of Maui…shall be perpetually dedicated and set apart as a public park or pleasure ground for the benefit and enjoyment of the people of the United States…" Between 2014 and 2017, the park averaged 1.2 million visitors annually (NPS 2018). Visitation was approximately 850,000 in 2021 (NPS 2021). Within the park, the Summit District sees approximately 3–4 times as much visitation as the Kīpahulu District. Most visitors enter the park in vehicles or tour buses.

The majority of the project area within the park is within the Kīpahulu Biological Reserve, which is closed to the public. Access is restricted to authorized scientists and land managers conducting research and management. The absence of public access to the reserve helps conserve the fragile biodiversity of the area (NPS 2018).

The lower portion of the Kīpahulu District (~766 acres) is the second most visited destination in the park. Approximately 325,000 visitors come to the lower Kīpahulu District annually (NPS 2021, FY 2018–2019). Recreational activities in the lower Kīpahulu Valley area include hiking, ranger-led interpretive hikes, commercial vehicle tours of the area, and camping, which all generate noise (NPS 2018). The Kīpahulu Campground has 21 designated sites for camping. Several popular trails include the Pīpīwai and Kūloa Point trails. The Kīpahulu District Visitor Center is open daily (usually from 9 a.m. to 5 p.m.) and the Kīpahulu campground (also open daily) offers 15 drive-up campsites, one group site, and five walk-in campsites.

Other than the lower Kīpahulu District, only 124 acres of the park-managed land within the project area has public visitation, including Hosmer Grove, located in the northwest corner of the project area. Hosmer Grove provides

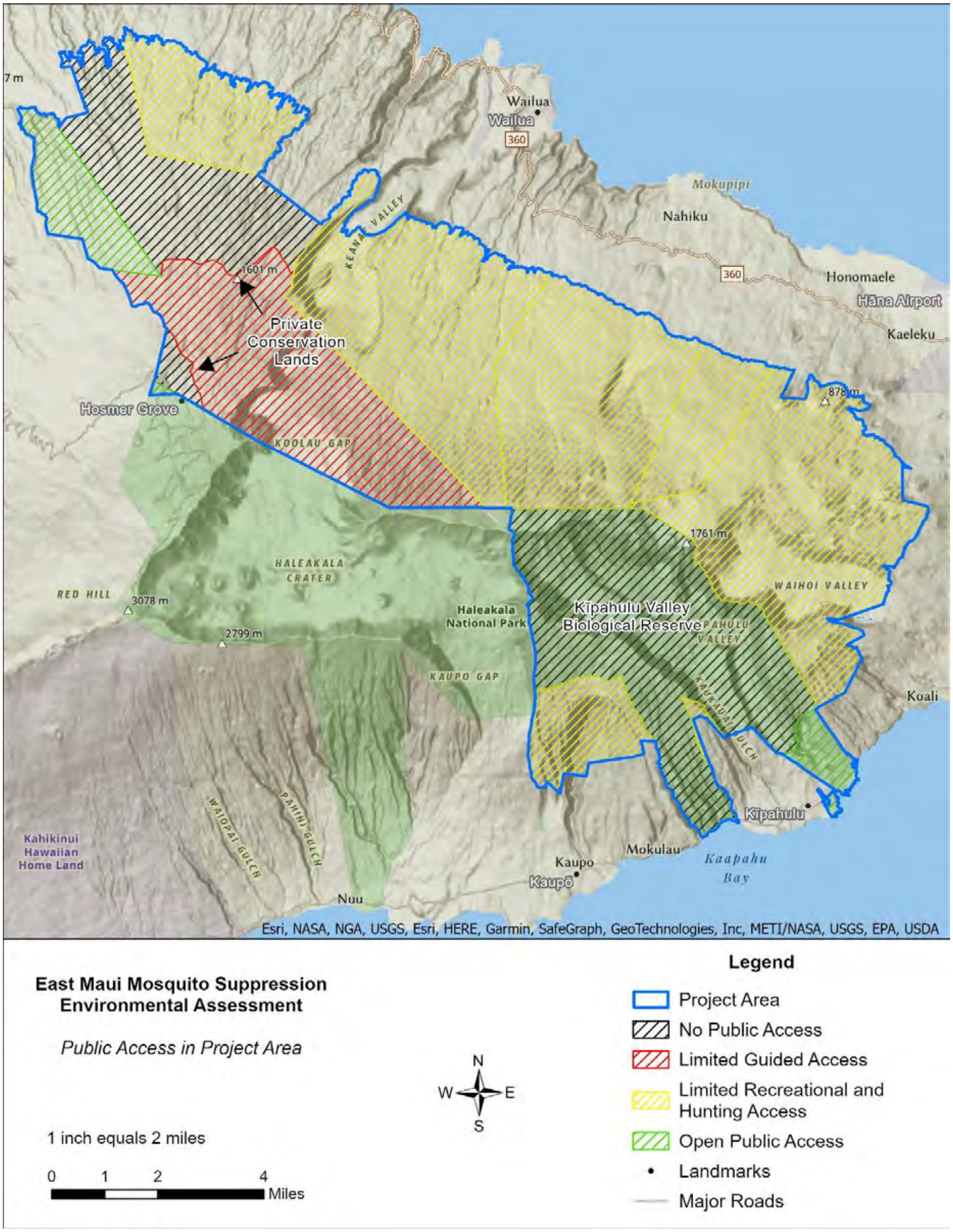

FIGURE 9: PUBLIC ACCESS IN THE PROJECT AREA

opportunities to camp, picnic, hike and birdwatch throughout the year, and is considered a birdwatching "hot spot" (eBird 2022). Neither the lower Kīpahulu Valley or Hosmer Grove areas are within the core mosquito release area.

Commercial air tours and recreational activities such as birdwatching and hiking are anticipated to continue or increase in or near portions of the project area. Periodic management helicopter flights on park lands are also anticipated to continue to facilitate resource stewardship projects and scientific research. Visitor use and experience may be disrupted by helicopter use for park management, but audible and visual impacts are intermittent and average 2-4 hours in duration. Commercial air tours operate year-round and may be constant near visitor use and recreational areas; within the project area air tours are most common above the Kīpahulu District. Visitors to the park may experience disruptions from ongoing infrastructure updates or health and safety management operations. For park projects, disturbance and impacts on visitor experience are assessed and efforts are made to reduce the duration of impacts, for example by avoiding use of load machinery during peak visitation hours or using an alternate tool. Improvements to trails would periodically occur, such as along the Pīpīwai Trail in the lower Kīpahulu District where a new viewing platform is planned. Visitors may also encounter park staff performing surveys or conducting invasive plant or wildlife control projects. Under the no-action alternative, mosquitoes would continue to spread avian malaria. This occurrence would severely impact native forest birds and lead to considerable mortality and likely extinction, which would diminish the experience of visitors seeking to enjoy these birds.

State Natural Area Reserves

The Natural Area Reserves System (NARS) was created to preserve and protect representative samples of Hawaiian biological ecosystems and geological formations (DLNR 1997). The Natural Area Reserves (NARs) are managed by DLNR DOFAW Native Ecosystem and Protection Program. Areas that are designated as NARs are protected by rules and management activities designed to maintain and restore native ecosystems intact, so a sample of that natural community would be preserved. NARs are some of Hawai‘i's most valued, pristine, and biologically diverse forests, coastal areas, and marine ecosystems. Visitor use in the Hanawī NAR is low as there are no legal access locations through adjacent lands. A small number of permits are issued each year for scientific research.

The Nature Conservancy and Private Lands

Public access to TNC's Waikamoi Preserve is limited to guided hikes, educational and service trips, and scientific research. TNC typically leads public hikes into Waikamoi Preserve one to two times per month throughout the year with a maximum of 15 participants. Approximately one volunteer work trip is additionally conducted once a month, and TNC typically provides trips into the preserve twice a month, once for local groups, and once a month for donors or other special guests. Visitation to the preserve by visitors is approximately 1,000 persons per year (TNC 2021). East Maui Irrigation/Mahi Pono lands are closed to the public unless given permission by land managers (M. Vaught, *pers. comm., 10/27/21*). Mosquitoes would continue to spread avian malaria, which would severely impact native forest birds and lead to considerable mortality and likely extinction., This occurrence would diminish the experience of visitors seeking to experience these birds, particularly in this preserve where birdwatching and visitation is popular.

Effects of the Proposed Action on Visitor Experience

Methods and Assumptions

Potential impacts to visitor use and experience under the proposed action would include noise and visual distractions from drones and helicopters. The baseline for evaluating potential impacts to visitor experience was developed using an assumption that visitors are seeking an experience in nature and that proposed management activities would impact that experience. Without a survey of visitors, there is an assumption that most visitors would consider activities under the proposed action to be a distraction, though they may possibly be of interest, as some visitors may not interpret seeing helicopter or drones engaged in conservation activities as an adverse impact to their experience. Given the similarity of conditions on state and TNC-managed lands outside the park, it is assumed existing noise levels in these areas would be similar to those occurring within national park boundaries. Impacts were evaluated based on the potential for incompatible mosquito release activities through any of the release methods to create impacts that could affect the visitor experience.

Analysis Area

The area of analysis for impacts of the alternatives on visitor use and experience includes the portions of the park, state, and TNC lands where management activities overlap with visitor use (**Figure 9**). Although the project area encompasses 64,666 acres, any considerable visitor use is largely limited to a much smaller area including the lower Kīpahulu District in the park (3,706 acres), Makawao Forest Reserve and scattered hunting in remote areas of State Forest Reserves, and guided hikes in some areas of TNC's Waikamoi Preserve (on less than approximately 10 percent of this preserve). The Summit District outside of the mosquito release area is discussed in this section to account for drone and helicopter flights over this area to reach the mosquito release areas under the proposed action. Much of the project area is remote, roadless, and consists of steep topography, deep ravines, and dense vegetative cover inhibiting sound and sight.

With the exception of Makawao Forest Reserve, which has popular hiking trails, hunting is the primary visitor use in much of the State Forest Reserves (343 sq mi). The Kīpahulu Biological Reserve within the park is closed to visitors. It is assumed for this analysis that the type of impacts on visitor use and experience would be similar for management activities occurring on NPS and adjacent state and private lands within the project area.

Analysis

Under the proposed action, visitor impacts would primarily be associated with some disturbance from aerial operations and pedestrian teams during project implementation. The noise disturbance and other visitor experience impacts vary with release method, location of release activities, and level of visitor access. As incompatible mosquito releases would only occur during the daytime on weekdays, there would be no impacts to visitor experience at night or on weekends. Potential intermittent disturbance may be offset by potentially successful mosquito suppression and conservation of Hawaiian honeycreepers, which would result in a long-term beneficial impact on the visitor experience, especially for visitors that would enjoy hearing and seeing the iconic Hawaiian honeycreepers.

Drone Release

As described in Chapter 2, drones would be the primary mosquito release method and it is conservatively estimated that the proposed action would require up to 49–72 hours of drone flight time per week (depending on the time of year) to achieve the desired incompatible mosquito release rate for mosquito suppression. Drone flight paths would vary substantially depending on the release locations being treated each day, and drones would likely pass over a specific location twice per week or less. Visitors at or near release locations would experience noise (maximum of 47–59 dBA at 100–200 feet AGL) and visual disturbance while the drone is flying above. This method would have minimal adverse impacts on visitor experience on park, state, and TNC lands as the visual and auditory disturbance would be short in duration, likely from 15 seconds to a few minutes (drones may hover for less than 15 seconds over a particular location). Drone mosquito releases would occur in areas largely inaccessible to visitors and would not occur at night or on weekends. Drone noise could potentially be heard (above approximately 27 dBA) up to 0.5 mile of the drone. Notably, the extensive tree canopy cover and rugged terrain can have a dampening effect on sound and may reduce the distance (likely by half or more based on anecdotal experience of park and state staff working in the project area) where sound is heard. The nearest recreational areas where people could experience drone noise are in Makawao Forest Reserve and lower Kīpahulu District. People in these areas could very briefly experience drone noise if drones pass within 0.5 mile of recreational trails or other public use areas. Most of the areas where drones would be conducting releases would be out of earshot for hikers along the Pīpīwai Trail to Waimoku Falls.

Helicopter Longline Release

When drones are not available, intermittent adverse impacts on visitor experience from helicopter overflights would occur. Adverse impacts would result from elevated sound levels along helicopter flight paths while accessing the project area. Impacts to the visitor experience could occur over a relatively short duration (15 seconds to a few minutes) primarily due to noise (maximum of 82 dBA at 150 feet AGL). Brief visual impacts would also be likely. As described in previous sections, under the worst-case scenario, helicopter noise could be audible up to 3.5 miles from a given helicopter flight path, and noise levels could be above existing ambient up to 1.8 miles from the flight path. Speech interference could occur at 0.47 miles from the flight path. Visitors in the Summit District of the park outside of the core mosquito suppression area could see an increase of helicopter flights, approximately one or two additional flights per week for up to two months, over this area to reach the incompatible mosquito release locations. However, the vast majority of flights (by helicopter or drone) would likely be based out of temporary helibases outside of the Summit District and flights would not cross the area. For flight paths to and from ʻOheʻo, helicopter noise would be audible at Waimoku Falls for a duration of less than four minutes. Speech interference would likely not occur, and noises would rarely exceed existing ambient in lower Kīpahulu Valley as a result of project-related flights. Additionally, lower Kīpahulu Valley already experiences consistent helicopter noise disturbance from commercial air tours. The Kīpahulu acoustic monitoring station recorded noise from helicopters approximately 28 percent of the time (likely up to 10 commercial tour flights per day). Therefore, the limited use of helicopters under the proposed action would likely not be noticeable to the public.

There would be no mosquito releases at night or on the weekends, so noise impacts would only occur during daylight hours (between civil sunrise and civil sunset) on weekdays. During helicopter longline releases, adverse impacts on visitor use and experience would primarily occur along flight paths, at helibases, and when hovering over mosquito release locations. Helicopters would hover for less than 15 seconds over each mosquito release location. At any given location in the core area, the perceived noise levels from helicopter operations would fluctuate for visitors because helicopters would be moving through the area quickly (22 mph during releases and up to 115 mph during transit). The core area contains many places where there is little to no public use. The most well-used areas with established public trails include Makawao Forest Reserve and the lower Kīpahulu District area where many people use the Pīpīwai Trail to access Waimoku Falls. The proposed action would only minimally elevate noises experienced by visitors in the vicinity of lower Kīpahulu Valley.

Pedestrian Release

Using this method, mosquito releases would result in a minimal adverse impact on visitor experience from the use of helicopters, mechanized and motorized equipment, and human activity associated with mosquito release and monitoring activities. Only Makawao Forest Reserve, TNC's Waikamoi Preserve, and other private lands within the project area could be subject to consistent pedestrian releases up to twice per week. Adverse noise impacts on visitor experience from helicopters (maximum of 82 dBA at 150 feet AGL) would be variable but would not be sustained, as ground teams and equipment would only be dropped off and picked up on a quarterly basis at the beginning and end of each monitoring effort (when some pedestrian releases could occur) at five remote LZs in the project area that do not see consistent visitor use other than occasional hunting. Generator noise (maximum of 52–58 dBA at 23 lateral feet) could be audible for up to 3 hours per day and 7 days per week on a quarterly basis. Generators, however, would only be used during mosquito monitoring activities that occur in remote areas and out of earshot of public visitors. Popular birdwatching areas in the project area include Waikamoi (TNC), Hosmer Grove (park), and Kahakapao Trail (Makawao Forest Reserve, state). While the Summit District does offer backpacking and hiking opportunities, mosquito release activities within the project area would be largely shielded from the Summit District by the rim of the crater and only limited visitors, if any, would hear or see helicopters operating in the project area. Overall, noise from this release method would be minimal and would include noise of up to 75 dBA at 50 feet from vehicles approaching and leaving trailheads up to 2 hours per day, 2 days per week. Noise impacts from vehicles would blend into the vehicle traffic/noise already occurring at trailheads.

Mosquito Monitoring

Motorized vehicles (SUVs or trucks) would assist in the transportation of field teams and gear to reach three ground-accessible monitoring sites in Makawao Forest Reserve and TNC's Waikamoi Preserve. Noise from vehicles used during monitoring would primarily occur along the Flume Road shown (in brown) on **Figure** 5 and is not expected to exceed 4 hours per day for up to 7 days on a quarterly basis. It should be noted that vehicles would not be running constantly during that 4-hour time period because crews would be stopping periodically to check mosquito traps. As previously mentioned, ground vehicles can reach 75 dBA at 50 feet from the source but would be muffled by the surrounding canopy and would not be expected to exceed 60 dBA at 50 feet from the source of noise.

Overall, the noise from helicopters and generators would be focused at the five helicopter-only accessible monitoring camps and LZs. Noise from approaching or departing vehicles would occur at trailheads in Makawao Forest Reserve and TNC's Waikamoi Preserve. Adverse impacts on visitor use and experience during monitoring activities from helicopters, generators, and vehicles would be highly variable and not sustained (would only occur every three months). In addition, it is unlikely that any visitors or recreationists would be aware of the helicopter landings or generator noise due to the remoteness of the LZs used during monitoring and the infrequency of trips required for quarterly monitoring.

Cumulative Impacts

The impacts of past, present, and reasonably foreseeable future actions (see Appendix E) and the no-action alternative are described in the section titled "Current and Expected Future Condition of Visitor Use and Experience if No Action is Taken". Because the no-action alternative would not result in any new actions that would have indirect or direct impacts to visitor use and experience, there would be no cumulative effects associated with the no-action alternative. Under the proposed action, there would be impacts to visitor experience mostly from the use of drones and helicopters to release mosquitoes. These impacts to visitor experience, however, would be limited to drone and helicopter flight paths and landing zones because much of the core mosquito release areas are closed to the public.

When the impacts of the proposed action are added to the impacts of present and reasonably foreseeable actions, an adverse cumulative impact on visitor experience would continue for visitors, due to ongoing actions, primarily in the form of commercial air tours. The proposed action would add a limited incremental adverse impact to visitor use and experience from increased drone and helicopter overflights. The suppression of invasive mosquitoes would additionally result in a long-term beneficial impact on the visitor experience, especially for visitors that would enjoy hearing and seeing the iconic Hawaiian honeycreepers. Overall, cumulative impacts would remain adverse, primarily due to the other past, present, and reasonably foreseeable future actions.

Conclusion

Under the no-action alternative, conditions and trends would remain the same or similar as existing conditions. Visitors would not experience additional disturbances from mosquito release activities and invasive mosquitoes would continue to spread avian malaria, which would severely impact native forest birds leading to considerable mortality and likely extirpation and extinction. Compared to the no-action alternative, mosquito release activities under the proposed action would contribute additional periodic adverse impacts on visitor experience near LZs, helibases, flight paths, and trails from the use of drones, mechanized equipment, and helicopters largely in the form of noise and visual intrusion. Adverse impacts from the pedestrian release method would be confined to a small portion of the overall project area. Because the majority of the project area is closed to the general public, there will be only minimal impacts to visitor experience from mosquito release and monitoring activities. A permanent beneficial impact on the visitor experience is anticipated under the proposed action, assuming the mosquito control effort is successful and native forest bird populations stabilize or recover. For those who are visiting portions of the analysis area to enjoy a unique native rainforest ecosystem or birdwatching, the beneficial impact could be considered substantial. Though considerable analysis is presented here, overall adverse impacts to visitor use and experience would be brief and minimal.

THREATENED AND ENDANGERED PLANT SPECIES AND STATE PLANT SPECIES AT RISK

Plant species listed as threatened or endangered receive federal and state protection under the ESA and Chapter 195D, Hawai‘i Revised Statutes, respectively, and are characterized as those that are in danger of or threatened with extinction throughout all or a significant portion of their range. State plant species at risk include species that are not federally or state listed but are recognized as imperiled or vulnerable by the state and have been identified as important to protect and manage by biologists or land managers as there are fewer than 50 individuals remaining in the wild. While some plant species at risk may be considered vulnerable to population declines, or extinction, by state or global metrics (e.g., NatureServe Global Conservation Rank), others are lacking enough information to make a status determination.

The analysis area for listed plant species, designated critical habitat, and plant species at risk includes portions of the project area that would be used for ground-based pedestrian mosquito releases and monitoring activities. This includes a 20-foot buffer around management trails, fence lines, and established helicopter LZs and camps that would be used to support ground-based activities. Although there are a few listed plant species and plant species at risk that grow on trees and occur within the project area, these are extremely unlikely to be affected by aerial activities (e.g., rotor wash during helicopter longline release). Therefore, portions of the project area that only include aerial activities (e.g., drone and helicopter longline flights) are not included in the analysis area, as these activities would not affect listed plant species, designated critical habitat, and plant species at risk.

Current and Expected Future Condition of Threatened and Endangered Plant Species and State Plant Species At Risk if No Action is Taken

The current condition of threatened and endangered plant species and state plant species at risk is described below. A detailed discussion of past, present, and reasonably foreseeable future projects within the park contributing to the existing conditions and current trends for threatened and endangered plant species, designated critical habitat, and state plant species at risk are described in more detail in Appendix E. The description below provides an overview of how these ongoing and future actions would affect threatened and endangered plant species and state plant species at risk.

Under the no-action alternative, conditions for threatened and endangered plant species and state plant species at risk would continue to be the same or similar to existing conditions with the same trends and impacts from past, present, and foreseeable planned actions. Therefore, the affected environment and impacts of no-action are the same and discussed only once here.

Currently, 425 plant species in Hawaiʻi are federally and state listed as threatened or endangered (USFWS 2022c). Many of these plant species persist at very low numbers and are in rapid decline (USFWS 2021a). Existing threats to listed plant species across the Hawaiian Islands include habitat loss, degradation, and modification of habitat by non-native invasive plants and animals, and disease (USFWS 2021a). While plant species at risk are not currently protected under the ESA and Hawaiʻi Revised Statues Chapter 195D, they face the same threats as listed species.

Climate change is exacerbating and accelerating threats to listed and at-risk animal and plant species across the Hawaiian Islands. Rapid climate change, including the global trend of atmospheric warming, is an important factor expected to contribute to numerous extinctions across the globe (Thomas et al. 2004). Changes in environmental conditions, such as increasing storm intensities and temperatures and decreasing precipitation, can result in changes to the microclimate of a species' habitat, and may lead to the loss of the species or of other native species associated with that species habitat (USFWS 2021a).

In addition, natural ecosystems in Hawaiʻi rely on the pollination services of native birds and insects, in particular native honeycreepers and yellow-faced bees (UH Honeybee Project 2022). Native Hawaiian lobeliads coevolved with Hawaiian honeyeaters and honeycreepers. Five genera of Hawaiian lobeliads (*Clermontia*, *Delissea*, *Cyanea*, *Lobelia*, and *Trematolobelia*) are believed to have evolved flowers adapted for pollination by Hawaiian honeycreepers and honeyeaters (Pender et al. 2014). Although information on specific plant-pollinator relationships is limited (Barton et al. 2021), the relationship between native Hawaiian birds and plants is threatened by the loss or functional extinction of much of Hawaiʻi's avifauna (Pratt et al. 2009). Due to the extinction of all native Hawaiian honeyeaters, most honeycreepers, and the decline of remaining nectar-feeding honeycreepers, reproduction in some lobeliads may now be limited (Barton et al 2021). Native birds are increasingly infrequent visitors to lobeliads in many Hawaiian forests, especially at low and mid elevations where introduced avian malaria has decimated native bird populations (Cory et al., 2015 as cited in Barton et al. 2021).

Federal Threatened and Endangered Plant Species and Designated Critical Habitat Within Analysis Area

Twenty-seven plant species listed as endangered under the federal ESA and HRS Chapter 195D occur within the plant analysis area. Table F-1 in Appendix F lists these species and their habitat, as well as the locations of known occurrences of these species within the analysis area. Fourteen of these species are found on park land within the analysis area, 11 on state land, and 11 are found on TNC-managed lands. One of these 27 listed plant species, hāhā (*Cyanea kunthia*), is known to occur on lands managed by all three entities (i.e., park, state, and TNC) within the analysis area. The majority of the listed plant species occurring in the analysis area are found in lowland or montane, wet to mesic forests.

The analysis area includes designated critical habitat for 37 federally listed plant species on park, state, and TNC-managed lands (USFWS 2022b; Appendix F). However, only 19 of the listed plant species with designated critical habitat that overlap the analysis area also have known occurrences within the analysis area (Table F-2 in Appendix F).

Within the analysis area, listed plant species and designated critical habitat have been and would likely continue to be affected by ongoing management activities. Under the no-action alternative, ongoing and future management activities expected within the analysis area include implementing ground and aerial herbicide spray techniques to help control or eradicate invasive plant species, as well as manual removal of invasive plant species; ungulate, predator, and pest control; trail maintenance; fence construction and maintenance; landing zone and shelter maintenance; fire management; and collection, reintroduction, and monitoring of endangered plants. These activities have the potential to inadvertently introduce and spread invasive species through movement of personnel and equipment, which can negatively affect listed plants and designated critical habitat. Invasive plants can outcompete or reduce habitat availability for listed plants. Similarly, feral ungulates can degrade native habitat required for listed plants, including designated critical habitat (USFWS 2021a). Other potential adverse effects from these activities include the accidental trampling of plants or inadvertent harm to listed plant species and designated critical habitat during application of herbicides for invasive plant control.

In addition to ongoing and future management activities, ongoing and future visitor use of the park, state, and TNC lands has the potential to affect listed plants and designated critical habitat. Pedestrian visitors within the plant analysis area have the potential to trample these species or their habitat. However, public access to much of the plant analysis area is generally limited or restricted (**Figure 9**) and visitors are likely to stay on designated hiking trails. Pedestrian visitors also have the potential to introduce or spread invasive species or pathogens, which may adversely affect listed plants and designated critical habitat. Future actions within the analysis area include the Pīpīwai Trail Viewing Platform and inventory and monitoring vegetation plots projects in the park, watershed resource monitoring on state-lands, and installation of cell tower infrastructure within TNC-managed lands have the potential to affect listed plants and designated critical habitat through accidental trampling or introduction of invasive species or pathogens.

Ongoing management actions and pedestrian visitation to the analysis area are discussed in the existing conditions for listed plant species and designated critical habitat. The effects of these activities are included in the affected environment, and the no-action alternative would therefore not result in any new direct or indirect impacts to listed plant species or designated critical habitat. As there would be no new direct and indirect impacts as a result of the no-action alternative, there would be no cumulative effects associated with the no-action alternative. If no action is taken, however, avian malaria would continue to devastate native forest bird populations, which could potentially affect listed plant species due to the loss of pollination services of these native birds. However, information on specific plant-pollinator relationships is limited (Barton et al. 2021) and the likelihood and extent of potential impacts to listed plant species from the continued loss of native forest birds is therefore unknown.

State Plant Species at Risk and Habitat Within Analysis Area

Twenty-three State plant species at risk occur in the plant analysis area. These species are listed in Table F-3 in Appendix F, along with their habitat, and locations within the analysis area. Four of these 23 species are found on park land within the analysis area, none on state land, and 19 are found on TNC-managed lands. None of these plant species at risk occur on lands managed by all three entities (i.e., park, state, and TNC) within the analysis area, and only two species, Hawai'i jewel-orchid (*Anoectochilus sandvicensis*) and awapuhiakanaloa (*Liparis hawaiensis*), are known to occur on lands managed by both the park and TNC within the analysis area. Most plant species at risk within the analysis are found within mesic to wet forest habitats.

The impacts to plant species at risk and their habitat within the analysis area from ongoing and future actions would be the same as described above for federal threatened and endangered plant species. The no-action alternative would not result in any new direct or indirect impacts to plant species at risk or their habitat within the analysis area. As a result, there would be no cumulative effects associated with the no-action alternative. If no action is taken, however, avian malaria would continue to devastate native forest bird populations, which could potentially affect plant species at risk due to the loss of pollination services of these native birds. As noted above, however, information on specific plant-pollinator relationships is limited (Barton et al. 2021) and the likelihood and extent of potential impacts to plant species at risk from the continued loss of native forest birds is therefore unknown.

Effects of the Proposed Action on Threatened and Endangered Plant Species and State Plant Species At Risk

Methods and Assumptions

Potential impacts on listed plant species and plant species at risk and their habitat, including designated critical habitat, were evaluated based on resource expert knowledge and professional judgment, review of available research, and anticipated locations where ground-based activities under the proposed action would occur. Listed plant species, designated critical habitat, and plant species assessed to be at risk assessed include those species with known occurrences or designated critical habitat that overlap the analysis area, as defined below. The locations of existing populations of these species within the analysis area were provided by park, state, and TNC staff. Additional sources of data included the locations of designated critical habitat for federally listed species within the analysis area (USFWS 2022b).

Analysis Area

The area of analysis to assess impacts of the alternatives on listed plant species, designated critical habitat, and plant species at risk includes the following:

- Up to 11 miles of fence lines and 100 miles of trails within the project area that may be used during pedestrian mosquito release and monitoring activities, plus a 20-foot buffer; and
- Up to 10 existing LZs and camps that would be used to support pedestrian mosquito release and monitoring activities.

The plant analysis area, including the buffers around the fence lines, trails, existing LZs and camps, was identified in consultation with the USFWS (USFWS 2022d) and internal discussions with park and state staff and encompasses the area where both direct and indirect effects to listed plant species and plant species at risk from the proposed action are likely to occur. As described earlier in this section, 27 listed plant species and 23 plant species at risk are known to occur within the analysis area (Appendix F). The analysis area also includes designated critical habitat for 37 federally listed plant species.

Threatened and Endangered Species Section 7 Determination Definitions

The Endangered Species Act (ESA), NPS Management Policies 2006, NEPA, and applicable regulations require the analysis of potential impacts on special-status species (federal or state endangered, threatened, candidate, or species at risk). According to section 4.4.2.3 of NPS Management Policies 2006, NPS must additionally "manage critical habitat […] to maintain and enhance their value for the recovery of threatened and endangered species" (NPS 2006).

This analysis serves as both a NEPA assessment of impacts on federally listed species (federal endangered, threatened, or candidate) that could be impacted by the project and a biological assessment as required by Section 7 of the ESA.

The USFWS guidance for implementing Section 7 consultation under the ESA (USFWS 2017) uses the following terminology to assess impacts on federally listed species:

No Effect

This conclusion is reached if the proposed action and its interrelated and interdependent actions would not directly or indirectly affect federally listed species or destroy/adversely modify designated critical habitat. Formal section 7 consultation is not required when the no effect conclusion is reached.

May Affect, but Not Likely to Adversely Affect

This conclusion is appropriate when effects to federally listed species or designated critical habitat are expected to be beneficial, discountable, or insignificant. Beneficial effects are contemporaneous positive effects without any adverse effects to the species or habitat. Insignificant effects relate to the size of the impact, while discountable effects are those that are extremely unlikely to occur. Based on best judgment, a person would not: (1) be able to meaningfully measure, detect, or evaluate insignificant effects; or (2) expect discountable effects to occur. If the project scientist making the determination and the project manager agree that the project "is not likely to adversely affect" federally listed species or designated critical habitat, the intra-service Section 7 consultation process is completed.

May Affect, Likely to Adversely Affect

This conclusion is reached if any adverse effect to federally listed species or designated critical habitat may occur as a direct or indirect result of the proposed action or its interrelated or interdependent actions, and the effect is not discountable or insignificant. In the event the overall effect of the proposed action is beneficial to the federally listed species or designated critical habitat but may also cause some adverse effect on individuals of the listed species or segments of the critical habitat, then the determination should be "is likely to adversely affect." Such a determination requires formal Section 7 consultation.

Analysis

This section discusses the potential effects to federally listed plant species, designated critical habitat, and plant species at risk from each of the three mosquito release methods and mosquito monitoring.

As noted above in the Current and Expected Future Condition of Threatened and Endangered Plant Species and State Plant Species at Risk section, natural ecosystems in Hawaiʻi rely on the pollination services of native birds and insects, in particular native honeycreepers, and yellow-faced bees (UH Honeybee Project 2022). Under the proposed action, actions taken to control mosquito populations that carry avian malaria would support recovery of native Hawaiian forest birds, reducing the likelihood for extirpation or extinction of these species. This could potentially have a beneficial impact on native Hawaiian plants, including listed plant species and plant species at risk, which rely on native forest birds for pollination. Information on specific plant-pollinator relationships is limited (Barton et al. 2021) and the potential benefit and level of benefit to listed plant species and plant species at risk from implementation of the proposed action is therefore unknown.

<u>Drone Releases</u>

As noted earlier in this section, the analysis area for listed plant species, designated critical habitat, and plant species at risk is limited to the portions of the project area that would be used for ground-based mosquito release and monitoring activities. The only ground-based activities associated with drone releases would be the use of temporary helibases for drone launch locations. However, as noted in Chapter 2, these drone launch locations would be existing "front country" locations accessible by major roads. As no vegetation clearing would occur at these drone launch locations, there would be no impact to listed plants, designated critical habitat, or plant species at risk from vegetation clearing.

The use of temporary helibases for drone launch locations could result in the introduction or spread of invasive plant species or pathogens (e.g., fungal pathogens responsible for rapid ʻōhiʻa death) through the spread of invasive plant pathogens, seeds, spores, or propagules on equipment or clothes of personnel. As outlined in Chapter 2, to minimize the risk of introducing and spreading invasive plant species or pathogens all vehicles, equipment, clothes, and footwear would be regularly inspected and cleaned and personnel would implement existing protocols, such as the PIFWO Office Invasive Species Biosecurity Protocols (USFWS 2022a). With implementation of mitigation measures, potential adverse impacts to listed plant species, designated critical habitat, and plant species at risk from the introduction or spread of invasive plant species or pathogens under the drone release method would be negligible.

Helicopter Longline Releases

Similar to the drone release method, the only ground-based activities associated with short-term (up to two months), temporary helicopter longline releases would be the use of temporary helibases for attachment of the longline and release mechanisms by ground teams. As no vegetation clearing would occur at these temporary helibases, there would be no impact to listed plants, designated critical habitat, or plant species at risk from vegetation clearing at these locations. Although the helicopter longline release method could result in the introduction or spread of invasive plant species or pathogens at temporary helibase locations, implementation of mitigation measures outlined in Chapter 2, such as regularly inspecting and cleaning vehicles, equipment, and clothes and implementing the PIFWO Invasive Species Biosecurity Protocols (USFWS 2022a), would minimize the transport and establishment of invasive plant species or pathogens at these locations. With implementation of mitigation measures, potential adverse impacts to listed plant species, designated critical habitat, and plant species at risk from the introduction or spread of invasive plant species or pathogens under the helicopter longline release method would be negligible.

Pedestrian Releases

Potential adverse impacts to listed plant species, designated critical habitat, and plant species at risk from pedestrian releases could occur through:

- Removal or trampling of individual plants, physical damage to plant parts (e.g., roots, stems, flowers, fruits, seeds), or damage to habitat, including designated critical habitat, from clearing, maintenance, and increased use of existing management trails and fence lines, helicopter LZs, and camps for mosquito release activities; and
- Introduction or spread of invasive plant species or pathogens from pedestrian or helicopter teams during mosquito release and monitoring activities.

As outlined in Chapter 2, the only consistent pedestrian release would be in Makawao Forest Reserve and Waikamoi Preserve; pedestrian releases within upper Kīpahulu Valley Biological Reserve and Hanawī Natural Area Reserve would likely only occur on a quarterly basis, simultaneous with ground-based mosquito monitoring (discussed below). Vegetation clearing around existing management trails and fence lines or LZs, and increased use of existing trails, fence lines, camps, and LZs have the potential to result in physical damage to listed plant species or plant species at risk. Cutting and removal of vegetation surrounding listed plants or plant species at risk has the potential to alter microsite conditions (e.g., light, moisture, temperature), which could alter habitat, including designated critical habitat for these species. Although there is the potential for listed plant species or plant species at risk to be removed or harmed during trail clearing and vegetation removal, implementation of mitigation measures (outlined in Chapter 2) such as flagging the boundaries of areas occupied by listed plant species prior to any clearing, would make any direct harm to these species unlikely.

Vegetation clearing and increased use of existing trails, fence lines, camps, and LZs can increase the risk of invasion or spread of invasive plants or pathogens. This could occur through the direct spread of invasive plants or pathogen seeds, spores or propagules on clothes or equipment of personnel, or indirectly through removal of existing vegetation, which allows openings for invasive plants to colonize. Existing protocols, however, such as the PIFWO Invasive Species Biosecurity Protocols (USFWS 2022a) and other measures outlined in Chapter 2, would be implemented to minimize the risk of introducing and spreading invasive species and pathogens. With implementation of these protocols and mitigation measures, adverse effects from the potential spread of invasive plants and pathogens are anticipated to be negligible.

Temporary disturbances such as vegetation removal around existing trails and LZs may affect the Primary Constituent Elements (PCEs) of designated critical habitat units. These impacts would be minimized by following the mitigation measures such as providing personnel with maps showing the locations of designated critical habitat areas and training them how to avoid unnecessary adverse impacts within critical habitat. With implementation of mitigation measures, the impacts to designated critical habitat are expected to be negligible.

Mosquito Monitoring

Potential impacts of mosquito monitoring to listed plant species, designated critical habitat, and plant species at risk could occur through; a) vegetation clearing, b) the removal or trampling of individual plants, c) physical damage to plant parts (e.g., roots, stems, flowers, fruits, seeds), d) introduction or spread of invasive plants or pathogens, or e) damage to habitat, including designated critical habitat, from clearing, maintenance, and increased use of existing management trails and fence lines, helicopter LZs, and camps. The adverse impacts of these activities would be as described for pedestrian releases.

Monitoring would likely occur quarterly (four times/year) and in some cases, such as within upper Kīpahulu Valley Biological Reserve and Hanawī Natural Area Reserve, could potentially be concurrent with pedestrian releases. As only established trails, fence lines, camps, and helicopter LZs proposed for use under pedestrian releases would be used for monitoring activities, no additional adverse impacts from vegetation removal or trampling in these areas would be anticipated. With implementation of mitigation measures outlined in Chapter 2, such as flagging and avoiding individuals or populations of federally listed plant species and plant species at risk and implementing invasive species biosecurity protocols, potential impacts to federally listed plant species, designated critical habitat, and plant species at risk during mosquito monitoring would be negligible.

Cumulative Impacts

Overall, the impacts on listed plant species, designated critical habitat, and plant species at risk from past, present, and reasonably foreseeable future actions would be as described earlier in the section titled “Current and Expected Future Condition of Threatened and Endangered Plant Species and State Plant Species at Risk”. There would be no new impacts to plants under the no-action alternative. Under the proposed action, steps taken to suppress mosquito populations that carry avian malaria would support recovery of native Hawaiian honeycreepers, reducing the likelihood for extirpation or extinction of these species. This could potentially have a beneficial impact on native Hawaiian plants, including listed and at-risk plant species, which rely on native forest birds for pollination. The proposed action would potentially have an adverse impact on listed plant species, designated critical habitat, and plant species at risk through vegetation clearing and trampling and increased risk of invasion or spread of invasive plants or pathogens. With implementation of mitigation measures described in Chapter 2, however, adverse impacts under the proposed action would be negligible. The incremental impacts of the proposed action would have only a small contribution to overall cumulative impacts.

Conclusion

Under the no-action alternative, conditions would remain the same or similar to existing conditions, including trends and impacts from past, present, and foreseeable future actions. This includes the potential extirpation or extinction of native forest bird species due to uncontrolled avian malaria, which could potentially have a detrimental impact on native Hawaiian plants, including listed plants and plant species at risk due to the loss of pollinators. Compared to the no-action alternative, the proposed action would potentially result in adverse impacts to federally listed plant species, designated critical habitat, and plant species at risk through removal or physical damage to plants, damage or modification of habitat, and the introduction of invasive plant species or pathogens. However, with implementation of mitigation measures, these adverse impacts are anticipated to be negligible. Additionally, the proposed action would likely support recovery of native Hawaiian forest birds, which may benefit native Hawaiian plants, including listed plants and plant species at risk. Though considerable analysis is presented here, overall adverse impacts to listed plants and plant species at risk would be minimal.

Section 7 Determination Summary

Based on the analysis, project activities under the proposed action, incorporating mitigation measures described in Chapter 2, *may affect, but is not likely to adversely affect*, all analyzed federally listed plant species and their designated critical habitat, as applicable (Appendix F).

THREATENED AND ENDANGERED WILDLIFE SPECIES AND WILDLIFE SPECIES OF CONCERN

Federally and state listed wildlife species receive protection under the ESA and Chapter 195D, Hawai‘i Revised Statutes, respectively, and are characterized as those that are in danger of extinction throughout all or a significant portion of their range. State protected wildlife species include all indigenous wildlife, which are protected under state law (Section 13-124-3, HAR). Other species of concern may be bird species protected under the Migratory Bird Treaty Act, and other species at risk such as species proposed for listing, or species considered globally threatened by organizations other than USFWS (e.g., IUCN, State of Hawai‘i, NPS 2017). Although all threatened and endangered wildlife species and wildlife species of concern in the project area were considered, only those species that have the potential to be impacted by the no-action alternative or the proposed action are described in this EA.

Current and Expected Future Condition of Threatened and Endangered Wildlife Species and Wildlife Species of Concern if No Action is Taken

The current condition of threatened and endangered wildlife species and wildlife species of concern is described below. A detailed discussion of past, present, and reasonably foreseeable future projects within the park contributing to the existing conditions and current trends for threatened and endangered wildlife species and wildlife species of concern are described in more detail in Appendix E. The description below provides an overview of how these ongoing and future actions would affect threatened and endangered wildlife species and wildlife species of concern.

Under the no-action alternative, conditions for threatened and endangered wildlife species and wildlife species of concern would continue to be the same as or similar to existing conditions with the same trends and impacts from past, present, and foreseeable planned actions, including the potential for continued declines in several threatened and endangered forest bird species. The affected environment and impacts of no-action are therefore the same and discussed only once here.

Federally and State Listed Wildlife Species and Habitat within Project Area

Island species co-evolved in isolation over millions of years with unique adaptations to their environments. Hawai‘i’s endemic plants, birds, and insect pollinators are remarkably co-specialized (Carlquist 1974). Habitat destruction, invasive plants, non-native predators and competitors, introduced ungulates, and introduced diseases have decimated the diverse, endemic native animal community of the Hawaiian archipelago (Pratt 2009).

The ecosystems of East Maui (and the project area) include numerous intermittent and perennial streams, bogs, small montane lakes, and rainforest that provide habitat for native birds, bats, invertebrates, and aquatic organisms. The upper elevation habitats, from approximately 3,900 feet to 6,400 feet, are characterized as very wet, high-quality native-dominated rainforest (Price et al. 2007). Nine species of federally listed threatened and endangered wildlife (one insect, eight bird species, and one mammal) are known to occur within the project area. Three of these listed bird species are Hawaiian honeycreepers—kiwikiu, ‘ākohekohe and ‘i‘iwi—and are declining rapidly due to mosquito-borne avian malaria and other threats. Threatened and endangered wildlife species and their trends are described in the following pages.

Birds

Hawaiian Honeycreepers

The introduction of the first mosquitoes to Maui, reported in 1826 (Van Dine 1904), has been devastating to the endemic Hawaiian forest bird species, particularly the Hawaiian honeycreepers (family Fringillidae, subfamily Carduelinae, tribe Drepanidini), in the last half century. The invasive southern house mosquito is highly adaptive and transmits at least two bird diseases in Hawaiʻi including avian pox (*Avipoxvirus*) and avian malaria (Atkinson and LaPointe 2009a, Harvey-Samuel et al. 2021). Avian malaria was introduced more than 100 years ago to the avifauna of Hawaiʻi and has caused massive endemic forest bird population declines, limited the elevational distribution of Hawaiian forest birds, and caused extinctions across the archipelago (including from the analysis area) as recently as the last two decades (Warner 1968, van Riper et al. 1986, Atkinson and Samuel 2010, USFWS 2021). Avian malaria's acute phase of infection causes anemia (loss of red blood cells and oxygen to the vital organs), with symptoms of weakness, loss of appetite, weight loss, organ failure, and death to susceptible birds after a single infected mosquito bite (Atkinson et al. 1995 and 2000). Highlighting the urgency of action needed to prevent avian malaria transmission, three Hawaiian honeycreeper species that disappeared from the project area within the last two decades were recently declared extinct at least in part due to avian malaria: Maui ʻākepa (*Loxops ochraceus*), poʻouli (*Melamprosops phaeosoma*), and Maui nukupuʻu (*Hemignathus affinis*) (USFWS 2021). As discussed in earlier sections of this document, three species of honeycreeper within the project area are federally listed as threatened or endangered and are at risk of continued population decline and/or extinction the next 20 years: kiwikiu, ʻākohekohe, and ʻiʻiwi.

The endangered kiwikiu is a stout yellow and olive-green honeycreeper with a large, hooked bill. Endemic to the islands of Maui and Molokaʻi, the species is currently only found on East Maui and is ranked as one of the most imperiled Hawaiian birds and is very susceptible to avian malaria (Warren et al. 2019, American Bird Conservancy 2022, USFWS 2019). Kiwikiu may nest all year but primarily breeds between January and June and are primarily insectivorous, using their disproportionately large bill to probe and excavate woody plant material (and, to a lesser extent, fruits) to eat mostly the larvae of beetles (Coleoptera) and caterpillars (Lepidoptera) found on or within native plants and lichens (Mountainspring 1987, Peck et al. 2015, Simon et al. 2020). Critical habitat has been designated for kiwikiu (**Figure 10**; USFWS 2016a), and the majority of the project area lies within it. Their habitat is characterized by wet-mesic and ʻōhiʻa-dominated rainforest above 5,280 feet (Judge et al. 2021).

The endangered Maui-endemic ʻākohekohe is a striking forest pollinator with a distinctive crest on its head. Critical habitat has been designated for ʻākohekohe (**Figure 10**; USFWS 2016a), which entirely overlaps the critical habitat of the kiwikiu; the majority of the project area lies within the critical habitat for these species. The ʻākohekohe persists on less than 7,400 acres of native rainforest above 5,280 feet (Judge et al. 2021), with breeding typically occurring between November and June (Wang et al 2020). Elevational range contraction and risks associated with avian malaria have been well documented for the species (Scott et al. 1986, Berlin and VanGelder 2020, Wang et al. 2020).

The ʻiʻiwi (federally listed as threatened) is a honeycreeper historically widespread and occurring at all elevations, but now persists only in the high-elevation forests primarily of Hawaiʻi, Maui, and Kauaʻi (Scott et al. 1986, Fancy and Ralph 2020, USFWS 2016b). Breeding may occur all year but peaks from February through June (Fancy and Ralph 2020). The ʻiʻiwi is a strong flier with high, long flights to locate nectar sources (Guillaumet et al 2017, Fancy and Ralph 2020) and makes seasonal movements in response to patchy availability of flowering ʻōhiʻa (*Metrosideros polymorpha*), māmane (*Sophora chrysophylla*), and other native plants. As the ʻiʻiwi is highly susceptible to avian malaria (Atkinson et al. 1995), the species' seasonal movement patterns across the landscape negatively affect its long-term population dynamics (Guillaumet et al. 2017). On the island of Hawaiʻi the movements into low elevations occur primarily during the post-breeding season (Guillaumet et al. 2017), an important time of year for the proposed action to target reducing southern house mosquito densities, thereby decreasing the risk of malaria infections. The USFWS is in the process of designating critical habitat for ʻiʻiwi.

Most honeycreeper species currently persist only in high-elevation forests where the risk of malaria transmission is lower due in large part to colder temperatures that limit both the reproduction of the malaria parasite and its mosquito vector (van Riper et al. 1986, Scott et al. 1986, Atkinson and LaPointe 2009b, Atkinson et al. 2014). Even though much of the high elevation threatened and endangered bird habitat in the project area is largely protected from feral ungulates

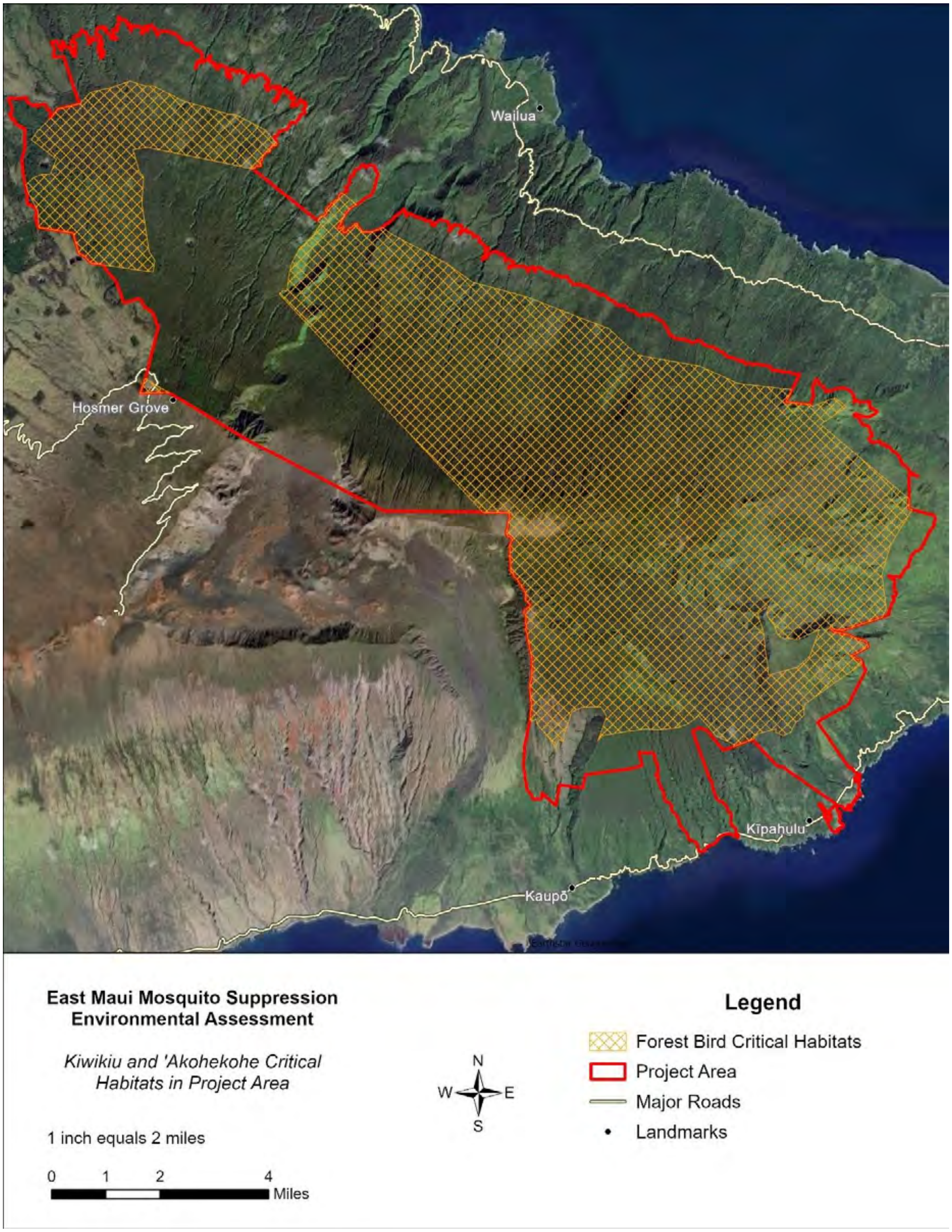

FIGURE 10: DESIGNATED FOREST BIRD CRITICAL HABITAT WITHIN THE PROJECT AREA

and direct human-caused habitat loss, there is evidence of continuing range contraction and population declines, especially from lower-elevation portions of their ranges since 1980 (Baker and Baker 2000, Camp et al. 2009, Vetter et al. 2012, Judge et al. 2021). Precipitous negative population trends have been observed for kiwikiu and ʻākohekohe across their small ranges (Judge et al. 2013, 2017, 2021). Kiwikiu and ʻākohekohe population estimates from surveys in 2017 are 157 individuals (44–312 individuals [95 percent confidence interval]) and 1,768 individuals (1193–2411), respectively (Judge et al. 2021). Kiwikiu and ʻākohekohe abundance has declined by more than 70 percent since 2001 (Judge et al. 2021), and a predicted range loss of more than 90 percent may occur by the end of this century under moderate climate change scenarios (Fortini et al. 2015).

ʻIʻiwi has disappeared from most of its historic range and is extremely susceptible to mortality from avian malaria (Atkinson et al.1995, USFWS 2016b). The species, however, is still common at elevations above 5,250 feet in the project area. Recent surveys in 2017 resulted in a population estimate of 50,252 (43,908–57,146 individual [95 percent confidence interval]) ʻiʻiwi on East Maui (Judge et al. 2019). A long- term trend analysis of the national park's ʻiʻiwi population showed stability in the park's upper Kīpahulu Valley, but with a declining population trend elsewhere in the park (Brink 2020). Surveys revealed an increasing trend of ʻiʻiwi between 2011 and 2017 within the project area outside the park (Judge et al. 2019). The majority of Maui's ʻiʻiwi population occurs within the project area (Judge et al. 2019).

Southern house mosquito and avian malaria parasite lifecycles are influenced by rainfall and temperature. Warming temperatures, increasing storm and drought intensity (Thomas et al. 2004), and fluctuating rainfall patterns (Krushelnycky et al. 2016) associated with climate change are intensifying avian malarial infections at mid-elevations and expanding the transmission of avian malaria to higher-elevation forests (Atkinson et al. 2014, Liao et al. 2015, Fortini et al. 2015). Increases in both mosquito abundance and prevalence of avian malaria indicate that disease transmission has indeed expanded to higher elevations (Atkinson et al. 2014, Glad and Crampton 2015, Warren et al. 2019, Fortini et al. 2020). As a result, the high-elevation forest habitats are no longer a safe refuge from avian malaria transmission, and it is becoming increasingly important to act quickly to suppress mosquito populations before Hawaiian honeycreeper populations decline even further (Atkinson et al. 2014, Liao et al. 2015, 2017). The continued and increasing threat of avian malaria means that under the no-action alternative, it is likely that additional Hawaiian honeycreeper species will go extinct in the next few years. Additional conservation actions to recover endangered bird species such as captive rearing and future reintroductions as well as translocations to Hawaiʻi Island are under consideration, but long-term conservation of these species is contingent on suppression of mosquitoes and malaria (Paxton et al. 2022).

Within the project area, land managers of the NPS, DLNR, and TNC are currently implementing ground and/or aerial treatment operations to manage invasive species and promote native plant species survival. Weed, predator and ungulate control benefit honeycreeper populations and their habitats (Banko et al. 2019) by protecting native habitat and forage plant availability for honeycreeper species. Monitoring of mosquitoes, avian malaria, and forest birds have been conducted and will continue into the future. Other management activities within the project area that could potentially affect wildlife habitat (including designated critical habitat for honeycreeper species) include trail maintenance, maintenance of LZs and campsites, fencing and fence maintenance and fire management. These activities are primarily beneficial because they also enable weed, predator, and ungulate control, yet have the potential to accidentally introduce invasive species or forest pathogens through movement of personnel, gear, and equipment, which can negatively, indirectly affect listed honeycreeper species. The NPS Inventory & Monitoring Vegetation Monitoring project at the park, watershed resource monitoring on state lands, and installation of cell tower infrastructure within TNC-managed lands have the potential to affect honeycreeper habitat (and designated critical habitat) with accidental trampling or introduction of invasive species or forest pathogens that tend to degrade listed honeycreeper habitat. The park, state, and TNC would continue current management actions and respond to future needs and conditions without major changes in the present course.

As discussed in the "Acoustic Environment" section of this document, helicopter use for park administrative activities averaged approximately 200 hours/year (approximately 100 operations), state administrative flights also averaged approximately 200 hours/year within or immediately near the project area, and approximately 60 helicopter operations are conducted per year (estimated 75 flight hours/year) into and out of the TNC's Waikamoi Preserve. The Hughes

500D helicopter (used for most of these administrative operations) has a small rotor diameter, yet rotor wash has some potential risk to disturb nesting birds during takeoff, landing, and while hovering, depending on the proximity, terrain, tilt, wind, and altitude of the helicopter relative to the habitat feature. Honeycreeper disturbance and displacement risk in this case depends on the proximity of trees to the active helicopter and duration of the disturbance. As discussed in the "Acoustic Environment" section of this document, commercial air tours also generally occur seven days a week year-round and have averaged approximately 10-13 air tours per day in recent years (approximately 2 hours per day or 750 hours per year). These flights intersect the project area primarily in its southernmost reaches, including around Kīpahulu Valley, Ka'āpahu, and Kaupō Gap. Tour operator helicopters and administrative flights for the park, state, and TNC in the project areas averaging more than 1000 hours per year have not reported listed or native migratory bird collisions.

The noise levels and honeycreeper disturbance risks within the project area associated with ongoing administrative activities, helicopter flights, and air tours would likely continue at current levels. Helicopters, mechanized equipment, and work crews would generate noise during overflights/landings/takeoffs, fencing activities, and maintenance of trails and LZs. There are no anticipated changes to public access within the project area, so ongoing impacts to honeycreepers from visitors would remain unchanged in the foreseeable future. Overall, current and reasonably foreseeable actions would continue to result in minimal adverse direct and indirect impacts to honeycreeper species and their habitat. The effects of these activities are included in the affected environment and the no-action alternative would therefore not result in any new direct or indirect impacts to listed honeycreeper species or designated critical habitat. As there would be no new direct and indirect impacts resulting from the no-action alternative, there would be no cumulative effects associated with this alternative. If no action is taken, however, avian malaria would continue to devastate native Hawaiian honeycreeper populations, resulting in significant adverse impacts.

Nēnē

The federally threatened nēnē, or Hawaiian Goose (*Branta sandvicensis*), was extirpated from all islands except Hawai'i by the early 1900s and was reestablished on the island of Maui through a captive-breeding and release program (Banko et al. 2020). The nēnē, the official state bird that is state listed as endangered, typically nests in the national park between October and April (*personal communication, J. Tamayose, April 6, 2021*). The species uses diverse habitats including sub-alpine grasslands, open native shrubland and grasslands, as well as mid- and low-elevation pasture and managed grasslands, to forage on leaves of grass, berries, seeds, and flowers. Some individuals or pairs make elevational movements for breeding, foraging, and molting (USFWS 2019, Banko et al. 2020, Leopold and Hess). Nēnē require intensive species management to protect breeding (ground-nesting) birds from introduced predators on Maui, especially the mongoose (*Herpestes javanicus*). Nēnē on Maui are also susceptible to vehicle collisions, wind turbine collisions and human or vehicle-related injuries and trauma, toxoplasmosis (a pathogen carried by feral cats), and mosquito-borne avian pox virus (Work et al. 2015).

The Maui nēnē population is relatively small, fluctuating around approximately 250 breeding pairs (USFWS 2019), is supplemented with captive-bred and translocated birds, and is reliant on breeding pens that exclude predators and predator control at breeding sites on NPS, state, and privately managed lands. In 2020 and 2021, respectively, there were 223 and 164 nēnē individuals outside the park, and 254 and 190 in the park. Breeding failures occur often during drought conditions (Black et al. 1997), and increasing drought or other extremes in climate variability, expanding invasives species, and associated climate change scenarios are likely to negatively affect nēnē. Climate change may disrupt seasonal movements and some habitats used by nēnē for molting, breeding, and foraging.

Ongoing and planned actions that could affect the nēnē population are nearly the same as those actions described in the "Hawaiian Honeycreepers" section of this chapter. Forest-based activities, however, do not necessarily impact nēnē since suitable habitat does not exist for them in forested areas. The park, state, and TNC would continue current management actions and respond to future needs and conditions without major changes in the present course. These activities are primarily beneficial because they also enable weed, predator, and ungulate control, yet have the potential to accidentally introduce invasive species or pathogens through movement of personnel, gear, and equipment, which can negatively, indirectly affect listed wildlife. The noise levels and nēnē disturbance risks within the project area associated with ongoing administrative activities, administrative helicopter flights, and air tours (see description of these impacts in the "Hawaiian Honeycreepers" section above) would likely continue at current levels. Helicopters, mechanized equipment, and work crews would generate noise during overflights/landings/takeoffs, fencing activities, and maintenance of trails and LZs. There are no anticipated changes to public access within the project area, so ongoing impacts to nēnē from visitors would remain unchanged in the foreseeable future. Overall, current and reasonably foreseeable actions would continue to result in minimal adverse direct and indirect impacts to nēnē and their habitat. The effects of these activities are included in the affected environment and the no-action alternative would therefore not result in any new direct or indirect impacts to nēnē, there would be no new direct and indirect impacts as a result of the no-action alternative, and no cumulative effects associated with the no-action alternative. With current climate variability and projected climate change trends, however, exposure to and transmission of avian pox would also be expected to increase under the no-action alternative. Under the no-action alternative the nēnē population is therefore expected to remain stable or continue to decline as a result of climate change impacts to the species' habitat.

Seabirds

Many tubenosed seabirds (albatrosses, petrels, shearwaters, and storm-petrels; order Procellariiformes) live at sea and return to the Hawaiian Islands to pair-bond and breed between February and November, laying a single egg cared for by both parents. These species fly into and out of their nests at night. During their breeding season, listed seabirds commute between the ocean for foraging and their cryptic underground burrows to feed their young (Ainley et al. 2019, Slotterback 2020). Feral cats, other invasive predators, and light pollution are the primary threats to Hawai'i's nocturnal ground-nesting seabirds (Raine et al. 2020).

The largest breeding colony of the endangered seabird 'ua'u or Hawaiian Petrel (*Pterodroma sandwichensis*), is within the park near the summit of Haleakalā, mostly outside the project area. Two other listed seabird species, the 'ake'ake (Band-rumped Storm-Petrel, *Oceanodroma castro*), and 'a'o (Newell's Shearwater, *Puffinus newelli*) are known to occur in or transit through the project area, but their nesting distributions and abundance are not known (Aruch 2006, Krushelnycky et al. 2019).

The breeding colony of 'ua'u in and around the Summit District of the park has been monitored since the 1960s and mammalian predator populations have been managed/reduced since 1982 (Krushelnycky et al. 2019). Most nests are known to occur in the higher and drier habitats outside the project area; however, 'ua'u fly through the project area and breeding pairs are known to occur within the crater at the edge of the project area and other nearby areas with suitable habitat (Krushelnycky et al. 2019). The 'ua'u population in the park is estimated at 3,000–4,000 breeding pairs and a total of 8,000–9,000 individual birds. The most recent count of known burrows within the park is 2,784 (*personal communication, J. Tamayose, April 6, 2021*); the 'ua'u population in the park has grown since the 1980s due to invasive predator control (Hodges and Nagata 2001).

Climate change affects seabirds' breeding success with increasing variability in the distribution and availability of at-sea prey, which is being affected by rising ocean temperatures. Little, however, is known about the potential effects of climate driven changes on the availability of prey for 'ua'u. Range expansions of invasive species are also associated with climate change scenarios, which can degrade the breeding habitat of the 'ua'u. (Ainley et al. 2019). Invasive Hymenoptera, for example, have caused seabird nest failures and burrow abandonment (Plentovich et al. 2008, Raine and McFarland 2015).

Ongoing and planned actions that could affect seabirds are nearly the same as those actions described in the "Hawaiian Honeycreepers" section of this chapter. Forest-based activities, however, do not necessarily apply to seabirds on Maui since the vast majority of known nesting sites on the island are in subalpine habitat. The park, state, and TNC would continue current management actions and respond to future needs and conditions without major changes in the present course. The noise levels and seabird disturbance risks within the project area associated with ongoing administrative activities, administrative helicopter flights, and air tours (see description of these impacts in the "Hawaiian Honeycreepers" section above) would likely continue at current levels. Helicopters, mechanized equipment, and work crews would generate noise during overflights/landings/takeoffs, fencing activities, and maintenance of trails and LZs. There are no anticipated changes to public access within the project area and ongoing impacts to seabirds from visitors would remain unchanged in the foreseeable future. Overall, current and reasonably foreseeable actions would continue to result in minimal adverse direct impacts to seabirds and their habitats. The effects of these activities are included in the affected environment and the no-action alternative would therefore not result in any new direct or indirect impacts to seabirds. As there would be no new direct and indirect impacts as a result of the no-action alternative, there would be no cumulative effects associated with the no-action alternative.

ʻŌpeʻapeʻa, Hawaiian Hoary Bat

The ʻōpeʻapeʻa, or Hawaiian Hoary Bat, is the only fully terrestrial native mammal in the Hawaiian Islands and is state and federally listed as endangered. ʻŌpeʻapeʻa are found from sea level to 11,800 feet, with most observations occurring in native rain forests up to at least 6,000 feet (Bonaccorso et al. 2015). Data indicate that ʻōpeʻapeʻa commonly traverse and forage in large parts of the project area and likely roost there. A summary of detections reported from within the national park, or the vicinity of the project area are documented in Krushelnycky et al. (2019), and include Pīpīwai Trail, Hosmer Grove, and numerous locations bordering the park (Krushelnycky et al. 2019, and Todd 2016).

Females typically give birth to twin pups from June to August and juveniles reach independence by November. ʻŌpeʻapeʻa are known to roost alone in tree foliage in a variety of tree species and in an assortment of habitats and elevations (native and non-native habitats). Roost trees are usually larger than randomly selected trees (Montoya-Aiona 2020). ʻŌpeʻapeʻa are vulnerable to roost disturbance while resting during the day and during pupping and pup care (June-November).

ʻŌpeʻapeʻa are insectivores, and prey items include a variety of night-flying insects, primarily moths and beetles (Whitaker and Tomich 1983, Pinzari et al. 2019). Acoustic detection studies show seasonal patterns of habitat occupancy with increased activity in the higher elevations (higher than 3,300 feet) during the non-breeding season (November to April), and increased activity in the low elevations during the breeding season (Bonaccorso et al. 2015).

Due to its solitary and cryptic roosting behavior (Bonaccorso et al. 2015), robust estimates of the population size and trends of the ʻōpeʻapeʻa are currently unavailable. ʻŌpeʻapeʻa can be injured and killed from collisions with man-made structures including barbed wire fences, wind turbines, and communication towers; limiting factors, however, are poorly understood.

Ongoing and planned actions that could affect ʻōpeʻapeʻa are nearly the same as those actions described in the "Hawaiian Honeycreepers" section of this chapter. The park, state, and TNC would continue current management actions and respond to future needs and conditions without major changes in the present course. The noise levels and ʻōpeʻapeʻa disturbance risks within the project area associated with ongoing administrative activities, administrative helicopter flights, and air tours (see description of these impacts in the "Hawaiian Honeycreepers" section above) would likely continue at current levels. Helicopters, mechanized equipment, and work crews would generate noise during overflights/landings/takeoffs, fencing activities, and maintenance of trails and LZs potentially impacting roosting ʻōpeʻapeʻa. Management actions are unlikely to affect foraging bats because bats are nocturnal and management activities would occur during the day. There are no anticipated changes to public access within the project area and ongoing impacts to ʻōpeʻapeʻa from visitors would therefore remain unchanged in the foreseeable future. Overall, current and reasonably foreseeable actions would continue to result in minimal adverse direct impacts to ʻōpeʻapeʻa and their habitat. Tthe effects of these activities are included in the affected environment and the no-action alternative would therefore not result in any new direct or indirect impacts to ʻōpeʻapeʻa. As there would be no new direct and indirect impacts as a result of the no-action alternative, there would be no cumulative effects associated with the no-action alternative.

Wildlife Species Of Concern

Wildlife species of concern (NPS 2017), also designated as State Protected Wildlife Species (Section 13-124-3, HAR), within the project area include at least 30 protected wildlife species of concern; 13 insect species, 3 snail species, 1 shrimp species, 5 fish species, and 8 native bird species protected under the Migratory Bird Treaty Act (MBTA). Some species may be considered vulnerable to population declines or extinction by state or global metrics (e.g., Nature Serve Global Conservation Rank), or are lacking information to make a status determination. Only a few of these species could potentially be impacted by the proposed action and are generally described below. Wildlife species of concern are found on or transiting park land, state land, and TNC-managed lands within the analysis area.

Birds

Three Hawaiian honeycreeper species (in addition to the three federally protected species described earlier) found within the project area are protected under the MBTA and HAR section 13-124-3: ‘apapane (*Himatione sanguinea*), Hawai‘i ‘amakihi (*Chlorodrepanis virens wilsoni*), and Maui ‘alauahio (*Paroreomyza montana*). Although ‘apapane and Hawai‘i ‘amakihi are most common in native forests above 3,000 feet in elevation, they are also found in lower elevation forests. The Maui ‘alauahio occurs only on Maui in forests between 3,900 to 7,500 feet (Baker and Baker 2020, Judge et al. 2021). ‘Apapane, Hawai‘i ‘amakihi, and Maui ‘alauahio are susceptible to avian malaria, avian pox, and extreme weather associated with climate change, as well as the ecosystem threats common to native forest across Hawai‘i (Pratt 2009, Atkinson and Samuel 2010, Harvey-Samuel et al. 2021). ‘Apapane annual mortality during seasonal avian malaria outbreaks was estimated at 50 percent of juveniles and 25 percent of adults (Atkinson and Samuel 2010). Likewise, Hawai‘i ‘amakihi exposed to a single infective mosquito bite experienced 65 percent mortality (Atkinson et al 2000). There is strong evidence that the Maui ‘alauahio population is dramatically declining (Brink 2020, Judge et al. 2021), and the species is known to be extremely susceptible to avian malaria (Atkinson et al. 2001). Recent estimates indicate a 48 percent decline in population abundance for the species within the analysis area (Judge et al. 2021).

The pueo or Hawaiian Short-eared Owl (*Asio flammeus sandwichensis*) is listed as endangered by the State of Hawai‘i only on the island of O‘ahu. This species is not federally listed but is protected under the MBTA and HAR section 13-124-3. Pueo are found on all the main Hawaiian Islands, at elevations ranging from sea level to 8,000 feet. Pueo occupy a variety of habitats, including agricultural lands, grasslands, wetlands, shrublands, and native forests. Ground nests are well concealed and lined with grasses and feather down (Price and Cotín 2018). Threats to this species include loss and degradation of habitat, predation by invasive mammals, vehicle and wind turbine collisions, and other human interaction (Pueo Project 2019). Pueo potentially forage and nest within and around the project area, yet their abundance and distribution has not been well studied on Maui.

Migrant or transiting birds that may occur in the project area include the kōlea or Pacific Golden-Plover (*Pluvialis fulva*), an overwintering migrant shorebird that occasionally may rest and forage within the project area; the noio or Hawaiian Black Noddy (*Anous minutus melanogenys*), which nests on the coasts; and ‘iwa or the Great Frigatebird (*Fregata minor palmerstoni*) and koa‘e kea or White-tailed Tropicbirds (*Phaethon lepturus*), both of which fly over the project area. All are protected under the MBTA and HAR section 13-124-3.

Changes in environmental conditions in the project area expected as a result of global climate change include increasing temperatures, decreasing precipitation, increasing storm intensities, and increasing variability in weather patterns (Thomas et al. 2004, Frazier and Giambelluca 2017). Existing trends of declining populations of species of concern are expected to continue.

Ongoing and planned actions that could affect wildlife species of concern are nearly the same as those actions described in the “Hawaiian Honeycreepers” section of this chapter. These ongoing and planned actions could result in minimal adverse indirect or direct impacts to most species of concern. Without action to suppress mosquitoes and reduce avian malaria transmission, native forest birds of concern would be subject to continuing exposure to southern house mosquitoes and resultant mortality from avian malaria. With current climate variability and projected climate change trends, exposure to and transmission of avian malaria and avian pox would also be expected to increase under the no-action alternative for wildlife species of concern. The no-action alternative, therefore,is expected to adversely affect native Hawaiian forest birds and possibly other native birds of concern that are vulnerable to mosquito borne diseases.

The park, state, and TNC would continue current management actions and respond to future needs and conditions without major changes in the present course. The noise levels and disturbance risk to wildlife species of concern within the project area associated with ongoing administrative activities, administrative helicopter flights, and air tours (see description of these impacts in the “Hawaiian Honeycreepers” section above) would likely continue at current levels. Helicopters, mechanized equipment, and work crews would generate noise during overflights/landings/takeoffs, fencing activities, and maintenance of trails and LZs. There are no anticipated changes to public access within the project area and ongoing impacts to wildlife species of concern from visitors would remain unchanged in the foreseeable future. Overall, current and reasonably foreseeable actions would continue to result in minimal adverse direct impacts to wildlife and their habitats. The effects of these activities are included in the affected environment and the no-action alternative would therefore not result in any new direct or indirect impacts to wildlife species of concern. As there would be no new direct and indirect impacts as a result of the no-action alternative, there would be no cumulative effects associated with the no-action alternative. As stated earlier, however, if no action is taken, avian malaria would continue to devastate native forest bird populations resulting in permanent, long-term adverse impacts.

Effects of the Proposed Action on Threatened and Endangered Wildlife Species and Species of Concern

Methods and Assumptions

Impacts to federally listed wildlife species, designated critical habitat, and state-protected wildlife species of concern and their habitats occurring or possibly occurring in the analysis area were analyzed using expert opinions from park, state, USFWS, and contractor staff. Information from published scientific literature, technical reports, monitoring, observations, and databases managed by the NPS and NatureServe were also taken into consideration and used for this analysis. General assumptions for impacts on federally listed wildlife species, designated critical habitat, and wildlife species of concern are described below.

The area of analysis for impacts of alternatives on federally listed wildlife species, designated critical habitat, and wildlife species of concern is the proposed project area including 64,666 acres of park, state, TNC-managed lands, and private conservation lands where mosquito control releases would occur.

The following analysis includes a description of direct impacts primarily associated with drone and helicopter flights, motorized equipment, vehicles, and pedestrian teams during the proposed project's implementation, and risks of indirect negative impacts associated with biosecurity lapses (accidental harmful invasive species introductions into the project area) on 44 possible species, 12 of which are federally listed as threatened or endangered. Six listed species known to occur in the analysis area are emphasized: five bird species, and one mammal. Approximately 32 wildlife species of concern potentially occur in the analysis area but only eight native and migratory bird species protected under the MBTA that occur or transit NPS, state, and TNC/private lands could possibly be impacted by the proposed action.

For a description of the USFWS terminology to assess impacts on federally listed species, refer to the "Threatened and Endangered Species Section 7 Determinations" subsection of the "Threatened and Endangered Plant Species and State Plant Species at Risk" section of this EA.

Potential Impacts and Relevant Studies

Aircraft Impacts on Wildlife

Aircraft disturbance (e.g., from noise or visual detection) can be defined as any aircraft activity that changes the behavior or physiology of wildlife. Impacts of various aircraft on birds have been found to include increased heart rate, changes in energy conversion, feeding times, alert behaviors, agitated behaviors, and protective or escape behaviors (Drewitt 1999). The response of wildlife to aircraft may depend on both the properties (aircraft size and engine) and flight pattern of the aircraft, and the attributes and context of the wildlife (species, life-history stage and aggregation or flock size). Owing to their low-altitude capabilities, helicopters have been widely viewed as the most disturbing type of aircraft for birds (Drewitt 1999). Although birds may not be always affected by helicopters more than other types of disturbance, chronic noise disturbance may change vocalization behaviors. Distance, speed, trajectory, frequency and previous exposure/habituation to aircraft, species, breeding status and colony or flock size have also been described as key factors influencing birds' response or disturbance to normal behaviors from various aircraft (Burger 1981; Hoang 2013, van der Kolk et al. 2020).

Noise Impacts on Wildlife

Sound levels can vary greatly, depending on location, topography, vegetation, biological activity, weather conditions, and other factors. The magnitude of sound levels is usually described by its sound pressure; the dBA scale is commonly used to describe sound levels. For a detailed discussion on noise impacts see “Acoustic Environment” section of this document. The potential exists for human-caused sounds to adversely impact wildlife under any of the release methods described in Chapter 2 because many animals rely on auditory cues for predator avoidance, mate attraction, obtaining nesting territories, and finding prey (Dufour 1980). Sound levels greater than 60 dBA may approach disturbance levels in some sensitive birds with the duration and frequency of the of the noise and vibrational movement interacting. Wildlife reactions to human-caused sounds can range from no reaction to mild reactions, such as a temporary increase in heart rate, to more severe reactions, such as damaging effects on metabolism and hormone balance (Kleist et al. 2018, Gallardo Cruz et al. 2021, Francis et al. 2011). Behavioral and physiological responses could potentially cause injury, energy loss and decreased food intake (resulting from continual movement away from a noise source or reduced foraging), impeded communication, habitat avoidance and abandonment, and reproductive losses (NPS 1994, Halfwerk et al. 2011, Shannon et al. 2015, Gallardo Cruz et al. 2021). Some wildlife, however, becomes accustomed to air traffic and other human caused noises if it occurs regularly (Kempf and Hüppop 1998) and the extent to which birds may be disturbed by aircraft may depend in part on their ability to habituate to them. Birds may learn that a stimulus does not pose a danger after repeated exposure and, as a result, may not display any substantial signs of behavior change. The ability to habituate may be a function of the species of bird as well as the frequency of aircraft overflights and the amplitude of the noise (Hoang 2013, Gallardo Cruz et al. 2021).

Hawaiian forest birds at Hawaiʻi Volcanoes National Park exposed to frequent helicopter overflights (4 passes per hour) at noise levels above 75 dBA showed a decrease in vocalizing behavior, which may limit communication between birds and therefore possibly affect breeding success (Gallardo Cruz et al. 2021). Aircraft operating at higher altitudes (e.g., over 328 feet AGL) where noise is attenuated and aircraft emit less than 75 dBA may be of less disturbance (Mulero-Pázmány 2017, Gallardo Cruz et al. 2021).

Aircraft Wildlife Collisions

Helicopters present the potential for bird collision (Lyons et al. 2018), but under the proposed action, helicopter use would be short-term with drones being the primary mosquito release vehicle. The FAA database on aircraft bird collision (https://wildlife.faa.gov/search) reports that the most common native species aircraft bird strikes on Maui primarily occur with larger commercial aircraft at the OGG airport, involving the seasonal migrant kōlea and resident pueo. Other species potentially using the project area involved in aircraft collisions across Hawaiʻi included koaʻe kea and nēnē (FAA 1990 wildlife strike database 1990 accessed July 5, 2022). Although it is possible that a drone could inadvertently fly into a flock of birds, there have not yet been any reported instances of accidental drone-bird strikes or midair collisions. Seasonally flocking birds include the migratory kōlea in late April-early May, and nēnē, which may form small flocks in June-August; both species may occur in open grassy fields outside of the core area but within or near portions of the project area. Tour operator helicopters and administrative flights for the park, state, and TNC in the project areas averaging more than 1000 hours per year have not reported listed or native migratory bird collisions.

Rotor Wash Impacts

Helicopters flying at low altitudes can create a vertical down wash of air (rotor wash) that can cause a ground surface wind. Helicopter rotor wash is influenced by the mass of the helicopter and the diameter of the helicopter rotor, height above the ground, and various terrain or environmental conditions. As mentioned earlier, the Hughes 500D helicopter (the likely aircraft proposed for release operations) has a small rotor diameter. Associated rotor wash, however, has some potential risk to disturb wildlife using the tree canopy (birds and possibly roosting bats) during takeoff, landing, and while hovering, depending on the proximity, terrain, tilt, wind, and altitude of the helicopter relative to the habitat. Wildlife disturbance and displacement risk in this case is dependent on proximity of trees and the duration of the disturbance.

Drone–Wildlife Interactions

Data are accumulating on the behavior of some wildlife species around drones used for natural resource applications in natural areas. The behavioral responses of birds are variable by species, season, and habitat. Typically, the effect of drones on Hawai'i's federally listed wildlife species, designated critical habitat, and wildlife species of concern is anecdotal, observational, or non-existent. Potential adverse impacts from drones are influenced by the engine type and size of the drone, as well as the flight pattern (Mulero-Pázmány et al. 2017). Target-oriented flight patterns (such as those used for wildlife photography), larger drone sizes, and fuel-powered (noisier) engines evoked stronger reactions in wildlife whereas electric-powered drones and "lawn-mower" pattern flights performed at higher altitudes and following regular trajectories were found less likely to affect wildlife (Mulero-Pázmány et al. 2017). This agrees with observations of wildlife responses to traditional aircraft indicating that directness of aircraft approach influences wildlife responses and could be related to anti-predator behavior, since animals perceive higher risks when the threat is on a trajectory towards them (Mulero-Pázmány et al. 2017). Flushing of waterfowl flocks and aggression by territorial birds of prey (usually hawks and eagles) have been described in other ecosystems (Lyons et al. 2018).

Analysis

Under the proposed action, impacts to federally listed wildlife and wildlife species of concern could be generated by drone–wildlife interaction, aircraft disturbance, rotor wash and collisions, accidental invasive species dispersal, pedestrian teams, motorized vehicles, and noise from helicopters, drones, and generators. The frequency and duration of these impacts would be dependent on the release method employed.

Drone Release

Under the proposed action, drones would systematically release incompatible mosquitoes at each location to achieve complete coverage of the core area. Drones would fly approximately 50–100 feet above the tree canopy during mosquito releases but no higher than 500 feet AGL when ferrying between release locations and the operator, as described in Chapter 2. The proposed action would require an estimated 49–72 hours of drone flight-time per week to achieve the desired incompatible mosquito release rate for mosquito suppression. Depending on the drone model in use, noise levels experienced by wildlife where the drone is flying at 100 feet AGL could range from 47 to 65 dBA and from 41 to 59 dBA at 200 feet AGL (**Table 10**). Drones are considerably less noisy than helicopters and would likely present fewer indirect impacts to federally listed species and wildlife species of concern.

Hawaiian forest birds have not demonstrated a change in vocalization rates with aircraft noise levels lower than 75 dBA, suggesting the adverse effects of noise increase with intensity (Gallardo Cruz et al. 2021). Gallardo Cruz et al. (2021) found thatHawaiian forest birds changed their vocalization behavior with four helicopter overflights per hour, illustrating that frequency of disturbance is also a key factor influencing adverse effects to forest birds. Less frequent passes as proposed for this project are expected to have less adverse effects to Hawaiian honeycreepers in the project area, especially with a drone that would produce far less intense noise than a helicopter. Drone flight paths would vary substantially depending on the release locations being treated, and drones would likely pass over a specific location only twice per week. Although drone flight speeds during transiting may reach 62 mph, the estimated speeds during incompatible mosquito releases are slower (less than 25 mph), thus reducing potential wildlife collision risks. As drones would move swiftly through the project area above the tree canopy during incompatible mosquito releases, wildlife responses to drones in the project area are expected to be minimal and short term (15 seconds to a few minutes). Given this information and the expected minimal and short-term exposure to noise, disturbance under this release method would be infrequent and of short duration to individual federally listed forest bird species and forest bird species of concern.

Studies of bird behavior in other regions during drone monitoring suggest that breeding raptors initiated aggressive interactions with drones (Lyons et al. 2018). The only native bird of prey in the project area is the pueo, which is most active around sunrise and sunset, nests primarily in grasslands, and is not expected to interact with drones flying above the forest canopy (Pueo Project 2019). Drone–pueo interactions have not been documented and mosquito suppression operations would only occur after sunrise and before sunset, reducing the opportunity for drone–pueo interactions. The risk of birds accidentally colliding with drones is considered low when compared to other aircraft. From studies conducted in other ecosystems, large flocks of migrating birds or waterbirds are expected to have a higher risk of aircraft collision (Mulero-Pázmány 2017). Endangered waterbirds do not generally flock to or use the project area, but they occur nearby. The one exception is nēnē, which tend to form small flocks seasonally in open grassy areas in portions of the project area or proximity thereof during the summer. Migratory kōlea (a species of concern) also form flocks in seasonally in fields and open areas. There maybe a low risk of collision with drones for these species, primarily because of the tendency of flocking birds to take flight together. Listed ʻōpeʻapeʻa and burrow-nesting seabirds are active at night, but drone releases would only occur during the day. To direct interactions or impacts to flying bats or birds would therefore occur. Day roosting and breeding ōpeʻapeʻa are unlikely to be disturbed by drones flying more than 50 feet above the canopy for 15 seconds to a few minutes.

Overall, with implementation of mitigation measures (i.e., daytime releases, higher flight altitudes, etc.) and with the exceptions of potential minimal impacts listed above (the low risk of collision with nēnē and unlikely risk of disturbance to native forest birds), listed wildlife and wildlife species of concern are unlikely to be adversely affected by drone activity (see **Table 14**). Over the long term, drone releases would benefit all six remaining Hawaiian honeycreeper species on Maui in the project area by contributing to the successful suppression of mosquito populations and the associated transmission of avian malaria.

Helicopter Longline Release

Under the proposed action, helicopters would be used as a short-term (up to two months), temporary release method, if needed, and flown approximately 150–200 feet AGL to release incompatible mosquitoes via a 50–100-foot longline. Short-term, temporary helicopter longline releases (up to 6 hours of flight time per day, 5–7 days per month for up to two months per year) could produce a maximum of 82 dBA at 150 feet AGL for less than 15 seconds at any given release location in the core area. This altitude would reduce rotor wash experienced in the tree canopy and on the ground (see Chapter 2).

Under this release method, the potential exists for limited indirect noise-related impacts to Hawaiian honeycreeper species during helicopter longline releases. Helicopter noise-related impacts could include interference with avian communication and breeding success (Halfwerk et al. 2011). Other species of nesting birds in other ecosystems have been observed flushing from nests in response to noise (Meillere et al. 2015). However, most incompatible mosquito releases in higher elevation habitat would be less frequent (outside of the core nesting areas of endangered kiwikiu and ʻākohekohe), occurring only as a short-term temporary release method on an as needed basis.

There is a low risk of bird collisions during helicopter longline operations. The native species most commonly affected by aircraft collisions are kōlea, koaʻe kea, and nēnē (FAA 1990); collisions with pueo, ʻiwa, and ʻuaʻu are possible but would be very rare. There is also a low risk of potential disturbance to roosting ʻōpeʻapeʻa or pueo, particularly if there are roosts near flight paths. Except for breeding females roosting with their pups, these bats roost alone in trees and roost locations and roost abundances are not known within the project area.

Overall, the disturbance exposure to federally listed wildlife species and wildlife species of concern (birds and bats) from helicopters in the project area would be short term and of limited duration, as release locations would shift with each helicopter flight, and the helicopter is estimated to spend less than 15 seconds over each release location during each flight. Any potential minimal impacts described in this section (i.e., a low risk of kōlea, koaʻe kea, pueo, ʻiwa, ʻuaʻu, and nēnē collision, low risk of pueo disturbance and/or collision, low risk of Hawaiian honey creeper disturbance, and low risk of roosting ʻōpeʻapeʻa disturbance) would be substantially limited by the infrequency and temporary nature of helicopter longline release. Over the long term, helicopter longline releases would benefit all six remaining Hawaiian honeycreeper species on Maui in the project area by contributing to the successful suppression of mosquito populations and the associated transmission of avian malaria.

Pedestrian Release

Consistent pedestrian release is possible over a very limited portion (less than 10%) of the project area. Potential impacts from pedestrian releases conducted concurrently with monitoring at helicopter-only accessible backcountry sites is discussed under “Mosquito Monitoring” on the following page. Vehicles would be used for pedestrian access

TABLE 14: THREATENED AND ENDANGERED WILDLIFE SPECIES AND WILDLIFE SPECIES OF CONCERN (SOC) WITH POTENTIAL RISK FOR ADVERSE IMPACTS AND SUGGESTED MITIGATION.

Scientific Name	Common Name	Status	Potential Impact (Direct or Indirect)	Mitigation Measures
Lasiurus cinereus semotus	ʻŌpeʻapeʻa, Hawaiian Hoary Bat	ESA Federally Endangered	Direct: Very low risk of aircraft collision as this is a nocturnal species and drones and helicopters would only be used during daylight hours. Indirect: Small risk of pup and day roost disturbance with helicopter rotor wash and LZ use. Most LZs are in open areas away from potential roost trees. Low risk of drone disturbance at day roosts. Helicopter noise may result in infrequent mild reactions such as a temporary increase in heart rate or brief flight.	Standard helicopter and mechanized equipment BMPs would be implemented. Drones flying 50-500 feet above canopy.
Asio flammeus sandwichensis	Pueo, Hawaiian Short-eared Owl	SOC; Migratory Bird Treaty Act	Direct: Low risk of aircraft or vehicle collision. Low risk of drone interaction. Helicopter noise may result in infrequent mild reactions such as a temporary increase in heart rate or brief flight.	Standard flight and vehicular operation BMPs would be implemented.
Family Fringillidae, Subfamily Carduelinae,	Hawaiian honeycreepers	ESA Federally Threatened and Endangered Species, SOC/Migratory Bird Treaty Act	Reduction in avian malaria and avian pox transmission would be a substantial beneficial impact. Low risk of disturbance by drones; Helicopter noise may result in infrequent mild reactions such as a temporary increase in heart rate or brief flight.	Standard flight operation BMPs would be implemented
Branta sandvicensis	Nēnē, Hawaiian Goose	ESA Federally Threatened	Reduction in avian pox infections would be a beneficial impact. Direct: Low risk of helicopter, drone and vehicle disturbance or interaction-collisions Indirect: Low risk of flock or brood disturbance Helicopter noise may result in infrequent mild reactions such as a temporary increase in heart rate or brief flight.	Standard flight and vehicular operation BMPs would be implemented.
Frigata minor palmerstoni	ʻIwa, Great Frigatebird	SOC; Migratory Bird Treaty Act	Direct: Low risk of aircraft collision; infrequent transit through project area	Standard flight and vehicular operation BMPs would be implemented.
Phaethon lepturus	Koaʻe kea, White-tailed Tropicbird	MBTA, SOC	Direct: Low risk of aircraft collision infrequently transits across project area.	Standard flight operation BMPs would be implemented
Pluvialis fulva	Kōlea, Pacific Golden-Plover	SOC	Direct: Low risk of aircraft collision, low risk of drone disturbance	Standard flight operation BMPs would be implemented.
Pterodroma sandwichensis	ʻUaʻu, Hawaiian Petrel	ESA Federally Endangered	Direct: Very Low risk of aircraft collision. Nocturnal in project area. Aerial operations under the proposed action would only occur during daylight hours.	Standard flight and vehicular operation BMPs would be implemented.

(*) indicates species is not known to occur in the project area (to reach trailheads) in portions of the project area with roads and readily accessible trails in Makawao Forest Reserve and Waikamoi Preserve (less than 2 percent of the project area) and noise from these vehicles may briefly induce a “flight” response in wildlife nearby. There is biosecurity risk associated with trail maintenance, trail use, and four-wheel drive vehicles delivering pedestrians and gear (e.g., invasive seeds, insects, fungal pathogens, and animals contaminating gear). There is also a very low risk of vehicle collision with nēnē and pueo (bird species susceptible to vehicle collisions) as driving would be limited to short distances and slow speeds in one small section of the project area (see **Figure 5**).

The potential direct and indirect adverse effects of pedestrian release on federally listed wildlife species, designated critical habitat, and wildlife species of concern that may result from the use of trails/fence lines and vehicles are detailed in Error! Reference source not found.. Field operations under this method may result in a minimal risk of a dverse impacts to some federally listed wildlife species and species of concern (primarily Hawaiian honeycreeper species and ʻōpeʻapeʻa) and their habitats from the human activity and noise in fragile habitats. Established roads and trails would be used, however, and species in the areas where pedestrian release could occur are likely accustomed to at least low levels of human presence and noise because the trails proposed for use are well-established and/or traveled by the public. Indirect impacts are possible if a biosecurity lapse introduces harmful invasive species or pathogens. For example, introduction of forest pathogens such as *Ceratocystis huliohia* and *C. lukuohia* (causes of Rapid ʻŌhiʻa Death) could result in adverse effects. Strict oversight and mitigation measures would be applied for effective biosecurity protocol implementation including appropriate cleaning, storage, and inspections of field equipment to reduce these risks and prevent adverse impacts.

Overall, the pedestrian release method is likely to have minimal impacts on wildlife and their habitats due to the limited area for pedestrian release activities and the mitigation measures that will be implemented. Pedestrian releases would benefit all six remaining Hawaiian honeycreeper species in the project area on Maui by contributing to the successful suppression of mosquito populations and the associated transmission of avian malaria.

Mosquito Monitoring

Motorized vehicles (SUVs or trucks) would assist in the transportation of field teams and gear to reach three ground-accessible monitoring sites in Makawao Forest Reserve and TNC’s Waikamoi Preserve. Noise from vehicles used during monitoring would primarily occur along the Flume Road shown in brown on **Figure 5** and is not expected to exceed 4 hours per day for up to 7 days on a quarterly basis. It should be noted that vehicles would not be running constantly during that 4-hour time period because crews would stop periodically to check mosquito traps. As previously mentioned, ground vehicles can reach 75 dBA at 50 feet from the source but would be muffled by the surrounding canopy and would not be expected to exceed 60 dBA at 50 feet from the source of noise. Noise from vehicles may briefly induce a “flight” response in nearby wildlife as vehicles pass through.

To conduct monitoring at the five helicopter-only accessible monitoring camps and LZs, helicopters would transport teams to the backcountry sites and crews would need to use quiet generators to power equipment necessary for mosquito monitoring activities. The duration and frequency of helicopter flights required for monitoring (2–6 hours per day for a total of approximately 17.5 hours per week for one week each quarter), and therefore the amount of time wildlife could experience helicopter noise impacts, would be brief, inconsistent, and vary by distance from the source. Noise levels along helicopter flight paths would reach less than 72 dBA at 500 feet AGL during overflights at the beginning and end of each monitoring session and helicopters would be flying faster than 62 mph, thereby decreasing the time exposure to noise. The most pronounced noise impacts from helicopter use would be focused at the five monitoring sites accessible only by helicopter and could reach 82–93 dBA during pick-ups and drop-offs (less than 10 minutes each). During the 7-day quarterly monitoring sessions, adverse noise impacts from generators would be limited to less than 58 dBA at 23 feet for up to 3 hours per day at five monitoring camps. Many of these camps are within or near sensitive habitat for Hawaiian forest birds, and noise from helicopter activity and generators could cause mild annoyance to birds nesting, roosting, or foraging in the area. Owing to the thick canopy that surrounds the camps, however, noise impacts would be limited to a very small radius around each camp and noise impacts would not be constant during that 7-day period.

The potential exists for limited indirect noise-related impacts to Hawaiian honeycreeper species during helicopter operations especially when hovering or taking off and landing with noise levels likely exceeding 82 dBA. Those noise related impacts include interference with avian communication and breeding success associated with chronic human-caused noise disturbance (Halfwerk et al. 2011). Other species of nesting birds in other ecosystems have been observed flushing from nests in response to noise (Meillere et al. 2015). There is a low risk of bird collisions during helicopter flights to and from monitoring locations. The most common native species affected by aircraft collisions are kōlea, koaʻe kea, and nēnē (FAA 1990); collisions with pueo, ʻiwa, and ʻuaʻu are possible but very rare. There is also a low risk of potential disturbance to roosting ʻōpeʻapeʻa or pueo, particularly if there are roosts near take-off and landing sites. Except for breeding females roosting with their pups, these bats roost alone in trees and roost locations and roost abundances are not known within the project area. As mentioned under "Pedestrian Release" above, increased trail use during monitoring and associated mosquito pedestrian releases could lead to higher risk of introducing and spreading invasive species during monitoring.

Overall, adverse impacts on wildlife during monitoring activities from helicopters, generators, and vehicles would be highly variable and not sustained (effects would only occur for up to 7 days every three months). Any potential minimal impacts described in this section (a low risk of kōlea, koaʻe kea, pueo, ʻiwa, ʻuaʻu, and nēnē collision, low risk of pueo disturbance and/or collision, low risk of Hawaiian honey creeper disturbance, and low risk of roosting ʻōpeʻapeʻa disturbance) would be substantially limited by the infrequency and temporary nature of helicopter flights for monitoring. Mosquito monitoring is therefore unlikely to have long-term adverse impacts on wildlife and their habitats because of its limited frequency and implementation of best practices to mitigate adverse effects from biosecurity lapses. Mosquito monitoring would indirectly benefit all six remaining Hawaiian honeycreeper species in the project area on East Maui by contributing to the successful suppression of mosquito populations and the associated transmission of avian malaria.

Cumulative Impacts

Overall, reasonably foreseeable actions would continue to result in minimal adverse direct impacts to wildlife and their habitats. With mitigation actions and best management practices described in Chapter 2, the ongoing and planned actions described in the "Hawaiian Honeycreeper" section of "Current and Expected Future Condition of Threatened and Endangered Wildlife Species and Wildlife Species of Concern if No Action is Taken" and in Appendix E, would result in minimal impacts to federally listed and most wildlife species of concern. Under the proposed action, adverse impacts would be intermittent and of short duration and would infrequently affect individual birds and other wildlife. Although there would be temporary and localized impacts to wildlife from mosquito release activities, the population and health of federally listed species and wildlife species of concern and their habitats would improve or remain stable. As previously described, the proposed action would directly reduce mortality of listed Hawaiian honeycreeper species due to the suppression of mosquitoes that spread avian malaria. The proposed action along with other park, state and TNC management actions, including invasive plant control, feral ungulate control, and fence maintenance, would enhance survival of native forest bird species by reducing stressors. Over time, the populations of these listed bird species may increase due to the combined actions of the park, state, and TNC to manage for avian malaria and other threats. The overall cumulative impacts of the proposed action, therefore, would be substantially beneficial.

Conclusion

Under the no-action alternative, there would be no direct impacts to ʻōpeʻapeʻa nor to most wildlife species of concern. Without action to suppress mosquitoes and reduce avian malaria transmission, six Hawaiian honeycreeper species (kiwikiu, ʻākohekohe, ʻiʻiwi, ʻapapane, Hawaiʻi ʻamakihi, and Maui ʻalauahio) would be subject to continuing exposure to southern house mosquitoes and resultant mortality from avian malaria. With current climate variability and projected climate change trends, exposure to and transmission of avian malaria and avian pox would also be expected to increase under the no-action alternative, likely causing the extinction of kiwikiu, ʻākohekohe, and Maui ʻalauahio, extirpation of ʻiʻiwi, and increased risk to nēnē and seabirds to avian pox virus. The no-action alternative is therefore expected to substantially and permanently adversely affect Hawaiian honeycreepers and to a lesser extent, other native birds.

The proposed action would result in limited adverse impacts to federally listed wildlife species, designated critical habitat, and wildlife species of concern and their habitats, The proposed action would primarily include a risk of wildlife noise disturbance from drones, helicopters, and generators, but a minimal risk of wildlife collision, and an indirect impact of increased risk of invasive species introduction from failed biosecurity during field operations. The most pronounced risk of impacts from noise disturbance, risk of collision, or biosecurity lapses would occur in the vicinity of LZs, helibases, fence lines, roads, and trails. Under the proposed action, noise from drones could occur throughout the 48,164-acre core area for 49–72 hours per week. Noise levels from drones could reach a maximum of 47–59 dBA at 100–200 feet AGL (the altitude where most releases would occur) for less than 15 seconds as the drone passes over any given location in the core area one to two times per week. Helicopter noise would only occur for 2–6 hours per day potentially spread over the course of 7 days for a total of approximately 17.5 hours per week for quarterly monitoring trips. Most helicopter flight noise would be highly variable depending on the flight height and lateral distance to a person or wildlife but could reach a maximum of 82–93 dBA during pick-ups and drop-offs at LZs. Short-term, temporary helicopter longline releases (with up to 6 hours of flight time per day, 5–7 days per month for up to two months per year) could produce a maximum of 82 dBA at 150 feet AGL for less than 15 seconds at any given release location in the core area. Generator noise (maximum of 52–58 dBA at 23 lateral feet) could occur for up to 3 hours per day for up to 7 consecutive days on a quarterly basis at the five backcountry monitoring locations. Noise from vehicles (maximum of 75 dBA at 50 feet from the source) would occur intermittently in Makawao Forest Reserve and TNC’s Waikamoi Preserve for up to 4 hours per day for up to 7 days during quarterly monitoring and up to 2 hours per day, up to 2 times per week for pedestrian releases that are scheduled to occur in those areas.

Impacts may decline over time as releases are needed less frequently and/or become more efficient. Potential minimal adverse effects to federally listed wildlife or wildlife species of concern from mosquito releases and monitoring include a low risk of the following:1) disturbance from the presence of drones and drone/helicopter/generator noise to Hawaiian honeycreeper species; 2) aircraft, drone, or vehicle collision with or noise disturbance to pueo; 3) pup and day roost disturbance with helicopter rotor wash, drone use, and LZ/camp use to‘ōpe‘ape‘a; 4) flock or brood disturbance and helicopter drone or vehicle interaction-collisions to nēnē; and 5) drone or helicopter collision with or disturbance to transiting seabirds (‘iwa, koa‘e kea, kōlea, and ‘ua‘u). Potential impacts to Hawaiian honeycreeper species would be minimized by the planned flight elevations, speed of release operations, use of drones, and limited ground or helicopter activity in critical habitats. The risk of roosting bat or pup disturbance or displacement with the presence of drones or helicopters is reduced given the planned flight elevations and the use of general best management practices, and the proposed action is unlikely to affect foraging bats because bats are nocturnal and release activities would only occur during the day. Daytime helicopter and drone activities are very unlikely to influence listed seabirds that generally fly near the project area at night. Pedestrian release teams are unlikely to encounter endangered seabird nests on established trails but should be aware of their possible existence in the project area and should report any nocturnal seabird vocalizations heard.

All six remaining Hawaiian honeycreeper species (both federally listed and species of concern) on Maui in the project area would substantially benefit from the proposed action to suppress mosquito populations and thereby avian malaria transmission. Indirect beneficial impacts include conservation biodiversity and reduced exposure by Hawaiian honeycreepers, nēnē, and other disease-susceptible birds to avian pox virus. More broadly, the proposed action may help restore ecosystem integrity of the rainforest (including designated critical habitat) by substantially reducing the extinction risk of culturally significant and vital avian pollinators and seed dispersers (the Hawaiian honeycreepers).

Though considerable analysis is presented here, adverse impacts to listed wildlife and wildlife species of concern would minimal because very few direct impacts are anticipated, and indirect impacts would be limited in duration, frequency, and intensity. Over the long term, there would be a beneficial impact to listed birds and bird species of concern due to anticipated suppression of the mosquito population that transmits avian malaria to forest birds in the project area.

ESA Section 7 Determination Summary

Threatened and endangered species Section 7 determination definitions were previously defined. Based on the analysis, the project activities under the proposed action and the incorporation of mitigation measures described in Chapter 2, *may affect, but are not likely to adversely affect*, all analyzed federally listed wildlife species and their designated critical habitat, as applicable. **Table 15** provides Section 7 determinations for listed wildlife species under the proposed action.

TABLE 15: THREATENED AND ENDANGERED WILDLIFE SECTION 7 DETERMINATIONS

Scientific Name	Common Name	Proposed Action Sec. 7 Determination
Branta sandvicensis	Nēnē, Hawaiian Goose	*May affect, but not likely to adversely affect*
Drepanis coccina	‘I‘iwi	*May affect, but not likely to adversely affect*
Palmeria dolei	‘Ākohekohe	*May affect, but not likely to adversely affect*
Pseudonestor xanthrophys	Kiwikiu or Maui Parrotbill	*May affect, but not likely to adversely affect*
Lasiurus cinereus semotus	‘Ōpe‘ape‘a, Hawaiian Hoary Bat	*May affect, but not likely to adversely affect*
Oceanodroma castro	'Akē'akē, Band-rumped Storm-Petrel	*May affect, but not likely to adversely affect*
Pterodroma sandwichensis	‘Ua‘u, Hawaiian Petrel	*May affect, but not likely to adversely affect*
Puffinus auricularis newelli	‘A‘o, Newell’s Shearwater	*May affect, but not likely to adversely affect*

CHAPTER 4: CONSULTATION AND COORDINATION

This chapter describes the civic engagement and agency consultation during the preparation of this EA. A combination of activities, including internal and public scoping, helped guide NPS and DLNR in developing this EA.

PLANNING

NEPA regulations require an "early and open process to determine the scope of issues for analysis" (40 CFR 1501.9). The internal scoping process for the project began in early 2021. Internal and external scoping associated with this EA has been extensive and has included numerous interdisciplinary team meetings and reviews and bi-weekly project meetings. Planning and public input for this project has also been in compliance with HEPA regulations at HRS Chapter 343.

A Pre-NEPA Workshop, "Addressing Avian Malaria and other Threats to Endangered Forest Birds at Haleakalā National Park," was conducted virtually from February 9-11, 2021. Representatives from NPS, DLNR, State of Hawaiʻi Department of Health, USFWS, and contractors Tetra Tech and JE Fuller participated and contributed. Day 1 of the workshop covered project background and a law and policy overview; Day 2 covered project issues, purpose and need, and potential management actions; and Day 3 addressed outreach, preliminary proposed action, available data, and next steps.

CIVIC ENGAGEMENT

Staff from the NPS, DLNR, and other partner agencies led civic engagement efforts with the local community and interested stakeholders prior to initiating the NEPA/HEPA process. The intent of civic engagement was to connect with and inform the public and stakeholders about proposed efforts on East Maui to reduce populations of mosquitoes and thus the effects of avian malaria among threatened and endangered bird populations. Civic engagement efforts were conducted through the use of informative websites, videos/multimedia, social media, virtual and in-person meetings, media kits, newsletters, meetings and webinars, and direct email/mail/phone campaigns. Park staff also participated in a series of civic engagement calls to notify the public and stakeholders via the outreach strategy of this project before the NEPA process began.

PUBLIC SCOPING

The NPS and DLNR held a 45-day public scoping period from December 6, 2021, to January 20, 2022, which initiated the NEPA/HEPA process. Virtual public scoping meetings were held on December 14, 2021, and January 6, 2022. Public notices of the comment period and meetings were distributed through the following sources:

- A news release posted on the park website
- A project newsletter posted to the NPS's Planning, Environment and Public Comment (PEPC) website: https://parkplanning.nps.gov/HALE-mosquito
- A news release sent electronically (via email) to various stakeholders, agencies, and media groups
- A news release posted on the park's social media accounts (Facebook and Instagram) and postings to the Hawaiʻi DLNR newsfeed, as well as the Oahu and Kauai DOFAW Facebook pages.

In total, 51 people attended the virtual public scoping meetings, including 34 on December 14, 2021, and 17 on January 6, 2022. The content was the same for both meetings and included a presentation followed by a "question and answer" session. Video recordings of the public scoping meetings were posted on the project's PEPC website. The project team received 72 correspondences during the 45-day scoping period. All 72 were submitted through the NPS PEPC system.

The comments received were reviewed by the NPS and DLNR and considered in developing this EA. A public scoping report documenting the process is available on the NPS PEPC project site at https://parkplanning.nps.gov/HALE-mosquito.

AGENCY CONSULTATION

NPS and DLNR initiated consultation with relevant agencies and organizations during the preparation of this EA. Copies of correspondence between NPS and other agencies, and responses from the agencies, if applicable, will be provided in the decision document.

Section 7 of the Endangered Species Act

Section 7 of the ESA requires federal agencies to ensure that the actions they authorize, fund, or carry out do not jeopardize the continued existence of listed species or destroy or adversely modify critical habitat. NPS is coordinating with the USFWS Pacific Islands Field Office to ensure compliance with Section 7 of the ESA. An official Species List and associated avoidance and minimization measures from the USFWS Pacific Islands Fish and Wildlife Office was received on January 20, 2022 and aided in developing mitigation measures and assessing potential impacts of the project. The USFWS reviewed and commented on an internal draft EA and a call with the NPS was held on October 24, 2022, to discuss potential impacts to threatened and endangered species. The NPS and DLNR will continue to work closely with the USFWS throughout the NEPA, HEPA, and Section 7 ESA processes. This EA is serving as a Biological Assessment with Section 7 determinations provided for federally listed plant and wildlife species.

Section 106 of the National Historic Preservation Act

Compliance with section 106 of the National Historic Preservation Act is being conducted in consultation with the Hawai‘i State Historic Preservation Division (SHPD), Native Hawaiian Organizations, and individuals with familial/traditional ties to Haleakalā concurrently during the NEPA/HEPA planning process. The expected determination of effect is *No adverse effect* under Section 106 and *No historic properties affected* under HRS Chapter 6e. In December 2021, NPS sent initial letters establishing the Area of Potential Effect (APE) and identifying historic properties to the Hawai‘i SHPD and consulting parties. SHPD replied on January 5, 2022. The SHPD had no objections to the APE. The SHPD noted that the APE is a very large area and requested "additional information pertaining to what type of work, if any, will be conducted on the ground that may impact historic properties, if present, and the location of that work" (Project No. 2021PR01527; Doc No. 2201SH01). No substantial comments were received by consulting parties. In August 2022, NPS sent preliminary determination of effect letters to the Hawai‘i SHPD and consulting parties, including additional information pertaining to what type of work, if any, will be conducted on the ground that may impact historic properties, if present, and the location of that work. No comments have been received to date. The project is under review by the Hawai‘i SHPD History and Culture Branch. A third letter, describing refinements to the proposed action based on new information gathered during the EA process, as well as final determination of effect, will be sent to consulting parties with this EA and Cultural Impact Assessment when released to the public.

APPENDIX A:
References

APPENDIX A: REFERENCES

Ainley, D.G, T.C. Telfer, M.H. Reynolds, and A.F. Raine. 2019. Newell's Shearwater (*Puffinus newelli*). Birds of North America. Birdsna.org. Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi.org/10.2173/bna.towshe2.02.

AirborneDrones.co (Airborne Drones). 2020. Drone Noise Levels. https://www.airbornedrones.co/drone-noise-levels/. Accessed May 3, 2022.

American Bird Conservancy. 2022. Five Rarest Hawaiian Birds. https://abcbirds.org/blog/five-rarest-hawaiian-birds/. Accessed May 3, 2022.

Anderson, S.B. 2003. Introduced axis deer (*Axis axis*) on Maui, Hawai'i: history, current status, home range, grouping patterns, and a species account. Doctoral Dissertation, University of California, Davis. https://search.library.ucdavis.edu/permalink/f/1osgl34/01UCD_ALMA21205181240003126

Anderson, S.H., D. Kelly, J.J. Ladley, S. Molloy, and J. Terry. 2011. Cascading effects of bird functional extinction reduce pollination and plant density. *Science* 331: 1068-1071. https://doi.org/10.1126/science.1199092. Epub 2011 Feb 3. PMID: 21292938.

Antaky, C.C, N.K. Galase, and M.R. Price. 2019. Nesting ecology in the Hawaiian population of an endangered seabird, the Band-rumped Storm-Petrel (*Oceanodroma castro*). *Wilson Journal of Ornithology* 131: 402-406 https://doi.org/10.1676/18-123

Aruch, S. 2006. Appendix A: Haleakalā National Park resource overview. In: HaySmith, L., F.L. Klasner, S.H. Stephens, and G.H. Dicus (eds). Pacific Island Network vital signs monitoring plan. Natural Resource Report NPS/PACN/NRR—2006/003 National Park Service, Fort Collins, Colorado.

Aslan, C.E., E.S. Zavaleta, B. Tershy, and D. Croll. 2013. Mutualism Disruption Threatens Global Plant Biodiversity: A Systematic Review. *PLoS ONE* 8: e66993. https://doi.org/10.1371/journal.pone.0066993

Atkinson, C.T., K.L. Woods, R.J. Dusek, L.S. Sileo, and W.M. Iko. 1995. Wildlife disease and conservation in Hawai'i: pathogenecity of avian malaria (*Plasmodium relictum*) in experimentally infected 'I'iwi (*Vestiaria coccinea*). *Parasitology* 111: S59-S69.

Atkinson, C.T., and D.A. LaPointe. 2009. Ecology and pathogenicity of avian malaria and pox. *In*: Conservation Biology of Hawaiian Forest Birds: Implications for island avifauna (T.K. Pratt, C.T. Atkinson, P.C. Banko, J.D. Jacobi, and B.L. Woodworth, eds.). Yale University Press, New Haven, CT. pp. 234-252

Atkinson, C.T., J.K. Lease, B.M. Drake, and N.P. Shema. 2001. Pathogenicity, serological responses, and diagnosis of experimental and natural malarial infections in native Hawaiian thrushes. *Condor* 103: 209–218.

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*. *Journal of Avian Biology* 41: 357–366.

Atkinson, C.T, R.J. Dusek, K.L. Woods, and W.M. Iko. 2000. Pathogenicity of avian malaria in experimentally infected Hawaii Amakihi. *Journal of Wildlife Disease* 36: 197-204.

Atkinson C.T, W. Watcher-Weatherwax, and D. LaPointe. 2016. Genetic diversity of *Wolbachia* endosymbionts in *Culex quinquefasciatus* from Hawai'i, Midway Atoll and American Samoa. University of Hawai'i at Hilo Technical Report HCSU-074.

Atkinson, C.T., and D.A. LaPointe. 2009a. Ecology and pathogenicity of avian malaria and pox. *In*: Conservation biology of Hawaiian forest birds: Implications for island avifauna (T.K. Pratt, C.T. Atkinson, P.C. Banko, J.D. Jacobi, and B.L. Woodworth, eds.). Yale University Press, New Haven, CT. pp. 234-252

Atkinson, C.T., and D.A. LaPointe. 2009b. Introduced Avian Diseases, Climate Change, and the Future of Hawaiian Honeycreepers. *Journal of Avian Medicine* 23: 53-63.

Atkinson, C.T., K.L. Woods, R.J. Dusek, L.S. Sileo, and W.M. Iko. 1995. Wildlife disease and conservation in Hawai'i: pathogenicity of avian malaria (*Plasmodium relictum*) in experimentally infected Iiwi (*Vestiaria coccinea*). *Parasitology* 111: S59-S69.

Atkinson,C.T., R.B. Utzurrum, D.A. LaPointe, R.J. Camp, L.H. Crampton, J.T. Foster, 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. *Global Change Biology* 20: 2426-2436. https://doi.org/10.1111/gcb.12535

Atyame, C.M., J. Cattel, C. Lebon, O. Flores, J.S. Dehecq, M. Weill, L.C. Gouagna, and P. Tortosa. 2015. Wolbachia-based population control strategy targeting *Culex quinquefasciatus* mosquitoes proves efficient under semi-field conditions. *PLoS ONE* 10: e0119288 https://doi.org/10.1371/journal.pone.0119288

Bailey, T., National Park Service, Haleakalā National Park. 2022. Personal Communication. May 26, 2022.

Baker, P.E. and H. Baker. 2020. Maui Alauahio (*Paroreomyza montana*), version 1.0. *In* Birds of the World (A. F. Poole and F. B. Gill, Eds). Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi.org/10.2173/bow.mauala.01

Banko, P.C., J.M. Black, and W.E. Banko. 2020. Hawaiian Goose (*Branta sandvicensis*), version 1.0. *In*: Birds of the World (A.F. Poole and F.B. Gill, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA.

Banko, P.C., K.A. Jaenecke, R.W. Peck, and K.W. Brinck. 2019. Increased nesting success of Hawai'i 'Elepaio in response to the removal of invasive black rats. *Condor* 121:1-12.

Banko, W.E., and P.C. Banko. 2009. Historic decline and extinction. *In*: Conservation biology of Hawaiian forest birds: Implications for island avifauna (T.K. Pratt, C.T. Atkinson, P.C. Banko, J.D. Jacobi, and B.L. eds.) Conservation Biology of Hawaiian Forest Birds: Implications for island avifauna. Yale University Press, New York, U.S.A.

Beebe, N.W., D. Pagendam, B.J. Trewin, A. Boomer, M. Bradford, A. Ford, C. Liddington, A. Bondarenco, P.J. De Barro, J. Gilchrist, C. Paton, K.M. Staunton, B. Johnson, A.J. Maynard, G.J. Devine, L.E. Hugo, G. Rasic, H. Cook, P. Massaro, N. Snoad, J.E. Cawford, B.J. White, Z. Xi, and S.A. Ritchie. 2021. Releasing incompatible males drives strong suppression across populations of wild and Wolbachia-carrying *Aedes aegypti* in Australia. *Proceedings of the National Academy of Sciences of the United States of America* 118: https://doi.org/10.1073/pnas.2106828118

Barton, K.E., A. Westerband, R. Ostertag, E. Stacy, K. Winter, D.R. Drake, L.B. Fortini, C.M. Litton S. Cordell, P. Krushelnycky, K. Kawelo, K. Feliciano, G. Bennet, and T. Knight. 2021. Hawai'i forest review: synthesizing the ecology, evolution, and conservation of a model system. *Perspectives in Plant Ecology, Evolution, and Systematics* 52: (2021) 125631

Beeco, J.A., D. Joyce, and S.J. Anderson. 2020. Evaluating the use of spatiotemporal aircraft data for air tour management planning and compliance. *Journal of Park and Recreation Administration*. https://doi.org/10.18666/JPRA-2020-10341.

Bennett, G.M., Pantoja, N.A. and O'Grady, P.M. 2012. Diversity and phylogenetic relationships of Wolbachia in *Drosophila* and other native Hawaiian insects. *Fly* 6: 273-283.

Berger, A.J. 1972. Hawaiian Birdlife. University Press of Hawaiʻi. 253 pp.

Berglund B, T. Lindvall, and D.H. Schwela. 1999 Guidelines for Community Noise. World Health Organization, Geneva, Switzerland. http://www.who.int/docstore/peh/noise/guidelines2.html.

Berlin, K.E. and E.M. Vangelder. 2020. Akohekohe (Palmeria dolei), version 1.0. *In*: Birds of the World (A.F. Poole and F.B. Gill, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi.org/10.2173/bow.crehon.01

Birds of the World. 2022. The Cornell Lab of Ornithology, Cornell University. Website: https://birdsoftheworld.org/bow/species/maupar/cur/introduction. Accessed September 22, 2022.

Bonaccorso, F.J., C.M. Todd, A.C. Miles, and P.M. Gorresen. 2015. Foraging range movements of the endangered Hawaiian hoary bat, *Lasiurus cinereus semotus* (Chiroptera: Vespertilionidae). *Journal of Mammalogy* 96: 64-71.

Bouyer, J., N. Culbert, A. H. Dicko, M. G. Pacheco, J. Virginio, M. C. Pedrosa, L. Garziera, T. Macedo Pinto, A. Klaptocz, J. Germann, T. Wallner, G. Salvador-Herranz, R. Argiles Herrero, H. Yamada, F. Balestrino, and M. J. B. Vreysen. 2020. Field performance of sterile male mosquitoes released from an unteamed aerial vehicle. *Science Robotics* 5: https://doi.org/10.1126/scirobotics.aba6251.

Brinck, K.W. 2020. Forest bird population trends within Haleakalā National Park. Hawaiʻi Cooperative Studies Unit Technical Report HCSU-097. University of Hawaiʻi at Hilo. 31 pp.

Burger, J. 1981. Behavioral responses of Herring Gulls *Laurs argentatus* to aircraft noise. *Environmental Pollution Series A* 24: 177-184

Camp, R.J., M.H. Reynolds, B.L. Woodworth, T.K. Pratt, and P.M. Gorresen. 2009. Monitoring Hawaiian forest birds. *In*: Conservation Biology of Hawaiian Forest Birds: Implications for island avifauna (T.K. Pratt, C.T. Atkinson, P. Banko, J. Jacobi, and B.L. Woodworth, eds.). Yale University Press, New York, U.S.A.

Carlquist, J. 1974. Island biology. Columbia University Press, New York.

Cohan, A. Hawaiʻi Terrestrial Director, The Nature Conservancy. Personal Communication. September 30, 2022.

Cole, F.R., A.C. Medeiros, L.L. Loope, and W.W. Zuehlke. 1992. Effects of the Argentine Ant on Arthropod Fauna of Hawaiian High-Elevation Shrubland. *Ecology* 73: 1313-1322. https://doi.org/10.2307/1940678

Cooper, B.A., and R.H. Day. 2003. Movement of the Hawaiian Petrel to inland breeding sites on Maui Island, Hawaiʻi. *Waterbirds* 26: 62-71.

Crawford, J.E., D.W. Clarke, V. Criswell, M. Desnoyer, D. Cornel, B. Deegan, K. Gong, K.C. Hopkins, P. Howell, J.S. Hyde, J. Livni, C. Behling, R. Benza, W. Chen, K.L. Dobson, C. Eldershaw, D. Greeley, Y. Han, B. Hughes, E. Kakani, J. Karbowski, A. Kitchell, E. Lee, T. Lin, J. Liu, M. Lozano, W. MacDonald, J.W. Mains, M. Metlitz, S.N. Mitchell, D. Moore, J.R. Ohm, K. Parkes, A. Porshnikoff, C. Robuck, M. Sheridan, R. Sobecki, P. Smith, J. Stevenson, J. Sullivan, B. Wasson, A.M. Weakley, M. Wilhelm, J. Won, A. Yasunaga, W.C. Chan, J. Holeman, N. Snoad, L. Upson, T. Zha, S.L. Dobson, F.S. Mulligan, P. Massaro, and B.J. White. 2020. Efficient production of male Wolbachia-infected *Aedes aegypti* mosquitoes enables large-scale suppression of wild populations. *Nature Biotechnology* 38: 482-492.

DLNR (State of Hawai‘i, Department of Land and Natural Resources). 1989. Hanawī Natural Area Reserve Management Plan. https://dlnr.hawaii.gov/ecosystems/files/2013/07/Hanawi-Management-Plan.pdf. Accessed January 12, 2022.

DLNR (State of Hawai‘i, Department of Land and Natural Resources). 1997. Management Policies of the Natural Area Reserves System. https://dlnr.Hawai'i.gov/ecosystems/files/2013/08/Management-Policies-of-the-Natural-Area-Reserves-System.pdf. Accessed January 12, 2022.

DLNR (State of Hawai‘i, Department of Land and Natural Resources). 2003. Rules Regulating Game Mammal Hunting. https://dlnr.Hawai'i.gov/huntered/files/2013/05/MammalHuntingRegs_Chap123.pdf. Accessed January 12, 2022.

DLNR (State of Hawai‘i, Department of Land and Natural Resources). 2021. Hunting on Maui. https://dlnr.Hawai'i.gov/recreation/hunting/maui. Accessed January 12, 2022.

Drewitt. A. 1999. Disturbance effects of aircraft on birds. English Nature Birds Network Information Note.

Dufour, P.A. 1980. Effects of Noise on Wildlife and Other Animals: Review of Research Since 1971. US Environmental Protection Agency. Washington, D.C. 97 pp.

Dutra, H.L.C., M.N. Rocha, F.B.S. Dias, S.B. Mansur, E.P. Caragata, and L.A. Moreira. 2016. Wolbachia blocks currently circulating Zika virus isolates in Brazilian *Aedes aegypti* mosquitoes. *Cell Host and Microbe* 19: 771-774.

Dyck, V. A., J. Hendrichs, and A. S. Robinson. 2021. Sterile insect technique: principles and practice in area-wide integrated pest management. Second edition. CRC Press, Boca Raton, Florida. Dordrecht, The Netherlands: Springer; 2021.

EBird. 2022. Cornell Lab of Ornithology. https://ebird.org/home. Accessed January 12, 2022.

Elphick, C.S., D.L. Roberts, and J.M. Reed. 2010. Estimated dates of recent extinctions for North American and Hawaiian birds. *Biological Conservation* 143: 617-624.

Environmental Protection Agency (EPA). 1974. Information on Levels of Noise Requisite to Protect the Public Health and Welfare with an Adequate Margin of Safety. Report No. 550/9-74-004. Prepared by the EPA Office of Noise Abatement and Control. Washington, D.C.

Fancy, S. and C.J. Ralph. 2020a. Apapane (*Himatione sanguinea*), version 1.0. *In*: Birds of the World (A.F. Poole and F.B. Gill, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA.

Fancy, S.G. and C.J. Ralph. 2020b. Iiwi (*Drepanis coccinea*), version 1.0. *In*: Birds of the World (A.F. Poole and F.B. Gill, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA.

Fay, K., personal communication, October 28, 2022

Federal Aviation Administration. 1990. Wildlife Strike Database. Retrieved on 5 July 2022 from https://wildlife.faa.gov/search.

Federal Highway Administration (FHWA). 2006. FHWA Highway Construction Noise Handbook. U.S. Department of Transportation. FHWA-HEP-06-015, DOT-VNTSC-FHWA-06-02, NTIS No. PB2006-109012. Final Report, August 2006.

Fleischer, R.C., C.E. McIntosh, and C.L. Tarr. 1998. Evolution on a volcanic conveyor belt: using phylogeographic reconstructions and KAr-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. *Molecular Ecology* 7:533-545.

Fortini, L.B., A.E. Vorsion, 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.

Fortini L.B., L.R., Kaiser, D. LaPointe. 2020. Fostering real-time climate adaptation: Analyzing past, current, and forecast temperature to understand the dynamic risk to Hawaiian honeycreepers from avian malaria. *Global Ecology and Conservation* 23: e01069.

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. https://doi.org/10.1111/j.1365-294X.2007.03550.x.

Francis C.D., J. Paritsis, C.P. Ortega, and A. Cruz. 2011. Landscape patterns of avian habitat use and nest success are affected by chronic gas well compressor noise. *Landscape Ecology* 26: 1269–1280.

Frazier, A.B., and T.W. Giambelluca. 2017. Spatial trend analysis of Hawaiian rainfall from 1920 to 2012. *International Journal of Climatology* 37: 2522–2531.

Gallardo Cruz, K.V., K.L. Paxton, and P.J. Hart. 2021. Temporal changes in songbird vocalizations associated with helicopter noise in Hawai‘i’s protected natural areas. *Landscape Ecology* 36: 829–843.

Gambino, P., A. Medeiros, and L. Loope. 1987. Introduced vespids *Paravespula pensylvanica* prey on Maui's endemic arthropod fauna. *Journal of Tropical Ecology* 3: 169-170. https://doi.org/10.1017/S0266467400001942

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. *Bulletin of the American Meteorological Society* 94: 313-316, https://doi.org/10.1175/BAMS-D-11-00228.1

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.

Glad, A., and L.H. Crampton. 2015. Local prevalence and transmission of avian malaria in the Alakai Plateau of Kaua‘i, Hawai‘i, U.S.A. *Vector Ecology* 40: 221-9. https://doi.org/10.1111/jvec.12157. PMID: 26611954.

Gon, S.M., III, S.L. Tom, and U. Woodside. 2018. ‘Āina momona, honua au loli—productive lands, changing world: using the Hawaiian footprint to inform biocultural restoration and future sustainability in Hawai‘i. *Sustainability* 10: 3420.

Guillaumet, A., W. Kuntz, M. Samuel, and E. Paxton. 2017. Altitudinal migration and the future of an iconic Hawaiian honeycreeper in response to climate change and management. *Ecological Monographs* 87. https://doi.org/10.1002/ecm.1253.

Halfwerk, W., L.J.M. Holleman, C. M. Lessells, and H. Slabbekoorn. 2011. Negative impact of traffic noise on avian reproductive success. *Journal of Applied Ecology* 48: 210-219.

Harabaldis A. S., K. Dimakopoulou, F. Vigna-Taglianti, M. Giampaolo, A. Borgini, M. Dudley, G. Pershagen, G. Bluhm, D. Houthuis, W. Babisch, V. Manolis, K. Katsouyanni, and L. Jarup. 2008. Acute effects of night-time noise exposure on blood pressure in populations living near airports. *European Heart Journal* 29: 658-664.

Harvey-Samuel, T., T. Ant, and J. Sutton. 2021. *Culex quinquefasciatus*: status as a threat to island avifauna and options for genetic control. *CABI Agriculture and Bioscience* 2: 9. https://doi.org/10.1186/s43170-021-00030-1

Hegland, S.J., A. Nielsen, A. Lázaro, A.-L. Bjerknes, and Ø Totland. 2009. How does climate warming affect plant-pollinator interactions? *Ecology Letters* 12: 184195. https://doi.org/10.1111/j.1461-0248.2008.01269.x

Hess, S.C., and J.D. Jacobi. 2011. The history of mammal eradications in Hawai‘i and the United States associated islands of the Central Pacific. *In*: Island Invasive: Eradication and Management. (Eds. C.R. Veitch, M.N. Clout, and D.R. Towns.). IUCN: Gland, Switzerland. pp. 67-73.

Hoang, T. 2013. A Literature Review of the Effects of Aircraft Disturbances ion Seabirds, Shorebirds and Marine Mammals. Presented to NOAA, Greater Farallones National Marine Sanctuary and The Seabird Protection Network, August 2013.

Hodges, C.S.N. and Nagata, R.J. 2001. Effects of predator control on the survival and breeding success of the endangered Hawaiian Dark-rumped Petrel. *Studies in Avian Biology* 22: 308-318.

Hoffmann, A.A., B.L. Montgomery, J. Popovici, I. Iturbe-Ormaetxe, P.H. Johnson, F. Muzzi, M. Greenfield, M. Durkan, Y.S. Leong, Y. Dong, H. Cook, J. Axford, A.G. Callahan, N. Kenny, C. Omodei, E.A. McGraw, P.A. Ryan, S.A. Ritchie, M. Turelli and S.L. O’Neill. 2011. Successful establishment of Wolbachia in *Aedes* populations to suppress dengue transmission. *Nature* 476: 454-457.

Honda. 2022. Noise Level, Decibel Chart. Available at: https://powerequipment.honda.com/generators/selecting-a-generator. Accessed October 15, 2022.

Interagency Visitor Use Management Council (IVUMC). 2016. Visitor Use Management Framework: A Guide to Providing Sustainable Outdoor Recreation. Edition One, July 2016.

International Union for Conservation of Nature. 2021. IUCN Red List of Threatened Species. Available at: http://www.iucnredlist.org. Accessed May 23, 2022.

Jarvi, S.I., C.T. Atkinson, and R.C. Fleischer. 2001. Immunogenetics and resistance to avian malaria in Hawaiian honeycreepers (Drepanidinae). *Studies in Avian Biology* 22: 254-263.

Job, J.R., A.R. Pipkin, and J.A. Beeco. 2018. Haleakalā National Park Acoustic Monitoring Report. Natural Resource Report NPS/NRSS/NSNS/NRR-2018/1678. National Park Service, Natural Sounds and Night Skies Division. Fort Collins, Colorado.

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. 2021. Population estimates and trends of three Maui Island-endemic Hawaiian Honeycreepers. *Journal of Field Ornithology* 92: 115-126.

Judge, S.W., R.J. Camp, and P.J. Hart. 2013. Pacific Island landbird monitoring annual report, Haleakalā National Park, 2012. Natural Resource Technical Report NPS/PACN/NRTR—2013/740. National Park Service, Fort Collins, Colorado. (11) (PDF) Pacific Island Landbird Monitoring Annual Report, Haleakalā National Park, 2012.

Kaushik, M, L. Pejchar, and L.H. Crampton. 2018. Potential disruption of seed dispersal in the absence of a native Kauai thrush. *PLoS ONE* 13: e0191992. https://doi.org/10.1371/journal.pone.0191992

Keith, L.M., R.F. Hughes, L.S. Sugiyama, W.P. Heller, B.C. Bushe, and J.B. Friday. 2015. First report of Ceratocystis wilt on ‘ohi‘a (*Metrosideros polymorpha*). *Plant Disease* 99: 1276.

Kempf, N. and O. Hüppop. 1998. How do airplanes affect birds? – An evaluative overview in nature conservation and landscape planning 30: 17-28

Keir, M., personal communication. November 15, 2022

Kitayama K. and D. Mueller-Dombois. 1992. Vegetation of the Wet Windward Slope of Haleakalā, Maui, Hawai‘i. *Pacific Science* 46: 197-220

Krushelnycky, P.D., C.G. Chimera, and E.A. VanderWerf. 2019. Natural resource condition assessment: Haleakalā National Park. Natural Resource Report NPS/HALE/NRR—2019/1977. National Park Service, Fort Collins, Colorado.

Krushelnycky, P.D., F. Starr, and K. Starr. 2016. Change in trade wind inversion frequency implicated in the decline of an alpine plant. Clim Chang Responses 3: https://doi.org/10.1186/s40665-016-0015-2

Lady Bird Johnson Wildflower Center (LBJWC). 2022. Plant Database Available online at: https://www.wildflower.org/plants/.

Landres, P., C. Barns, S. Boutcher, T. Devine, P. Dratch, A. Lindholm, L. Merigliano, N. Roeper, and E. Simpson. 2015. Keeping It Wild 2: An Updated Interagency Strategy to Monitor Trends in Wilderness Character Across the National Wilderness Preservation System. Gen. Tech. Rep. RMRSGTR-340. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 114 pp.

LaPointe, D.A. 2000. Avian malaria in Hawai‘i: the distribution, ecology, and vector potential of forest-dwelling mosquitoes. 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., J.M. Gaudioso-Levita, C.T. Atkinson, A. Egan, and K. Hayes. 2016. Changes in the prevalence of avian disease and mosquito vectors at Hakalau Forest National Wildlife Refuge: a 14-year perspective and assessment of future risk. Hawai‘i. Hawai‘i Cooperative Studies Unit Technical Report HCSU-073.

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.

Lawler, J.J., S.L. Shafer, D. White, P. Kareiva, E.P. Maurer, A.R. Blaustein, and P.J. Bartlein. 2009. Projected climate-induced faunal change in the Western Hemisphere. *Ecology* 90: 588-597.

Lee, C.S.Y., G.G. Fleming, C.J. Roof, J.M. MacDonald, C.J. Scarpone, A.R. Malwitz, G. Baker. 2016. Haleakalā National Park: Baseline Ambient Sound Levels 2003. DOT-VNTSC-FAA-06-09 or DOT/FAA/AEE/2016-06. U.S. Department of Transportation, Federal Aviation Administration and U.S. Department of Interior, National Park Service.

Leopold, C.R., and S.C. Hess. 2014. Corridor- and stopover-use of the Hawaiian goose (*Branta sandvicensis*), an intratropical altitudinal migrant. *Journal of Tropical Ecology* 30: 67-78. http://www.jstor.org/stable/43831696

Lerner, H.R.L., M. Meyer, H.F. James, M. Hofreiter, and R.C. Fleischer. 2011. Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian Honeycreepers. *Current Biology* 21: 1838-1844.

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

Liao W., C.T. Atkinson, D.A. LaPointe, and M.D. Samuel. 2017. Mitigating Future Avian Malaria Threats to Hawaiian Forest Birds from Climate Change. *PLoS ONE* 12: e0168880. https://doi.org/10.1371/journal.pone.0168880

Lignell, B.W. 2020. Reporting Information for Commercial Air Tour Operations over Units of the National Park System, 2019 Annual Report. Natural Resource Report NPS/NRSS/NSNSD/NRR—2020/2193. National Park Service, Natural Sounds & Night Skies Division / Overflights Program. Fort Collins, CO.

Loope, L.L. and A.C. Medeiros. 1995. Strategies for long-term protection of biological diversity in rainforests of Haleakalā National Park and East Maui, Hawai‘i. *Endangered Species Update* 12: 1– 5.

Loope, L.L., and D. Mueller-Dombois. 1989. Characteristics of invaded islands, with special reference to Hawai‘i. *In*: Ecology of biological invasions: a global synthesis (J.A. Drake, H.A. Mooney, F. DiCastri, R.H. Groves, F.J. Kruyer, M. Rejmanek, and M. Williamson, eds.). John Wiley and Sons, New York, NY. pp. 257-280

Lynch, E. 2012. Haleakalā National Park: Acoustical monitoring 2008. Natural Resource Technical Report NPS/NRSS/NRTR—2012/549. National Park Service, Fort Collins, Colorado.

Malachowski, C., B. Dugger, K. Uyehara, and M.H. Reynolds. 2018. Nesting ecology of the Hawaiian Duck *Anas wyvilliana* on northern Kaua‘i, Hawai‘i, USA. *Wildfowl* 68: 123-139.

Mallinson, J., personal communication. December 14, 2021

Maui Forest Bird Recovery Project. 2021. Hawaiian Honeycreepers – Native Forest Birds of Maui. Available at https://mauiforestbirds.org/Hawaiian-honeycreepers/

McClure, K. M. 2020. Mosquito suppression models to inform Wolbachia implementation strategies. Birds Not Mosquitoes, Research Subcommittee Meeting, Hilo. HI.

Medeiros, A.C, L.L. Loope, and R.A. Holt. 1986. Status of the native flowering plant species on the south slope of Haleakalā, East Maui Cooperative National Park Studies Unit, University of Hawai`i Technical Report 59.

Meillère, A., Brischoux, F., and Angelier, F. 2015. Impact of chronic noise exposure on antipredator behavior: an experiment in breeding house sparrows. *Behavioral Ecology* 26: 569–577.

Medeiros, M.J., J.A. Eiben, W.P. Haines, R.L. Kaholoaa, C.B.A. King, P.D. Krushelnycky, K.N. Magnacca, D. Rubinoff, F. Starr, and K. Starr. 2013. The importance of insect monitoring to conservation actions in Hawai‘i. *Proceedings of the Hawaiian Entomological Society* 45: 149-166.

Meyer W.M., III, R. Ostertag, and R.H. Cowie. 2013. Influence of Terrestrial Mollusks on Litter Decomposition and Nutrient Release in a Hawaiian Rain Forest. *BioTropica* 45: 719-727.

Montoya-Aiona, K.M. 2020. Roosting Ecology and Behavior of the Solitary and Foliage-roosting Hawaiian Hoary Bat (*Lasiurus cinereus semotus*). Master’s Thesis University of Hawai‘i, Hilo.

Moreira, L.A., I. Iturbe-Ormaetxe, J.A. Jeffery, G. Lu, A.T. Pyke, L.M. Hedges, B.C. Rocha, S. Hall-Mendelin, A. Day, M. Riegler, L.E. Hugo, K.N. Johnson, B.H. Kay, E.A. McGraw, A.F. van den Hurk, P.A. Ryan, and S.L. O'Neill. 2009. A Wolbachia symbiont in *Aedes aegypti* limits infection with dengue, chikungunya, and *Plasmodium*. *Cell* 139: 1268-1278.

Mounce, H.L., C.C. Warren, C.P. McGowan, E.H. Paxton, and J.J. Groombridge. 2018. Extinction risk and conservation options for Maui Parrotbill, an endangered Hawaiian honeycreeper. *Journal of Fish and Wildlife Management* 9: 367-382.

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

Mulero-Pázmány M, Jenni-Eiermann S, Strebel N, Sattler T, Negro JJ, et al. 2017. Unmanned aircraft systems as a new source of disturbance for wildlife: A systematic review. *PLoS ONE* 12: e0178448. https://doi.org/10.1371/journal.pone.0178448

NatureServe. 2021. NatureServe Explorer. Available online at: https:/explorer.natureserve.org/. Accessed September – October 2021.

NPS (National Park Service). 1994. Report to Congress on the Effects of Aircraft Overflights on the National Park System. Prepared pursuant to Public Law 100-91, The National Park Overflights Act of 1987. National Park Service. http://www.nonoise.org/library/npreport/intro.htm. Accessed May 3, 2022.

NPS (National Park Service). 1995. General Management Plan/Environmental Impact Statement for Haleakalā National Park. U.S. Department of the Interior.

NPS (National Park Service). 1995. Report on effects of aircraft overflights on the National Park System: Report to Congress. Washington, DC.

NPS (National Park Service). 2006. Management Policies 2006. U.S. Department of the Interior.

NPS (National Park Service). 2010. Soundscapes Management Plan for Zion National Park. National Park Service. http://www.nps.gov/zion/parkmgmt/upload/ZNP-Soundscape-Plan_Sep_2010.pdf. Accessed October 6, 2021.

NPS (National Park Service). 2015a. Wilderness Building Blocks 1 & 2, Wilderness Basics & Wilderness Character Assessment, Haleakalā Wilderness, Haleakalā National Park

NPS (National Park Service). 2015b. Foundation Document, Haleakalā National Park. U.S. Department of the Interior.

NPS (National Park Service). 2018. Kīpahulu Comprehensive Plan and Environmental Assessment, Maui, Hawai`i. Haleakalā National Park. U.S. Department of the Interior.

NPS (National Park Service). 2002. Notice of Conversion of Potential Wilderness as Designated Wilderness, Haleakala National Park. Federal Register 67: 6944.

NPS (National Park Service). 2021. Visitor use statistics. https://irma.nps.gov/STATS/Reports/Park/HALE. Accessed October 6, 2021.

NPSpecies. 2017. The National Park Service Biodiversity Database. IRMA Portal version. Certified species lists. Available from https://irma.nps.gov/NPSpecies/. Accessed October 6, 2021.

NSNSD (Natural Sounds and Night Skies Division). n.d. Attachment 1 – Acoustic Environment and Soundscape. U.S. Department of the Interior, National Park Service. Natural Resource Stewardship and Science Directorate.

NSNSD (Natural Sounds and Night Skies Division). 2018. The Power of Sound Interpretive Handbook. U.S. Department of the Interior, National Park Service, Natural Resource Stewardship and Science Directorate. Fort Collins, CO. Available at: https://www.nps.gov/subjects/sound/upload/PowerofSound_May2018updated-508.pdf/.

NSNSD (Natural Sounds and Night Skies Division). 2021. Natural Sounds – Policies and Authorities. U.S. Department of the Interior, National Park Service. Available at: https://www.nps.gov/subjects/sound/policy.htm. Accessed May 3, 2022.

Occupational Safety and Health Administration (OSHA). 2013. OSHA Technical Manual – Noise. https://www.osha.gov/dts/osta/otm/new_noise/index.pdf. Accessed May 3, 2022.

Oregon.gov (Oregon). 2022. Off-Road Recreational Vehicle Standards. DEQ OAR 340-035-0083 (Excerpt from Table 4). https://www.oregon.gov/oprd/ATV/Pages/ATV-Sound.aspx Accessed May 3, 2022.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics* 37: 637-669.

Paxton, E.H, R. J. Camp, P. M. Gorresen, L. H. Crampton, D. L. Leonard, and E. A. Vanderwerf. 2016. Collapsing Avian Community on A Hawaiian Island. *Science Advances* 2: e1600029

Paxton, E.H., M. Laut, J.P. Vetter, and S.J. Kendall. 2018. Research and management priorities for Hawaiian forest birds. *Condor* 120: 557-565.

Paxton, E.H., P.M. Gorresen, and R.J. Camp. 2013. Abundance, distribution, and population trends of the iconic Hawaiian Honeycreeper, the ʻIʻiwi (*Vestiaria coccinea*) throughout the Hawaiian Islands. U.S. Geological Survey Open-File Report 2013-1150, 59 pp.

Paxton, E.H., M. Laut, S. Enomoto, and M. Bogardus. 2022. Hawaiian forest bird conservation strategies for minimizing the risk of extinction: Biological and biocultural considerations. Hawaiʻi Cooperative Studies Unit Technical Report HCSU-103. University of Hawaiʻi at Hilo, Hawaiʻi, USA. 125 pp. http://hdl.handle.net/10790/5386.

Peck, R.W., P.C. Banko, J. Cappadonna, C. Steele, D.L. Leonard, H.L. Mounce, C.D. Becker, and K. Swinnerton. 2015. An assessment of arthropod prey resources at Nakula Natural Area Reserve, a potential site of reintroduction for Kiwikiu (*Pseudonestor xanthophrys*) and Maui ʻAlauahio (*Parareomyza montana*). Hawaiʻi Cooperative Studies Unit Technical Report. TR HCSU-0059, 43 pp.

Pender, R.J., C.W. Morden, and R.E. Paull. 2014. Investigating the pollination syndrome of the Hawaiian lobeliad genus *Clermontia* (Campanulaceae) using floral nectar traits. *American Journal of Botany* 101: 201-205

Perroy, R.L., T. Sullivan, D. Benitez, R.F. Hughes, L.M. Keith, E. Brill, K. Kissinger, and D. Duda. 2021. Spatial Patterns of ʻOhiʻa Mortality Associated with Rapid ʻOhiʻa Death and Ungulate Presence. Forests 12: 1035. https://doi.org/10.3390/f12081035

Pinzari, C.A. 2019a. Genetic variation, population structure, and morphology of an endemic bat, *Lasiurus cinereus semotus* (Chiroptera: Vespertilionidae) across the Hawaiian Islands. Master's Thesis. University of Hawaiʻi at Hilo.

Pinzari, C.A., R.W. Peck, T. Zinn, D. Gross, K. Montoya-Aiona, K.W. Brinck, P.M. Gorresen, and F.J. Bonaccorso. 2019b. Hawaiian hoary bat (*Lasiurus cinereus semotus*) activity, diet and prey availability at the Waihou Mitigation Area, Maui. Hawaiʻi Coop. Stud. Unit Tech. Rep. 90:1-68.

Plentovich, S. A. Hebshi, and S. Conant. 2008. Detrimental effects of two widespread invasive ant species on weight and survival of colonial nesting seabirds in the Hawaiian Islands. *Biological Invasions* 11: 289–298.

Polhemus D.A. 1993. Damsels in distress: a review of the conservation status of Hawaiian *Megalagrion* damselflies (Odonata: Coenagrionidae). *Aquatic Conservation* 3: 343-349.

Polhemus D.A. 2004. Critical species of Odonata in the Hawaiian Islands. *International Journal of Odonatology* 7: 133-138.

Polhemus D.A. 2019. East Maui Irrigation Ditch System, East Maui, Hawaiʻi, Stream Diversion Abandonments, Native Damselfly Baseline Survey. USFWS Field Survey Report Final Draft.

Pratt, H.D. 2005. The Hawaiian honeycreepers: Drepanidae. Oxford University Press, New York, NY.

Pratt, T.K., C.T. Atkinson, P.C. Banko, J.D. Jacobi, B.L. Woodworth, and L.A. Mehrhoff. 2009. Can Hawaiian Forest Birds be Saved? *In*: Conservation Biology of Hawaiian Forest Birds: Implications for Island Avifauna (T. K. Pratt, C. T. Atkinson, P. C. Banko, J. D. Jacobi, and B. L. Woodworth, eds.). University of Hawaiʻi Press, Honolulu, HI. pp. 137-158

Pratt, L.W. and J.D. Jacobi. 2009. Loss, degradation, and persistence of habitats. *In*: Conservation Biology of Hawaiian Forest Birds: Implications for Island Avifauna (T. K. Pratt, C. T. Atkinson, P. C. Banko, J. D. Jacobi, and B. L. Woodworth, eds.). University of Hawai‘i Press, Honolulu, HI. pp. 137-158

Price, J.S. M. Gon III, J.D. Jacobi, and D. Matsuwaki. 2007. Mapping Plant Species Ranges in the Hawaiian Islands: Developing a Methodology and Associated GIS layers. Hawai`i Cooperative Studies Unit Technical Report HCSU-008. University of Hawai`i at Hilo. 58 pp., incl. 16 Figures and 6 Tables. https://hilo.hawaii.edu/hcsu/documents/Priceetal008pdfFinal.pdf

Price, M., and J. Cotín. 2018. The Pueo Project. Final Report: April 2017 – March 2018. Population size, distribution, and habitat use of the Hawaiian Short-eared Owl (*Asio flammeus sandwichensis*) on O‘ahu. Available at: https://www.pueoproject.com/resources.

Pueo Project. 2019. Pueo Distribution and Sightings Map. Available at https://www.pueoproject.com/distribution-map Accessed July 15, 2020.

Ritchie, S. 2014. Rear and release: a new paradigm for dengue control. *Austral Entomology* 53: 363-367.

Safecom. 2022. Aviation Safety Database. The Department of the Interior (DOI) and the U.S. Forest Service (USFS) aviation safety reporting system. Available at https://www.safecom.gov/. Accessed May 3, 2022.

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 modeling approach. *Ecological Applications* 21: 2960-2973.

Schäffer, B., R. Pieren, K. Heutschi, J.M. Wunderli, and S. Becker. 2021. Drone Noise Emission Characteristics and Noise Effects on Humans—A Systematic Review. *International Journal of Environmental Research and Public Health* 18: 5940. https://doi.org/10.3390/ijerph18115940

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: 1-431.

Shannon G., M. McKenna, L. Angeloni, K. Crooks, K. Fristrup, E. Brown, K. Warner, M. Nelson, C. White, J. Briggs, S. McFarland, and G. Wittemyer. 2015. A Synthesis of Two Decades of Research Documenting the Effects of Noise on Wildlife. Cambridge Philosophical Society, Biological Reviews.

Simberloff, D. 2010. Invasive Species, in Conservation Biology for All, N.S. Sodhi, P.R. Ehrlich, Eds. Oxford Univ. Press Inc., 2010). pp. 131-152.

Simon, J. C., P. E. Baker, and H. Baker. 2020. Maui Parrotbill (*Pseudonestor xanthophrys*), version 1.0. *In*: Birds of the World (A. F. Poole and F. B. Gill, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi.org/10.2173/bow.maupar.01

Simons, T.R., and C.N. Bailey. 2020. Hawaiian Petrel (*Pterodroma sandwichensis*), version 1.0. In: Birds of the World (A.F. Poole and F.B. Gill, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi.org/10.2173/bow.hawpet1.01. Last updated 1998.

Slotterback, J.W. 2021. Band-rumped Storm-Petrel (*Hydrobates castro*), version 1.1. *In*: Birds of the World (A.F. Poole, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi.org/10.2173/bow.barpet.01.1

Tamayose, J., personal communication. April 6, 2021

Thomas, C.D., A. Cameron, R.E. Green, M. Bakkenes, L.J. Beaumont, Y.C. Collingham, B.F.N. Erasmus, M.F. de Siqueira, A. Grainger, L. Hannah, L. Hughes, B. Huntley, A.S. van Jaarsveld, G.F. Midgley, L. Miles, M.A. Ortega-Huerta, A.T. Peterson, O.L. Phillips, and S.E. Williams. 2004. Extinction risk from climate change. *Nature* 427: 145-148.

Todd, C., C.A. Pinzari, F.J Bonaccorso. 2016. Acoustic surveys of Hawaiian hoary bats in Kahikinui Forest Reserve and Nakula Natural Area Reserve on the island of Maui. HSCU report, University of Hawaiʻi at Hilo.

Tuttle, N. C., K. H. Beard, and R. Alchokhachy. 2008. Aerially applied citric acid reduces the density of an invasive frog in Hawaii, USA. Wildlife Research 35: 676–683

Uchida, S., H. Mori, T. Kojima, K. Hayama, Y. Sakairi, and S. Chiba. 2016. Effects of an invasive ant on land snails in the Ogasawara Islands. *Conservation Biology* 30: 1330-1337. https://doi.org/10.1111/cobi.12724. Epub 2016 Jun 15. PMID: 27027403.

UH Honeybee Project. 2022. Pollinators in Hawaii, https://kohalacenter.org/docs/resources/hpsi/PollinatorsInHawaii.pdf. Accessed May 3, 2022.

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

USFWS (U.S. Fish and Wildlife Service). 2010. Endangered and Threatened Wildlife and Plants; Listing the Flying Earwig Hawaiian Damselfly and Pacific Hawaiian Damselfly as Endangered Throughout Their Ranges. Federal Register 75: 35990.

USFWS (U.S. Fish and Wildlife Service). 2012. Endangered and Threatened Wildlife and Plants; Listing 38 Species on Molokai, Lanai, and Maui as Endangered and Designating Critical Habitat on Molokai, Lanai, Maui, and Kahoolawe for 135 Species; Proposed Rule. FR 77(112) 34464-34775.

USFWS (U.S. Fish and Wildlife Service). 2013. Endangered and Threatened Wildlife and Plants; Determination of Endangered Status for 38 Species on Molokai, Lanai, and Maui; Final Rule. Federal Register 78: 32014-32065.

USFWS (U.S. Fish and Wildlife Service). 2016a. Designation and Non-designation of Critical Habitat on Molokaʻi, Lanaʻi, Maui, and Kahoʻolawe for 135 Species: Final Rule. FR 81(61) 17790-18110.

USFWS (U.S. Fish and Wildlife Service). 2016b. Endangered Status for 49 Species from the Hawaiian Islands: Final Rule. Federal Register 81: 67786-67860.

USFWS (U.S. Fish and Wildlife Service). 2017. Threatened species status for the ʻiʻiwi (*Drepanis coccinea*). Federal Register 82: 43873–43885

USFWS (U.S. Fish and Wildlife Service). 2019. Recovery Outline for the Islands of Maui, Molokaʻi, Kahoʻolawe, and Lanaʻi (Maui Nui). Pacific Islands Fish and Wildlife Office, Honolulu, HI. Available online at: https://ecos.fws.gov/docs/recovery_plan/Maui_Nui_Recovery_Outline_20191031.pdf. Accessed September 2021.

USFWS (U.S. Fish and Wildlife Service). 2021a. Draft Recovery Plan for 50 Hawaiian Archipelago Species. U.S. Fish and Wildlife Service, Portland, OR.

USFWS (U.S. Fish and Wildlife Service). 2021. U.S. Fish and Wildlife Service Proposes Delisting 23 Species from Endangered Species Act Due to Extinction [Press Release, September 29, 2021]. Available at https://www.fws.gov/press-release/2021-09/us-fish-and-wildlife-service-proposes-delisting-23-species-endangered-species.

USFWS (U.S. Fish and Wildlife Service). 2021. Endangered and Threatened Wildlife and Plants; Removal of 23 Extinct Species from the Lists of Endangered and Threatened Wildlife and Plants. Proposed Rule. Federal Register 86: 54298-54338. https://www.federalregister.gov/documents/2021/09/30/2021-21219/endangered-and-threatened-wildlife-and-plants-removal-of-23-extinct-species-from-the-lists-of

USFWS (U.S. Fish and Wildlife Service). 2022s. Invasive Species Biosecurity Protocols. Pacific Islands Fish and Wildlife Office (PIFWO). Updated, February 2022.

USFWS (U.S. Fish and Wildlife Service). 2022b. ECOS Environmental Conservation Online System, Threatened & Endangered Species Active Critical Habitat Report, https://ecos.fws.gov/ecp/report/table/critical-habitat.html

USFWS (U.S. Fish and Wildlife Service). 2022c. ECOS Environmental Conservation Online System, Listed species believed to or known to occur in Hawai'i, https://ecos.fws.gov/ecp/report/species-listings-by-state?stateAbbrev=HI&stateName=Hawaii&statusCategory=Listed. Accessed May 3, 2022.

USFWS (U.S. Fish and Wildlife Service). 2022d. Project Consultation Letter, Pacific Islands Fish and Wildlife Office, January 20, 2022.

U.S. Geological Service (USGS). 2007. Hawaiian Duck's Future Threatened by Feral Mallards. https://pubs.usgs.gov/fs/2007/3047/fs2007-3047.pdf. Accessed May 3, 2022.

Van Dine, D.L. 1904. Mosquitoes in Hawai'i. *Hawai'i Agricultural Experimental Station Bulletin* 6: 7-30.

van Dyk, K.N., K.L. Paxton, P.J. Hart, and E.H. Paxton. 2019. Seasonality and Prevalence of Pollen Collected from Hawaiian Nectivorous Birds. *Pacific Science* 73: 187-197

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, and J.M. Scott. 2001. Limiting factors affecting Hawaiian native birds. *Studies in Avian Biology* 22: 221-233.

van der Kolk, Henk-Jan & Allen, Andrew & Ens, Bruno & Oosterbeek, Kees & Jongejans, Eelke & van de Pol, Martijn. 2020. Spatiotemporal variation in disturbance impacts derived from simultaneous tracking of aircraft and shorebirds. *Journal of Applied Ecology* 57: 10.1111/1365-2664.13742.

Vaught, M. Director, Water Resources, Mahi Pono. 2021. Personal Communication. October 27, 2022.

Vetter, J.P., K.J. Swinnerton, E.A. VanderWerf, J.C. Garvin, H.L. Mounce, H.E. Breniser, D.L. Leonard, and J.S. Fretz. 2012. Survival Estimates for Two Hawaiian Honeycreepers. *Pacific Science* 66: 299-309.

Videvall, E., K.L. Paxton, M.G. Campana, L. Cassin-Sackett, C.T. Atkinson, and R.C. Fleischer. 2021. Transcriptome assembly and differential gene expression of the invasive avian malaria parasite *Plasmodium relictum* in Hawai'i. *Ecology and Evolution* 11: 4935– 4944. https://doi.org/10.1002/ece3.7401

Virginio, J.F., Gomez, M., Almeida, F., Pedrosa, M.C., Pinto, A.T., Garziera, L., Klaptocz, A., Germann, J., Ponsot, W., Lopez, O. et al. 2018. Drone-Based Aerial Release Mechanism for Mosquitoes; WeRobotics: Geneva, Switzerland.

Wang, A.X., E.H. Paxton, H.L. Mounce, and P.J. Hart. 2020. Divergent movement patterns of adult and juvenile ʻAkohekohe, an endangered Hawaiian Honeycreeper. *Journal of Field Ornithology* 91: 346-353. https://doi.org/10.1111/jofo.12348

Warner, R.E. 1968. The Role of Introduced Diseases in the Extinction of the Endemic Hawaiian Avifauna. The Condor 70: 101-120. https://doi.org/10.2307/1365954

Warren, C., personal communication. October 27, 2022

Warren, C.C., L.K. Berthold, H.L. Mounce, P. Luscomb, B. Masuda, and L. Berry. 2020. Kiwikiu Translocation Report 2019. Internal Report. Pages 1-101.

Warren, C.C., L.K. Berthold, H.L. Mounce, J.T. Foster, and L.C. Sackett. 2019. Evaluating the risk of avian disease in reintroducing the endangered Kiwikiu (Maui Parrotbill: *Pseudonestor xanthophrys*) to Nakula NAR, Maui, Hawaiʻi. Pacific Cooperative Studies Unit Technical Report #201. University of Hawai`i at Mānoa, Department of Botany. Honolulu, HI. 50 pp.

Wells, C.P., P. Lavretsky, M.D. Sorenson, J.L. Peters, J.M. DaCosta, S. Turnbull, K.J. Uyehara, C.P. Malachowski, B.D. Dugger, J.M. Eadie, and A. Engilis. 2019. Persistence of an endangered native duck, feral mallards, and multiple hybrid swarms across the main Hawaiian Islands. *Molecular Ecology* 28: 5203-5216.

Whitaker, J.O., P.Q Tomich. 1983. Food Habits of the Hoary Bat, Lasiurus cinereus, from Hawaiʻi. *Journal of Mammalogy* 64: 151-152, https://doi.org/10.2307/1380766

Wood, L. 2015. Acoustic Environment and Soundscape Resource Summary, Haleakalā National Park. Natural Sounds & Night Skies Division. https://irma.nps.gov/DataStore/DownloadFile/534087

Work, T., J. Dagenais, R. Rameyer, and R. Breeden. 2015. Mortality patterns in endangered Hawaiian geese (Nene; Branta sandvicensis). *Journal of Wildlife Diseases* 51: 688-695.

Yorinks, N., and C.T. Atkinson. 2000. Effects of malaria Plasmodium relictum on activity budgets of experimentally infected juvenile ʻapapane Himatione sanquinea. *Auk* 117: 731-738.

Zheng, X., D. Zhang, Y. Li, C. Yang, Y. Wu, X. Liang, Y. Liang, X. Pan, L. Hu, Q. Sun, X. Wang, Y. Wei, J. Zhu, W. Qian, Z. Yan, A.G. Parker, J.R.L. Gilles, K. Bourtzis, J. Bouyer, M. Tang, B. Zheng, J. Yu, J. Liu, J. Zhuang, Z. Hu, M. Zhang, J.-T. Gong, X.-Y. Hong, Z. Zhang, L. Lin, Q. Liu, Z. Hu, Z. Wu, L.A. Baton, A.A. Hoffman, and Z. Xi. 2019. Incompatible and sterile insect techniques combined eliminate mosquitoes. *Nature* 572: 56–61. https://doi.org/10.1038/s41586-019-1407-9

Zimmerman, E.C. 1970. Adaptive Radiation in Hawaiʻi with Special Reference to Insects. *BioTropica* 2: 32-38. https://doi.org/10.2307/2989786.

APPENDIX B:
Issues, Impact Topics, and Alternatives Dismissed from Detailed Analysis

APPENDIX B: ISSUES, IMPACT TOPICS, AND ALTERNATIVES DISMISSED FROM DETAILED ANALYSIS

ISSUES AND IMPACT TOPICS DISMISSED FROM DETAILED ANALYSIS

Section 4.2 E of the National Park Service (NPS) NEPA Handbook (NPS 2015) states that, generally, issues should be discussed in detail in an Environmental Assessment (EA) if any of the following apply:

- the environmental impacts associated with the issue are central to the proposal or of critical importance
- a detailed analysis of environmental impacts related to the issue is necessary to make a reasoned choice between alternatives
- the environmental impacts associated with the issue are a big point of contention among the public or other agencies
- there are potentially significant impacts to resources associated with the issue

The NPS NEPA Handbook further states that if the considerations above do not apply, issues should be dismissed from detailed analysis. The following issues and impact topics were not fully addressed in the EA because the listed resources are not in the project area; the environmental impacts associated with the issue are not central to the proposal, pivotal, or of critical importance; a detailed analysis of environmental impacts related to the issue is not necessary to make a reasoned choice between alternatives; or the resource would not be or only negligibly impacted and there is no potential for significant impacts. More details about the dismissal for these issues and impact topics are provided in the sections below.

Air Quality, Greenhouse Gas Emissions, and Climate Change

The park regularly monitors air quality in the frontcountry (headquarters area) and baseline data is available. Air quality in the project area is typically very good, and Maui is in attainment for National Ambient Air Quality Standards (EPA 2021).

Under the proposed action, there are several release methods, ranging from pedestrian release with relatively limited helicopter flight times to helicopter long line and drone dispersal, but none of these would perceptibly adversely affect air quality. The primary mosquito release method would use drones, which do not burn fossil fuels.

Although some management actions would result in emissions of criteria pollutants pursuant to the Clean Air Act and greenhouse gases due to the use of helicopters and other motorized vehicles, contributions would be extremely low and would result in impacts on air quality and greenhouse gas emissions that would be below *de minimis* levels. Overall, any effects resulting from the proposed alternatives would be negligible. The regional effects of climate change are evident in the Hawaiian archipelago, and after a minor lull in the rate of climactic change in the early 2000s, a rapid warming trend appears to have resumed in 2014 (McKenzie et al. 2019). As suggested by some climate change models, the mean temperatures in Hawaiʻi may increase by 2°– 3°C by 2100 (IPCC 2007). The effects of climate change can result in increased stress to natural systems through altered temperatures and rainfall patterns (Alexander et al. 2016). Frazier and Giambelluca (2017) examined trends by elevation and showed that the highest rates of drying during dry season months were found in high-elevation areas where populations of threatened or endangered populations of forest birds are still able to persist.

Though climate change and associated adverse impacts have and will continue to affect specific resources on Maui and within the project area (Alexander et al. 2016, Pauchard et al. 2016), greenhouse gases from helicopter and motor vehicle emissions are not expected to have a measurable effect on local climatic conditions. For example, the management activities proposed to release mosquitoes would result in fossil fuel consumption from helicopters, but the greenhouse gas emissions associated with these activities would be negligible because of the comparatively limited number of flights anticipated, compared to ongoing commercial and administrative flights on Maui.

Based on the considerations discussed above, air quality, greenhouse gas emissions, and climate change were dismissed from detailed analysis as an impact topic. However, climate change was addressed in terms of impacts on the existing conditions of resources, and their long-term trends, as applicable.

Vegetation (Non-threatened/Endangered)

The Kīpahulu Valley and other portions of the project area above approximately 1,650 feet (502 meters) in elevation include important rainforest. The native koa (*Acacia koa*), and in some areas, invasive guava (*Psidium* spp.), dominate the forest from 2,000 to approximately 4,000 feet (610–1,219 meters), while ‘ōhi‘a (*Metrosideros polymorpha*) dominates the forest above 4,000 feet. Tree ferns (*Cibotium* spp.) are important in the understory. Lobelioids (*Cyanea* spp., *Clermontia* spp., *Lobelia* spp., and *Trematolobelia macrostachys*) and mints (*Stenogyne* spp. and *Phyllostegia* spp.) are among the rare and spectacular endemic plant species of the Kīpahulu Valley.

If rare forest birds recover through the release of incompatible mosquitoes, project activities could indirectly benefit East Maui’s native vegetation. Hawaiian honeycreepers play a critical role in ecosystem function by dispersing seeds and pollinating native plants. Maintaining populations of these species benefits the native plant community and preserves ecosystem function.

There is potential under the proposed action for minimal adverse impacts to vegetation from localized plant removal or disturbance along trails, fencelines, and at landing zones and camps by ground crews. These impacts would be temporary in nature and largely occur in previously disturbed locations. In addition, these activities have been cleared through previous environmental compliance conducted by the state or park. To help mitigate any vegetation/ground disturbance, monitoring efforts and the dispersal of incompatible mosquitoes via ground-based pedestrian releases would be conducted on existing resource management trails and fence lines to avoid disturbance of soils and plant communities. Additionally, best management practices (BMPs) would be implemented to reduce or remove the threat of introducing invasive plants within the project area; however, a risk of introduction still exists. Crews would be trained to follow BMPs to minimize this risk. Given previous environmental compliance of proposed activities and anticipated negligible impacts, this issue was considered and dismissed from further analysis.

Wildlife and/or Wildlife Habitat

There may be *de minimis* adverse impacts to general wildlife (those not federally listed or deemed as species of concern) or wildlife habitats that would result from the presence of people, drones, or helicopters used for implementation of the proposed action. In general, if the project were to be successful at reducing the prevalence of non-native *Culex* mosquitoes in the environment of East Maui, there would be periodic, short-term adverse impacts due to increased air and foot traffic, but long-term beneficial indirect impacts to general wildlife or wildlife habitat from the suppression of non-native mosquito populations and in turn avian malaria. Given the anticipated negligible impacts on general wildlife and/or wildlife habitat, this topic was dismissed from further analysis.

Museum Collections

No impacts to museum collections would result from the proposed action as none are present within the project area. This issue was considered and dismissed from further analysis.

Prehistoric/Historic Structures

No impacts to prehistoric or historic structures are anticipated to result from the proposed action. Much of the project area has not been surveyed, but no new ground disturbance would occur. To help mitigate potential effects of ground-based activities on previously undiscovered prehistoric or historic structures, pedestrian releases and monitoring would only be conducted via existing, previously disturbed resource management trails and fence lines, as well as camping at established remote camps or helicopter landing zones for overnight stays, to avoid new ground disturbance. Helicopter operations would utilize existing, previously disturbed landing zones. These existing areas (trails, fence lines, and landing zones or camps) have been cleared through previous environmental compliance conducted by the state or park. Therefore, this issue was considered and dismissed from further analysis.

Archeological Resources

As defined by NPS *Management Policies 2006*, the term “archeological resources” refers to any material remains or physical evidence of past human life or activities and includes precontact and historic sites and features. The project area within Haleakalā encompasses the entirety of the Kīpahulu Historic District. The Kīpahulu Historic District was determined eligible and nominated for listing on the National Register of Historic Places (NRHP) in 1976. Kīpahulu Historic District was listed on the Hawaiʻi State Inventory of Historic Places (SIHP) in 1977 (Site# 50-50-17-299). The historic district encompasses 327 hectares (810 acres) of lands around ʻOheʻo Gulch, from sea level to about 1,640 feet (500 meters) above sea level. The Kīpahulu Historic District encompasses the lower sections of five traditional ahupuaʻa within the larger ancient moku (district) of Kīpahulu. These are Kaumakani, Papauluana, ʻAlae Iki, ʻAlae Nui, and Kakalehale. The individual archeological sites in the Kīpahulu District, all of which have been determined eligible for listing in the National Register of Historic Places through consultation with the Hawaiʻi State Historic Preservation Division, represent occupational periods from pre-historic (pre-1778) through to the modern period (1850–present) and are associated with agriculture and animal husbandry, permanent residences, temporary encampments, and ceremonial purposes. Site types include mounds, terraces, walls, burials, platforms, enclosures, walled shelters, trails, and rock shelters, with rock mounds, walls, and terraces making up the majority of recorded archeological features.

The proposed action would occur just within the boundaries of the Crater Historic District, which was listed on both the State Inventory of Historic Places SIHP (SIHP 50-50-11-12-1739) and on the National Register of Historic Places (NRHP) on November 1, 1974. The Crater Historic District encompasses 17,000 acres within Haleakalā National Park, encompassing the original park boundaries and lands throughout the crater wilderness and “frontcountry” shrubland on the northwest-facing slopes of Haleakalā. The district consists of 56 recorded archeological sites and is significant under Criterion D because it has yielded, and is likely to yield, information important in prehistory or history. No known individual archeological sites in the Crater Historic District are within the project area.

Individual archeological sites are also present and have been documented in the coastal area of Kaʻāpahu Ahupuaʻa within Kalepa, ʻAlelele, Lelekēa, and Kukuiʻula Valleys. Portions of the Nuʻu parcel, including the proposed road corridor and helicopter landing zone, were surveyed between 2012 and 2014, with over 1,600 features identified and grouped into 76 archeological sites (Tomonari-Tuggle et al. 2015).

In addition, limited surveys have been completed in the upper elevations of Haleakalā National Park. Previous archeological surveys between the 1,800-foot and 4,600-foot elevation contour levels of the

Kīpahulu District of the park conducted by NPS Pacific Archeologist Gary Somers between 1985 and 1989 encountered no surface archeological sites. Similarly, a previous archaeological survey between the 2,400-foot and 5,000-foot elevation contour levels of the Kīpahulu District of the park conducted by Haleakalā National Park Archeologist Elizabeth Gordon in 2004 encountered no surface archeological sites. In 2015, Haleakalā National Park Archeologist Rachel Hodara Nelson surveyed portions of the Nuʻu parcel between the 2,500-foot and 1,400-foot contours. No new sites were identified during that survey.

Overall, no impacts to archeological resources are anticipated to result from the proposed action. Much of the project area has not been surveyed, but no new ground disturbance would result from the proposed action. To help mitigate potential effects of ground-based activities on previously undiscovered archaeological resources, pedestrian releases and monitoring would only be conducted via existing, previously disturbed resource management trails and fence lines, as well as camping at established remote camps or helicopter landing zones for overnight stays, to avoid new ground disturbance. Helicopter operations would utilize existing, previously disturbed landing zones. These existing areas (trails, fence lines, and landing zones or camps) have been cleared through previous environmental compliance conducted by the state or park. Therefore, this issue was considered and dismissed from further analysis.

Cultural Landscapes

The NPS defines cultural landscapes as geographic areas associated with historic events, activities, or people that reflect the history of the park unit, development patterns, and the relationship between people and the park. Portions of the Haleakalā Highway Historic District, Puʻunianiau Area, and Hosmer Grove Campground and Picnic Area cultural landscapes are within the project area near the entrance to Haleakalā National Park. No impacts to cultural landscapes are anticipated to result from the proposed action. Much of the project area has not been surveyed, but no new ground disturbance would result from the proposed action. To help mitigate potential effects of ground-based activities on cultural landscapes, pedestrian releases and monitoring would only be conducted via existing, previously disturbed resource management trails and fence lines, as well as camping at established remote camps or helicopter landing zones for overnight stays, to avoid new ground disturbance. Helicopter operations would utilize existing, previously disturbed landing zones. These existing areas (trails, fence lines, and landing zones or camps) have been cleared through previous environmental compliance conducted by the state or park. The proposed action will result in limited visual and noise impacts to the feeling and setting of historic period cultural landscapes. However, these noise and visual impacts have been minimized in order to limit negative impacts to cultural landscapes. The proposed action has minimized the use of helicopters with the increased focus on the use of drones, which are smaller and quieter than helicopters. Therefore, this issue was considered but dismissed from further analysis.

Ethnographic Resources and Traditional Cultural Practices

As defined in NPS Director's Order 28: Cultural Resource Management Guidelines (NPS 1998), ethnographic resources can be both natural and cultural resources that have been identified as having cultural significance by culturally associated users. They can include subsistence and ceremonial sites, structures, objects, and rural and urban landscapes assigned cultural significance by traditional users. The Summit of Haleakalā, including the Kīpahulu Valley and Kaupō Gap, has been determined eligible for the National Register of Historic Places as a traditional cultural property (TCP) "for its association with the cultural landscape of Maui and because it has known uses, oral history, mele, and legends, was a source for both traditional materials and sacred uses, and is a place exhibiting spiritual power. The sacred essence of the mountain includes the sky above (Prasad and Tomonari-Tuggle 2008).

Approximately 300–500 Hawaiians enter the Lower Kīpahulu Valley area of the park for traditional cultural practices annually; the Upper Kīpahulu Valley is a designated Biological Reserve closed to all public access. Archeological resources, recorded and oral histories, and Native Hawaiian traditions provide valuable information related to cultural land use, settlement patterns, and ethnographic practices within the Kīpahulu District. Cultural practices known to occur in this section of the park include performance of ceremonies and spiritual training, and farming. Native Hawaiians have strong cultural and spiritual connections to the resources and land located within the park as well as on DLNR and private lands within the project area.

Section 5.3.5.3 of NPS *Management Policies 2006* commits the NPS to adopt "a comprehensive approach towards appreciating the diverse human heritage and associated resources that characterize the national park system." The proposed action will result in limited visual and noise impacts to the feeling and setting of ethnographic resources, including the Haleakalā Summit, Kīpahulu Valley, and Kaupō Gap Traditional Cultural Property. Noise associated with helicopter or drone flights and their visual intrusion could potentially be a disturbance to the traditional users of park or state areas and could potentially detract from their enjoyment and use. However, these noise and visual impacts have been minimized in order to limit negative impacts to ethnographic resources. Park operations, e.g., flight times and flight paths, would be planned to balance efficiency and any potential impacts. The proposed action will minimize the use of helicopters and focus on the use of drones, which are smaller and quieter than helicopters. Any necessary helicopter flights would be planned to avoid the park's annual commercial-free days. As specified in the park's Commercial Services Plan, commercial-free days are opportunities for Kānaka Maoli (Native Hawaiians) to conduct traditional cultural practices in the park without commercial tours present. In 2023, the commercial-free days will occur on January 6 (end of Makahiki); May 24 (Zenith Noon); June 21 (Summer Solstice); July 18 (Zenith Noon); October 27 (start of Makahiki); and December 21 (Winter Solstice). The commercial-free days are designated prior to the start of the calendar year and change slightly each year. They are determined in consultation with the Native Hawaiian Community. The NPS consulted with the Native Hawaiian Community to identify any impacts from the proposed action and no substantial comments have been received to date. DLNR prepared a Cultural Impact Assessment (CIA) as part of compliance with the Hawaiʻi, Environmental Policy Act (HEPA). The CIA states:"Due to the size of the project area, this cultural impact assessment did not identify or inventory individual historic sites within the project area. Due to the nature of the activities, it is not anticipated that these activities could impact, modify, or effect historic properties in the project area" (Watson 2022: 43).

In the CIA (Honua Consulting 2022: 25-26) Kepā Maly describes the significance of natural resources to Native Hawaiians:

> We find in native traditions and beliefs, that Hawaiians shared spiritual and familial relationships with the natural resources around them. Each aspect of nature from the stars in the heavens, to the winds, clouds, rains, growth of the forests and life therein, and everything on the land and in the ocean was believed to be alive. Indeed, every form of nature in ancient Hawaiʻi was believed to be a body-form of some god or lesser deity. In the Hawaiian mind, care for each aspect of nature, the kino lau (myriad body-forms) of the elder life forms, was a way of life. This concept is still expressed by Hawaiian kūpuna (elders) through the present day and passed on in many native families. Also, in this cultural context, anything which damages the native nature of the land, forests, ocean, and kino lau therein, damages the integrity of the whole. Thus, caring for, and protecting the land and ocean resources, is a way of life. Furthermore, in the traditional context above referenced, we find that the mountain landscape, its' native species, and the intangible components therein, are a part of a sacred Hawaiian landscape. Thus, the natural landscape itself is a highly valued cultural property. It's protection, and the

continued exercise of traditional and customary practices, in a traditional and customary manner, are mandated by native custom, and State and Federal Laws.

Based on the research and ethnographic data within the CIA report, it was found that it would be unlikely that the proposed action would adversely impact traditional or customary practices. Yet, it is clear that additional education and outreach is needed, particularly to the practitioner community. Hunters use the project area extensively, and they hunt for subsistence. This subsistence lifestyle provides critical protein and food resources to families in East Maui" (Watson 2022: 84). Thus far the NPS has conducted two virtual public meetings to collect initial comments in the development of the draft EA. Information may be found here: Park planning - Suppression of Non-native Mosquito Populations to Address the Impacts of Avian Malaria on Threatened and Endangered Forest Birds (nps.gov) and here: Birds Not Mosquitoes. The state DLNR and Birds not Mosquitoes, a public-private partnership, plans to do additional outreach to East Maui communities to educate about this project. To mitigate potential public concerns regarding Wolbachia-incompatible mosquito releases, the IIT project team consulted with the DLNR Maui Branch Manager to identify areas on state lands commonly used by hunters or cultural practitioners. Most public hunting areas within the East Maui project area are only open on weekends, when it's unlikely that mosquito release operations will take place. Further, most treatment area points on public hunting lands are in remote upland areas rarely visited by hunters. The one exception is the Makawao Forest Reserve, where there are 60 release points, which would take approximately 1-2 hours to treat by aerial methods. The reserve is open for hunting and other recreational activities daily. Those activities may include plant and flower gathering for lei making and other traditional Hawaiian practices. The project team met with the DLNR Na Ala Hele trail advisory committee on July 27, 2022, to discuss potential concerns and how best to communicate IIT implementation plans in that popular recreational area. The project team will work with DLNR to post signage on trails communicating release plans, and to participate in public outreach events. On DLNR lands, Native Hawaiian organizations would be notified prior to any planned release efforts.The CIA also found that native birds could be considered a cultural resource as they are entwined in both Hawaiian culture and tradition across the islands. The history of the birds in Hawaiʻi is one of tremendous adaptive radiation due to geographic isolation resulting in numerous species of birds found nowhere else on earth. The use of helicopters and drones under the proposed action could temporarily disturb native forest birds, but over the long term there would be substantial benefits by minimizing the spread of avian malaria and reducing bird mortality.

In conclusion, any minimal impacts to ethnographic resources and traditional cultural practices would likely be temporary at any given location, though releases would likely occur over the long term. In addition, reduction of avian malaria as proposed would conserve numerous rare birds important to Native Hawaiian culture providing a beneficial impact. This issue was considered and dismissed from further analysis in the EA but was assessed in the aforementioned CIA which is included as an appendix to the EA.

Geological Features and Soils

No impacts to geological features are anticipated to result from the proposed action. Any disturbances to bedrock geology or soils from pedestrian release activities and monitoring would be minimal, and therefore have negligible effects on soils. To help mitigate any effects of ground disturbance, ground-based monitoring efforts and pedestrian mosquito releases would be conducted on existing resource management trails and fence lines to avoid disturbance. Helicopter operations would utilize existing, previously disturbed landing zones. For these reasons, impacts to geology and soils were considered and dismissed from further analysis.

Lightscapes

No impacts to lightscapes are anticipated to result from the proposed action. All work would be conducted during daylight hours. This issue was considered and dismissed from further analysis.

Land Use

No impacts to land use are anticipated to result from the proposed action. All current land uses would continue as is under the proposed action. This issue was considered and dismissed from further analysis.

Environmental Justice

Environmental justice is the fair treatment and meaningful involvement of all people regardless of race, color, national origin, or income, with respect to the development, implementation, and enforcement of environmental laws, regulations, and policies. Executive Order 12898, Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations, provides that "each Federal agency shall make achieving environmental justice part of its mission by identifying and addressing, as appropriate, disproportionately high and adverse human health or environmental effects of its programs, policies, and activities on minority and low-income populations." A minority population exists within an affected area when either the minority population exceeds 50%, or the minority population is meaningfully greater than the minority population of the general population (CEQ 1997).

According to EJScreen, EPA's Environmental Justice Screening and Mapping Tool, census block groups within and around the project area on East Maui are comprised of populations where at least 50 percent of the population is considered a minority. Therefore, environmental justice communities exist in the study area. The proposed action involves the use of drones and helicopters to release incompatible mosquitoes for the purpose of suppressing the spread of avian malaria. The mosquitoes that would be released provide no threat to the public as they would be male mosquitoes (which don't bite) and do not transmit disease to humans. While the suppression of avian malaria should result in a positive overall effect on the East Maui ecosystem, mosquito release methods would involve the use of aircraft which could adversely impact the public who are recreating on public and conservation lands during project implementation. These potential impacts would mostly be due to the noise or visual disturbance from aircraft. Similar aerial operations are already ongoing on state and federal lands on East Maui. There would be minimal or no adverse effects on the public outside of the project area. Because noise and visual impacts could primarily affect only those members of the public that are actively recreating in the project area during implementation, there would be no low income or minority populations that would be disproportionately affected by project activities. Therefore, this issue was considered and dismissed from further analysis.

Socioeconomics

The economy of Maui County has a high reliance on the visitor industry, with 34,400 jobs or approximately 41 percent of all jobs in the county being visitor-related in the categories of food services, accommodation, retail trade, and arts, entertainment, and recreation (Department of Business, Economic Development & Tourism 2018). The majority of visitors travel to Kīpahulu by way of the state- and county-maintained Hāna Highway through the community of Hāna. The Kīpahulu District can receive over 500 cars per day and as many as 1,500 to 1,800 people per day during peak times (NPS 2022).

Tourism is the largest single source of private capital for Hawaiʻi's economy. Tourism in Maui contributed $14.0 million per day to the local economy in 2019. The Hawaiʻi Tourism Authority anticipates continued growth in tourism from "upgrades" to natural resources and increased distribution of visitors to the "neighbor" islands. In 2007 $35 million in tourism spending in the State of Hawaiʻi supported 172,000 jobs; in 2017 these figures had grown to $46 million in spending and 203,000 jobs supported (Hawaiʻi Tourism Authority 2019). Birding significantly drives visitation within The Nature Conservancy's (TNC's) Waikamoi Preserve because much of the pristine forest habitat where rare forest birds can be viewed within state forest reserves and the park is inaccessible to birders seeking a glimpse of rare forest birds. Exceptions would be Hosmer Grove and Palikū, which is adjacent to Kīpahulu Valley, in the park where ʻiʻiwi can be seen. The Waikamoi Preserve can only be accessed with permission from TNC. Public trips for birding typically occur once per month and have a maximum of 15 participants.

The proposed action could potentially adversely impact birding trips within the Waikamoi Preserve, but only minimally due to occasional noise from field crews, helicopters, or drones; however, tourism related to birding only comprises a small portion of local tourism, and there would be a beneficial impact to birding from suppression of avian malaria and increased viability of native forest bird populations. No measurable impact to the local economy would occur as a result of the proposed action. Therefore, this issue was considered and dismissed from further analysis.

Viewsheds

Under the proposed action, helicopters and drones would be visible above the tree canopy for very limited periods of time during flights to release mosquitoes, but the visual intrusion would be temporary, perhaps a few minutes at a time in each location and impacts would be considered *de minimis*. There would be no permanent impacts to viewsheds. Therefore, this issue was considered and dismissed from further analysis.

Floodplains

No impacts to floodplains are anticipated to result from the proposed action because the project would not result in disturbance to designated floodplains which are primarily located downstream of the project area. According to the State of Hawaiʻi, DLNR, Flood Hazard Assessment Tool, the project area overlaps with many streams originating on the slopes of the park that have designated floodways. However, only pedestrian release routes via existing trails and fence lines and helicopter or drone landing zones or camps would be used. Therefore, this issue was considered and dismissed from further analysis.

Marine or Estuarine Resources

No impacts to marine or estuarine resources are anticipated to result from the proposed action as the project area is in terrestrial areas only. Therefore, this issue was considered and dismissed from further analysis.

Water Quality or Quantity

The proposed action would not affect water quality in any measurable manner because care would be taken to avoid water sources during pedestrian, helicopter, or drone releases of mosquitoes. This project would involve no change to water quantity in East Maui as water is not required for implementation of this project. Therefore, this issue was considered and dismissed from further analysis.

Wetlands

No impacts to wetlands are anticipated to result from the proposed action because pedestrian release routes and helicopter and drone landing sites would avoid wetland areas. Ground-based monitoring efforts and mosquito releases would be conducted on existing resource management trails and fence lines. Helicopter operations would utilize existing, previously disturbed landing zones. These existing areas (trails, fence lines, and landing zones or camps) have been cleared through previous environmental compliance conducted by the state or park. No protected wetland areas would be disturbed during implementation of the proposed action. Therefore, this issue was considered and dismissed from further analysis.

Human Health and Safety

Under the proposed action, pedestrian release, helicopter long line, and drone operations would present some risk of accidents or injuries to employees and contractors during ground crew transportation or mosquito release. In addition, ground crews would be subject to some risk of injury from hiking in remote areas and through difficult terrain. The NPS and State of Hawaiʻi have strict guidelines and safety/training standards that are followed on all management projects and would be followed under the proposed action. Safety is paramount to all missions.

The released mosquitoes pose no risk to human health. Only male mosquitoes will be released and only female mosquitoes bite animals or humans. The risk of females being accidentally released is estimated to be 1 out of 900 million (Crawford et al. 2020). Even if a female is released, a bite from a released female will pose no risk to humans and no greater risk to wildlife than a wild female mosquito currently in the environment. Wolbachia cannot live within vertebrate cells and cannot be transferred to humans even through the bite of an infected mosquito (Popovici et al. 2010). Additionally, no new organisms would be introduced to Hawaiʻi by the proposed action. Humans are commonly bitten by the Asian tiger mosquito, Aedes albopictus, infected with the strain of Wolbachia that would be transfected into the southern house mosquitoes for release. The southern house mosquito also bites humans, and this species is also naturally infected with Wolbachia. Thus, humans in Hawaiʻi are regularly bitten by mosquitoes containing Wolbachia, including the strain that would be used in the proposed action, and no ill effects have ever been reported nor would there be a mechanism for this to occur. Further, there is no indication that the released mosquitoes would be any better at transmitting disease to humans or wildlife. Popovici et al. (2010) addresses many potential concerns regarding releasing Wolbachia-infected mosquitoes.

Aerial mosquito release operations would be carried out by only trained personnel and contractors approved by the U.S. Department of Interior Office of Aviation Services and would be required to observe proper safety protocols and use proper personal protective equipment. Equipment would be well-maintained and helicopter flights would only occur during favorable weather conditions. In addition, an aviation safety plan specific to this project would be developed and implemented. A safety briefing would be performed for each flight. Agencies would seek to minimize the risk of accident or injury during helicopter-based release activities and temporarily cease operations if unsafe conditions exist. Given the proposed action includes activities that are routinely carried out already and there would be no or only minimal risk to visitors, and that released mosquitoes pose no risk to human health, this issue was considered and dismissed from further analysis.

Alternatives Potentially Considered but Dismissed from Further Consideration

During the development of the proposed action and refinement of the project’s purpose and need statement, the NPS and DLNR considered numerous alternatives that were ultimately dismissed from detailed analysis. A summary of these alternatives and reasons for their dismissal from further consideration are provided below. The NPS and DLNR dismissed alternatives determined to be infeasible and as such would not accomplish the purpose and need of the project, which is to substantially suppress or eliminate wild mosquito populations and thus avian malaria in threatened and endangered forest bird populations on East Maui. The following alternatives were therefore considered but dismissed from further consideration in the EA.

Sterile Insect Technique

The Sterile Insect Technique (SIT) aims to sterilize male insects and release them into the wild population to reduce reproductive output and suppress insect populations. The technique has been successfully applied globally to several species of pests, including some species of mosquito (Dyck et al. 2021). The primary method for sterilizing male mosquitoes is through gamma ray exposure, which induces random breaks in the DNA to cause infertility (Klassen and Curtis 2021). Captive reared gamma-irradiated males are released into a population to mate with wild females, which in turn would lay non-viable eggs. Initial *Culex* SIT field trials demonstrated success in inducing modest to high levels of sterility in wild females by releasing irradiated males in small areas of India and Florida (Patterson et al. 1975, 1977). Larger SIT field trials were complicated by mated female immigration (Yasuno et al. 1975) and several *Aedes* SIT trials indicated that irriadiated males had reduced mating competitiveness (Bellini et al. 2013, Yamada et al. 2014). Concerns regarding the quality of SIT males and their mating competitiveness were alleviated by several successful *Aedes* SIT trials (Ageep et al. 2014, Madakacherry et al. 2014), but uncertainties remained for *Culex*. During the "To Restore a Mosquito-Free Hawai'i" workshop in 2016, experts weighed the advantages and disadvantages of both the Incompative Insect Technique (IIT) and SIT methods and cited evidence of reduced fitness of SIT male *Aedes* mosquitoes when compared to IIT *Wolbachia* males (Atyame et al. 2016). The group expressed the need for additional laboratory research for identifying the irradiation dose that would fully sterilize males and maintain competitiveness with wild *Culex* males in Hawaiian rainforests. Elsewhere, SIT has been applied in conjunction with several *Aedes* IIT programs, primarily as a means for ensuring that no sterile *Wolbachia* females are released accidentally with *Wolbachia* males (Zhang et al. 2015, Bourtzis et al. 2016). Advancements in sex-sorting techniques reduced the need for integrated SIT and IIT programs and help propel IIT as the primary means for suppressing *Culex* populations in Hawai'i. Researchers are working to overcome the complication of reduced competitiveness in irradiated *Culex* males and their findings should determine if SIT will be a viable tool worth considering in the future. Because this is not a viable tool at this time, this alternative would not meet the purpose and need for taking action, and therefore has been dismissed from detailed consideration.

Introducing Self-Limiting Male Mosquitoes with Edited Genes

Male mosquitoes may be engineered to contain a self-limiting gene that, when passed to offspring, prevents the offspring from developing into adulthood. This method has been proposed for implementation in Florida, and an Experimental Use Permit was issued by the Food and Drug Administration. After extensive evaluation of the best available science and public input, the U.S. Environmental Protection Agency (EPA) granted an experimental use permit to Oxitec Ltd. to field test the use of genetically engineered *Aedes aegypti* mosquitoes as a way to reduce mosquito populations to protect public health from mosquito-borne illnesses (EPA 2020). However, this technology is not currently available for near-term implementation of *Culex* mosquitoes. There may also be considerable public resistance to this method as has been seen in Florida. Because this technology is not currently available, this alternative would not meet the purpose and need for taking action, and therefore has been dismissed from detailed consideration.

Gene Drive

The gene drive method involves introducing a novel DNA sequence that permanently transfers a useful trait into a wild population to eliminate the population or render it inert for the threat it poses. In this system, this would be done by engineering *Culex* mosquitoes to carry a certain gene and releasing those mosquitoes into the wild to spread that particular trait. The gene in the released mosquitoes may theoretically code for any number of traits including mutations resulting in mortality or even alter vector-parasite compatibility. This method has the ability to eliminate mosquito populations island-wide or alter the population in a lasting manner. Although there would be up-front development costs, there may be no need to repeatedly deploy treatment mosquitoes once introduced as this is not a self-limiting method. However, this technology is still approximately 10–20 years away from viability and has not been proven or tested in the field. Safeguards would also need to be developed and there may be some public resistance to a tool using genetic modification. Because this technology is not currently available, this alternative would not meet the purpose and need for taking action, and therefore has been dismissed from detailed consideration.

Mosquito Habitat Source Reduction

Alteration or removal of water bodies has long been used to control mosquito numbers through reduction in larval habitat. Draining or channelizing waterways has been an effective method of reducing standing water and thus suppressing mosquito reproduction for centuries. However, alteration of the natural hydrology of an area can have significant effects, impacting numerous species and entire ecosystems. The hydrology of the mountainous regions of Hawai‘i, including the project area considered here, is driven by rainfall patterns and little ground water is maintained for long periods in lakes, ponds, or wetlands that could act as breeding grounds for mosquitoes. Thus, there are few wetland/marsh habitats to drain or alter in the project area, even if such an action was considered. Additionally, enumerable species depend on the natural flow of water on the landscape and there is a high likelihood of significant adverse impacts to other listed species or species of concern. Because this alternative would not meet the purpose and need for taking action and due to the potential for severe environmental impacts, it has been dismissed from detailed consideration.

Biological Larvicide Controls

Bacterial and other biological larvicides have been developed and are commercially available for the control of mosquito populations. One such bacterial control agent, *Bacillus thuringiensis* var. *israelensis* (Bti), can be effective for reducing mosquito larvae abundance. When applied to larval habitat, the microbe produces a toxin that is lethal when ingested by developing mosquito larvae. Bti larvicides (e.g., Vectomax® FG, Vectobac®, MosquitoDunks®) have demonstrated success for reducing *Culex* larvae abundance in areas of Kaua‘i Island, where pedestrian crews could access and apply the granular pesticide to standing pools of water (LaPointe et al. 2021). Because *Culex* are capable of breeding in a variety of habitats, including habitats rich in organic matter, the species can take advantage of pooled water in tree wells, pig wallows, and stagnant ground pools far from streams; thus, it is difficult to locate and treat these sources that are diffusely spread throughout native forest bird habitat. *Culex* mosquitoes can travel up to 3 kilometers in less than 12 days (LaPointe 2008), thus individuals can infiltrate relatively small locally treated areas. In 2019, standing pools of water were treated with Vectomax® FG in a 170-ha area where 14 kiwikiu birds were translocated and nearly every individual bird suffered mortality because of exposure to avian malaria (Warren et al. 2021). Scaling up Bti treatments to a landscape level in wet and steep environments could be logistically infeasible. Bti has been aerially broadcasted in several parts of the world, but its application in densely forested areas of Hawai‘i has not been tested. Additionally, Bti degrades under ultraviolet exposore (Zogo et al. 2019) and active ingredients can be flushed or diffused during rain events, thus the frequency of treatments could depend on local conditions and readily available resources, which may be impractical in most cases. Further, while Lapointe et al. (2021) observed no evidence of population level impacts to two non-target invertebrates, effects to several endemic flies, midges, and gnats have not been tested. Bti has potential for reducing larval abundance in combination with an IIT program, but the method alone is inadequate for suppressing mosquito populations within the entire East Maui project area. Because this alternative would not meet the purpose and need for taking action, it has been dismissed from detailed analysis.

Chemical Controls

Successful control and eradication of disease-carrying mosquitoes has been accomplished globally using several pesticides, such as organophosphate or organochloride insecticides. Widescale application of insecticides, in addition to removal of larval habitat, is responsible for the eradication of human malaria throughout the United States. However, there are no mosquito-specific insecticides available and most of the available insecticides are indiscriminate and could cause mortality of non-target native and listed insects and arthropods in the treatment area. Insecticides have also proven to have higher adverse effects through bioaccumulation (e.g., DDT in raptor eggs). Organophosphate and pyrethroid adulticides are among the most used insecticides used to control mosquitoes. However, resistance to these chemical agents has been documented in *Culex* spp. mosquitoes over the past several decades, potentially reducing the efficacy of these chemicals (Pasteur et al. 1984, Raymond et al. 2001, Liu et al. 2009). Targeted application of larvicides would be expected to impact federally listed damselflies. . Because implementation of this alternative could result in greater environmental effects to species in the project area, it has been dismissed from detailed consideration.

Translocation of Birds to Mosquito-free Areas

Translocation is the intentional effort to transport organisms from their current range to distinct locations to establish a second sustaining population. The practice has been applied with variable success for a number of rare birds in Hawaiʻi. Successful translocations are primarily restricted to the Northwestern Hawaiian Islands where *Culex* mosquitoes and avian malaria are absent. The U.S. Fish and Wildlife Service successfully established new populations of the Laysan Finch (*Telespiza cantans*) and ulūlu (Nihoa Millerbird; *Acrocephalus familiaris*) on Pearl and Hermes Atoll and Laysan Island, respectively (Morin and Conant 2020 a, b). However, most translocations in the main Hawaiian Islands have failed, including the recent translocation of wild and captive kiwikiu to a restored area of Nakula Natural Area Reserve on Leeward Maui. Nearly every bird died of avian malaria shortly after being released (Warren et al. 2021). Several efforts to reintroduce the endangered Palila (*Loxioides bailleui*) to former areas of its range in high montane and sub-alpine forests on Hawaiʻi Island failed, primarily because birds quickly returned to their native range where they had established pair bonds and territories (Banko et al. 2014). Because of the current conservation crisis, the translocation of four critically endangered honeycreepers (including kiwikiu and ʻākohekohe) to high elevation forests (>1,500 meters in elevation) on Hawaiʻi Island, where birds may be less vulnerable to disease because of cooler annual mean temperatures, was assessed by a group of translocation experts, cultural practitioners, and resource managers (Paxton et al. 2022). A panel of experts scored the probability of success for each species, and native Hawaiians, with strong connections to native birds, shared perspectives regarding moving birds from their endemic range to a separate island. The probability of success for each species ranged from 38 percent to 51 percent, meaning most experts predicted that the translocations would fail, except for the endangered ʻākohekohe, which had a near equal probability of failure and success. Cultural practitioners shared concerns about losing the cultural and familial connection to native avifauna and the potential suffering to individual birds during capture and transport efforts. The lack of remaining individuals in the wild to move and start a new population was one of the biggest factors in the decision process and there was little indication that translocated birds would be free from the threat of avian malaria, because of evidence that species vulnerable to the disease, such as the threatened ʻiʻiwi, were in decline throughout most of their range on Hawaiʻi island (Paxton et al. 2013, Kendall et al. 2022). Further, climate projections reduce current ranges of endangered birds on Hawaiʻi Island by more than 75 percent by years 2080–2100 and those species and translocated species would face similar challenges (Fortini et al. 2015). Translocation may be considered a complementary approach to the proposed action, potentially buying time for species in the wild while the threat of disease-carrying mosquitoes is being addressed, but the action would not meet the urgent need of preventing extinction of several endangered birds. Because this alternative would not meet the purpose and need for taking action, it has been dismissed from detailed analysis.

Treatment of Birds with Acute Infections using Anti-malarial Drugs

Vulnerable bird populations could be treated with injections of anti-malarial drugs (e.g., chloroquine, artesunate, primaquine, doxycycline). This approach could be effective in reducing the adverse effects of malaria in treated birds for a short period of time. The efficacy of anti-malarial drugs has been tested with variable success on poultry and captive penguins (Chitty 2011, Sohsuebngarm et al. 2014). Infected Hawaiian honeycreepers have also been successfully treated with these medications as well (Warren et al. 2021). This option is generally not feasible on a landscape or population scale because each individual bird would require repeated treatment. Individuals would need to be captured and identified for acute malaria with rapid testing techniques. Infected birds would be transported to a captive facility where a veterinarian could administer multiple doses of anti-malarial drugs. The birds' health and measures of malaria parasitemia would need to be monitored for several weeks until experts are confident to release individuals back into the wild, whereupon individuals would again be vulnerable to re-infection. The capture and transportation of infected birds, as well as the stress of captivity, could cause fatalities of sick individuals. It would be extremely labor intensive and impractical for reducing the impact of malaria among an entire community of threatened and endangered forest birds on East Maui. The approach could result in considerable environmental impact and possibly adverse impacts to threatened and endangered forest bird species. This approach was dismissed from further consideration because it does not meet the purpose and need and is technologically and economically infeasible.

Genetic Modification of Forest Birds

Under this scenario, forest bird genetic information would be modified to promote resistance to malarial infections. The practice of gene editing with CRISPR-Cas9 technology has been applied to domestic animals (Novak et al. 2018); for example, the genome of pigs was edited to enhance resistance to porcine reproductive and respiratory syndrome virus (Whitworth et al. 2016). Recently, the CRISPR-Cas9 tool was assessed in the conservation and recovery of the endangered black-footed ferret, a species vulnerable to sylvatic plague. The U.S. Fish and Wildlife Service approved an Endangered Species Recovery Permit for the foundational laboratory research for the genetic rescue of the species (Revive and Restore 2021), but the tool has not been applied to wild populations yet. Similarly, CRISPR-Cas9 could be applied to enhance resistance to avian malaria in Hawaiʻi. This facilitated adaptation through gene editing has been a modeled approach, but the tool has not been developed for honeycreepers in Hawaiʻi (Samuel et al. 2020). Technology for this approach is not available for near-term implementation. Genetic modification of culturally significant species could be highly controversial. This approach would not meet the purpose and need and is technologically infeasible at this time and has been dismissed from detailed analysis.

Ground Release of Mosquitoes using Cars, Trucks, or ATVs

Under this approach, *Wolbachia*-incompatible male mosquitoes are released into the wild via motor vehicles on the ground; wild female mosquitoes who mate with incompatible males lay eggs that do not hatch. Similar to the proposed action for this project, the regulatory path to obtain approval is defined and approvals are in place to use the approach to control mosquitoes of public health concern. The proposed project area covering the targeted birds' current and historic range is nearly entirely roadless. To release *Wolbachia*-incompatible male mosquitoes at the intervals necessary to achieve effective control, this approach would require construction of a vast network of roads that would be cost-prohibitive and would result in adverse environmental impacts to various natural and cultural resources. Roads and vehicles can create more larval habitat for mosquitoes as well as fragment critical habitat for endangered plants and animals present in the proposed project area. This approach, given current infrastructure, would not meet the project purpose and need and would likely result in significant adverse environmental impacts, and has therefore been dismissed from detailed analysis.

Pedestrian Release of Mosquitoes Without the Use of Helicopters

The project area in East Maui is 64,666 acres and is characterized by very remote, heavily forested, and exceptionally rugged terrain. Only a few roads penetrate this area and the only established trails accessible without the use of helicopters occur in Makawao Forest Reserve, Waikamoi Preserve, and the Lower Kīpahulu Valley comprising less than 2% of the overall project area. In order to release mosquitoes using only the pedestrian release method, a massive trail system would need to be developed over the entire East Maui project area at great cost and with resultant environmental impacts. In addition to the current lack of infrastructure that would potentially allow for mosquito releases without the use of helicopters, the short life span of the incompatible mosquitos would require rapid dispersal following shipment to Maui from the mainland, on the order of 24 hours. Pedestrian releases could not feasibly release mosquitoes throughout the project area within the required timeframe. Therefore, this alternative has been dismissed from detailed analysis.

REFERENCES

Ageep, T.B., D. Damiens, B. Alsharif, A. Ahmed, E.H.O. Salih, F.T.A Ahmed, A. Diabaté, R.S. Lees, and J.R.L. Gilles. 2014. El Sayed BB: participation of irradiated *Anopheles arabiensis* males in swarms following field release in Sudan. *Malaria Journal 13*: 484

Alexander, J.M., J.J. Lembrechts, L.A. Cavieres, C. Daehler, S. Haider, C. Kueffer, F. Liu, K. McDougall, A. Milbau, A. Pauchard, L. Rew, and T. Seipel. 2016. Plant invasions into mountains and alpine ecosystems: current status and future challenges. *Alpine Botany 126*: 89–103.

Atyame, C.M., N. Pasteur, E. Dumas, P. Tortosa, M.L. Tantely, N. Pocquet, S. Licciardi, A. Bheecarry, B. Zumbo, M. Weill, and O. Duron. 2011. Cytoplasmic incompatibility as a means of controlling *Culex pipiens quinquefasciatus* mosquito in the islands of the south-western Indian Ocean. *PLoS Neglected Tropical Diseases 5(12):* e1440. Available at https://doi.org/10.1371/journal.pntd.0001440

Atyame, C.M., J. Cattel, C. Lebon, O. Flores, J.-S. Dehecq, M. Weill, L.C. Gouagna, and P. Tortosa. 2015. *Wolbachia*-based population control strategy targeting *Culex quinquefasciatus* mosquitoes proves efficient under semi-field conditions. *PLoS ONE 10(3):* e0119288 Available at https://doi.org/10.1371/journal.pone.0119288.

Atyame, C.M., P. Labbé, C. Lebon, M. Weill, R. Moretti, F. Marini, L.C. Gouagna, M. Calvitti, and P. Tortosa. 2016. Comparison of Irradiation and *Wolbachia* Based Approaches for Sterile-Male Strategies Targeting *Aedes albopictus*. *PLoS ONE 11(1):* e0146834. Available at https://doi.org/10.1371/journal.pone.0146834

Banko, W.E., and P.C. Banko. 2009a. Historic decline and extinction. In: Conservation Biology of Hawaiian Forest Birds (T.K. Pratt, C.T. Atkinson, P.C. Banko, J.D. Jacobi, and B.L. Woodworth, eds.), pp. 25– 58. Yale University Press, New Haven, CT.

Banko, P.C., and W.E. Banko. 2009b. Evolution and ecology of food exploitation. In: Conservation biology of Hawaiian forest birds (T.K. Pratt, C.T. Atkinson, P.C. Banko, J.D. Jacobi, and B.L. Woodworth, eds.), pp. 159-193. Yale University Press, New Haven, CT.

Banko, P.C., K.A. Jaenecke, R.W. Peck, and K.W. Brinck. 2019. Increased nesting success of Hawai‘i ‘Elepaio in response to the removal of invasive black rats. *Condor 121:* 1–12.

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 endangered Maui parrotbill (*Pseudonestor xanthophrys*) with implications for recovery. *Endangered Species Research 278:* 189–194.

Bellini, R., F. Balestrino, A. Medici, G. Gentile, R. Veronesi, and M. Carrieri. 2013. Mating competitiveness of *Aedes albopictus* radio-sterilized males in large enclosures exposed to natural conditions. *Journal of Medical Entomology 50:* 94–102. Available at https://doi.org/10.1603/ME11058.

Chitty, J. 2011. Use of doxycycline in treatment of malaria in penguins. In E. Bergman (Ed.), *Proceedings of the Association of Avian Veterinarians 32nd Annual Conference & Expo with the Association of Exotic Mammal Veterinarians* (pp. 63–65). Seattle, WA.

Department of Business, Economic Development & Tourism (DBEDT). 2018. State of Hawaiʻi Quarterly Statistical and Economic Report, 1st Quarter 2018.

Department of Land and Natural Resources (DLNR). 2021, July 23. One Small Bird Provides Glimmer of Hope for Saving a Species [News release]. Available at https://dlnr.hawaii.gov/blog/2021/07/23/nr21-140/

Dyck V. A, J. Hendrichs, and A. S. Robinson. 2021. Sterile Insect Technique: Principles and Practice in Area-wide Integrated Pest Management. Springer.

Environmental Protection Agency (EPA). 2020. "EPA Approves Experimental Use Permit to Test Innovative Biopesticide Tool to Better Protect Public Health" Press Release, May 1. Available at: https://www.epa.gov/pesticides/epa-approves-experimental-use-permit-test-innovative-biopesticide-tool-better-protect.

EPA. 2021. Current Nonattainment Counties for All Criteria Pollutants. Available at https://www3.epa.gov/airquality/greenbook/ancl.html.

Fortini, L. B., A. E. Vorsion, 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.

Frazier, A.B. and T.W. Giambelluca. 2017. Spatial trend analysis of Hawaiian rainfall from 1920 to 2012. *International Journal of Climatology 37:* 522–2531.

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 Hawaiʻi. Final report submitted to the U.S. Army Corps of Engineers—Honolulu District, and the Commission on Water Resource Management, State of Hawaiʻi.

Hawaiʻi Tourism Authority. 2019. Fact Sheet: Benefits of Hawaiʻi's Tourism Economy

Honua Consulting. 2022. Cultural Impact Assessment Report for the Proposed Activities Associated with the Suppression of Non-Native Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on Maui. 88 pages.

Intergovernmental Panel on Climate Change (IPCC). 2007. Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri, R.K and Reisinger, A.(eds.)]. IPCC, Geneva, Switzerland, 104 pp.

Kendall, S. J., R. A. Rounds, R. J. Camp, and A. S. Genz. 2022. Forest bird populations at the Big Island National Wildlife Refuge Complex, Hawaiʻi. Hawaiʻi Cooperative Studies Unit Technical Report HCSU-102. University of Hawaiʻi at Hilo, Hawaiʻi, USA. 141 pages.

Klassen, W., C. F. Curtis, and J. Hendrichs. 2021. History of the sterile insect technique. In: Dyck VA, Hendrichs J, Robinson AS, editors. Sterile insect technique: principles and practice in area-wide integrated pest management. Berlin: Springer; 2005. p. 3–36.

LaPointe, D. A. 2008. Dispersal of *Culex quinquefasciatus* (Diptera: Culicidae) in a Hawaiian Rain Forest. *Journal of Medical Entomology 45:* 600-609.

LaPointe, D. A., T.V. Black, M. Riney, K.W. Brinck, L.H. Crampton, and J. Hite. 2021. Field trials to test new trap technologies for monitoring *Culex* populations and the efficacy of the biopesticide formulation VectoMax® FG for control of larval *Culex quinquefasciatus* in the Alaka'i Plateau, Kaua'i, Hawaii.

Liu, N. Q. Xu, T. Li, L. He, and L. Zhang. 2009. Permethrin resistance and target site insensitivity in the mosquito *Culex quinquefaciatus* in Alabama. *Journal of Medical Entomology 46:* 1424–1429.

Madakacherry O., R.S. Lees, J.R.L. Gilles. 2014. *Aedes albopictus* (Skuse) males in laboratory and semi-field cages: release ratios and mating competitiveness. *Acta Tropica 2014, 132S:* 124S-129S.

McKenzie, M. M., T. W. Giambelluca, and H.F. Diaz. 2019. Temperature trends in Hawai'i: A century of change, 1917–2016. *International Journal of Climatology 39(10):* 3987–4001.

Morin, M. P. and S. Conant. 2020a. Laysan Finch (*Telespiza cantans*), version 1.0. In Birds of the World (A. F. Poole, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA. Available at https://doi.org/10.2173/bow.layfin.01.

Morin, M.P., S. Conant, and P. Conant. 2020b. Millerbird (*Acrocephalus familiaris*), version 1.0. In Birds of the World (A. F. Poole and F. B. Gill, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA. Available at https://doi.org/10.2173/bow.miller.01

National Park Service (NPS). 1998. U.S. Department of the Interior. NPS-28: Cultural Resource Management Guidelines. Available at https://irma.nps.gov/DataStore/DownloadFile/466037

National Park Service (NPS). 2006. U.S. Department of the Interior. Management Policies 2006. Available at https://www.nps.gov/subjects/policy/upload/MP_2006.pdf.

National Park Service (NPS). 2015. U.S. Department of the Interior. National Park Service NEPA Handbook. Director's Order 12. Available at http://www.nps.gov/applications/npspolicy/DOrders.cfm.

National Park Service (NPS). U.S. Department of the Interior. 2018. National Park Service Visitor Use Statistics. Available at https://irma.nps.gov/Stats/Reports/Park/HALE

National Park Service (NPS). 2022. NPS Stats. National Park Service Visitor Use Statistics. Available at https://irma.nps.gov/Stats/Reports/Park/HALE (accessed 11 February 2022).

Novak B.J., T. Maloney, and R. Phelan. 2018. Advancing a New Toolkit for Conservation: From Science to Policy. *The CRISPR Journal 11-15.* Available at https://doi.org/10.1089/crispr.2017.0019.

Pasteur, N., G.P. Georghiou, and A. Iseki. 1984. Variation in organophosphate resistance and esterase activity in *Culex quinquefasciatus* Say from California. *Génétique Sélection Évolution 16:* 271–284.

Patterson R.S., V.P. Sharma , K.R.P. Singh, G.C. Labrecque, P.L. Seetheram, and K.K. Grover. 1975 Use of radiosterilized males to control indigenous populations of *Culex-Pipiens Quinquefasciatus* say—laboratory and field studies. *Mosquito News 35(1):* 1–7.

Patterson R.S., R.E Lowe., B.J.Smittle, D.A. Dame, M.D. Boston, and A.L. Cameron. 1977. Release of radiosterilized males to control *Culex Pipiens-Quinquefasciatus* (Diptera Culicidae). *Journal of Medical Entomology 14(3):* 299–304.

Pauchard, A., A. Milbau, A. Albihn, J. Alexander, T. Burgess, C. Daehler, G. Englund, F. Essl, B. Evengård, G. Greenwood, S. Haider, J. Lenoir, K. McDougall, E. Muths, M. Nuñez, J. Olofsson, L. Pellissier, W. Rabitsch, L. Rew, M. Robertson, N. Sanders, and C. Kueffer. 2016. Non-native and native organisms moving into high elevation and high latitude ecosystems in an era of climate change: new challenges for ecology and conservation. *Biological Invasions 18:* 345–353.

Paxton, E.H., P.M. Gorresen, and R.J. Camp. 2013. Abundance, distribution, and population trends of the iconic Hawaiian Honeycreeper, the ‘I‘iwi (*Vestiaria coccinea*) throughout the Hawaiian Islands: U.S. Geological Survey Open-File Report 2013–1150. 59 pages.

Paxton, E.H., R.J. Camp, P.M. Gorresen, L.H. Crampton, D.L. Leonard, and E.A. VanderWerf. 2016. Collapsing avian community on a Hawaiian island. *Science Advances 2:* e1600029.

Paxton, E.H., M. Laut, J.P. Vetter, and S.J. Kendall. 2018. Research and management priorities for Hawaiian forest birds. *Condor 120:* 557– 565.

Paxton, E.H., M. Laut, S. Enomoto, and M. Bogardus. 2022. Hawaiian forest bird conservation strategies for minimizing the risk of extinction: Biological and biocultural considerations. Hawai‘i Cooperative Studies Unit Technical Report HCSU-103. University of Hawai‘i at Hilo, Hawaii, USA. 125 pages. Available at http://hdl.handle.net/10790/5386.

Raymond, M., C. Berticat, M. Weill, N. Pasteur, and C. Chevillon. 2001. Insecticide resistance in the mosquito *Culex pipiens*: what have we learned about adaptation? *Genetica 112–113:* 287–296.

Revive & Restore. 2021. The Black-Footed Ferret Project. Available at: https://reviverestore.org/projects/black-footed-ferret.

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 modeling approach. Ecological Applications 21: 2960–2973.

Sohsuebngarm, D., Sasipreeyajan, J., Nithiuthai, S., and Chansiripornchai, N. 2014. The efficacy of artesunate, chloroquine, doxycycline, primaquine and a combination of artesunate and primaquine against avian malaria in broilers. *The Journal of Veterinary Medical Science 76(6):*813–817. Available at https://doi.org/10.1292/jvms.13-0455

Tomonari-Tuggle, M.J., T.M. Rieth, M. Bell, D. Filimoehala, and E.E. Cochrane, 2015. Archaeological Inventory and Reconnaissance Surveys of Nu‘u, Haleakalā National Park, Maui Island, Hawai‘i, TMK (2) 1-8-001:002,Volume I, Narrative. Report Prepared for the National Park Service. Honolulu: International Archaeological Research Institute, Inc.

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, Hawai‘i. Pacific Cooperative Studies Unit Technical Report 201. University of Hawai‘i at Mānoa, Department of Botany. Honolulu, HI. 50 pages.

Warren, C.C., L.K. Berthold, H.L. Mounce, P. Luscomb, B. Masuda. L. Berry. 2020. Kiwikiu Translocation Report 2019. Internal Report. p. 1–101.

Warren, C.C., L.K. Berthold, H.L. Mounce, P. Luscomb, B. Masuda. L. Berry. 2021. Kiwikiu Translocation Report 2020. Internal Report. p. 1–101.

Whitworth K.M., R.R. Rowland, C.L. Ewen, B.R Trible, M.A. Kerrigan, A.G. Cino-Ozuna, M.S. Samuel, J.E. Lightner, D. G. McLaren, A.J. Mileham, K.D. Wells, and R.S. Prather. 2016. Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. *Natural Biotechnology 34:* 20–22. https://DOI.org/10.1038/nbt.3434.

Yamada, H., A.G. Parker, C.F. Oliva, F. Balestrino, and J.R.L. Gilles. 2014. X-ray-induced sterility in *Aedes albopictus* (Diptera: Culicidae) and male longevity following irradiation. *Journal of Medical Entomology 51(4):* 811–816. PMID: 25118413

Yasuno M., Macdonald W.W., Curtis C.F., Grover K.K., Rajagopalan P.K., Sharma L.S., Sharma V.P., Singh D., Singh K.R.P., Agarwal H.V., Kazmi S.J., Menon P.K., Menon R., Razdan R.K., Samuel D., Vaidyanathan V. 1975. A control experiment with chemosterilized male *Culex pipiens fatigans* Wiedemann in a village near Delhi surrounded by a breeding-free zone. Japanese *Journal of Sanitary Zoology 29:* 325–343.

Zhang, D., Zheng, X., Xi, Z., Bourtzis, K. and Gilles, J. R. L. 2015. Combining the Sterile Insect Technique with the Incompatible Insect Technique: I-Impact of *Wolbachia* Infection on the Fitness of Triple- and Double-Infected Strains of *Aedes albopictus*. *PLoS One 10:* e0121126.

APPENDIX C:
Cultural Impact Assessment

Cultural Impact Assessment Report for the Proposed Activities Associated with the Suppression of Non-Native Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on Maui

Prepared for

State of Hawaiʻi Department of Land and Natural Resources

Prepared by

HONUA CONSULTING

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Note on Hawaiian Language Use

In keeping with other Hawaiian scholars, we do not italicize Hawaiian words. Hawaiian is both the native language of the pae'āina of Hawai'i and an official language of the State of Hawai'i. Some authors will leave Hawaiian words italicized if part of a quote; we do not. In the narrative, we use diacritical markings to assist our readers, except in direct quotes, in which we keep the markings used in the original text. We provide translations contextually when appropriate. Unless otherwise noted, all translations are by Honua Consulting authors.

Front Cover Credit

Hawaii State Archives

n.d. Image of 'I'iwi bird, Jack Jefferies

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Executive Summary

At the request of Tetra Tech, Inc,, Honua Consulting, LLC prepared a Cultural Impact Assessment (CIA) for the State of Hawaiʻi Department of Land and Natural Resources for the Proposed Activities Associated with the Suppression of Non-Native Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on Maui. The "Project Area" includes 262-square kilometers in East Maui on various Tax Map Keys (TMKs).

Research in preparation of this report consisted of a thorough search of Hawaiian language documents, including but not limited to the Bishop Museum Mele Index and Bishop Museum archival documents, including the Hawaiian language archival cache. All Hawaiian language documents were reviewed by Hawaiian language experts to search for relevant information to include in the report. Documents considered relevant to this analysis are included herein, and translations are provided when appropriate to the discussion. Summaries of interviews and information on other oral testimonies are also provided herein.

Based on the information gathered and the assessment of the resources conducted, the project is not anticipated to have any adverse impacts on cultural resources, traditions, customs, or practices.

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Abbreviations and Acronyms

AIS: Archaeological Inventory Survey
BMP: Best Management Practice
CIA: Cultural Impact Assessment
DC: Direct Current
EA: Enviornmental Assessment
ESP: Environmental Review Project, Office of Planning and Sustainable Development
HAR: Hawaii Administrative Rules
HRS: Hawaii Revised Statutes
ILK: Indigenous Local Knowledge
Ka Pa'akai: *Ka Pa'akai O Ka 'Āina v. Land Use Commission*, 94 Haw. 31 (2000)
LRFI: Literature Review and Field Investigation
NPS: U.S. National Park Service
NRHP: National Register of Historic Places
OEQC: Office of Enviornmental Quality and Control
ROI: Range of Influence
SHPD: State Historic Preservation Division
SIHP: State Inventory of Historic Places
SLH: Session Laws of Hawaii
TEK: Traditional Ecological Knowledge
TMK: Tax Map Key
UH: University of Hawai'i
USGS: U.S. Geological Survey

1.0 Project Description and Compliance

At the request of Tetra Tech, Inc., Honua Consulting, LLC prepared a Cultural Impact Assessment (CIA) for the State of Hawaiʻi Department of Land and Natural Reesources for the Proposed Activities Associated with the Suppression of Non-Native Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on Maui. The “Project Area” includes 262-square kilometers in East Maui on various Tax Map Keys (TMKs).

The proposed action consists of releasing *Wolbachia*-infected male *Culex quinquefasciatus* mosquitos within an approximately 262-square-kilometer (64,660-acre) project area on East Maui, Hawaiʻi. *Wolbachia* is a naturally occurring bacterium. This approach renders the *Wolbachia*-infected male mosquitos incapable of producing viable offspring after mating with wild-type females, thus providing landscape-scale control of the *Culex* population.

1.1 Project Description and Proposed Action

The Project Area Overview map (Figure 1) illustrates the overall project area. As noted, this project will involve releasing *Wolbachia*-infected male *Culex quinquefasciatus* mosquitos. There is no ground disturbance or construction activities associated with these activities. It is unique among cultural assessments in that these activities are largely programmatic in nature and involve a large area rather than project-based involving only a defined project area.

Additionally, species are not limited to physical boundaries. Therefore, while there is a specified project area in east Maui, depending on where these individuals are released, they may travel, to the extent they are physically capable, within the region as a whole.

Therefore, this cultural assessment focuses primarily on the following:

1. Any potential cultural value of mosquitoes themselves, as the proposed activities would result in a decline of the population on Maui.
2. The cultural value of Hawaiian forest birds, which would positively benefit from a reduction in mosquitoes that carry diseases harmful to these birds.
3. Traditional or customary practices in the project area.

Due to the programmatic nature of this action, a more cursory background on the project area is provided. East Maui is a tremendously significant cultural environment, with an important political history. This history is provided to the extent appropriate to assess the proposed activities.

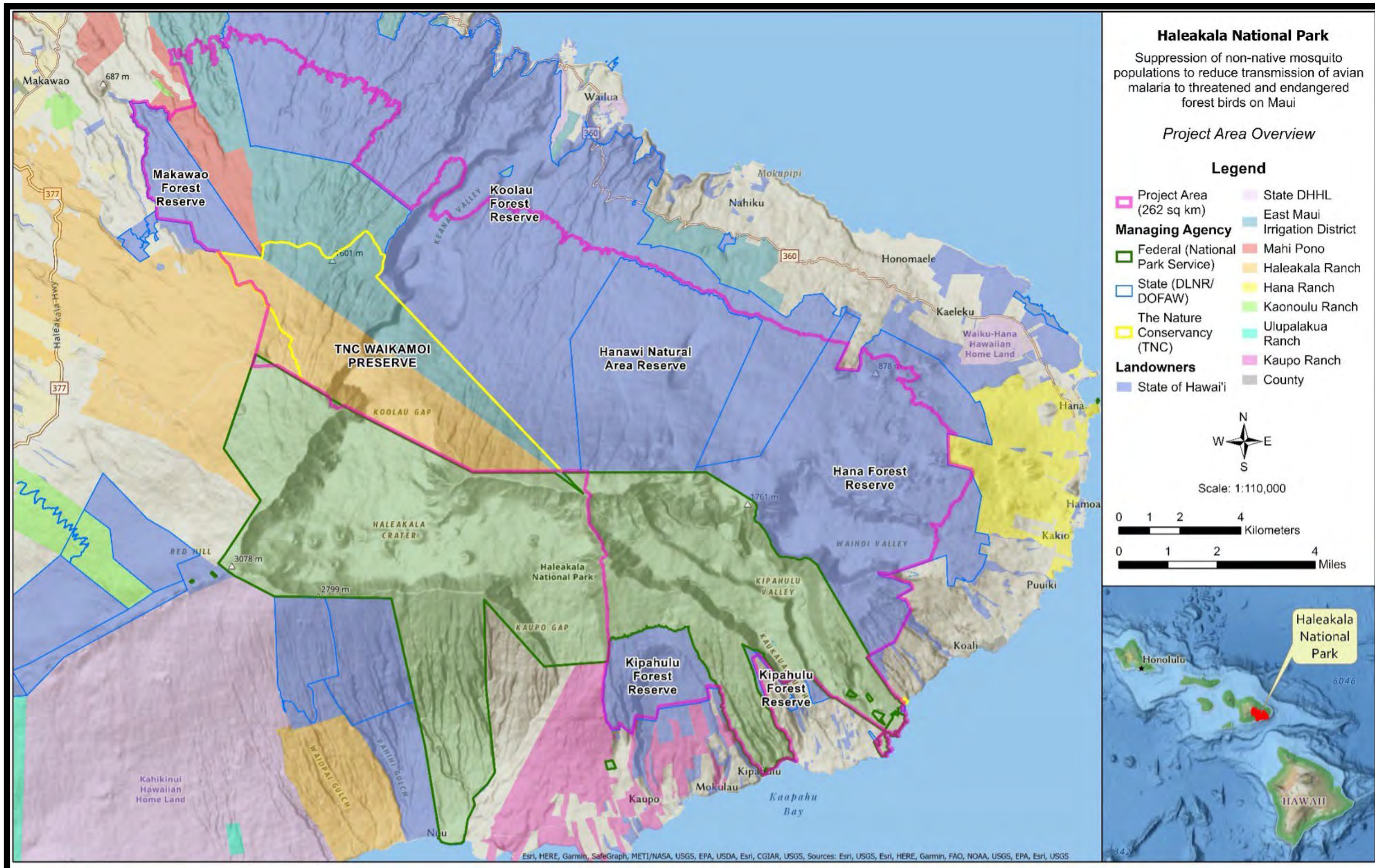


Figure 1. Project Area Overview (Tetra Tech)

1.2 Background

Articles IX and XII of the State Constitution, other state laws, and the courts of the state require government agencies to protect and preserve cultural beliefs, practices, and resources of Native Hawaiians and other ethnic groups. To assist decision makers in the protection of cultural resources, Chapter 343, HRS and Hawaiʻi Administrative Rules (HAR) § 11-200 rules for the environmental impact assessment process require project proponents to assess proposed actions for their potential impacts to cultural properties, practices, and beliefs.

This process was clarified by the Act 50, Session Laws of Hawaiʻi (SLH) 2000. Act 50 recognized the importance of protecting Native Hawaiian cultural resources and required some environmental review documents include the disclosure of the effects of a proposed action on the cultural practices of the community and state, and the Native Hawaiian community in particular. Specifically, the Environmental Council suggested the CIAs should include information relating to practices and beliefs of a particular cultural or ethnic group or groups. Such information may be obtained through public scoping, community meetings, ethnographic interviews, and oral histories.

It is important to note that while similar in their areas of studies, archaeological surveys and CIAs are concerned with distinct and different foci. Archaeological studies are primarily concerned with historic properties and tangible heritage, whereas CIAs look at cultural practices and beliefs, which can be associated with a specific location, but also often intangible in nature.

The State and its agencies have an affirmative obligation to preserve and protect Native Hawaiians' customarily and traditionally exercised rights to the extent feasible.[1] State law further recognizes that the cultural landscapes provide living and valuable cultural resources where Native Hawaiians have and continue to exercise traditional and customary practices, including hunting, fishing, gathering, and religious practices. In *Ka Paʻakai*, the Hawaiʻi Supreme Court provided government agencies an analytical framework to ensure the protection and preservation of traditional and customary Native Hawaiian rights while reasonably accommodating competing private development interests. This is accomplished through:

1) The identification of valued cultural, historical, or natural resources in the project area, including the extent to which traditional and customary Native Hawaiian rights are exercised in the project area;
2) The extent to which those resources—including traditional and customary Native Hawaiian rights—will be affected or impaired by the proposed action; and

1 Article XII, Section 7 of the Hawaiʻi State Constitution, Ka Paʻakai O Ka ʻĀina v. Land Use Commission, 94 Haw. 31 [2000] (Ka Paʻakai), Act 50 SLH 2000.

3) The feasible action, if any, to be taken to reasonably protect Native Hawaiian rights if they are found to exist.

The appropriate information concerning east Maui has been collected, focusing on areas near or adjacent to the project area. A thorough analysis of this project and potential impacts to cultural resources, historical resources, and archaeological sites is included in this assessment.

The CIA provides an overview of cultural and historic resources in the project area using thorough literature review, community and cultural practitioner consultation, and high-level, project-specific surveys. The CIA will focus on identifying areas in which disturbance should be avoided or minimized to reduce impacts to historic properties or culturally important features. The paramount goal is to prevent impacts through avoidance of sensitive areas and mitigating for impacts only if avoidance is not possible.

1.3 Geographic Extent

The geographic extent for impacts to cultural resources and historic properties includes the project area and localized surroundings. This CIA also reviews some of the resources primarily covered by the regulatory review. It primarily researches and reviews the range of biocultural resources identified through historical documents, traditional knowledge, information found in the Hawaiian language historical cache, and oral histories and knowledge collected from cultural practitioners and experts.

There is clear guidance from the Office of Environmental Quality and Control (OEQC), now known as the Environmental Review Project, Office of Planning and Sustainable Development (ESP), that recommends a geographic extent beyond the identified or typical boundaries of the geographic project area. The recommended area is typically the size of the traditional land area (ahupuaʻa) or region (moku), but this can be larger or smaller depending on what best helps to identify the resources appropriately.

The geographic extent of the CIA is based on the position that the “project area” is part of a cultural landscape or cultural landscapes that therefore it is most appropriate to set and study the proposed alternatives within that cultural context.

In this case, the project area includes most of east Maui.

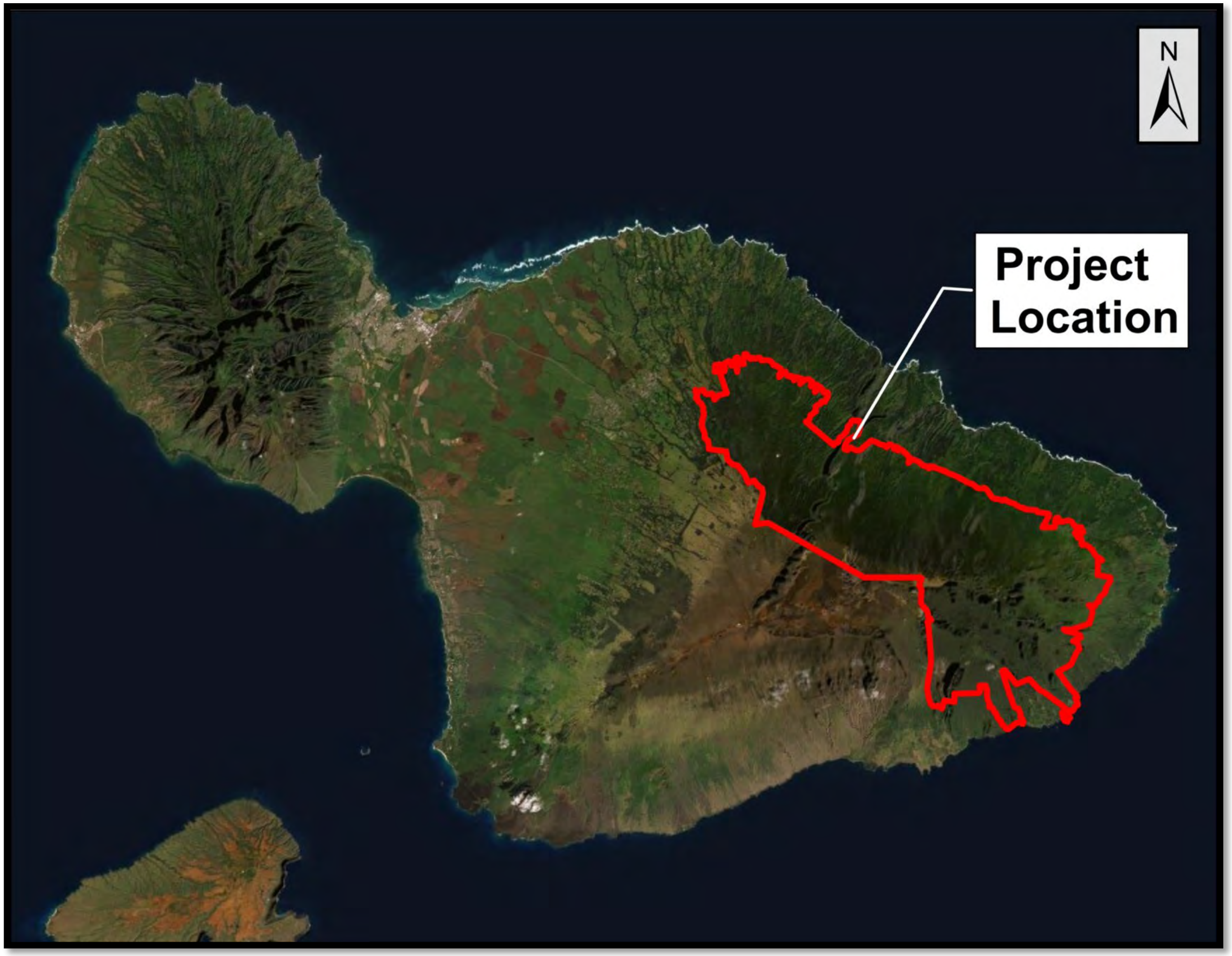


Figure 2. Project Area in relationship to the Island of Maui

Figure 3. Enlarged image of project area

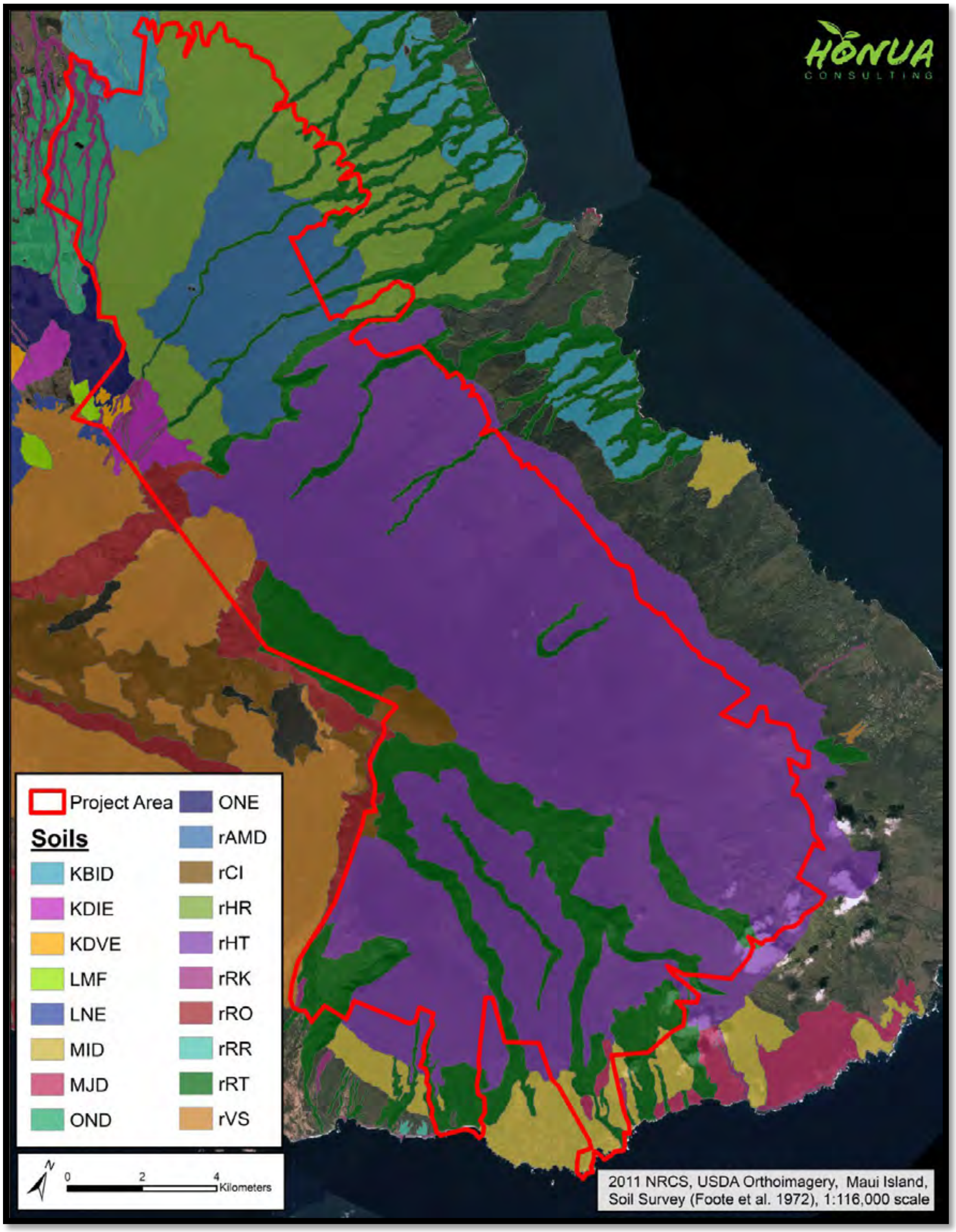


Figure 4. Soil types throughout project area

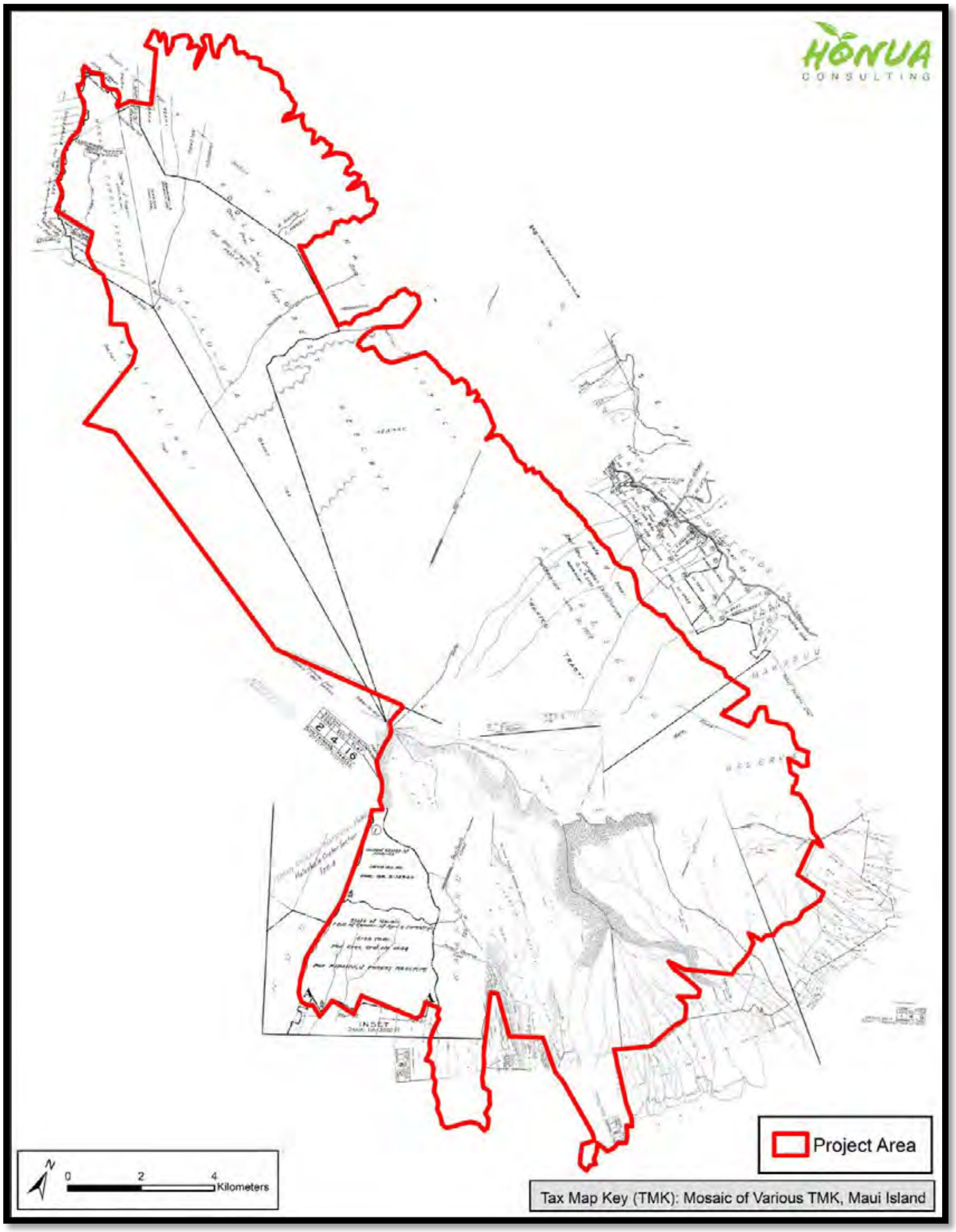


Figure 5. Project area with corresponding TMKs

1.4 Goal of Cultural Impact Assessment

This cultural impact assessment looks to partially fulfill the requirement of taking into account the Project's potential impacts on historic and cultural resources and, at a minimum, describe: a) any valued cultural, historic, or natural resources in the area in questions, including the extent to which traditional and customary native Hawaiian rights are exercised in the area, b) the extent to which those resources – including traditional and customary native Hawaiians rights – will be affected or impaired by the Project; and c) the feasible action, if any, to be taken to reasonably protect native Hawaiian rights if they are found to exist.

1.5 Regulatory Background

Articles IX and XII of the State Constitution, other state laws, and the courts of the state require government agencies to protect and preserve cultural beliefs, practices, and resources of Kānaka ʻŌiwi (Native Hawaiians) and other ethnic groups. To assist decision makers in the protection of cultural resources, Chapter 343, HRS and Hawaiʻi Administrative Rules (HAR) § 11-200 rules for the environmental impact assessment process require project proponents to assess proposed actions for their potential impacts to cultural properties, practices, and beliefs.

This process was clarified by the Act 50, Session Laws of Hawaiʻi (SLH) 2000. Act 50 recognized the importance of protecting Native Hawaiian cultural resources and required that EAs include the disclosure of the effects of a proposed action on the cultural practices of the community and state, and the Native Hawaiian community in particular. Specifically, the Environmental Council suggested the CIAs should include information relating to practices and beliefs of a particular cultural or ethnic group or groups. Such information may be obtained through public scoping, community meetings, ethnographic interviews, and oral histories.

It is important to note that while similar in their areas of studies, archaeological surveys and CIAs are concerned with distinct and different foci. Archaeological studies are primarily concerned with historic properties and tangible heritage, whereas CIAs look at cultural practices and beliefs, which can be associated with a specific location, but also often intangible in nature.

1.6 Compliance

The State and its agencies have an affirmative obligation to preserve and protect Native Hawaiians' customarily and traditionally exercised rights to the extent feasible.[2] State law

2 Article XII, Section 7 of the Hawaiʻi State Constitution, Ka Paʻakai O Ka ʻĀina v. Land Use Commission, 94 Haw. 31 [2000] (Ka Paʻakai), Act 50 SLH 2000.

further recognizes that the cultural landscapes provide living and valuable cultural resources where Native Hawaiians have and continue to exercise traditional and customary practices, including hunting, fishing, gathering, and religious practices. In *Ka Paʻakai*, the Hawaiʻi Supreme Court provided government agencies an analytical framework to ensure the protection and preservation of traditional and customary Native Hawaiian rights while reasonably accommodating competing private development interests. This is accomplished through:

4) The identification of valued cultural, historical, or natural resources in the project area, including the extent to which traditional and customary Native Hawaiian rights are exercised in the project area;
5) The extent to which those resources—including traditional and customary Native Hawaiian rights—will be affected or impaired by the proposed action; and
6) The feasible action, if any, to be taken to reasonably protect Native Hawaiian rights if they are found to exist.

While not attached to a HRS Chapter 343 action, this CIA was prepared under HRS Chapter 343 and Act 50 SLH 2000 as those are the prevailing standards and best practices for CIAs. The appropriate information concerning the ahupuaʻa has been collected, focusing on areas near or adjacent to the project area. A thorough analysis of this project and potential impacts to cultural resources, historical resources, and archaeological sites is included in this assessment.

The present analyses of archival documents, oral traditions (oli or chants, mele or songs, and/or hula or dance texts), and Hawaiian language sources including books, manuscripts, and newspaper articles, are focused on identifying recorded cultural and archaeological resources present on the landscape, including: Hawaiian and non-Hawaiian place names; landscape features (ridges, gulches, cinder cones); archaeological features (kuleana parcel walls, house platforms, shrines, heiau or places of worship, etc.); culturally significant areas (viewsheds, unmodified areas where gathering practices and/or rituals were performed); and significant biocultural resources. The information gathered through research helped to focus interview questions on specific features and elements within the project area.

Interviews with lineal and cultural descendants are instrumental in procuring information about the project area's transformation through time and changing uses. Interviews were conducted with recognized cultural experts and summaries of those interviews are included herein.

2.0 Methodology

The approach to developing the CIA is as follows:

1) Gather Best Information Available
    a) Gather historic cultural information from stories and other oral histories about the affected area to provide cultural foundation for the report;
    b) Inventory as much information as can be identified about as many known cultural, historic, and natural resources, including previous archaeological inventory surveys, CIAs, etc. that may have been completed for the possible range of areas; and
    c) Update the information with interviews with cultural or lineal descendants or other knowledgeable cultural practitioners.
2) Identify Potential Impacts to Cultural Resources
3) Develop Reasonable Mitigation Measures to Reduce Potential Impacts
    a) Involve the community and cultural experts in developing culturally appropriate mitigation measures; and
    b) Develop specific Best Management Practices (BMPs), if any are required, for conducting the project in a culturally appropriate and/or sensitive manner as to mitigation and/or reduce any impacts to cultural practices and/or resources.

While numerous studies have been conducted on this area, very few have effectively utilized Hawaiian language resources and Hawaiian knowledge. This appears to have impacted modern understanding of this location, as many of the relevant documents are native testimonies given by Kanaka Hawaiʻi (Hawaiians) who lived on this land.

While hundreds of place names and primary source historical accounts (from both Hawaiian and English language narratives) are cited on the following pages, it is impossible to tell the whole story of these lands in any given manuscript. A range of history, spanning the generations, has been covered. Importantly, the resources herein are a means of connecting people with the history of their communities—that they are part of that history. Knowledge of place will, in turn, promote appreciation for place and encourage acts of stewardship for the valued resources that we pass on to the future.

Background research for the literature review was conducted using materials obtained from the State Historic Preservation Division (SHPD) library in Kapolei and the Honua Consulting LLC. report library. On-line materials consulted included the Ulukau Electronic Hawaiian Database (www.ulukau.com), Papakilo Database (www.papakilodatabase.com), the State Library on-line (http://www.librarieshawaii.org/Serials/databases.html), and Waihona ʻĀina Māhele database (http://www.waihona.com). Hawaiian terms and place names were translated using the on-line Hawaiian dictionaries (Nā Puke Wehewehe ʻŌlelo Hawaiʻi) (www.wehewehe.com), *Place Names of Hawaiʻi* (Pukui et al. 1974), and *Hawaiʻi Place Names* (Clark 2002). Historic maps were obtained from the State Archives, State of Hawaiʻi Land Survey Division website (http://ags.hawaii.gov/survey/map-search/), UH-Mānoa Maps, Aerial Photographs, and GIS (MAGIS) website (http://guides.library.manoa.hawaii.edu/magis). Maps were geo-referenced for this report using ArcGIS 10.3. GIS is not 100% precise and historic maps were created with inherent flaws; therefore, geo-referenced maps should be understood to have some built-in inaccuracy.

While conducting the research, primary references included, but were not limited to: land use records, including the Hawaiian L.C.A. records from the Māhele ʻĀina (Land Division) of 1848; the Boundary Commission Testimonies and Survey records of the Kingdom and Territory of Hawaiʻi; and historical texts authored or compiled by: David Malo (1987); Samuel M. Kamakau (1964, 1991, 1992); records of the American Board of Commissioners of Foreign Missions (A.B.C.F.M.) (1820–1860); Charles Wilkes (1845); Alexander & Preston (1892–1894); Abraham Fornander (1916–1919); and many other native and foreign writers. The study also includes several native accounts from Hawaiian language newspapers (primarily compiled and translated from Hawaiian to English by K. Maly), and historical records authored by nineteenth century visitors, and residents of the region.

Historical and archival resources were located in the collections of the Hawaiʻi State Archives, Survey Division, Land Management Division, Survey Division, and Bureau of Conveyances; the Bishop Museum Library and Archives; the Hawaiian Historical Society and the Hawaiian Mission Children's Society Library; University of Hawaiʻi-Hilo Moʻokini Library; the National Archives and Records Administration (NARA), Maryland; the Library of Congress, Washington D.C.; the National Oceanic and Atmospheric Administration National Library, Maryland; the Smithsonian Institution Natural History and National Anthropological Archives libraries, Washington, D.C.; the Houghton Library at Harvard; the United States Geological Survey (USGS) Library, Denver; the Paniolo Preservation Society and Parker Ranch collections; private family collections; and in the collection of Kumu Pono Associates LLC. This information is generally cited in categories by chronological order of the period depicted in the narratives.

M. P. Nogelmeier (2010) discusses the adverse impacts of methodology that fails to properly research and consider Hawaiian language resources. He strongly cautions against a mono-rhetorical approach that marginalizes important native voices and evidence from consideration, specifically in the field of archaeology. For this reason, Honua Consulting consciously employs a poly-rhetorical approach, whereby all data, regardless of language, is researched and considered. To fail to access these millions of pages of information within the Hawaiian language cache could arguably be a violation of Act 50, as such an approach would fundamentally fail to gather the best information available, especially considering the voluminous amounts of historical accounts available for native tenants in the Hawaiian language.

Hawaiian culture views natural and cultural resources as largely being one and the same: without the resources provided by nature, cultural resources could and would not be procured. From a Hawaiian perspective, all natural and cultural resources are interrelated, and all natural and cultural resources are culturally significant. Kepā Maly (2001), ethnographer and Hawaiian language scholar, points out, “In any culturally sensitive discussion on land use in Hawaiʻi, one must understand that Hawaiian culture evolved in close partnership with its natural environment. Thus, Hawaiian culture does not have a clear dividing line of where culture ends and nature begins” (Maly 2001:1). As a leading researcher and scholars on Hawaiian culture, Maly, along with his wife, Onaona, have conducted numerous ground-breaking studies on cultural histories throughout Hawaiʻi. A substantial part of the archival research utilized in this study was previously compiled and published by Kepā and Onaona Maly, who have granted their permission to use this important work and are identified properly as associated authors and researchers to this study.

This study also specifically looks to identify intangible resources. Tangible and intangible heritage are inextricably linked (Bouchenaki 2003). Intangible cultural resources, also identified as intangible cultural heritage (ICH), are critical to the perpetuation of cultures globally. International and human rights law professor Federico Lenzerini notes that, “At present, we are aware on a daily basis of the definitive loss—throughout the world—of language, knowledge, knowhow, customs, and ideas, leading to the progressive impoverishment of human society” (Lenzerini 2011:12). He goes on to warn that:

> the rich cultural variety of humanity is progressively and dangerously tending towards uniformity. In cultural terms, uniformity means not only loss of cultural heritage—conceived as the totality of perceptible manifestations of the different human groups and communities that are exteriorized and put at the others’ disposal—but also standardization of the different peoples of the world and of their social and cultural identity into a few stereotyped ways of life, of thinking, and of perceiving the world. Diversity of cultures reflects diversity of peoples; this is particularly linked to ICH, because such a heritage represents the living expression of the idiosyncratic traits of the different communities. Preservation of cultural diversity, as emphasized by Article 1 of the UNESCO Universal Declaration on Cultural Diversity, ‘is embodied in the uniqueness and plurality of the identities of the groups and societies making up humankind’. Being a ‘source of exchange, innovation and creativity’, cultural diversity is vital to humanity and is inextricably linked to the safeguarding of ICH. Mutual recognition and respect for cultural diversity—and, *a fortiori*, appropriate safeguarding of the ICH of the diverse peoples making up the world—Is essential for promoting

harmony in intercultural relations, through fostering better appreciation and understanding of the differences between human communities. (Lenzarini 2011:103)

Therefore, tradition and practice, as elements of Hawaiian ICH, are essential to the protection of Hawaiian rights and the perpetuation of the Hawaiian culture.

2.1 Identifying Traditional or Customary Practices

It is within this context that traditional or customary practices are studied. The concept of traditional or customary practices can often be a challenging one for people to grasp. Traditional or customary practices can be defined as follows:

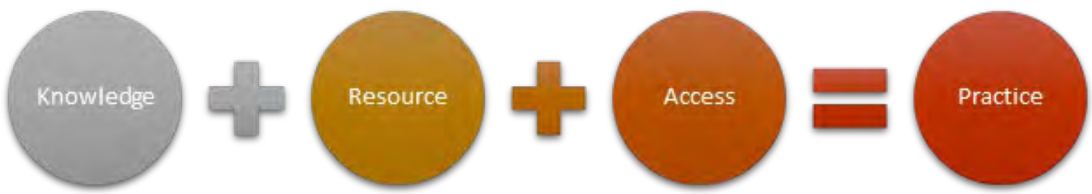


Figure 6. Diagram of elements that contribute to traditional or customary practices (Honua Consulting)

The first element is knowledge. This has been referred to as traditional ecological knowledge (TEK), Indigenous local knowledge (ILK), or ethnoscience. In the context of this study, it is the information, data, knowledge, or expertise Native Hawaiians or local communities possessed or possess about an area's environment. In a traditional context, this would have included information Hawaiians possessed in order to have the skills to utilize the area's resources for a range of purposes, including, but not limited to, travel, food, worship or habitation. This element is largely intangible.

The second element are the resources themselves. These are primarily tangible resources, either archaeological resources (i.e., habitation structures, walls, etc.) or natural resources (i.e., plants, animals, etc.). These can also be places, such as a sacred or culturally important sites or wahi pana. Sometimes these wahi pana are general locations, this does not diminish their importance or value. Nonetheless, it is important to recognize that potential eligibility as a "historic site" on the National Register of Historic Places (NRHP) would require identifiable boundaries of a site.

The third element is access. The first two elements alone are not enough to allow for traditional or customary practices to take place. The practitioners must have access to the resource in order to be able to practice their traditional customs. Access does not just mean the ability to physically access a location, but it also means access to resources. For example, if a particular plant is used for medicinal purposes, there needs to be a sufficient amount of that plant available to practitioners for us. Therefore, an action that would adversely impact the population of a particular plant with cultural properties would impact practitioners' ability to access that plant. By extension, it would adversely impact the traditional or customary practice.

Traditional or customary practices are, therefore, the combination of knowledge(s), resource(s) and access. Each of these individual elements should be researched and identified in assessing any potential practices or impacts to said practices.

2.2 Traditional Knowledge, or Ethnoscience, and the Identification of Cultural Resources

The concept of ethnoscience was first established in the 1960s and has been defined "the field of inquiry concerned with the identification of the conceptual schemata that indigenous peoples use to organize their experience of the environment" (Roth 2019). Ethnoscience includes a wide range of subfields, includes, but is not limited to, ethnoecology, ethnobotany, ethnozoology, ethnoclimatology, ethnomedicine and ethnopedology. All of these fields are important to properly identify traditional knowledge within a certain area.

Traditional Native Hawaiian practitioners were scientists and expert natural resource managers by necessity. Without modern technological conveniences to rely on, Hawaiians developed and maintained prosperous and symbiotic relationships with their natural environment for thousands of years. Their environments were their families, their homes, and their laboratories. They knew the names of every wind and every rain. The elements taught and inspired. The ability of Indigenous people to combine spirituality and science led to the formation of unique land-based methologides that spurred unsurpassed innovation. Therefore, identifying significant places requires a baseline understanding of what made places significant for Hawaiians.

Hawaiians were both settlers and explorers. In *Plants in Hawaiian Culture,* B. Krauss explains: "Exploration of the forests revealed trees, the timber of which was valuable for building houses and making canoes. The forests also yielded plants that could be used for making and dying tapa, for medicine, and for a variety of other artifacts" (Krauss 1993). Analysis of native plants and resource management practices reveals the depth to which Hawaiians excelled in their environmental science practices:

> [Hawaiians] demonstrated great ability in systematic differentiation, identification, and naming of the plants they cultivated and gathered for use. Their knowledge of the gross morphology of plants, their habits of growth, and the requirements for greatest yields is not excelled by expert agriculturists of more complicated cultures. They worked out the procedures of cultivation for every locality, for all altitudes, for different

> weather conditions and exposures, and for soils of all types. In their close observations of the plants they grew, they noted and selected mutants (spores) and natural hybrids, and so created varieties of the plants they already had. Thus over the years after their arrival in the Islands, the Hawaiians added hundreds of named varietis of taro, sweet potatoes, sugarcane, and other cultivated plants to those they had brought with them from the central Pacific (Krauss 1993).

Thus, Native Hawaiians reinforced the biodiversity that continues to exist in Hawaiʻi today through their customary traditional natural resource management practices.

The present analyses of archival documents, oral traditions (oli or chants, mele or songs, and/or hula dances and haʻi moʻolelo or storytelling performances), and Hawaiian language sources including books, manuscripts, and newspaper articles, are focused on identifying recorded cultural resources present on the landscape, including: Hawaiian and non-Hawaiian place names; landscape features (ridges, gulches, cinder cones); archaeological features (kuleana parcel walls, house platforms, shrines, heiau [places of worship], etc.); culturally significant areas (viewsheds, unmodified areas where gathering practices and/or rituals were performed); and significant biological, physiological, or natural resources. This research also looks to document the wide range of Hawaiian science that existed within the geographic extent.

2.3 Moʻolelo ʻĀina: Native Traditions of the Land

Among the most significant sources of native moʻolelo are the Hawaiian language newspapers which were printed between 1838 and 1948, and the early writings of foreign visitors and residents. Most of the accounts that were submitted to the papers were penned by native residents of areas being described and noted native historians. Over the last 30 years, Kepā Maly has reviewed and compiled an extensive index of articles published in the Hawaiian language newspapers, with particular emphasis on those narratives pertaining to lands, customs, and traditions. Many traditions naming places around Hawaiʻi are found in these early writings. Many of these accounts describe native practices, the nature of land use at specific locations, and native moʻolelo (history, narrative, story). Thus, we are given a means of understanding how people related to their environment and sustained themselves on the land.

2.4 Historic Maps

There are also numerous, informative historic maps for the region. Surveyors of the eighteenth and nineteenth centuries were skilled in traversing land areas and capturing important features and resources throughout Hawaiʻi's rich islands. Historic maps were carefully studied, and the features detailed therein were aggregated and categorized to help identify specific places, names, features, and resources throughout the study area. From these, among other documents, new maps were created that more thoroughly capture the range of resources in the area.

2.5 Ethnographic Methodology

Information from lineal and cultural descendants is instrumental in procuring information about the project area's transformation over time and its changing uses. The present analyses of archival documents, oral traditions (including oli or chants, mele or songs), and/or hula dance), and Hawaiian language sources including books, manuscripts, and newspaper articles, are focused on identifying recorded cultural and archaeological resources present on the landscape, including: Hawaiian and non-Hawaiian place names; landscape features (ridges, gulches, cinder cones); archaeological features (kuleana parcel walls, house platforms, shrines, heiau or places of worship, etc.); culturally significant areas (viewsheds, unmodified areas where gathering practices and/or rituals were performed); and significant biocultural resources. The information gathered through research helped to focus interview questions on specific features and elements within the project area.

Information from lineal and cultural descendants are instrumental in procuring information about the project area's transformation through time and changing uses. A notice was placed in the *Ka Wai Ola*, published by the Office of Hawaiian Affairs (Figure 3). Additionally, letters were sent to area organizations inviting their participation. All the correspondence provided through these processes are included in the appendices.

Cultural Impact Assessment Notice: East Maui

Honua Consulting, LLC, on behalf of Tetra Tech, is conducting a Cultural Impact Assessment (CIA) for the National Park Service on 262-square-kilometers in Each Maui, Maui Island, Various TMKs. The project is for the suppression of non-native mosquito populations to reduce transmission of Avian Malaria to threatened and endangered forest birds on Maui. Potential areas where the project would occur include Western Waikamoi, Hanawī, and Kīpahulu (priority areas); and Western Waikamoi, and Manawainui (second tier priority areas). The CIA team is seeking consultation with practitioners, Native Hawaiian Organizations, stakeholders, and other individuals. Specifically, consultation is sought on a) identification of an appropriate geographic extent of study, b) historic or existing cultural resources that may be impacted by the proposed project, c) historic or existing traditional practices and/or beliefs that may be impacted by the proposed project, and d) identification of individuals or organizations that should be sought out for consultation on the CIA. Individuals or organizations interested in participating can contact the CIA team at community@honuaconsulting.com or (808) 392-1617.

Figure 7. Copy of the public notice placed in the *Ka Wai Ola* in November 2021

3. Historic Background

The purpose of this section is to characterize the Hawaiian cultural landscape within which the project area is located; this includes a description of east Maui's relevant and representative inoa ʻāina (place names), moʻolelo (oral-historical accounts), wahi pana (legendary places), and other natural and cultural resources. A general (ahupuaʻa-wide) summary is followed by a project-area specific discussion.

3.1 Traditional Period

Maui has a unique geography; it is considered to be two islands, joined together by an isthmus. Land divisions on Maui are unlike those on other islands (Sterling 1998). Ancient names for Maui include Ihikapalaumaewa and Kulua (Sterling 1998: 2).

The forested regions of Maui Hikina (East Maui) are made up of several forest reserves and conservation areas including the Makawao Forest Reserve (located in the district of Hāmākualoa), the Koʻolau Forest Reserve, the Waikamoi Preserve (located in the district of Hāmākuapoko), the Hanawī Natural Area Reserve, the Hāna Forest Reserve, the Kīpahulu Valley Biological Preserve, and the Kīpahulu Forest Reserve. These upland regions are situated in the wao akua, distant mountain regions surrounded by wilderness and believed to be inhabited by the gods. They are also situated on the eastern slopes of Haleakalā. These lands form the rich watershed forests of Maui Hikina, collecting rains (ua) and mists (ʻohu) from the koʻolau or windward weather systems.

Abundant rains from rich forests like those in the upper regions of Maui Hikina form hundreds of streams (kahawai) that form large valleys and gulches. These well-watered valleys in Maui Hikina have been home to many endemic life forms, including native birds, and have sustained Native Hawaiian communites for centuries. The wao akua supplies vital natural resources for plant and wildlife. Kepā Maly describes the signifiance of natural resources to Native Hawaiians:

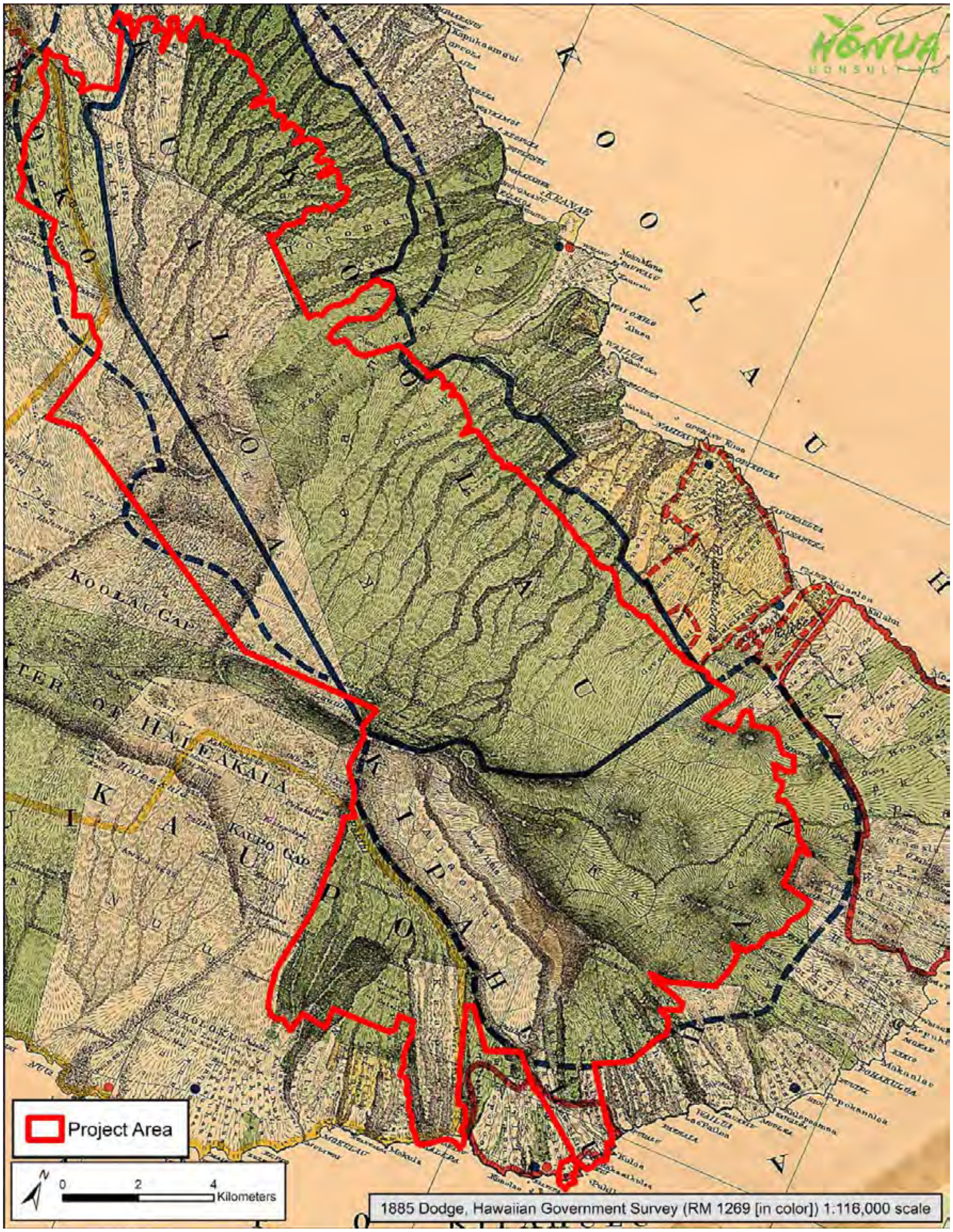
> We find in native traditions and beliefs, that Hawaiians shared spiritual and familial relationships with the natural resources around them. Each aspect of nature from the stars in the heavens, to the winds, clouds, rains, growth of the forests and life therein, and everything on the land and in the ocean was believed to be alive. Indeed, every form of nature in ancient Hawaiʻi was believed to be a body-form of some god or lesser deity. In the Hawaiian mind, care for each aspect of nature, the kino lau (myriad body-forms) of the elder life forms, was a way of life. This concept is still expressed by Hawaiian kūpuna (elders) through the present day, and passed on in many native families. Also, in this cultural context, anything which damages the native nature of the land, forests, ocean, and kino lau therein, damages the integrity of the whole. Thus caring for, and protecting the land and ocean resources, is a way of life. Furthermore,

> in the traditional context above referenced, we find that the mountain landscape, its' native species, and the intangible components therein, are a part of a sacred Hawaiian landscape. Thus, the natural landscape itself is a highly valued cultural property. It's protection, and the continued exercise of traditional and customary practices, in a traditional and customary manner, are mandated by native custom, and State and Federal Laws (as those establishing the Maui Hikina Forest and Natural Area Reserves, and the Waikamoi Preserve). Maly, 2006: 3.

In the early 1900s, the Maui Hikina forest and watershed lands were determined to be some of the most significant in Hawaiʻi and in need of protection. Between 1907 and 1986 several different Forest Reserves, including the Haleakalā National Park, were established to protect the fragile ecosystem and the natural and cultural resources in the vicinity. These forested regions are home to several species of endangered or threatended native birds. Many of Hawaiʻi's native honeycreepers are restricted to East Maui as their only existing habitat.

Hawaiian moʻokūʻauhau (genealogical accounts) reveal that the Hawaiian islands were born from akua (gods) who also birthed the first Hawaiian people. One moʻokūʻauhau records that Wākea (the expanse of the sky– father) and Papahānaumoku (Papa—Earth-mother who gave birth to the islands), also called Haumeanuihānauwāwā (Great Haumea—Woman-earth born time and time again), and various akua gave birth to the islands. Maui, the second largest of the islands, was the second-born of these island-children. These same akua were also the parents of Hāloanakalaukapalili (long stalk quaking and trembling leaf). This Hāloa was born as a "shapeless mass" and buried outside the door of his parents' house (cf. Pukui and Elbert, 1981:382), and from his grave grew the very first kalo (taro) plant. When the next child was born to these akua, he was also named Hāloa (the long stalk or breath of life), and he is considered to be the progenitor of the Hawaiian race (cf. David Malo 1951:3, 242-243; Beckwith 1970; Pukui and Korn 1973). It was in this context of kinship that Native Hawaiians interacted with their environment and it is the basis of the Hawaiian system of land use.

There are several moʻolelo (traditional accounts, stories, histories) that discuss the uplands and forested regions of Maui Hikina. Ethnographer and researcher, Kepā Maly, has gathered and translated many traditions from Hawaiian language resources concerning Maui Hikina.


Project Area
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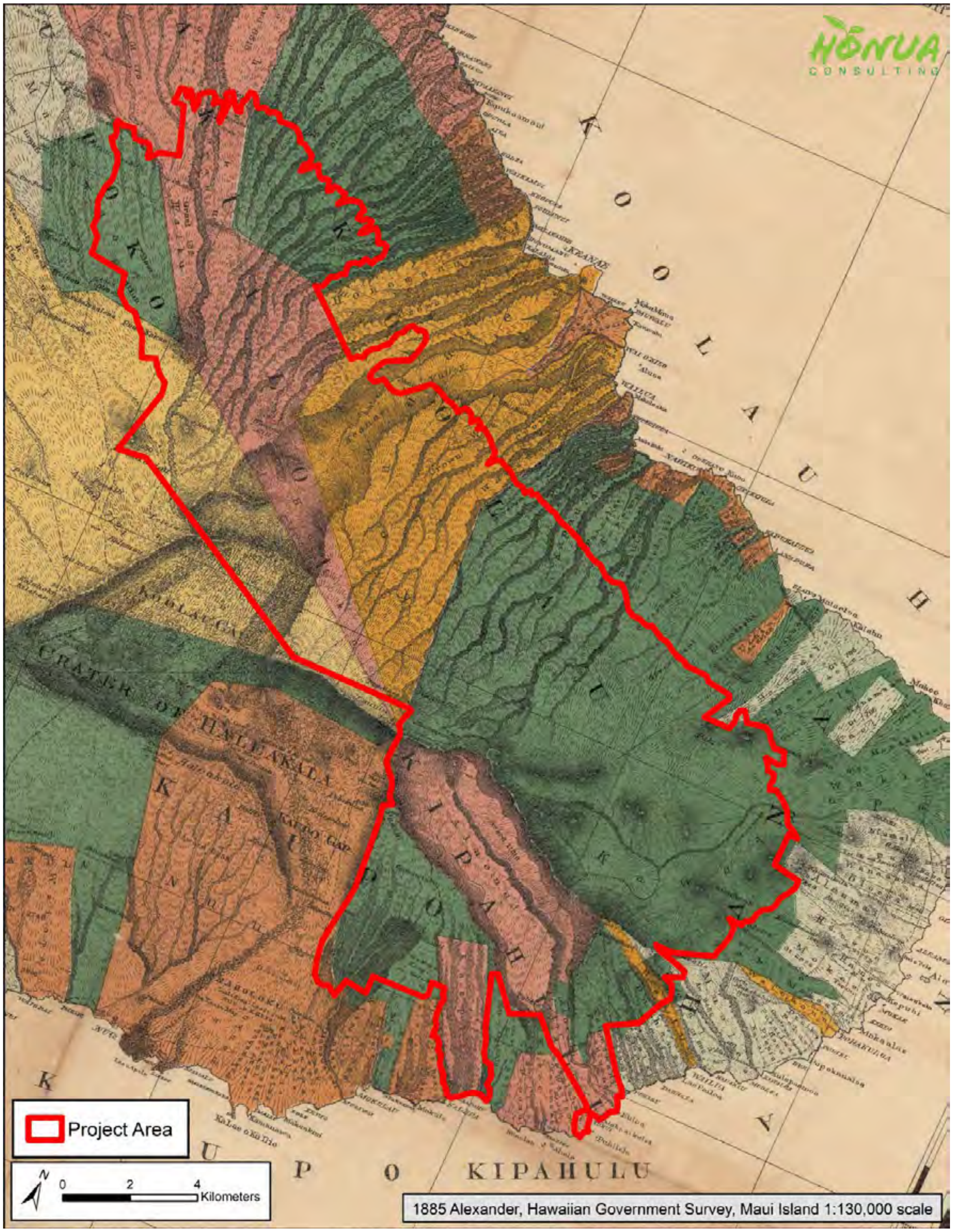
1885 Dodge, Hawaiian Government Survey (RM 1269 [in color]) 1:116,000 scale


Project Area
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N
1885 Alexander, Hawaiian Government Survey, Maui Island 1:130,000 scale
CRATER OF HALEAKALA
KIPAHULU

3.1.1 He Moʻolelo no Kamapuaʻa (1861)

"He Moolelo no Kamapuaa" (A Tradition of Kamapuaʻa) predates the twelfth century. This moʻolelo was submitted to the Hawaiian language newspress, *Ka Hae Hawaii*, in 1861 by G.W. Kahiolo who wrote from Kalihi, Oʻahu. Kamapuaʻa is a Hawaiian kupua (demigod) who could transform into a wide range of forms including a pig and a human form. Kamapuaʻa is associated with agriculture, rain, and the god Lono. The issue published on August 7, 1861 (Helu 7) includes the first written account of Kamapuaʻa's visit to Maui:

> …Kamapuaʻa's advances towards Pele, having been thwarted, he departed from Kilauea, following Kapo-maʻilele (Pele's sister who had taken her genitals off and thrown them across the land to distract Kamapuaʻa — thus the name, Kapo-of-the-flyinggenitals). It was in this way that Kapo-maʻilele saved Pele from Kamapuaʻa's advances. Traveling across the island of Hawaiʻi, and eating maiʻa (bananas), Kamapuaʻa met with Kapo-maʻilele at Kahuā in Kohala. Kapo-maʻilele then flew across the sea, and returned to her home on Maui, at Wailua-iki. From the heights of Kapaliiuka, Kamapuaʻa looked across the ocean, and decided to follow her. He crossed the channel and landed at Hāmoa, Hāna… He then traveled to Kawaikau which is near the boundary between Koʻolau and Hāna. From there, he traveled to Kaliae, and then arrived at Wailua-iki, where he found the house of Kapo-maʻilele. Looking shoreward, he saw Puoenui, the husband of Kapo, fishing. He then chanted:
>
> Kanikani hia Hikapoloa—e,
> *Hīkapōloa cries out loudly.*
> Ka la o Wailua-iki.
> *The day is at Wailua-iki.*
> Ka lai malino a Kapo i noho ai,
> *Kapo dwells in the calm,*
> A ka wahine a Pueonui,
> *The woman of Pueonui,*
> I noho nanea i ka lai a ke Koolau, aloha.
> *Dwelling with pleasure, in the peace of Koʻolau—aloha.*
>
> Kamapuaʻa then went to the kapa making house (hale akuku), and asked Kapo-maʻilele if they two might sleep together. She agreed, and they did. Now a man saw this and went to tell Pueonui that his wife was sleeping with another man. Pueonui returned to the house in anger, and he struck Kamapuaʻa on the back with a paddle. Kapo got angry, and he struck Kamapuaʻa again. Kapo told him "stop, don't do that, for he is not a man, but is Kamapuaʻa." Hearing this, he was afraid, for he had heard that he was a god and man of power. Kamapuaʻa then went to Hāmākua-loa,

Hāmākua-poko, and on to Wailuku... [Kahiolo in Ka Hae Hawaii, August 7, 1861. Maly, translator]

3.1.1 Inoa ʻĀina

Inoa ʻĀina or place names are critical in understanding how Hawaiians valued and understood their surrounding environment. A selection of place names from the project area are provided below.

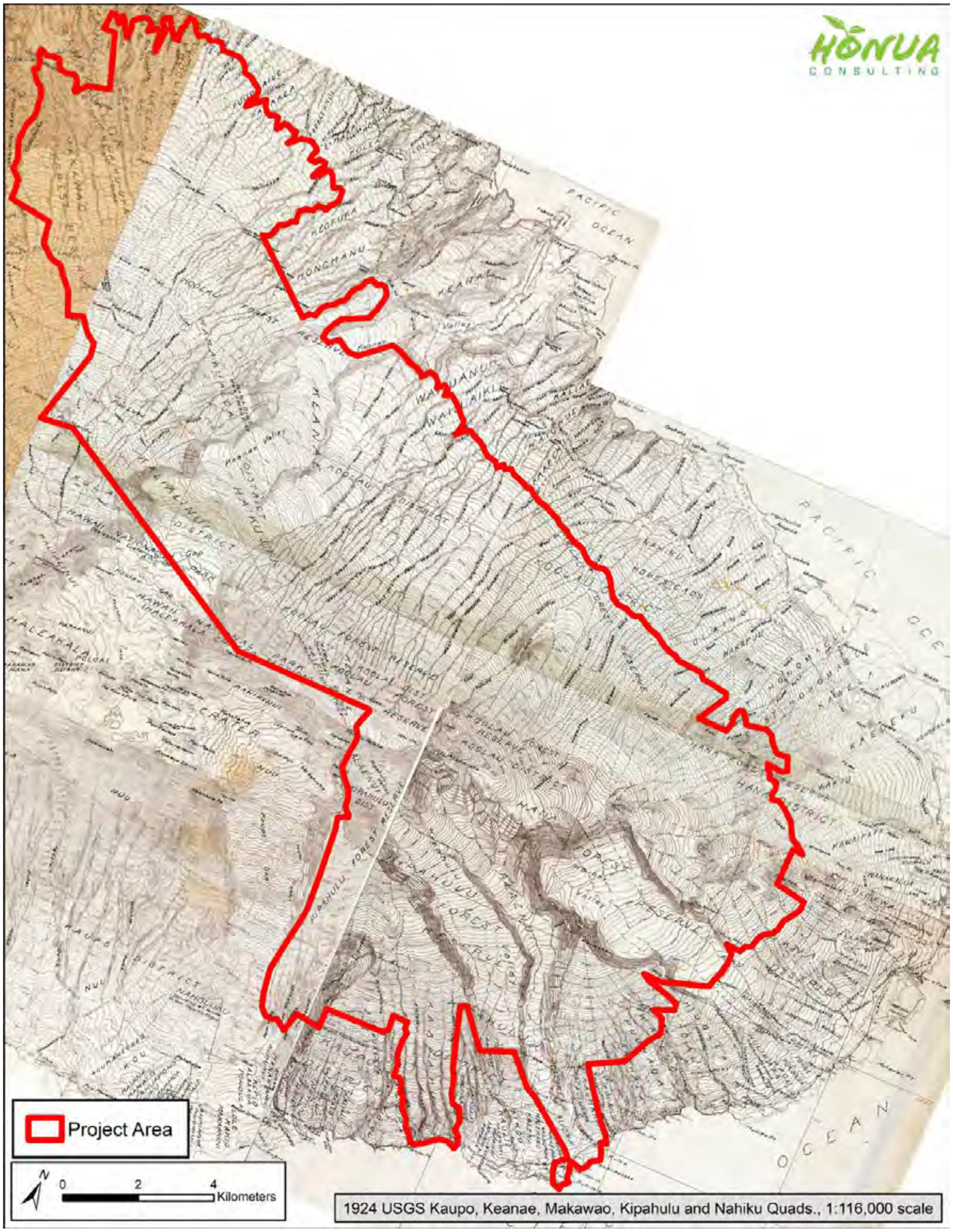
Table 1. Selected inoa ʻāina from the project area.

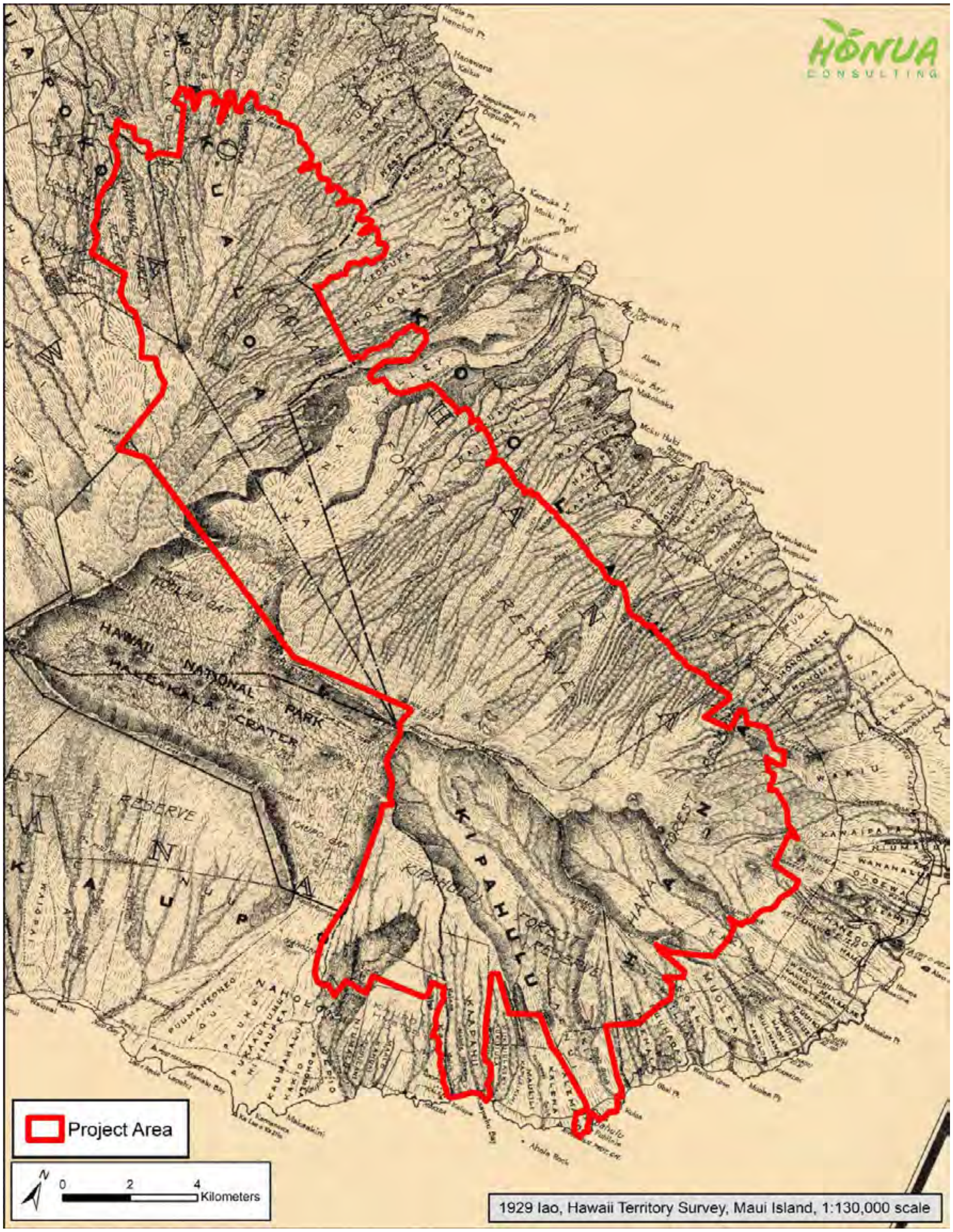
Inoa	Description
Makawao	Derivision of its name. The trade wind which blows from the ocean across the northwestern slope of Haleakala is highly charged with vapor, which is cooled by the cool mountain air, and falls in abundant rains over the region of Makawao. Along the western side of the mountain, about half way to the summit, lay a long line of cumulo stratus clouds, and between this and the nimbus there is but little space. The former lay along the side of the mountain, apparently immovable, while the latter would advance and recede, now coming very near and coquettishly scattering its shining rain drops beneath the very head of the immovable cumulus, and now retreating as though afraid of its more dignified companion. This the feature of the clouds which gave the place its name, Makawao, "makao" to be afraid, "wao" a cloud.
East Makaiwa	Eleio was universally noted for his great speed who was also known as a messenger for the great king of Maui, called Kakaʻaleneo. When Kakaʻaleneo was ready for his morning meal, he would order Eleio to go to Hana and bring him some awa. On one of his trips to Hana, he met a ghost named, Kaʻahualiʻi in the forest of Oʻopuloa. The ghost asked Eleio to give him some of the awa, and Eleio insulted the ghost by telling him to take the hairs of his body and use it for his awa.

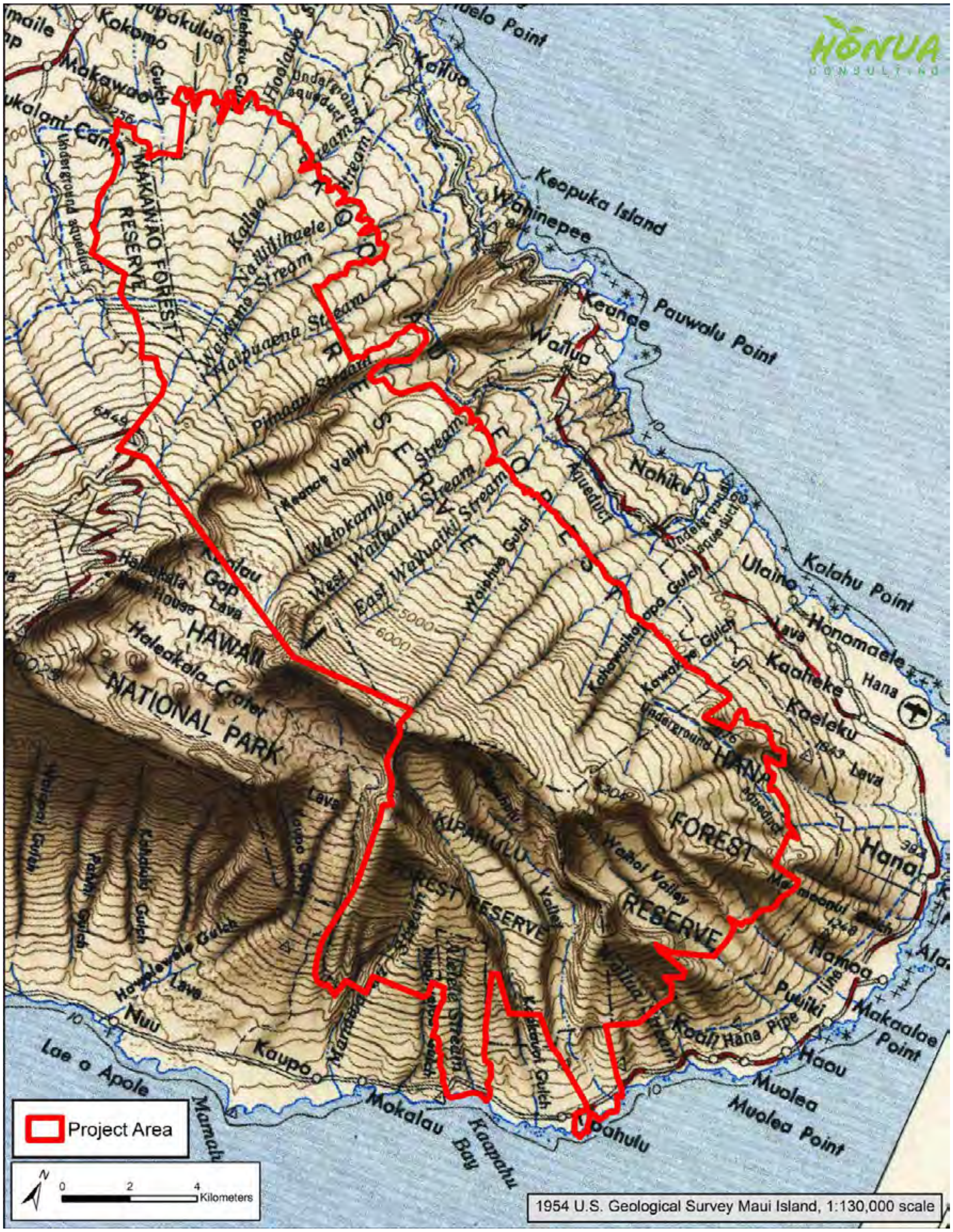
Inoa	Description
Ke'anae	In times of great famine, ti roots were gathered from the forest in large quantities and steamed in great ovens, then grated, mashed, and mixed with water, and drunk. It is said that there was a famous oven of this sort east of Honolulu at Kaimuki "the tea oven". At Ke'anae, there was likewise a great *imu ki,* a pit in the lava to make this famous drink.
Kawaipapa	After the war of Kapalipio, Kamehameha-nui remained ruling chief of Maui. Later in life, He was taken ill at Kawaipapa on a journey about the island, and at Nenewepua in Hana, he ceded his lands and the ruling power to Kahekili who became the ruler of of Maui. Reaching Hamakualoa, Kamehameha-nui died and was laid to rest at Pihana.
Kukui'ula	The stream where the rugged ridge and valley trail to Kaupo begins, waters several small groups of terraces which are still in use.
Ka'āpahu	About 1786 with Kahekili on O'ahu, Kamehameha decided to try and take the districts of Hana and Kīpahulu and sent his younger brother Kalanimalokuloku-i-Kapo'ikalani who accomplished this mission. As soon as Kalanikupule received tidings of this invasion, he immediately send Kamohomoho with what forces he could muster to drive the invaders out of Maui. The armies met on the Kīpahulu side of the Lelekea gulch, and the battle waged with great fierceness. The Hawai'i troops were driven back as ar as Maulili, in Kīpahulu, where they were joined by a reinforcement under Kahanaumaikai and the battle continued. But victory rested with the Maui troops, and what were not killed of the Hawaii expedition fled back to Kohala.

3.2 Historic Period

This section describes general land-use patterns and change in East Maui in the historic period, that is, following the disintegration of the traditional kapu system (circa 1820); some comments on how the project area, in particular, was affected by these changes; and historic maps and aerials that illustrate some of these temporal changes.


Project Area
0 2 4 Kilometers
1924 USGS Kaupo, Keanae, Makawao, Kipahulu and Nahiku Quads., 1:116,000 scale


Project Area
0 2 4 Kilometers
1929 Iao, Hawaii Territory Survey, Maui Island, 1:130,000 scale
HAWAII NATIONAL PARK
HALEAKALA CRATER
KIPAHULU


Project Area
0
2
4
Kilometers
1954 U.S. Geological Survey Maui Island, 1:130,000 scale
HONUA
Kokomo
Makawao
Keopuka Island
Wahinepee
Keanae
Pauwalu Point
Wailua
Nahiku
Ulaino
Kalahu Point
Honomaele
Kaeleku
Hana
HANA FOREST RESERVE
KIPAHULU FOREST RESERVE
HAWAII NATIONAL PARK
Haleakala Crater
Koolau Gap
Kaupo
Nuu
Lae o Apole
Mokulau
Kaapahu Bay
Muolea
Muolea Point
Makaalae Point
Haou
Hamoa
Kawakoe Gulch
Waihoi Valley
Keanae Valley
Waiokamilo
West Wailuaiki Stream
East Wailuaiki Stream
Haipuaena Stream
Puohokamoa Stream
Waikamoi Stream
Nailiilihaele Stream
Kailua
Aqueduct
Lava

3.2.1 Kahekili and Kalaniʻōpuʻu

In 1776 Kalaniʻōpuʻu (king of Hawaiʻi) invaded Maui, and met the warriors of Kahekili in battle on the plain of Kamaʻomaʻo. In 1778, Kalaniʻōpuʻu invaded the kingdom of Kahekili again, attacking Kaupō, Kahoʻolawe, and Lāhainā. During these battles the young Kamehameha exhibited his skill on the battlefield as well. Maui's forces eventually drove Kalaniʻōpuʻu's army from Maui, and they took Lānaʻi by force. The people and resources of Lānaʻi were abused and overtaxed, and a famine took place on the island. Kalaniʻōpuʻu then set sail to Koʻolau, Maui, and Kamakau described the events in the following narratives:

> Ka-lani-ʻopuʻu decided to go on to Koʻolau, Maui, where food was abundant. He went to Kaʻanapali and fed his soldiers upon the taro of Honokahua... At Hamakualoa Ka-lani- ʻopuʻu landed and engaged in battle, but Ka-hekili hastened to the aid of his men, and they put up such a fierce fight that Ka-lani-ʻopuʻu fled to his canoes. Landing at Koʻolau he slew the common people and maltreated the captives by urinating into their eyes. Descendants of people so treated are alive today. From Hana, Mahi-hele-lima, commander of the fortress Kaʻuiki, joined forces with Ka-lani-ʻopuʻu, and for six months the fighting continued. During this campaign, carried on for half a year, from 1778 to 1779, with fighting at Kaupo, Lahaina, Lanai, Hamakualoa, and Koʻolau, Kamehameha, as well as his master in warfare, Ke-ku-hau-piʻo, distinguished himself for skill and bravery in war... [Kamakau 1961:91]

Kamakau also recorded that while the battles were occurring on Maui Captain James Cook and his ships sailed along the coast of Maui Hikina. In Kamakau's version of the arrival, readers learn that Cook anchored near Haʻaluea Rock.

> While Ka-lani-ʻopuʻu was in Wailua in Koʻolau, Maui, on the evening of November 19, 1778, Captain Cook's ship was sighted northeast of Mokuhoʻoniki with the prow turned a little to the southeast. It was seen at Kahakuloa, and the news spread over the island, then at Hamakua, and at evening it was seen in Koʻolau. The night passed, and the next day the ship was anchored at Ha'aluea just below Wailua. When they saw that its appearance exactly fitted the description given by Moho, there was no end of excitement among the people over the strange object. "The tower of Lono! Lono the god of our fathers!" they exclaimed, redoubling their cries at the thought that this was their god Lono who had gone to Kahiki. The men went out in such numbers to visit the ship that it was impossible for all to get on board.
>
> When the canoes returned to shore, Kalaʻi-mamahu' persuaded Kamehameha and one other to remain on board, and that night the ship sailed away taking Kamehameha and his companions and by morning it had disappeared. Ka-lani-ʻopuʻu thought that

> Kamehameha must have gone away to Kahiki. He was displeased and ordered Ke-pa‘alani to bring them all back. Ke-pa‘a-lani took six paddlers and a large single canoe supplied with food and water. Puhie declared that within two days and two nights they would sight the ship. Maui disappeared, and Mauna Kea rose before them out of the waves. Kamehameha, looking out, saw a white object on the wave and said to Kala‘imamahu’, “Is that a canoe or only a wave?” “Where?” “Yonder.” As they watched it became clearly a canoe, and Kamehameha guessed that it was Ke-pa‘a-lani come to seek them. But Captain Cook had no intention of carrying them away; he only wanted them to guide him to a good harbor on Hawaii. Captain Cook may have sailed by a map made by the Spaniards, for how else could he have found the proper harbors at Waimea, Mahukona, and Kealakekua? As for Ke-pa‘a-lani he was relieved, for he had already sailed two [page 97] days and nights without sighting the ship. Kamehameha pointed out the canoe to Captain Cook and then pointed toward Maui. Cook would not consent; he pointed to the ship and then to Hawaii. Again Kamehameha pointed to Maui, and the ship turned about and reached Wailua in a single night… [Kamakau 1961:98]

3.2.2 He Mo‘olelo Ka‘ao Hawai‘i no Lauka‘ie‘ie

“He Moolelo Kaao Hawaii no Laukaieie…” (A Hawaiian Tradition of Lauka‘ie‘ie) was published in *Nupepa Ka Oiaio* between January 5th 1894 to September 13th 1895. The mo‘olelo was submitted to the paper by Moses Manu. The following narratives (translated by Maly), have been excerpted from the mo‘olelo, and include an overview of the tradition and the travels of Makanikeoe, one of the main figures in the mo‘olelo. During his travels, Makanikeoe sought out caves, and tunnels that served as underground trails, covering some of the important places and resources in the Ko‘olau-Hāmākua region. Maly summarizes:

> Later in the account, Makanikeoe returned to Maui, and traveled round the island. On his journey, he visited various places at Kahikinui, Kaupō, Kīpahulu, Hāna, Ko‘olau and Hāmākua. Having traveled through Hāna, Makanikeoe:
>
> [November 16, 1894]
>
> …looked to the uplands and saw many places where kalo was growing. The interesting thing about the kalo at this place was that it appeared to be flying along the edges of the cliffs at Hanawī. Desiring to understand how the kalo could grow along the cliffs in this manner, Makanikeoe climbed up to one of the places where these kalo ‘e‘epa (mysterious formed taro) was growing. It was a large place where the water flowed, and in the distance, he saw a man carrying a single large kalo.

> Makanikeoe then heard a voice calling out, and saw one of the small kalo plants growing along the side of the cliff talking, just as if it were a man. It called out to the large kalo:
>
> Make no ʻoe e kalo nui – Ola no au o kalo iki."
> (Say there large taro, you are to die –and I, the little taro, will live.)
>
> Makanikeoe chuckled to himself, hearing the words of the little kalo, and he understood the surprising nature of the kalo at this place. These places where the kalo grows on the cliffs may still be seen to this day.
>
> After seeing this kalo that speaks like a man, Makanikeoe then went to the top of the mountain ridge, where he could see the cliff of Lelekea (Kīpahulu) below. He then went down to Kahaleikalalea, where he turned his gaze to the calm sea, and the pond of Waihī, Kīpahulu… [Maly, translator]

3.3 Mosquitoes in Hawaiʻi

There is no evidence that mosquitos have any cultural significance. They are not native to the Hawaiian Islands, and since their introduction by foreigners, they have proved to be a devastating menance to the population and ecosystem in Hawaiʻi.

According to the U.S. National Library of Medicine, mosquitoes first arrived in Hawaii in 1826, when "European and American ships carry the first mosquitoes to Hawaii, where there are no blood-sucking insects. Although these mosquitoes cannot transmit malaria to humans, they carry avian malaria, which decimates birds native to the Hawaiian Islands. Over the next 150 years, four more mosquito species are introduced" (2021). It also believed that the first introduction of mosquitoes took place on Maui, in Lahaina, when a foreign vessel brought the species into Lahaina Harbor and they spread from there.

Eventually, four more species of mosquitoes would be introduced to the islands, likely again from foreign contact in the islands. They were quickly identified as a serious health concern. There would be various efforts to eradicate, or at least control, mosquitoes in Hawaiʻi. Even the effort to use biocontrol to eradicate mosquitoes was not a new concept in Hawaiʻi. The first biocontrol efforts were started in the early 1900s, nearly 120 years ago.

Mosquitoes have proven most devastating to native birds, likely contributing to the extinction of many species. The following historic newspaper accounts document previous eradication efforts throughout the Hawaiian Islands.

The Pacific Commercial Advertiser.

WEATHER FORECAST FOR TODAY—Light trades fair with occasional showers. Last 24 hours' rainfall .00; temperature, max. 85, min. 71.

Established July 2, 1856.

SUGAR—96° Centrifugals, 3.9375—Per Ton $78.75. 88 Analysis Beets, 9s 9d. Parity with Centrifugals, $82 Per Ton.

VOL. XXXIX., NO. 6855. HONOLULU, HAWAII TERRITORY, SATURDAY, JULY 30, 1904. PRICE FIVE CENTS.

RUSSIAN SHIPS OFF TOKIO BAY

Skrydloff's Ships Are Closing In---Russians Pessimistic Over the Loss of Newchwang and Retreat of Kuropatkin---Prize Arabia Arrives at Vladivostok.

(ASSOCIATED PRESS CABLEGRAMS.)

TOKIO, July 30.—The Vladivostok squadron has appeared off the entrance to Tokio bay.

ARABIA AT VLADIVOSTOK.

VLADIVOSTOK, July 30.—The captured prize Arabia has arrived here.

The cable on July 25th reported that the German steamer Arabia, from Oregon for Japan with a cargo of flour, had been captured by the Vladivostok squadron. The vessel was en route to Yokohama and at the time of her capture the Russian fleet was about sixty miles off Yokohama. From Yokohama to Vladivostok, via Tsugaru Strait, is about 800 miles.

TOO HEAVILY BURDENED.

LIAOYANG, July 30.—The Russian soldiers complain of their heavy accoutrements.

RUSSIANS DISCOURAGED.

ST. PETERSBURG, July 30.—The Japanese capture of Newchwang and the Russian retreat has caused a pessimistic feeling here. The withdrawal northward of the entire Russian force is inevitable. There is talk of making a diversion by invading Korea from Vladivostok.

AFTERNOON REPORT.

VLADIVOSTOK, July 29.—Viceroy Alexieff is on his way to this place.

LONDON, July 29.—The report of the fall of Port Arthur is not confirmed.

BERLIN, July 29.—Russia has agreed to indemnify the Germans for seizures of merchant ships and cargoes.

TOKIO, July 29.—The reported losses at Tatchekiao were 1,000 Japanese and 2,000 Russians. The Russian Generals Sakaloff and Condendovitch were wounded.

SHANGHAI, July 29.—Reports received here from Wei Hai Wei and Chefoo state that Port Arthur has been captured by the Japanese.

The British fleet is returning to Wei Hai Wei.

LONDON, July 29.—The Russian Government responding to the demands of Great Britain for ships captured and destroyed protests that the British ships taken carried contraband cargoes.

CHEFOO, July 29.—The refugees arriving from Port Arthur state that the Japanese lost a cruiser and gunboat Thursday in an attack on the Russian stronghold. A bombardment of the forts is now in progress.

WASHINGTON, July 29, 1904.
(Received at noon.)

To JAPANESE CONSUL, Honolulu:

General Oku reports on the battle near Tatchekiao and among other things, he states that, according to the statements made by the captured Russian officers, Kuropatkin was present on the battlefield. General Sakaloff and General Condendovitch were wounded and the Russian casualties were about two thousand. He also reports that our casualties were about one thousand and that the booty and prisoners taken by our forces are now under investigation.

TAKAHIRA.

INDICTMENTS FOLLOW DISASTER.

NEW YORK, July 30.—The captain of the ill-fated river steamer General Slocum and two inspectors of steamboats have been indicted.

EIGHT PROJECTED FORTS AND BATTERIES ARE TO SAFEGUARD HONOLULU

FORT AT PUULOA.
FORT AT BARBER'S POINT.
BATTERY AT OLD WAIKIKI.
BATTERY NEAR LIGHTHOUSE, DIAMOND HEAD.
BATTERY AT NIU.
MORTAR BATTERY AT HOME FOR INCURABLES.
BATTERY AT WAIANAE GAP.
PLATFORM FOR FIELD BATTERY AT PALI.

The above program is derived from high military sources. It bears out Lieut. Slattery's statement that when the plans of the War Department are carried out, Honolulu will be one of the most strongly fortified places in the world.

The War Department has had its eye on the Home for Incurables site for some time and intends to get it either by purchase or condemnation.

The defenses at Waikiki are said to contemplate a battery of two 12-inch guns and a secondary battery of eight six-inch guns.

Various estimates have been put on the Waikiki property and it is said that $100 per front foot is thought to be the price that will be asked by property owners. As 1300 feet frontage is required by the War Department, this would make the property valuation $130,000.

The Afong and McCandless homes are of a modern type and the improvements on all the property have given them a value far above the property lying to the rear, which is mostly in waterways at present.

BARTON RETURNS

Comes With a Party to Study Our Craters.

(Continued on Page 6.)

RIOT WAS SERIOUS

Over Fifty Men Were in the Waialua Fight.

(Continued on page 6.)

PASSENGERS PLAY BASEBALL ON MIDWAY

A GREAT SHOWING FOR ANTI-MOSQUITO CRUSADE

Another Year's Work Would Make the Stinging Insect a RARA AVIS---Interesting Addresses at Young Hotel Public Meeting.

That Honolulu can be rid of the mosquito pest by diligent co-operation on the part of citizens with the agents of the Board of Health was clearly shown at the annual meeting of the Citizens' Mosquito Committee held in the makai ballroom of the Alexander Young Hotel. The report of the committee's agent, Mr. A. D. Larnach, who has been at the head of the active war of extermination waged against the pest during the past year, was optimistic enough to arouse the most doubting Thomas to action. The opening address of President Pinkham of the Board of Health, the addresses on mosquitoes and their menace to humanity by Dr. C. B. Cooper, former president of the Board of Health, and by Dr. F. R. Day, and the lantern slide exhibition by D. L. Van Dine, entomologist of the Federal Experiment Station in this city, and the remarks of Federal Judge S. B. Dole, were all enthusiastic in upholding the extermination idea.

Owing to the sudden downpour of rain about 7 o'clock last evening the attendance at the meeting was not as large as anticipated but those there made up in enthusiasm what they lacked in numbers.

Throughout the meeting there was a feeling that an appeal was being made to the citizens of Honolulu to continue their efforts to assist the Board of Health and Mosquito Committee agents to rid the town of the pest. The past year's extraordinary success under the able management of A. D. Larnach was made apparent in all that the medical men had to say on the subject. Mr. Larnach himself believed that within another year a most appreciable difference in the number of mosquitoes even now present would be noticeable, judging by the past year's work. Following are the addresses:

PRESIDENT PINKHAM PRAISES.

The meeting was opened by President L. E. Pinkham of the Board of Health, who made the following remarks:

It is a privilege to preside at a gathering of citizens who are voluntarily intrusted in work for public improvement.

It is the every day petty annoyance that wears, and if there was ever an annoyance that not only destroyed good nature, wore on patience and deprived one of the mental equilibrium necessary to the peaceful pursuit of knowledge and happiness, it is the operations of the mosquito and its untamed bloodthirsty disposition.

The past year has given some hope of deliverance. It is generally conceded that the mosquito nuisance has abated very materially. Certainly the freedom from these pests has been very marked in some localities.

This campaign tends greatly toward the preservation of public health. All authorities agree that the pernicious activity of the mosquito is a means of widely spreading certain diseases.

In order to be fully informed as to those localities where the annoyance is specially prevalent, the Board of Health has caused to be inserted in each paper a notice requesting information be sent.

If the citizens will kindly comply with our request they will increase greatly the efficiency and amount of work your agents can accomplish.

The success of this campaign will serve as an example of what combined action can produce at little cost in the way of city improvement.

While any reference to the wonderful climate of the Hawaiian Islands is trite and rather tiresome, it is nevertheless, a most potent fact, and when we leave this home for a time, the realization comes back, and the longing for the charm of the beautiful out-door life nowhere else equalled or possible.

The one marring circumstance that detracts from the ideal of comfort and restfulness, your Citizens' Mosquito Campaign has undertaken to practically remove.

It is worth the money it has cost, and which it may cost.

It is sincerely hoped the year on which we have entered will find your support as equal to the past year.

We are busy, based on our own information, but we will get busy up to the limit if you will point out the spots needing attention.

DR. COOPER'S ADDRESS.

Dr. Cooper made an excellent address on the mosquito question, speaking to the subject, "The Board of Health's Attitude in the Organization of the Citizens' Mosquito Campaign in Honolulu:"

The mosquito problem in this Territory has been a much mooted question for many years. There have been discussions on this subject by the various Boards of Health, and papers have been read and discussed by the Territorial Medical Society, resulting always in good resolutions, but there the matter ended. About a year ago interest was revived and the subject actively agitated by a number of the progressive business and scientific men in our community, resulting in the organization of the Citizens' Mosquito Committee. As the presiding officer of the Territorial Board of Health I was approached by the committee, with a view of a permanent organization being formed to be operated to a certain extent under the auspices of the Board of Health. A meeting was called on August 19th, 1903, for the discussion of permanent organization and those present were: Dr. L. E. Cofer, Chief Quarantine Officer Public Health and U. S. M. H. Service; W. E. Rowell, Acting Assistant Superintendent of Public Works; C. H. Tracy, City Sanitary Officer; Sam'l Johnson, Superintendent Garbage Department; D. L. Van Dine, U. S. Experimental Station; F. W. Macfarlane, Chamber of Commerce, and Messrs. P. M. Pond and P. R. Helm, Research Club; Dr. C. B. Cooper, President Board of Health.

The president of the Board of Health was elected chairman pro tem and the object of the meeting stated, which was to formulate plans for the campaign, to devise ways and means of raising funds and to see what was necessary to be done in general to make a successful issue. Work had already been started in a general way by the sanitary inspectors of the Board of Health; they had been instructed to see that every householder kept their yards clear of tin cans, cesspools were petrolized and cov-

(Continued on page 3.)

Figure 8. Article from the Honolulu Advertiser (1904) covering efforts to eradicate the mosquito in Hawai'i. The article appeared on the front page of the newspaper and one a subsequent page.


A GREAT SHOWING FOR ANTI-MOSQUITO CRUSADE

(Continued from page 1.)

LARNACH'S GREAT WORK.

DR. DAY'S VIEWS.

BACKERS OF THE CRUSADE.

THE NEWLY DISCOVERED MOSQUITO DESTROYER

IT IS A HAIR-LIKE WORM WHICH IN ONE STAGE OF ITS EXISTENCE SEEKS THE LARVAE OF MOSQUITOES AND ENTERS THE ABDOMINAL CAVITY CAUSING THE DEATH OF ITS HOST.

NAKUINA'S DAMAGE SUIT

Figure 9. 1903 article discussion eradication efforts.

SIXTEEN HONOLULU STAR-BULLETIN, WEDNESDAY, NOVEMBER 15, 1950

Two Suits Filed By Householders For Fire Losses

Two suits by Punaluu householders against the city-county seeking $50,000 damages for fire losses in 1948 are on trial before Circuit Judge John E. Parks.

A freak fire, said to have been caused by electric current deflected through telephone wires November 28, 1948, is involved.

In one suit, Archibald and Nora Mark, Jen Fui and Eva Moo allege their two story home was completely destroyed in the blaze. They want $50,000 damages.

Richard L. and Marie H. Carter ask $800 compensation for damage to their house and furniture.

According to the complaints, corroded insulators installed by the city on telephone poles caused the current to roll through telephone wires which short-circuited.

Nicholas W. Y. Char is attorney for the complainants. Moon Chan, assistant city-county attorney, is in charge of the case for the government.

African Mosquito Is Hope Of Controlling Pest Here

Eighteen mosquito eating mosquitoes arrived in the territory recently after a nine day journey from their South African home. They were survivors of the 22 larvae which originally started the jaunt.

Known as the "elephant mosquito" in Africa, this insect confines its cannibalistic instincts to its larvae stage, feeding on larvae of other mosquitoes.

Its scientific name is Megarhinus Brevipalpis.

Dr. David D. Bonnet, medical entomologist with the territorial health department, says the first Hawaiian born larvae of the elephant mosquito have just been hatched.

They are to be used as breeding stock in the laboratory. But when production allows it, some are to be released in surroundings here suitable for their survival, according to Dr. Stephen M. K. Hu, chief of the bureau of mosquito control.

He adds that he eventually hopes they can be introduced on all the islands.

This particular mosquito's curved proboscis (beak), which is present in the adult stage, makes it impossible for them to bite humans. They feed on flower nectar and ripe fruits.

The elephant mosquito is strictly a forest insect and is rarely found near houses.

* * *

If it can survive in natural surroundings here, it will help control the Aedes albopictus (forest day mosquito), which breeds in great numbers in rock and tree holes and water holding plants.

Health officials say that although extensive control measures have been practiced, the presence of numerous breeding places that are inaccessible make the control of the forest day mosquito extremely difficult and costly.

It is hoped the elephant mosquito may provide the answer to that problem.

MOSQUITO-EATING MOSQUITOES, which are newcomers in Hawaii, are pictured in this photo. Their scientific name is Megarhinus Brevipalpis, and they come from South Africa. The shape of the proboscis (beak) in the adult stage has earned the insect the nickname of "elephant mosquitoes." During the larvae stage this mosquito feeds on other mosquito larvae. But in the adult stage the shape of the beak prevents it from biting humans. A penny is shown in this photo for size comparison.—Health department photo.

Figure 10. 1950 article discussing biocontrol method used in the islands.

4.0 Cultural Resources

This section identified cultural resources, including biological resources with cultural importance, within the project area.

4.1 Cultural and Historic Sites

Due to the size of the project area, this cultural impact assessment did not identify or inventory individual historic sites within the project area. Due to the nature of the activites, it is not anticipated that these activites could impact, modify, or effect historic properties in the project area.

4.2 Natural Resources with Cultural Significance

Hawaiians, like most indigenous and local communities, ascribe great cultural value to the natural resources in the environment around them. Due to the size of the project area, this cultural impact assessment did not identify or inventory the full range of biological resources within the project area, rather it noted and assessed natural resources with cultural significance through the ethnographic data. Based on this data, there are numerous plant resources used for cultural practices throughout the project area. There are also the native birds, which are highly valued and prized by practitioners. While there is no intent to collect these birds, their importance to moʻolelo and mele makes their preservation important to continuing cultural practices.

Multiple informants also identified game in the area that is regularly gathered by hunters for subsistence purposes. Hunting is a cultural practice. There has also been appellate court decisions in Hawaiʻi that have identified the hunting of non-native ungulates as cultural practices. This game is hunted by local practitioners and used to feed their families and communities.

4.2.1 Manu Mūkīkī

Hawaiian Honeycreepers

Manu mūkīkī is the general term for Hawaiian honeycreepers. At least 56 different species of manu mūkīkī are known to have existed throughout our islands however, all but 18 of them are now extinct. Manu mūkīkī are extremely endangered due to threatended habitats and invasive predators.

Manu mūkīkī are a very diverse species and are some of the rarest species of Hawaiian birds. The manu mūkīkī considered in this study are the ʻiʻiwi, ʻākohekohe, and the kiwikiu (Maui Parrotbill). These birds currently reside primarily in the uplands of East Maui, namely in the Makawao, Waikamoi, Koʻolau, Hanawī, Kīpahulu, and Hāna forest reserves. The kiwikiu and the ʻākohekohe are restricted to East Maui forest reserves. The ʻiʻiwi occupies high elevation forests on most islands, however, in recent years populations have decreased in these ranges.

The ʻākohekohe is the crested honeycreeper (Palmeria dolei) and was formerly endemic to Maui and Molokaʻi. It is critically endangered and rare. The ʻākohekohe is one of the largest manu mūkīkī measuring over seven inches long. ʻĀkohekohe, like many honeycreepers, survive on nectar sources from native trees like the ʻōhiʻa lehua. ʻĀkohekohe currently only reside on Maui in elevations above 5,000 feet. They are currently restricted to about 25 square kilometers of forest near Haleakalā volcano. Like many bird names in Hawaiʻi, ʻākohekohe is an onomatopoeia. The name ʻākohekohe is said to be associated with the bird's song.

The kiwikiu is the Maui Parrotbill (Pseudonestor xanthophrys) and was formerly found on Maui and Molokaʻi. Today it is also restricted to the forested ranges of East Maui, primarily within the Hanawī, Waikamoi, and Haleakalā preservation areas. It is estimated that fewer than 300 kiwikiu exist. Kiwikiu feed on the nectar of native plants like the ʻōhiʻa, ʻākala, koa, ʻōlapa, pilo, and kanawao. They will also use their beaks to split open berries for insect larvae. Kiwikiu is not a traditional name for this particular bird. In 2010 the Hawaiian Lexicon Committee renamed this bird after deeming that the original Hawaiian name was lost. Traditional plant and animal names can be forgotten due to the decline of the species as well as the decline of Native speakers and use of Hawaiian language. Specific species of plants and animals are also often referred to in a general sense rather than by sepcific names over time. In the case of the kiwikiu, it is possible that this bird was discussed generally as a manu mūkīkī or honeycreeper rather than its specific name. Kiwikiu is an onomatopoeia and is associated with the bird's unique song.

The ʻiʻiwi is the scarlet honeycreeper (Drepanis coccinea or Vestiaria coccinea). The ʻiʻiwi was once found on all of the main islands. The name ʻiʻiwi is generally associated with the scarlet honeycreeper, however there were once different varieties of ʻiʻiwi feather colors. The ʻiʻiwi pōlena was a yellowish honeycreeper unlike the familiar scarlet one. In Hawaiian language newspaper accounts, ʻiʻiwi, both scarlet and yellow, are commonly referred to as the ʻiʻiwimakapōlena, the ʻiʻiwi with yellow eyes. There was also the ʻiʻiwipōpolo, a darker variety of honeycreeper. The ʻakialoa was another group of manu mūkīkī that were said to be a type of ʻiʻiwi as well. ʻAkialoa have long curved bills and yellow-green plumage. They were called by this name on Oʻahu and were also found on Kauaʻi and Hawaiʻi. On Kauaʻi, certain ʻiʻiwi were referred to as olokele. The scarlet honeycreeper is one of the most recognizeable manu mūkīkī. They feed on the nectar of native plants and are commonly associated with lehua blossoms. The feathers of the ʻiʻiwi were used extensively in traditional featherwork. Scarlet and yellow ʻiʻiwi feathers were sought out for ʻahu ʻula (feather capes), lei hulu (feather garlands), mahiole (feather helmets), and other garments and crafts.

From May 2, 1863 to June 13, 1863, the Hawaiian language newspress, *Ka Nupepa Kuokoa,* published a series called "Ka Moolelo on Na Manu o Hawaii nei" (A Chronicle of the Birds of

Hawaiʻi). The series was authored by G.W. Kahiolo from Kalihi. The series was published in six installments and covered many different species of native birds found in Hawaiʻi. A total of 42 different species of birds were discussed in the series. Manu mūkīkī were not covered in this series as a general class of birds, but 9 different honeycreepers made the list including the ʻōʻū, ʻamakihi, ʻāʻā, ʻōʻō, ʻakakane, olokele, hulimaiʻa, mamo, and the ʻiʻiwimakapōlena. Many of the honeycreepers listed were compared to the ʻiʻiwi in size and resemblance. The beaks of the different honeycreepers discussed were often compared to that of the ʻiʻiwi. The fourth installment discusses the ʻiʻiwimakapōlena and provides the following narrative on this manu mūkīkī.

> "The ʻIʻiwimakapōlena Bird"
>
> The ʻIʻiwimakapōlena is similar to the Mamo, the ʻŌʻō, and the ʻŌʻū. Its beak is lightcolored and attractive and also long with a nice curve. Its head is beautifully small and narrow and its feathers are bright scarlet with dark black wings and tail feathers. Its legs, feet, and tallons are a glowing yellow, and its eyes are very yellow. The nectar of lehua blossoms is its food, as well as other blossoms, like the Mamo.
>
> The ʻIʻiwi has a delightful voice when it sings. When it sings it raises its head and billows out its neck and also raises its tail feathers and stands upright above and then sings out. Its song goes like this: "ko-koki," with a "ko" that follows.
>
> This is a very alert bird. Its activity and its movements are unusual as it searches for different areas to obtain its food with much pleasure, rushing without hesitation and doing lots of activity without fatigue. It perches in high places here and there, as if it would be treated with scorn if it were to be idle and rest in one place and was inactive or slept.
>
> It is also unusual how this bird takes pleasure in activity through weariness in the cold and damp without caring about the chill of the dewy sticks in the early morning, braving to work in the force of the cold in the drizzling rain of the uninhabited mountain as if it is fulfilling the decree, "work until the termination of his body."
>
> Something that this bird greatly desires as it sings up above on the peak of its throne is the topmost branches to exhibit its great joy at having passed above the loftly summits, singing perched high above and below, gazing about while going "ka-kaki" with a "ko" that follows with great delight.
>
> It already takes pleasure before but after it drinks a few small drops of nectar from the lehua blossom it does not complain for what little it got with all its weariness as if it were following the words of Solomon, "a simple meal with love is better…"

> There are three types of ʻIʻiwi. 1. The ʻIʻiwimakapōlena; 2. The ʻIʻiwialokele; 3. The ʻIʻiwipōpolo. The ʻIʻiwimakapōlena is what was described above and the ʻIʻiwialokele is the ʻIʻiwi with a desired red in its eyes. The ʻIʻiwipōpolo is the ʻIʻiwi with bright dark scarlet feathers. The ʻIʻiwi that was first described is an ʻiʻiwi with bright scarlet feathers as well, however, the extremeties are yellow and that is how this one is slightly different when you look at it.
>
> The ʻIʻiwi has an attractive bent and gentle beak. It was therefore given its name in accordance to its beak and the anture of its eyes and feathers, its superb majesty as well as the nobility of this bird. For these reasons it is called "the Diplomat of the birds of the uplands."
>
> Kahiolo, G.W. "Ka Moolelo on Na Manu o Hawaii nei." *Ka Nupepa Kuokoa,* Volume II, Number 21. May 23, 1863. Page 1. Translated by J. Uʻilani Au. January 2022.

Based on the description provided in *Ka Nupepa Kuokoa,* the ʻiʻiwimakapōlena is a general term for the scarlet honeycreeper, better known as the ʻiʻiwi. The name ʻiʻiwimakapōlena (the ʻiʻiwi with yellow eyes) indicates that the bird's yellow eyes were particularly admired and recognizeable. The author lists three different varieties of ʻiʻiwi: the ʻiʻiwimakapōlena, the ʻiʻiwialokele, and the ʻiʻiwipōpolo. The ʻiʻiwialokele is seemingly similar to the ʻiʻiwimakapōlena but with reddish eyes rather than yellow. In puke wehewehe "alokele" is defined as a red bird reported by Hawaiian historian and author, Kepelino. There does not appear to be a lot of historical information regarding the ʻiʻiwialokele specifically, however, the alokele is discussed later in the series. The author likens the alokele to a dove with a short beak and rosy red feathers. The alokele eats the nectar of lehua blossoms like the ʻiʻiwi and other honeycreepers. The ʻiʻiwipōpolo is described as looking similar to the scarlet ʻiʻiwi, but with a yellowish head. (See images below). According to the description provided, the ʻiʻiwimakapōlena has yellow extremeties while the ʻiʻiwipōpolo has red legs and feet.

The author provides the poetic term, "ke Kuhina o na manu o ka uka" (the Diplomat of the birds of the uplands) for the ʻiʻiwi. Of all of the manu mūkīkī described in the series, the ʻiʻiwi is the most detailed and most praised for its beauty and behavior, which the author notes is unusually active.

Figure 11. The ʻiʻiwimakapōlena or ʻiʻiwi. Photo from Maui Forest Bird Recovery Project.

Figure 12. The ʻiʻiwipōpolo is scarlet and black but has yellowish feathers on its head according to the 1863 description in Ka Nupepa Kuokoa. Photo from Maui Forest Bird Recovery Project.

4.2.3 Water

Fresh water (wai) is of tremendous significance to Native Hawaiians. It is closely associated with many Hawaiian gods. According to traditional accounts, Kāne and Kanaloa were the "water finders:" "Ka-ne and Kanaloa were the water-finders, opening springs and pools over all the islands, each pool known now as Ka-Wai-a-ke-Akua (The water provided by a god)" (Westervelt 1915:38). Kāne is widely known to be closely associated with all forms of water, as outlined in the mele "He Mele No Kane."

There was no element more important or precious than water. There was no god more powerful than Kāne. Pua Kanahele recounts the oli "ʻO Kāne, ʻo wai ia aliʻi o Hawaiʻi?" and notes of the oli: "The chant begins with Kāne and focuses on this deity as the connective force of all the poʻe akua, or god family. All the entities mentioned in each paukū, or verse, are a manifestation of Kāne" (Kanahele 2011:24). The association between water and Kāne is logical considering certain interpretations of Hawaiian mythology identify Kāne as the most powerful of all the Hawaiian gods.

Further investigation into the relationship between Kāne and Pele would be appropriate and helpful. Some interpretations identify Kāne as Pele's father (Westervelt 1915). A brief analysis is provided below.

He Mele No Kāne

E ui aku ana au iā ʻoe,	One question I ask of you:
Aia i hea ka Wai a Kāne?	Where flows the water of Kane?
Aia i lalo, i ka honua, i ka Wai hū,	Deep in the ground, in the gushing spring,
I ka wai kau a Kāne me Kanaloa-	In the ducts of Kane and Kanaloa,
He waipuna, he wai e inu,	A well spring of water, to quaff,
He wai e mana, he wai e ola,	A water of magic power- The water of life!
E ola no, ʻea!	Life! O give us this life!

This mele and other moʻolelo are clear: Kāne is water. It is deeply valued among the Hawaiian people. The only exceptions may be mist, known to be associated with Lilinoe, and snow, associated with Poliʻahu. There is an extensive body of traditional knowledge about the expeditions of Kāne and Kanaloa during which Kāne drove his ʻōʻō (digging stick) into the earth in search of water.

4.3 Intangible Cultural Resources

It is important to note that Honua Consulting's unique methodology divides cultural resources into two categories: biocultural resources and built environment resources. We define biocultural resources as elements that exist naturally in Hawaiʻi without human contact. These resources and their significance can be shown, proven, and observed through oral histories and literature. We define built environment resources as elements that exist through human interaction with biocultural resources whose existence and history can be defined, examined, and proven through anthropological and archaeological observation. Utilizing this methodology is critical in the preparation of a CIA as many resources, such as those related to akua, do not necessarily result in material evidence, but nonetheless are significant to members of the Native Hawaiian community.

Hawaiian culture views natural and cultural resources as being one and the same: without the resources provided by nature, cultural resources could and would not be procured. From a Hawaiian perspective, all natural and cultural resources are interrelated, and all natural and cultural resources are culturally significant. Kepā Maly, ethnographer and Hawaiian language scholar, points out, “In any culturally sensitive discussion on land use in Hawaii, one must understand that Hawaiian culture evolved in close partnership with its natural environment. Thus, Hawaiian culture does not have a clear dividing line of where culture ends and nature begins” (Maly 2001:1).

4.3.1 ʻŌlelo Noʻeau

ʻŌlelo noʻeau are another source of cultural information about the area. ʻŌlelo noʻeau literally means “wise saying,” and they encompass a wide variety of literary techniques and multiple layers of meaning common in the Hawaiian language. Considered to be the highest form of cultural expression in old Hawaiʻi, ʻōlelo noʻeau bring us closer to understanding the everyday thoughts, customs, and lives of those that created them. There are no identified ʻōlelo nōʻeau for the immediate project area.

4.3.2 Mele (Songs)

The *Buke Mele Lahui* (Hawaiian National Songbook), published in 1895, is “the largest number of political and patriotic Hawaiian songs ever printed in one place,” featuring mele that “echo the steadfast resilience of Hawaiians of that time as they weathered the political turbulence of the 1880s and 1890s that completely altered their world” through the overthrow and establishment of a foreign-led provisional goverment and subsequent annexation to the U.S. (Nogelmeier and Stillman 2003:xii). There are numerous mele and ʻoli composed for and inspired by the project area.

5.0 Traditional or Customary Practices Historically in the Study Area and Surrounding Area

In traditional (pre-western contact) culture, named localities served a variety of functions, informing people about: (1) places where the gods walked the earth and changed the lives of people for good or worse; (2) heiau or other features of ceremonial importance; (3) triangulation points such as ko‘a (fishing markers) for fishing grounds and fishing sites (4) residences and burial sites; (5) areas of planting; (6) water sources; (7) trails and trail side resting places (o‘io‘ina), such as a rock shelter or tree shaded spot; (8) the sources of particular natural resources/resource collections areas, or any number of other features; or (9) notable events which occurred at a given area. Through place names knowledge of the past and places of significance was handed down across countless generations. There is an extensive collection of native place names recorded in the mo‘olelo (traditions and historical accounts) published in Hawaiian newspapers.

This is not intended to be a comprehensive list of all the practices that historically or contemporaneously occur in East Maui. This is meant to show the range of traditional or customary practices that took place in the larger geographic extent. Many of these practices may not have taken place within the Study Area, and many of those that may have do not currently take place within the Study Area, although that may actively occur within the larger region.

5.1 Mo‘olelo

Mo‘olelo is the practice of storytelling and developing oral histories for the purpose of transmitting knowledge information and values intergenerationally. Mo‘olelo are particularly critical in protecting and preserving traditional culture in that they are the primary form through which information was transmitted over many generations in the Hawaiian Islands and particularly in the Native Hawaiian community.

Storytelling, oral histories, and oration are widely practiced throughout Polynesia and important in compiling the ethnohistory of the area. The Native Hawaiian newspapers were particularly valued for their regular publication of different mo‘olelo about native Hawaiian history. Were it not for the newspapers having the foresight to allow for the printing and publication of mo‘olelo, far less information about the cultural history of the Hawaiian people would be available today.

There are numerous mo‘olelo about the Study Area. These mo‘olelo are provided in **Sections 3.1 (Traditional Period)** and in **Section 4.0 (Cultual Resources)**.

5.2 Habitation

Hawaiians lived extensively throughout the islands. Handy, Handy, and Pukui (1991) identify how different kānaka and their ‘ohana lived in accordance with what the authors termed “occupational contrasts” (286), meaning that based on occupation (i.e., planter or fisherman, for example), habitation systems differed. They describe, “The typical homestead or *kauhale*…

consisted of the sleeping or common house, the men's house, women's eating house, and storehouse, and generally stood in relative isolation in dispersed communities. It was only when topography or the physical character of an area required close proximity of homes that villages exist. There was no term for village. *Kauhale* meant homestead, and when there were a number of *kauhale* close together the same term was used. The old Hawaiians, in other words, had no conception of village or town as a corporate social entity. The terrain and the subsistence economy natural created the dispersed community of scattered homesteads" (284).

5.3 Travel and Trail Usage

The ability to travel was essential to Hawaiians and enabled their sustainability. Travel, and the freedom to move throughout different areas, had different names, including huakaʻi, kaʻapuni, or kaʻahele. Traveling by sea had distinct names as well, like ʻaumoana. Traveling through the mountains was sometimes referred to as hele mauna. Travel, and moving throughout various places and regions was an essential practice and way of life in traditional Hawaiʻi.

The freedom to travel safely was so important that Kamehameha I would come to pass a well-known law protecting travelers, Ke Kānāwai Māmalahoe (The Law of the Splintered Paddle). It is explained by the William S. Richardson School of Law as follows:

> As a young warrior chief, Kamehameha the Great came upon commoners fishing along the shoreline. He attacked the fishermen, but during the struggle caught his foot in a lava crevice. One of the fleeing fishermen turned and broke a canoe paddle over the young chief's head. The fisherman's act reminded Kamehameha that human life was precious and deserved respect, and that it is wrong for the powerful to mistreat those who may be weaker.
>
> Years later when Kamehameha became ruler of Hawaiʻi, he declared one of his first laws, *Ke Kānāwai Māmalahoe* (the Law of the Splintered Paddle), which guaranteed the safety of the highways to all. This royal edict was law over the entire Hawaiian kingdom during the reign of Kamehameha the Great. Considered one of the most important *kānāwai* (royal edict), the law gave the Hawaiian people an era of freedom from violent assault (William S. Richardson School of Law 2021).

The kānāwai (law) reads:

E nā kānaka	O my people
E mālama ʻoukou i ke akua	Honor thy god
A e mālama hoʻi	Respect alike, the rights of
Ke kānaka nui a me kānaka iki	All men great and humble
E hele ka ʻelemakule	Se to it that our aged,
Ka luahine, a me ke kama	Our women, and children

A moe i ke ala	Lie down to sleep by the roadside
Aʻohe mea nana e hoʻopilikia	Without fear of harm
Hewa no, make	Disobey, and die

The law would have such long-lasting resonance that it would be expressly incorporated into the Hawaiʻi State Constitution.[3]

As traveling through traditional trails was the primary means by which people traveled on land throughout most of Hawaiian history, the traditional trail system is particularly important throughout the Hawaiian Islands. Throughout the islands, there were numerous trails that allowed for people to access different locations. This trail system was critical not only for maintaining a healthy population and managing this population, but it was also important for the traditional economic system of bartering. The trail system allowed for different localized communities to engage and interact. This also allowed for the trade of goods throughout island communities.

In 1862, L.W.K Kaaie shared information pertaining to ancient practices associated with interment of loved ones within the mountain landscape in the Hawaiian language newspress *Ka Hoku o ka Pakipika.* This is one of the earliest accounts documenting traditional access to the summit region of Haleakalā. Through this account we learn that families of Maui traveled along trails from the lowlands, through the forests, and to the summit Haleakalā where the remains of departed family members were hidden.

The narratives below are excerpted from the original Hawaiian (1862), with a translation from Fornander's "Hawaiian Antiquities" (1919, Volume V):

> No ke Kupapau. I ka wa e kaa mai ana a kokoke make, he oeoe mamua aku o ka make ana o kekahi mea, o na makamaka a me na mea e ae o ua mea nei ina he poe ua make, a ina he poe ua ola, a penei ka ka mea mai e olelo ai: "Eia ae o mea ke kii mai nei iaʻu e hele," a pela ia e olelo pinepine ai a hiki i kona wa e make ai. Aia hoi o kona poe makamaka a pau, uwe no lakou, a ina he mea ia i aloha nui ia, unuhi lakou i kekahi mea no ua mea make nei, ina he maiao, a ina he niho, a ina he lauoho…
>
> When confined with long illness, and death draws near, a person before his demise mutters in an indistinct and mumbling way, speaking of his relatives and his gods, whether they be dead or whether they be living in this manner: "So and so is coming to get me to go." And thus he would rave until he died. Whereupon all his relatives mourned, and if he was greatly beloved, they extracted something from his corpse, such as a nail, a tooth, or perhaps some hair…

[3] Article IX. Section 10 of the Hawaii State Constitution reads: "The law of the splintered paddle, mamala-hoe kanawai, decreed by Kamehameha I--Let every elderly person, woman and child lie by the roadside in safety--shall be a unique and living symbol of the State's concern for public safety."

I ka lawe ana e huna i ke kupapau, elua, a ekolu wale iho no mau kanaka, aole lehulehu. I ka po no hoi keia hana, aole i ke ao. I ka eli ana i ka lua, he lua poepoe no, ano like me ka lua maia. O ka hohonu kupono o ka lua i ka humemalo, he pahee ka inoa o ka lua; i ka eli ana, lawe ka lepo o ka lua i kahi e iloko o ka ahu, ipu, o ike ia ka me hou. Ina he hale hou, eli mai no ka poe nana ke kupapau mawaho mai, a komo iloko o ka hale, me ka ike ole o ka mea nona kela hale. Manao na kanaka ina e ike ia kahi i waiho ia, kii ia mai na iwi i mea makau; o ka io he mea kupalu mano. He mau lua huna no kekahi, ma ka pali paha, ma kahi papu paha. Aia no kekahi lua ma Haleakala, o ka Luaokaawa ka inoa, mauka pono o Nuu, ma Maui...

In taking the corpse to be hidden, it is done by two or three of his friends; not by many people. The burial is done at night, however, not in the day time. In digging the grave, it is dug round like a banana hole. The usual depth of the grave is up to one's waist, that is, up to the groin of a man. In olden time, this grave was called a pahee (smooth place). Upon digging, take dirt from the grave to another place in a fine mat, or a gourd, else the tracks would be shown. Should it be a new house, the friends of the dead would dig from the outside till they reached within, without the house owner knowing anything about it. The people thought that if the burial place was known, the bones would be taken for fish hooks, and the flesh for shark bait. There are some hidden graves among the precipices; and others are on plains. There is a hidden grave at Haleakala; it is called the grave of Kaawa; it is right mauka of Nuu, on Maui...

Eia no hoi na lua huna e kanu ia ai na alii, o Nuu, o Makaopalena, Kealaohia, o Puukilea, aia ma ke alo o Haleakala ma Maui Hikina lakou a pau. O Hamohamo, a me Alalakeiki kekahi; a ma Alalakeiki kahi i make ai o na kanaka mai Hawaii mai i lawe mai i ke kupapau ilaila e huna ia ai a pau kela poe kanaka no Hawaii i ke komo iloko o ka lua, hiki mai kekahi kanaka kamaaina, o Niuaiaawa ka inoa, a pani i ka waha o ka lua i ka pohaku, malaila lakou i noho ai a make. Aole kanaka e ola ana i ike i kekahi o keia mau lua huna. Ua nalowale loa ia lakou. Aole i pau a hoopuka hou aku no au, ke aloha ia mai nae.

Here are the secret graves wherein the chief of Nuu were buried: Makaopalena, Kealaohia and Puukilea, all on the side of Haleakala, on the eastern side of Maui. Hanohano and Alalakeiki are others. At Alalakeiki a number of men from Hawaii who brought a corpse to be hidden were killed. When those men from Hawaii had gone into the cave a man of the place, Niuaiaawa by name, came along and closed up the mouth of the cave with stones, and those people stayed in there until they died. There is no living man who knows any of these secret burial places, so well hidden are they.
L. W. K. KAAIE. Kamanuwai, Honolulu, Mar. 15, 1862. [In Ka Hoku o ka Pakipika, Malaki 20, 1862:4]

5.4 Hunting

Hunting is an important practice in the project area. Numerous individuals interviewed for this CIA are hunters and actively hunt in the project area. Informants identified deer, goat, wild cattle, and pigs/boar as some of the game they actively hunt within the project area.

5.5 Farming

Since poi was the staple food for Native Hawaiians, it was of the utmost priority for the first settlers to establish loʻi. Kalo's prominence in the Hawaiian diet derived from its nutritional value, but even more so from its mythological significance. According to Hawaiian traditions, the first human (male) was born from the taro plant:

> The first born son of Wakea and Papa was of premature birth and was given the name Haloa-naka. The little thing died, however, and its body was buried in the ground at one end of the house. After a while, a taro plant shot up from the child's body, the leaf of which was named lau-kapa-lili, quivering leaf; but the stem was given the name Haloa.
>
> After that another child was born to them, whom they called Haloa, from the stalk of the taro. He is the progenitor of all the peoples of the earth. (Malo 1951:244)

As discussed in **Section 3.2 (Traditional Period) and 3.3 (Historic Period)**, the area has an extensive history of farming that extends well back into the pre-European contact era.

5.6 Traditional Clothing (Clothes Making, Dyeing, and Lei Making)

Kapa (commonly known as barkcloth) was the traditional material made through a traditional method of gathering, treating, and beating plant fibers, often, but not limited to, wauke (*Broussonetia papyrifera*) to make fabric that was used to make lole (clothing). Pacific and Hawaiian kapa was known for its wide range of colors and the application of watermarks.

One article describes the process for making kapa:

> The finest kapa came from the paper of the mulberry tree. These trees were cultivated on plantations and grew to heights of more than twelve feet. As the tree grew, the branches were nipped off along the main trunk, insuring a long piece of bark which was easily peeled from the tree.

> The manufacture of kapa was an important occupation for women. After the bark had been peeled from the tree, the inner bark was separated and soaked in sea water to make it soft and pulpy. The softened bark was placed on an anvil and beaten with a cylindrical wooden beater. The first beating separted the fibers and produced strips about eight or nine feet long and ten to fourteen inches wide. These strips could be dried and stored until needed. When needed, the strips were soaked in water, placed in layers between banana leaves, and left for about ten days to mature by "retting" which is the decomposition and removal of softened tissues, leaving the finer fibers. These partially decomposed layered strips were beaten a second time with specially carved four-sided beaters. The patterns carved on the beaters were functional as they produced the necessary characteristics in the kapa for its end use. These carved designs left the equivalent of a watermark on the kapa.
>
> Kapa which was to be extremely soft and pliable, such as that used for the malo or loincloth, was subjected to an additional softening process. This process, which produced a finely ribbed fabric, was done by dampening the cloth, stretching it over a grooved board, and running a wooden grooving tool along the indentations in the board. When the cloth dried, permanent ribs remained. The hand was very similar to our crinkle gauze of today (Furer 1981:109-110).

Hawaiians were skilled at utilizing plants and materials to dye their clothing and other materials. Different methods would be employed to hōʻawa, extract dye colors from their source material(s). These dyes would be placed in a cup, known as a kā kāpala. Even foreign or exotic plants were utilized for this practice. Hawaiians used different words for the various types of dyeing activities and methods.

- Weʻa – a red dye or to print or dye red
- Hili – bark dye, as hili kukui, hili kōlea, hili noni; also kapa dyed with bark or the name for dyeing with the use of bark
- Kūhili – to dye (or stain) by soaking in water containing mashed bark, such as used for nets; also mulberry bark before it is beat into kapa
- Kūpenu – to dye by dipping material
- Kiʻolena – to dye kapa
- Hōlei –native tree (*Ochorosia compta*) related to the hao (*Rauvolfia*), which yields a yellow dye for kapa
- Kīheʻaheʻa palaʻā – dye made from the palaʻā (Sphenomeria chinensis syn. chusana) fern; palaʻā also references a kapa made from the māmaki (*Pipturus spp.*) bark which is then dyed a brownish-red with palaʻā fern

Hawaiians also had a lexicon for the various colors that could be achieved through this traditional practice.

- ‘Ōlenalena – yellow
- Hili – Dark-brown dye made from bark
- Puakai – red
- Nao – dark red
- Pōkohukohu – color made from the noni (*Morinda citrifolia*) root
- ‘Ākala – color made from raspberry or thimbleberry juice
- ‘Ōma‘oma‘o – light green color made from ma‘o leaves

Similarly, lei making was a regular occurance in traditional Hawaii. Anderson-Fung and Maly (2009) write about the traditional practice:

> In old Hawai‘i, lei could have important ceremonial functions, such as in religious offerings and for chiefly regalia, but lei were also enjoyed as personal adornment by Hawaiians of all levels of society. The ali‘i (chiefs) and the maka‘ainäna (the common people who tended the land) all wore lei. Even the akua (gods, deities, spirits), it was believed, sometimes wore lei when they walked the land in human form. The following observation by the French botanist Gaudichaud, who visited the islands in 1819, paints a picture of Hawai‘i as a place where the lei was an integral part of everyday life:
>
> > "It is indeed rare to encounter one of the natives of this archipelago who does not have an ornamental plant on his head or neck or some other part of his body...[The] women ... change [the plants they wear] according to the seasons, [and for them] all the fragrant plants, all flowers, and even the colored fruits, serve as attire, one after another. ...The young girls of the people, those of the island of Hawai‘i especially, seem to be fond of the [kou, Cordia subcordata], a tree very abundant in all the cultivated areas... The young girls of the mountains, who live near the forests, give their preference to the flowers of the [Erythrina (wiliwili) and a species of Canavalia, called ‘awikiwiki], the lively color of which makes magnificent garlands. Such natural attire is much more rich, much more striking, than all the dazzling creations of the elegant European ladies."
>
> This account and others like it suggest that lei worn for personal adornment were fashioned from the favorite plant materials that were readily available and abundant in the lei maker's environment (4).

Lei making continues as an important practice today, as the making and giving of lei as an expression of aloha to loved ones still regularly occurs throughout the Hawaiian Islands. Practitioners of these crafts actively practice in the project area, especially hula practitioners who use the forest to gather plants for their ceremonial purposes.

5.7 Lāʻau Lapaʻau

Lāʻau lapaʻau is the practice of traditional Hawaiian medicine. For centuries, native Hawaiians relied upon the environment around them to provide them medicine. It is still actively taught and practiced today. Medicinal experts or healers have intimate knowledge about plants and other resources to cure ailments illnesses and sicknesses. Traditional medicine is practiced by native peoples and local communities around the world. Similarly, Native Hawaiians, over many generations, have learned how to properly care for, utilize, and prepare plants to maintain the community's health.

It was important to not only have plants and have access to plants but to ensure that these plants were healthy and in good condition. These resources are cultural resources. They are critical to the ongoing practice of traditional medicine and healing within the Native Hawaiian community. There are still many traditional medicine practitioners in the Hawaiian community and throughout the Hawaiian Islands today. It is a practice that is still taught to the younger generation, and it is a practice that is still honored and utilized in many Hawaiian households throughout the state.

It was important that medicinal plants existed throughout the Hawaiian Islands so that when people traveled throughout different places on in the islands, they would always have access to the medicine they needed. In some cases, some plants were extremely rare, and, in those cases, it was particularly important to make sure that these populations were well protected and well cared for. There were also numerous gods associated with health, healing, and medicine. They are listed in **Table 2.**

Table 2. Hawaiian Gods Associated with Health, Healing and Medicine

Hawaiian gods associated with health, healing, and medicine (Pukui, 1971)
HiʻiakaikapolioPele
Lonopūhā
Maʻiola
Hiʻiakaikapuaʻenaʻena
Hauwahine
Hina
Hinaʻea
Hinalaulimukala
Kamakanuiʻahuʻilono
Kanaloa
Kū
Kūkeoloʻewa
Mauliola
ʻŌpeluhuikauhaʻailo

Maui has an active community of healing practitioners. These practitioners actively practice in the area, and due to its rural nature, there is likely to be a higher number of people who still utilize traditional medicine practices in this general area than in other, more urbanized areas. Additionally, this practice is enjoying a resurgence of activity throughout the islands, as more individuals are returning to cultural practices. Numerous practitioners interviewed for this assessment identified this practice as occurring in the area but did not specify specific plants used for these practices.

5.8 Kilo

Kilo are observational traditions and people who examine, observe, or forecast are identified as kilo and serve as traditional climate and weather experts. Kilo "references a Hawaiian observation approach which includes watching or observing [the] environment and resources by listening to the subtleties of place to help guide decisions for management and pono practices" ('Āuamo Portal 2021). The practice of kilo is seeing a resurgence on Hawai'i Island and in the Hawaiian Islands.

Kilo hōkū are traditional astronomers, or those who study the stars. A hale kilo or hale kilo hōkū were observatories or star observatories respectfully. Kilo makani were those who traditionally observed the winds. Kilo moana were traditionally oceanographers. Kilo 'uhane were those who observed and communicated with spirits.

Traditionally the practice of kilo or observation was critical to the management of traditional Hawaiian landscapes. This practice is very closely tied to traditional or customary access as observers would require access to specific vistas viewsheds or areas in order to observe environmental phenomenon.

As illustrated in the proceeding section, Native Hawaiians created a wide range of terms for the environment and understanding the ecosystems around them. These terms were often quite specific, and many were tied closely to a specific geographic area. This level of specificity illustrated the close kinship Hawaiians shared to their surrounding environment. The ability to observe and understand all elements of their ecosystem was essential to both the successful care of natural resources and the survival of the Hawaiian people.

The ability to effectively and accurately read weather phenomena was essential to the ability of Hawaiian people who farm, fish, navigate, and conduct any number of practices in a sustainable and successful manner. The knowledge Hawaiians acquired about their environment around them, including weather phenomena were the result of multi-generational observations that comprised an extensive body of information passed down through oral traditions. The following Hawaiians names and their descriptions of weather phenomena include words for clouds, rains, and winds that are utilized by kilo to help guide activities and practices:

ao akua – godly cloud, figurative representative of a rainbow.

ao loa – long cloud or high, distant cloud. Status cloud along the horizon.
ao ‘ōnohi – cloud with rainbow, ‘ōnohi, colors contained within it.
ao pua‘a – cumulus clouds of various sizes piled together, like a mother pig with piglets clustered around her. The Kona coast is famous for ao pua‘a, a sign of good weather and no impending storms.
ao pehupehu – continually growing cumulus typical of summer. Drifting with the tradewinds, these clouds pick up moisture and darken at their base, finally releasing their rain on the windward mountain cliffs.
ho‘omalumalu – sheltering cloud.
ho‘oweliweli – threatening cloud.
ānuenue – rainbow, a favorable omen.
ua loa – extended rainstorm.
ua poko – short rain spell.

5.9 Ceremonial Practices

The ceremonial practices of traditional Hawaiians are extensive. Throughout the course of Hawaii’s history, traditional Hawaiians have integrated religious, spiritual, and ceremonial practices in their daily lifestyle. Traditional or customary practices are then not distinct ceremonial practices but rather a part of their way of life. Therefore, it is challenging to define in discrete terms ceremonial practices associated with traditional Hawaiian customs. For the purpose of this section, the ceremonial practices discussed here focus primarily on customs carried out by general populations of Hawaiians, as opposed to activities or rituals carried out by trained and recognized specialists, kahuna. Those practices are discussed in a separate section.

Ceremonial practices are incorporated throughout numerous, if not all, of the activities identified in this section. For example, there is a great level of ceremonial practice and ritual associated with the care of the dead, burial remains, and funerary objects. Native Hawaiians as with most indigenous people integrated ceremony into most of their practices especially those that occurred out in the natural landscape or related to their way of life. There was no specific site or materials required for ceremony *per se*.

Nonetheless, shrines were sometimes associated with ceremonial practices. Shrines for the purpose of this assessment are distinct from heiau, which were places of worship. Again, the distinction is the nature in which these features or sites were created. Heiau required the advice and guidance of a kahuna, who would help ali‘i determine the best location in which to erect a heiau. Conversely, shrines were erected by maka‘āinana (working class) as part of their daily or occupational functions.

Makahiki is one example of a practice that has taken place prior to contact and continues post-contact and involves ceremonial elements. One of these elements is the akua loa, described by Malo as “the image of the Makahiki god, Lono-makua ... This work was called ku-i-ke-pa-a” (Malo 1951: 143). Further described by Malo:

> 22. This Makahiki idol was a stick of wood having a circumference of about ten inches and a length of about two fathoms. In form, it was straight and staff-like, with joints carved at intervals and resembling a horse’s leg; and it had a figure carved at its upper end.
>
> 23. A cross piece was tied to the neck of this figure, and to this cross piece, kea, were bound pieces of the edible pala[4] fern. From each end of this cross piece were hung feather lei that fluttered about, also feather imitations of the kaupu bird[5], from which all the flesh and solid parts had been removed.
>
> 24. The image was also decorated with a white tapa cloth made from wauke[6] kakahi[7], such as was grown at Kuloli[8]. ... One end of this tapa was basted to the cross piece, from which it hung down in one piece to a length greater than that of the pole. The width of this tapa was the same as the length of the cross piece, about sixteen feet.
>
> 25. The work of fabricating this image, I say, was called kuikepaa. The following night the chiefs and people bore the image in grand procession, and anointed it with cocoanut (sic) oil. Such was the making of the Makahiki god. It was called Lono-makua (father Lono), also the akua loa. This name was given it because it made the circuit of the land (Malo, 1951: 144-145).

The akua loa was taken to each ahupua‘a. This custom was important to the care, stewardship, and worship of the gods. These practices were intimately tied to the proper care and sustainable stewardship of all cultural and natural resources. Ethnographic data indicates that such practices take place within the Project Area or Study Area.

As with many concepts of traditional Hawaiian living and practices, the contemporaneous concept of the kahuna has been largely influenced by Western thought. The roles and responsibilities of the kahuna are well explained by Professor Terry Kanalu Young in his text, *Rethinking the Native Hawaiian Past*, in which he writes:

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[4] Native fern (*Marattia douglasii)* used for medicinal purposes as well as in ceremony.
[5] Laysan albatross (*Diomedea immutabilis*), written with diacritical markings as ka‘upu.
[6] Paper mulberry (Broussonetia papyrifera)
[7] Meaning outstanding or of high quality, as in reference to the white kapa (tapa) made from these fibers.
[8] Likely a reference to the place in Pelekunu Valley at Kamalō, Moloka‘i, located between the peaks of Kaunuohua and Pēpē‘ōpae.

> As recipients of hana lawelawe[9], the Ali‘i Nui were themselves serves of a sort. They were responsible for maintaining a positive spiritual relationship with the Akua through pono conduct. Pono was defined for individuals of that era within the context of a particular task specialty. Kahuna who functioned as experts in specific skill areas like medicinal healing, canoe building, or spiritual advising were consulted by leaders. The experts were looked to as responses for what was considered pono in their respective realms of knowledge (Young 1998).

Kahuna were critical to traditional Hawaiian lifeways as their extensive expertise helped to provide sound and strategic advice to ali‘i and other leaders on proper spiritual, cultural, and ecological management. There are numerous types of kahuna in Hawaiian traditions. including, but not limited to:

kahuna ‘anā‘anā - sorcerer who practices black magic and counter sorcery

kahuna a‘o - teaching preacher, minister, sorcerer.

kahuna hāhā - an expert who diagnoses, as sickness or pain, by feeling the body.

kahuna ha‘i‘ōlelo - preacher, especially an itinerant preacher.

kahuna ho‘ohāpai keiki - medical expert who induced pregnancy.

kahuna ho‘opi‘opi‘o - malevolent sorcerer, as one who inflicts illness by gesture.

kahuna ho‘oulu ‘ai - agricultural expert.

kahuna ho‘oulu lāhui - priest who increased population by praying for pregnancy.

kahuna hui - a priest who functioned in ceremonies for the deification of a king.

kahuna kālai - carving expert, sculptor.

kahuna kālai wa‘a - canoe builder.

kahuna ki‘i - caretaker of images, who wrapped, oiled, and stored them, and carried them into battle ahead of the chief.

kahuna kilokilo - priest or expert who observed the skies for omens.

[9] Hana lawelawe are defined by Young as “service tasks” by which kaukau ali‘i (lower ranked chiefs) served the Ali‘i Nui (high chiefs). These hana lawelawe were critical to the ability of the Ali‘i Nui to effective govern (Young 1989).

kahuna lapaʻau - medical doctor, medical practitioner, healer. Lit., curing expert.

kahuna makani - a priest who induced spirits to possess a patient so that he might then drive the spirits out.

kahuna nui - high priest and councilor to a high chief; office of councilor.

kahuna poʻo - high priest.

kahuna pule - preacher, pastor, minister, parson, priest. clergyman. Lit., prayer expert.

kahuna pule kaʻahele - preacher

kahuna pule wahine - priestess

In 1888, King David Kalākaua published a collection of Hawaiian traditions including the moʻolelo of the chief Hua. This moʻolelo references bird hunting on the upper mountain slopes of Haleakalā (Kalākaua, 1990). This moʻolelo is set in the district of Hāna and references the upper regions of Haleakalā crater. The practice of traveling into the mountain regions to catch birds extended into what is now the Waikamoi Preserve and throughout the forested regions of Maui Hikina. Kepā Maly summarizes:

> Some 1,000 years ago, in the district of Hāna, there lived a chief by the name of Hua, who grew abusive of his people. His elder kahuna nui (high priest), Luahoʻomoe, warned him to amend his ways, lest he incur the wrath of the gods. Hua wearied of this priest's warnings and devised a plan by which to rid himself of the elderly kahuna. The plot was laid out, in which the chief called upon his bird catchers to go to the uplands to fetch ʻuaʻu to be eaten by himself. When asked if he thought such birds would be found on the mountain lands, Luahoʻomoe told the chief that at that time of year, no ʻuaʻu would be found. Hua feigned indignity, and told Luahoʻomoe that if his bird catchers returned from the uplands with the desired ʻuaʻu, that he would be killed as a false prophet and seer.
>
> The bird catchers departed, as if traveling to the distant upland nesting grounds of the ʻuaʻu, while secretly going to the shore, where they found ʻuaʻu and other birds. When they returned to the Hua's court, they presented the birds to the king, with testimony that the birds had come from the mountains.

King Kalākaua's narratives tell us:

> Luahoomoe had two sons, Kaakakai and Kaanahua... Knowing that they would not be spared, Luahoomoe advised them to leave Hana at once and secrete themselves in

> the mountains, and suggested Hanaula, an elevated spur of the mighty crater of Haleakala, as the place they would be most likely to escape observation... [page 161]

Kalākaua then reported that the bird catchers:

> ...Returned, bringing with them a large number of birds, including uau and ulili, all of which they averred, had been caught in the mountains, when in reality they had been snared on the sea-shore.
>
> Hua summoned the high-priest, and, pointing to the birds, said: "All these birds were snared in the mountains. You are therefore condemned to die as a false prophet who has been abandoned by his gods, and a deceiver of the people..." Taking one of the birds in his hand, the priest calmly replied: "These birds did not come from the mountains; they are rank with odor from the sea..." [Kalakaua, 1990:162]

Kepā Maly summarizes:

> King Kalākaua's account continues, relating that Hua and his bird catchers denied the birds were from the shore. When Luahoʻomoe opened the stomachs of several of the birds, "all were found to be filled with small fish and bits of sea-weed" (ibid.) Outraged, Hua then took a spear and killed Luahoʻomoe. Immediately thereafter, rains stopped falling, rivers stopped flowing, and the land lay bare, parched by the sun. After a period of time, the king, Hua, himself wandered the mountains, and from district to district, until he died unattended, with his bones laying exposed to dry and rattle about in the sun, with no one to bury him—this being a great disgrace in ancient times (Kalākaua, 1990:165). Thus, the saying, "Nakeke nā iwi o Hua i ka lā" (The bones of Hua rattle in the sun).
>
> After Hua's death, a priest from Waimalu, on Oʻahu, by the name of Naula-a-Maihea (Naula). Naula discerned the cause of the drought and famine that had spread across the islands, as a result of Hua's evil treatment of Luahoʻomoe, and he also understood that the sons of the old priest could help relieve the people of their difficulty since Hua had died. Naula was led to the summit region of Haleakalā, and found Kaʻakakai and Kaanahua living in hiding. A black pig was offered with prayers and rituals, and the black, rain bearing clouds of Lono returned, bringing life giving rains to the islands (Kalākaua, 1990:169-173).

Kamakau's discussion on burial places on the Haleakalā mountain lands reveals how ancestral Hawaiians utilized and revered the wao akua. Kamakau's specific reference to "the rock that divides the lands" is in reference to the famed Pōhakuʻokiʻāina (also called "Pōhaku Pālaha"). This rock is a wahi pana (traditional storied place) and is situated just outside of the

Waikamoi Preserve/Hanawī NAR, and was accessed by various traditional trails, including those which passed through the Waikamoi Preserve. Pōhaku‘oki‘āina is situated at an elevation of 8,015 feet. Kamakau reported:

> The disposal pit of Ka‘a‘awa is a deep disposal pit inside the crater of Haleakala. It is on top of a lava mound in a pit (lua) on the north side, close to Wai‘ale‘ale [a swamp just outside the crater wall] and the rock that divides the lands [Pohaku Palaha, or Pohaku‘oki‘aina] on the eastern edge of the Ke'anae gap that opens at Ko'olau. It is a chasm, a nupa, or perhaps a deep pit, a lua meki, opened up from the foundations of the island by the forces of heaping lava, and may be several miles deep, with fresh or sea water at the bottom. Because of the insipid taste (‘ono ‘ole) of the waters, some people have supposed that the waters of Waiu and Waipu at Kaupo have their source at this pit of Ka‘a‘awa, or from some [page 39] disposal pits mauka of Pu‘umane‘one‘o. This pit of Ka‘a‘awa was like Waiuli; it was the disposal pit for the people of Makawao, Kula, and Kaupo. These pits could be visited in broad daylight because no evilly disposed people could get at the bones and take them away to work mischief. This is the character of nupa and lua meki—they are pits that mischievous people cannot get at. Burial caves, disposal pits, and caverns (ana huna, lua huna, nupa) were important from Hawaii to Kauai… [1968:40]

5.10 Haku Mele, Haku Oli, and Hula

This practice is related to the composition of song and chants. this is a practice that has existed for many centuries in the Hawaiian culture. When the Hawaiian culture primarily relied on an oral tradition to pass on knowledge and information, the ability to create songs and chants was essential to pass information from one generation to the next. As Donaghy (2013) notes, Hawaiians had hundreds of terms associated with this practice.

Songs and chants are largely influenced by the environment around them. As a pedagogical device it was important if not imperative that these songs or chants effectively captured data from the environment around the composer and passed on this information for others to utilize when managing natural resources. In a very real sense, the land and natural resources act as a muse for composers. The category of songs that provide information on or speak to natural resources are called mele ‘āina (songs of the land). As shown in the previous section, there are numerous traditional chants and songs about the project area and its surrounding landscape.

Much like mele and oli, hula serves as a way of both honoring place and telling the story of place. Many hula, especially those based on mele ‘āina, require intimate understanding of the place where the mele was composed, including the natural elements of that ‘āina. Hula hālau will regularly take huaka‘i, or journeys, to visit and honor the place a particular mele speaks of. The ability to visit the place and learn about it is important to the practice of hula.

Hula, as well as mele or oli, are also offered as gifts to kupuna or gods. This practice also requires access to traditional sites. Associated with hula would have been the practices of lei making and the use of plants to dye clothing.

5.11 Kia Manu

Kia manu (birdcatchers) were skilled individuals who would capture different birds to harvest their colorful hulu (feathers) for featherwork. Kia manu often sought after different manu mūkīkī, like the ʻiʻiwi, for their delicate and colorful feathers. Kia manu did not harm or kill birds for their feathers, but relied on a clever method of harvesting a few feathers from a single bird before releasing it. Kia manu set traps and decoys for birds like manu mūkīkī in the forest. Kia manu entails birdcatching by gumming. Birdcatchers would set decoys with fresh flowers like lehua tied on as bait. The decoys would be covered in kēpau, a sticky resin that birds would get stuck on while feeding. While the birds were feeding, trapped by the sticky kēpau, the kia manu would lower their traps and harvest the hulu.

Colorful birds like the ʻiʻwi, the kiwikiu, and the ʻākohekohe had desireable hulu for featherwork. Many feathered garments, like ʻahu ʻula (feather cloaks and capes), were reserved by aliʻi. ʻAhu ʻula were made using the feathers of the ʻiʻiwi and other birds. They were most commonly red and yellow with a black or greem trim. Kāhili (feather standards) were another form of featherwork to honor aliʻi, as well as lei hulu (feather lei).

In the moʻolelo of Laukaieie featured in *Ka Leo o ka Lahui* in June of 1894, a brief description of Hawaiian featherwork is provided. In this account the beauty of those adorned in ornate featherwork is described. These beautiful youth are kupua (supernatural beings) and the descendants of the akua Hina and Kū. It is clarifed that the wild forest in the mountains has become the home of these kupua of Hina and Kū.

> Not long after she went, she heard the voices of people talking. They were the voices of girls and she followed these voices until she could see two beauitful girls sitting outside of their house making bird feather lei with Mamo and ʻIʻiwi feathers and the yellow feathers of the ʻŌʻō. That is what they wore on their bodies night and day. Sitting in between these beauties residing in the forest was a handsome boy. His skin was a rosy pink and his eyes were like the bud of the ʻōhiʻa tree, and when joined with his feather garlands and his feather cape, it was like the yellow of a marigold. Just like the fine fragrant scent of this flower, the breath flowing from his nose was different. The familiar work of this very handsome one of these uplands was birdcatching. That is what he did regularly every day from the days of his youth to when he was grown. Those who resided in the forest of these uplands were constantly adorned with feathers.

Manu, Moses. “He Moolelo Kaao Hawaii No Laukaieie.” *Ka Leo o ka Lahui,* Volume II, Number 971. June 27, 1894. Page 1.

This practice no longer occurs, in part due to the decline of native birds and in part due to regulations that protect these species. There is no indicator from the ethnographic data that area practitioners continue this practice in secret or have a desire to revive the practice, but it is important to note that this was an important cultural practice that took place historically that was likely lost in large part due to the decline of Hawaiian forest birds. Kia manu supported a range of other cultural activities, like the weaving of lei hulu (feather lei) and other garments made for ali‘i. As such, the loss of birds and its associated practices had a significant adverse effect on Hawaiian traditional and customary practices.

6.0 Ethnographic Data

As discussed previously in **Section 2.5 (Ethnographic Methodology)**, information was collected from a wide range of individuals and sources. The findings of those efforts are discussed in this section. Ethnographic data is utilized to supplement the other research methods utilized. It is one in a range of research tools employed to gather information about the project area.

Honua Consulting was tasked with gathering information from individuals with lineal and cultural ties to the area and its vicinity regarding regional biocultural resources, potential impacts to these biocultural resources, and mitigation measures to minimize and/or avoid these impacts.

The bulk of the information available from practitioners and kūpuna were drawn from native testimonies and Hawaiian language sources and integrated into the cultural and historic overview section of this assessment. Those sources, along with responses to this project, were considered when researching the traditional or customary practices discussed in a previous section. Interviews were conducted with seven (7) individuals and additional mo‘olelo from a sixth individual was included in this section. This data helped to identify additional resources and practices in the area; this information also helped to confirm research conducted for this report.

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6.1 Interview with Pueo Pata

Interview with Pueo Pata

Interviewer: Mathew Sproat

Interviewee: Pueo Pata

Date: 2/9/2022

Location: via telephone

Biography

Mr. Pata is a kumu hula and cultural & language consultant. He was born in California but raised in Kihei, Maui. He has lived in upcountry Maui since 2003.

General Discussion

Mr. Pata is associated with the project area as a cultural practitioner and resident of the area. When asked if there was anything he would like to share at the end of the interview, Mr. Pata explained that mosquitos have been a problem in Hawai'i since they have been introduced.

As climate change warms environment and mosquitos are able to travel further upward in elevation, our remaining native bird populations will be placed at further risk.

Cultural Resources

Mr. Pata noted that the resources of the area are used for cultural practice, including native plants for laʻau lapaʻau. He also mentioned the myriad native birds that exist in the area.

Traditions and Customs

Mr. Pata noted that he and other cultural practitioners access the area for native plants, stones, and wood for various cultural practices including hula, altars, and ceremonies.

Impacts

Mr. Pata noted that if the bacteria used for the mosquito mitigation method cannot be spread elsewhere into the environment, he does not see a reason why he would object to the project. He specifically mentioned that mosquitos are not native to Hawaiʻi and have only caused damage.

Mitigation Measures & Recommendations

Mr. Pata recommended that the project minimize any restrictions of access to Native Hawaiians, specifically for native/natural resources. These resources are important for traditional and cultural practices.

(This area intentionally left blank.)

6.2 Interview with Piʻilani Lua

Interview with Piʻilani Lua

Interviewer: Mathew Sproat

Interviewee: Piʻilani Lua

Date: 2/9/2022

Location: via telephone

Biography

Ms. Lua is a kumu hula and Hawaiian studies teacher at Hana school. She was born and raised in Kailua on the island of Oʻahu. She currently lives in Hana, Maui.

General Discussion

Ms. Lua is associated with the project area as a community member and cultural practioner. Further, she is a lineal descendant of the area. She was also a cultural specialist at Kahanu Gardens, which has the largest heiau in all of Polynesia.

Cultural Resources

Ms. Lua mentioned that the area has many heiau and lo'i. She mentioned that the project area has many farmers and cultural practitioners. Specific to the water resources in the area, Ms. Lua mentioned there were hihiwai and opae, which are used for subsistence by some. Further, she also noted that cultural practitioners utilize native plants in the area for la'au lapa'au.

Traditions and Customs

Of the many traditions and customs associated with the large project area, Ms. Lua mentioned farming, fishing, hunting (pig and deer), lo'i, and la'au lapa'au.

Impacts

Ms. Lua raised concerns regarding the method of using bacteria to make mosquitos infertile. She asked if this method had been done elsewhere, or if Maui was the first experiment. Ms. Lua recounted the story of bringing the mongoose to kill the rat – thereby creating a new problem later on. Ms. Lua also asked if there will be an impact on birds.

Mitigation Measures & Recommendations

Ms. Lua recommended that, if the project is an experiment or pilot project, the project should be experimented/trialed somewhere else. She noted that the area is sensitive and people depend on native flora and fauna for their livelihoods. Further, Ms. Lua recommended that the project take place further away from people.

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6.3 Interview with Ikaika Blackburn

Interview with Ikaika Blackburn

Interviewer: Mathew Sproat

Interviewee: Ikaika Blackburn

Date: 2/9/2022

Location: via telephone

Biography

Mr. Blackburn is a firefighter and musician. He was born and raised in Wailuku, Maui, where he currently lives.

General Discussion

Mr. Blackburn is familiar with the project area, although he noted that he does not venture there often today. He explained that the area is very lush given the high amount of rainfall. He also explained that many tourists visit the area.

Cultural Resources

Mr. Blackburn noted that the large project area has a plethora of native species. There are native freshwater fish, native birds, native ferns, and native trees. There are also many freshwater streams which flow mauka/makai. Mr. Blackburn also mentioned that several heiau also exist in the project area.

Traditions and Customs

Mr. Blackburn explained that there are still many native farmers associated with the land on this side of Maui. He noted that these people utilize the area for many traditions and customs, such as laʻau lapaʻau. Further, he explained that the maintenance of the streams – to ensure they are going through the proper channels – is also a cultural practice.

Mr. Blackburn also shared that the area is popular for hunting, specifically boar in this area.

Impacts

Mr. Blackburn raised some concern about the potential for unintended impacts from the project. He noted that the project area is a fragile system. Using bacteria for mosquito control may work theoretically, but what will the actual impact be on the whole system?

Mitigation Measures & Recommendations

Ms. Blackburn recommended that, should the project proceed, it be monitored closely.

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6.4 Interview with Ho'o Cabanilla

Interview with Ho'o Cabanilla

Interviewer: Mathew Sproat

Interviewee: Ho'o Cabanilla

Date: 2/8/2022

Location: via telephone

Biography

Ms. Cabanilla is an operator trainee at the Lahaiana Wastewater Treatment Facility. She was born and raised in Lāhainā, Maui. She still lives on the west side of Maui in the Kahana area.

General Discussion

Ms. Cabanilla is associated with the project area through hunting. Coming from the west side of Maui originally, Ms. Cabanilla noted that the project area was an "experience". The area is vast and open, with very little human use or impact.

Cultural Resources

Ms. Cabanilla recounted that she hunted goats, deer, and wild cattle with her ʻohana in the project area. There are also wild pigs in the area which are hunted. As such, Ms. Cabanilla mainly discussed these huntable species as cultural resources.

Traditions and Customs

As discussed above, Ms. Cabanilla primarily mentioned hunting in the area. She noted that family members have hunted in the area throughout their entire lives.

Impacts

Ms. Cabanilla would assume that there could be a potential of impact to cultural resources and traditions and impacts from the project. She raised the question: will the affected male mosquitos in any way harm or impact other species? Will it affect humans?

Mitigation Measures & Recommendations

Ms. Cabanilla would like to know if there will be any negative impact or effect on people or other species by the project. She suggested that the project be done in phases to ensure that there is an understanding of the impacts before fully scaling the proajct.

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6.5 Interview with Kamaka Kukona

Interviewer: Mathew Sproat
Interviewee: Kamaka Kukona
Date: 2/17/2022
Location: via telephone

Biography

Mr. Kukona is a kumu hula, cultural advisor, entrepreneur, and musician. He was born and raised in Wailuku Maui where he still resides.

General Discussion

Mr. Kukona has ʻohana who reside in mauka areas of the region. He noted that in some places in the area, there is a lot of standing water in puddles and mud. He explained that it would be great to reduce mosquito populations if it were shown that there would be no negative side effects from the bacteria.

Cultural Resources

Other than native birds, Mr. Kukona noted that other cultural resources include maile, palapalai, ʻieʻie, and lehua which they have gathered in the area. However, there are a myriad native species in the area, including most of the plants that are used for hula.

Traditions and Customs

Mr. Kukona noted that one tradition and custom associated with the area is gathering. He explained that he and other practitioners gather native plants in the area for their various practices.

Impacts

Mr. Kukona could not think of any impacts to cultural resources or traditions and customs caused by the project. He did raise the question: what happens if an infected mosquito bites a person?

Mitigation Measures & Recommendations

Mr. Kukona recommended that perhaps the project could be contained to a smaller area first, before scaling.

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6.6 Interview with Mike Opgenorth

Interviewer: Dane Maxwell
Interviewee: Mike Opgenorth
Date: 2/8/2022
Location: via Zoom

Biography

Mike Opgenorth is the director at Kahanu Garden and Preserve of the National Tropical Botanical Garden. Mike has been at Kahanu Garden and Preserve for seven years. Mike is originally from Fallbrook, California, an agricultural community located in northern San Diego county. Mike's father works in horticulture which inspired the work he does now. Mike currently resides in Makaʻalae in Hana on Maui.

Overview

Mike Opgenorth provided information on various traditions, customs, and natural and cultural resources that exist nearby and within the project area.

General Discussion

Mike Opgenorth is associated with the project area through the conservation work he does at the Kahanu Garden and Preserve, which is located just ma kai of the project area.

Cultural Resources

Kahanu Garden and Preserve is home to a coastal hala preserve as well as cultural sites like Piʻilani Hale heiau, which is ma kai of the project area in the Honomaʻele ahupuaʻa. The project area includes the upper region of this ahupuaʻa, including the watershed areas of Honomaʻele. Mike emphasizes that anything that happens mauka, will impact the lower makai regions as well. Kahanu Garden and Preserve is adjacent to the project area and includes natural plant preserves, like hala, and other significant cultural sites like Waiʻānapanapa and Waiʻeleʻele. Both Waiʻānapanapa and Waiʻeleʻele are significant pools of fresh water that were historically visited by aliʻi like Piʻilani. Mike considers these waters to be sacred in that they are ancestral water sources.

Mike discusses that because the upper regions of Hana have such rich watersheds there is a lot of biodiversity in the areas including a diverse populace of native birds.

Traditions and Customs

Mike named the water sources, Waiʻānapanapa and Waiʻeleʻele as significant sites within the project area that are rooted deeply in Hawaiian tradition. Piʻilani Hale heiau is considered to be one of the most significant sites within Polynesia and is the one hānau (place of birth) of many aliʻi and people throughout Maui. Mike shared that ma kai of the project area there are many fishing practices associated with the Honomaʻele ahupuaʻa.

The upper regions of Honomaʻele is home to a hala preserve which reflects weaving traditions in the area. He also mentions that there are many lāʻau lapaʻau and mahi ʻai practioners in the area. Many people hold ʻaha ʻawa (ʻawa ceremonies) in the area as well. Mike was taught that the name Homomaʻele, meaning "numb bay" alludes to the practice of drinking and making ʻawa in the area. Honomaʻele is also home to a famous ʻōhiʻa ʻieʻie vine, referenced in Kamakau's work. Mike references the famous battle of Lono-a-Piʻilani and Kiha-a-Piʻilani which took place within the upper and lower regions of Hana.

Impacts

Mike had mentioned the significance of the Waiʻānapanapa and Waʻeleʻele pools and how those are potentially areas where mosquitoes breed and lay eggs. He notes that a lot of the water sources in the project area are streams and rivers, and not stagnant water sources where mosquitoes breed, however, the streams often run dry and pools of stagnant water collect, possibly attracting mosquitoes. Mike raised questions about how the Wolbachia bacteria might impact other forms of fauna when released in this way. He had questions about possible residual effects from releasing more Wolbachia into the environment through mosquitoes and how that will impact other animals like ʻōpae and ʻoʻopu that live in streams and their habitats. He asked if adequate studies and research has been done to determine possible residual effects from Wolbachia mosquitoes on the environment and other animals and insects. He also raised a question on whether or not the increase in Wolbachia bacteria could impact native plants that are used for lei making, weaving, and other cultural practices.

Mitigation Measures & Recommendations

Mike thinks that this project is set with good intentions and is hopeful it is successful in protecting native birds. His biggest recommendation is to keep the public informed and practice strong communication with the community so they know what is happening and if the project is successful. Because this project concerns Maui's water sources Mike pointed out that it also concerns the people, so keeping the public up to date on whether or not the project is successful and possible impacts discovered is critical. Mike recommended sharing other studies done with Wolbachia mosquitoes with the public so that they can be better informed.

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6.7 Interview with Edward Makahiapo Cashman

Interviewer: Dane Maxwell
Interviewee: Makahiapo Cashman
Date: 2/8/2022
Location: via Zoom

Biography

Makahiapo Cashman is the director of Ka Papa Loʻi o Kānewai at UH Mānoa. Makahiapo was born and raised on Oʻahu and shared that he spent a lot of time on Maui where his mom and grandparents are from. Makahiapo currently resides in Kāneʻohe.

Overview

Makahiapo Cashman provided information on various traditions, customs, and natural and cultural resources that exist nearby and within the project area.

General Discussion

Makahiapo considers Maui to be his second home as it is where his mother grew up and where he has spent a lot of his time since his youth. His association to the project area comes from these familial connections to the ʻāina of Maui.

Cultural Resources

Makahiapo discussed water sources as a valuable cultural resource that is formed in the ma uka regions and trickles down to the lower regions. He shared that his ʻohana on Maui are fishermen and discussed how water from the upper regions connects to fishing practices. The beaches in Hana have upwelling of freshwater from ma uka and that informs the fishing practices of the area.

Makahiapo discussed the fishponds in the area, like Kūmaka, that have water sources connected to the ma uka forest regions. He shared that Kamehameha was known to work on the fishponds in the Hana area becuase they were so significant to the entire ahupuaʻa.

Traditions and Customs

Makahiapo was taught that the water that feeds Kūmaka fishpond is conected to Kiha, the moʻo, who was associated with the water sources in the area. Kūmaka and other fishponds in the area were also connected to the traditions of Kūʻula, the fishing god, and his son ʻAiʻai.

Inland of the fishpond is a freshwater pool fed from the ma uka regions that has become overgrown. Makahiapo shared that because the pool is mostly stagnant, mosquitoes frequent the near shore pool and when dengue fever was first reaching Maui they had infected mosquitoes in that area. The areas were cleared to mitigate the spread of dengue fever. Makahiapo emphasized how near shore pools and ponds are fed by the streams in the upper regions and the need for people to protect and maintain water sources ma uka to ma kai.

Makahiapo shared that the ma uka regions of Maui Hikina including Kīpahulu are areas where hunting and gathering is prevalent. People utilize the rich upper regions to gather materials for lei, pōhaku for walls and imu, and for hunting.

Makahiapo shares a moʻolelo about how Māui gives the birds their colors. Māui and a friend are in the forest listening to the birds sing, but only Māui, who is friends with the birds, can see them. His friend shares that where he comes from the birds are bright and colorful so you can see them. Māui decides to color each bird so now the ʻiʻiwi and other honeycreepers are vivid and colorful.

Impacts

Makahiapo had shared that the project area has a history of issues with mosquitoes infected with disease. He is skeptical of the fact that the Wolbachia mosquitoes are sterile and raised concerns about what that might do to the people they bite, the native plant life, and the water.

Makahiapo discussed how water sources in the Maui Hikina area connect to other islands through tides and currents, so he wonders if any impacts from the mosquitoes could spread to other islands like Hawaiʻi, Kahoʻolawe, and Molokaʻi. He expressed the need to share more research and information on these mosquitoes so people can better understand the project and possible impacts.

Mitigation Measures & Recommendations

Makahiapo is concerned about native bird populations and agrees that something needs to be done to prevent their death and extinction.

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7.0 Findings and *Ka Paʻakai* Analysis

Based on the research and ethnographic data within this report this project is unlikely to adversely impact traditional or customary practices. Yet, it is clear that additional education and outreach is needed, particularly to the practitioner community. Hunters use the project area extensively, and they hunt for subsistence. This subsistence lifestyle provides critical protein and food resources to families in East Maui.

It has long been the law of the land that the State of Hawaiʻi has an "obligation to protect the reasonable exercise of customary and traditionally exercised rights of Hawaiians to the extent feasible" *Public Access Shoreline Hawaiʻi v. Hawaiʻi County Planning Commission ("PASH")* 79 Hawaiʻi 425, 450 n. 43, 903 P.2d 1246, 1271 n. 43 (1995). In 2000, in the *Ka Paʻakai* decision, the Court established a framework "to help ensure the enforcement of traditional and customary Native Hawaiian rights while reasonably accommodating competing private development interests." 94 Hawaiʻi 31, 35, 7 P.3d 1068, 1972 (2000). This analysis is used here to fulfill the goal of this CIA (**Section 1.4**).

Based on the guidelines set forth in *Ka Paʻakai*, the Hawaiʻi Supreme Court provided government agencies an analytical framework to ensure the protection and preservation of traditional and customary Native Hawaiian rights while reasonably accommodating competing private development, or other, interests. The Court has stated: "that in order to fulfill its duty to preserve and protect customary and traditional Native Hawaiian rights to the extent feasible, as required by Article XII, Section 7 of the Hawaiʻi Constitution, an administrative agency must, at minimum, make specific findings of fact and conclusions of law as to the following:

1) The identification of valued cultural, historical, or natural resources in the project area, including the extent to which traditional and customary Native Hawaiian rights are exercised in the project area.
2) The extent to which those resources—including traditional and customary Native Hawaiian rights—will be affected or impaired by the proposed action; and
3) The feasible action, if any, to be taken to reasonably protect Native Hawaiian rights if they are found to exist. *Ka Paʻakai*, 94, Hawaii at 47, 7 P.3d at 1084. Cited in *Matter of Contested Case Hearing Re Conservation District Use Application (CDUA) HA-3568 for the Thirty Meter Telescope at the Mauna Kea Science Reserve, Kaʻohe Mauka, Hāmākua, Hawaiʻi*, 143 Hawaiʻi 379, 431 P.3d 752 (2018) ("*Mauna Kea II*").

In order to complete a thorough CIA that complies with statutory and case law, it is necessary to fully consider information available from, and provided by, Native Hawaiian cultural practitioners and cultural descendants from the project area.

The *Ka Paʻakai* analysis is largely a legal analysis, as the applicable tests are legal standards. Therefore, a strong analysis will be conducted by someone with sufficient legal training. Additionally, at the core of a thoughtful *Ka Paʻakai* analysis is a comprehensive understanding of traditional and customary practices. In breaking down the Court's tests, it is important to the different elements that contribute to each test.

The first test - "The identification of valued cultural, historical, or natural resources in the project area, including the extent to which traditional and customary Native Hawaiian rights are exercised in the project area" – actually consists of two separate elements. First, the simple identification and existence of valued cultural, historical, or natural resources. These resources are tangible in nature. They can include sacred places, culturally valuable plants, or a religious or historic site. This assessment sought to identify the extent of resources that may exist in the project area or adjacent areas.

As to this test, this assessment shows there are potentially resources within the project area, the primary of which is access to other cultural sites. Interviews indicate there are otherwise no current traditional cultural resources in the area that are used for traditional or customary practices, but based on the ethnographic data, practitioners may access mauka lands and the resources located there through the project area.

The second element of this first test is access. Access requires two things to occur. One is the existence of a resource. Whether a plant, an animal, a place, or site, the resource must exist in order a practitioner to access it. The second thing is physical access. This includes, but it is not limited to, the ability to physically access a plant, animal, site, or location associated with a particular practice. This can also include the traditional and customary route or path taken to access the resource. This can also include cultural protocols that existed in accessing a resource. These are often temporal, in that access protocols can be at a certain time of day or year. Makahiki would be a good example of a traditional custom that has specific cultural protocols associated with access. In the case of Makahiki, the custom takes place at a certain time of year.

Therefore, the first test under *Ka Paʻakai* should include not only a listing of resources, but the identification of ways in which those resources are accessed and utilized in association with a traditional and customary practice.

Therefore, the second test – "The extent to which those resources—including traditional and customary Native Hawaiian rights—will be affected or impaired by the proposed action" – also looks at two separate elements. The first, does the proposed action and its alternatives have an adverse impact on the existence of resources? This would include the alteration, destruction, modification, or harm of sites, including biological resources, sacred places, burial sites, etc. It also includes a loss of species. Any adverse impact or harm to resources is alone an affect or impairment caused by the proposed action.

Under this element, adverse impacts to historic sites or culturally utilized plants would all be identified adverse impacts. Under this same element, any indirect or cumulative effects would create an adverse impact under *Ka Paʻakai* if those actions harmed resources.

In addition to this, any action that impacts traditional and customary access to resources, even if there is not direct adverse impact to the resource itself, would result in an affect or impairment resulting from the proposed action. Therefore, the limitations on access that could result from development or use of the project area could create an adverse impact under *Ka Paʻakai.*

There was concern expressed by informants that the project could potentially and adversely impact native flora and fauna. This is why the recommended education and outreach to the East Maui community, particularly hunters and other practitioners is critical. If the project and concerns about the use of this biocontrol discourage practitioners from conducting their traditional or customary practices, it would be an adverse effect to these cultural activities.

The third part of the *Ka Paʻakai* framework aims to identify “[t]he feasible action, if any, to be taken to reasonably protect Native Hawaiian rights if they are found to exist.” Determining whether or not action has been suitably “feasible” is a matter for the State. These feasible actions could include continued access to the project as needed to conduct cultural practices.

Extensive education and outreach would make this third step unnecessary, as it would resolve the concerns of practitioners and help to avoid any potential impacts to traditional or customary practices.

8.0 References

Akana C.L. and Gonzalez K. (2015) *Hānau Ka Ua: Hawaiian Rain Names*. Honolulu: Kamehameha Publishing.

Handy, E.S.C. (1940) *The Hawaiian Planter*, vol. 1. Bernice P. Bishop Museum Bulletin 161. Bishop Museum Press, Honolulu.

Handy, E.S.C., and E.G. Handy (1972) *Native Planters in Old Hawaii: Their Life, Lore, and Environment.* Bernice P. Bishop Museum Bulletin 233. Bishop Museum Press, Honolulu.

Malo D. (1951) *Hawaiian Antiquities (Moʻolelo Hawaiʻi).* Honolulu, HI: Bishop Museum Press.

Maly K. (2001) Mālama Pono i ka ʻĀina – An Overview of the Hawaiian Cultural Landscape. Hilo, HI: Kumu Pono Associates.

Maly K. and Maly O. (2003) Ka Hana Lawaiʻa a Me Nā Koʻa o Na Kai ʻEwalu: a History of Fishing Practices and Marine Fisheries of the Hawaiian Islands Compiled from Native Hawaiian Traditoins, Historical Accounts, Government Communications, Kamaʻāina Testimony and Ethnography. Hilo, HI: Kumu Pono Associates.

Maly K. and Maly O. (2006) He Moʻolelo no Maui Hikina-Kalialinui i uka a me Nā ʻĀina o lalo: A Cultural –Historical Study of Easy Maui – the uplands of Kalialinui, and the Lands that Lie Below, Island of Maui, "The Waikamoi Preserve". Hilo, HI: Kumu Pono Associates.

Pukui M.K. (1983) *ʻŌlelo Noʻeau: Hawaiian Proverbs & Poetical Sayings*. Bernice P. Bishop Museum Special Publication No. 17. Honolulu, HI: Bishop Museum Press.

Pukui M.K. and Elbert S.H. (1971) *Hawaiian Dictionary*. Honolulu, HI: University of Hawaii Press.

Pukui M.K., Elbert S.H. (1986) *Hawaiian Dictionary*. Honolulu, HI: University of Hawaiʻi Press.

Pukui M.K., Elbert S.H., and Mookini E.T. (1974) *Place Names of Hawaii (Revised and expanded edition).* Honolulu, HI: University of Hawaii Press.

Spriggs, M., and A. Anderson (1993). Late Colonization of East Polynesia, *Antiquity* 67:200–217.

Sterling, E.P., and C.C. Summers (1978) (compilers). *Sites of Maui.* Bernice P. Bishop Museum, Honolulu.

U.S. National Library of Medicine (2021) "1826: Mosquitoes arrive in Hawaii." Native Peoples' Concepts of Health and Illness. https://www.nlm.nih.gov/nativevoices/timeline/694.html

Appendix I: Glossary of Hawaiian Terms

The following list of terms were used frequently throughout this report. All definitions were compiled using Pukui and Elbert's *Hawaiian Dictionary* (1986).

Ahupuaʻa	Land division usually extending from the uplands to the sea, so called because the boundary was marked by a heap (ahu) of stones surmounted by an image of a pig (puaʻa), or because a pig or other tribute was laid on the altar as tax to the chief.
ʻĀina	Land, earth. *Lit.* That which feeds.
Akua	1. God, goddess, spirit, ghost. 2. Divine, supernatural, godly.
Ala	Path, road, trail.
Aliʻi	1. Chief, chiefess, ruler, monarch. 2. Royal, regal. 3. To act as chief, reign.
ʻAumakua	Family or personal gods, deified ancestors who might assume the shape of sharks, owls, hawks, dogs, plants, etc. A symbiotic relationship existed; mortals did not harm or eat them, and the ʻaumakua warned or reprimanded mortals in dreams, visions, and calls.
ʻAumākua	Plural of ʻaumakua.
ʻAuwai	Irrigation ditch, canal, waterway.
Hālau	1. Long house, as for canoes or hula instruction; meeting house. 2. Large, numerous; much.
Hale pili	House thatched with pili grass.
Heiau	Pre-Christian place of worship, shrine. Some heiau were elaborately constructed stone platforms, other simple earth terraces.
Hoʻi	1. To leave, go or come back; to cause to come back. 2. To enter, as an institution or last resting place. 3. A parting chant to which hula dancers dance as they leave the audience. 4. Marriage of a chief with the daughter of a brother or sister; to do so (a means of increasing offspring).
Hula	A Hawaiian dance form accompanied by chant or song.
ʻIli	Land section, next in importance to ahupuaʻa and usually a subdivision of an ahupuaʻa.
ʻIli kū	Shorted form of ʻili kūpono.
ʻIli kūpono	A nearly independent ʻili land division within an ahupuaʻa, paying tribute to the ruling chief and not to the chief of the ahupuaʻa. Transfer of the ahupuaʻa from one chief to another did not include the ʻili kūpono located within its boundaries. Sometimes shorted to ʻili kū.

Kanaka	Human being, person, individual, party, humankind, population; often used for man.
Kānaka	Plural of kanaka.
Kāne	Male, husband, male sweetheart, man; brother-in-law of a woman.
Kanikau	1. Dirge, lamentation, chant of mourning, lament. 2. To chant, wail, mourn.
Kapu	1. Taboo, prohibition. 2. Special privilege or exemption from ordinary taboo. 3. Sacredness, prohibited, forbidden, sacred, holy, consecrated. 4. No trespassing, keep out.
Kuleana	Right, privilege, concern, responsibility, title, business, property, estate, portion, jurisdiction, authority, liability, interest, claim, ownership, tenure, affair, province.
Kupuna	Grandparent, ancestor, relative or close friend of the grandparent's generation, grandaunt, granduncle.
Kūpuna	Plural of kupuna.
Limu	A general name for all kinds of plants living under water, both fresh and salt, also algae growing in any damp place in the air, as on the ground, on rocks, and on other plants; also mosses, liverworts, lichens.
Lo'i	Irrigated terrace, especially for taro, but also for rice and paddy.
Loko i'a	Traditional Hawaiian fishpond.
Makai	On the seaside, toward the sea, in the direction of the sea.
Mālama	To take care of, tend, attend, care for, preserve, protect, beware, save, maintain.
Mauka	Inland, upland, towards the mountain.
Mele	1. Song, anthem, or chant of any kind. 2. Poem, poetry. 3. To sing, chant.
Mele māka'ika'i	Travel chant.
Mō'ī	King, sovereign, monarch, majesty, ruler, queen.
Moku	1. District, island, islet, section, forest, grove, clump, fragment. 2. To be cut, severed, amputated, broken in two.
Mo'o	Lizard, reptile of any kind, dragon, serpent.
Mo'olelo	Story, tale, myth, history, tradition, literature, legend, journal, log, yard, fable, essay, chronicle, record, article.
Mo'owahine	Female lizard deity.
Nī'aupi'o	Offspring of the marriage of a high-born brother and sister, or half-brother and half-sister.
'Ōlelo no'eau	Proverb, wise saying, traditional saying.
Oli	Chant that was not danced to, especially with prolonged phrases chanted in one breath, often with a trill at the end of each phrase; to chant thus.

Piʻo	Marriage of full brother and sister of nīʻaupiʻo rank, presumably the highest possible rank. Their offspring had the rank of naha, which is less than piʻo but probably more than nīʻaupiʻo. Later piʻo included marriage with half-sibling.
Pueo	Hawaiian short-eared owl (*Asio flammeus sandwichensis*), regarded often as a benevolent ʻaumakua.
ʻŪniki	Graduation exercises, as for hula, lua fighting, and other ancient arts (probably related to niki, to tie, as the knowledge was bound to the student).
Wahi pana	A legendary place; a place made special celebrated in stories associated with it. Often sacred.
Wahine	Woman, lady, wife; sister-in-law, female cousin-in-law of a man, female.
Wao	1. Realm. 2. A general term for inland region usually forested but not precipitous and often uninhabited.

APPENDIX D:
USFWS Mitigation Measures and Biosecurity Protocols

D-1

Avoidance, Minimization, and Conservation Measures for listed plants in the Pacific Islands

Revised September 2020

Project activities may affect listed plant species by causing physical damage to plant parts (roots, stems, flowers, fruits, seeds, etc.) as well as impacts to other life requisite features of their habitat which may result in reduction of germination, growth and/or reproduction. Cutting and removal of vegetation surrounding listed plants has the potential to alter microsite conditions (e.g., light, moisture, temperature), damaging or destroying the listed plants and also increasing the risk of invasion by nonnative plants which can result in higher incidence or intensity of fire. Activities such as grazing, use of construction equipment and vehicles, and increased human traffic (i.e. trails, visitation, monitoring), can cause ground disturbance, erosion, and/or soil compaction which decrease absorption of water and nutrients and damage plant root systems and may result in reduced growth and/or mortality of listed plants. Soil disturbance or removal has the potential to negatively impact the soil seed bank of listed plant species if such species are present or historically occurred in the project area.

In order to avoid or minimize potential adverse effects to listed plants that may occur on the proposed project site, we recommend minimizing disturbance outside of existing developed or otherwise modified areas. When disturbance outside existing developed or modified sites is proposed, conduct a botanical survey for listed plant species within the project action area, defined as the area where direct and indirect effects are likely to occur. Surveys should be conducted by a knowledgeable botanist with documented experience in identifying native Hawaiian and Pacific Islands plants, including listed plant species. Botanical surveys should optimally be conducted during the wettest part of the year (typically October to April) when plants and identifying features are more likely to be visible, especially in drier areas. If surveys are conducted outside of the wet season, the Service may assume plant presence.

The boundary of the area occupied by listed plants should be marked with flagging by the surveyor. To avoid or minimize potential adverse effects to listed plants, we recommend adherence to buffer distances for the activities in the **Table below**. Where disturbed areas do not need to be maintained as an open area, restore disturbed areas using native plants as appropriate for the location. Whenever possible we recommend using native plants for landscaping purposes. The following websites are good resources to use when choosing landscaping plants: Landscape Industry Council of Hawai‘i Native Plant Poster (http://hawaiiscape.wpengine.com/publications/), Native Hawaiian Plants for Landscaping, Conservation, and Reforestation (https://www.ctahr.hawaii.edu/oc/freepubs/pdf/of-30.pdf), and Best Native Plants for Landscapes (https://www.ctahr.hawaii.edu/oc/freepubs/pdf/OF-40.pdf).

If listed plants occur in a project area, the avoidance buffers are recommended to reduce direct and indirect impacts to listed plants from project activities. However, where project activities will occur within the recommended buffer distances, additional consultation is required. The impacts to the plants of concern within the buffer area may be reduced by placing temporary fencing or other barriers at the boundary of the disturbance, as far from the affected plants as practicable.

The above guidelines apply to areas outside of designated critical habitat. If project activities occur within designated critical habitat unit boundaries, additional consultation is required.

All activities, including site surveys, risk introducing nonnative species into project areas. Specific attention needs to be made to ensure that all equipment, personnel and supplies are properly checked and are free of contamination (weed seeds, organic matter, or other contaminants) before entering project areas. Quarantines and or management activities occurring on specific priority invasive species proximal to project areas need to be considered or adequately addressed. This information can be acquired by contacting local experts such as those on local invasive species committees (Kauai: https://www.kauaiisc.org/; Oahu: https://www.oahuisc.org/; Maui Nui: https://mauiinvasive.org/; and Hawaii: https://www.biisc.org/

Table 1. Recommended buffer distances to minimize and avoid potential adverse impacts to listed plants from activities listed below.

Action	**Buffer Distance (feet (meters)) - Keep Project Activity This Far Away from Listed Plant**	
	Grasses/Herbs/Shrubs and Terrestrial Orchids	**Trees and Arboreal Orchids**
Walking, hiking, surveys	3 ft (1 m)	3 ft (1 m)
Cutting and Removing Vegetation By Hand or Hand Tools (e.g., weeding)	3 ft (1 m)	3 ft (1 m)
Mechanical Removal of Individual Plants or Woody Vegetation (e.g., chainsaw, weed eater)	3 ft up to height of removed vegetation (whichever greater)	3 ft up to height of removed vegetation (whichever greater)

Removal of Vegetation with Heavy Equipment (e.g., bulldozer, tractor, "bush hog")		2x width equipment + height of vegetation	820 ft (250 m)
Use of Approved Herbicides (following label)	**Ground-based Spray Application; hand application (no wand applicator; spot treatment)**	10 ft (3 m)	Crown diameter
	Ground-based Spray Application; manual pump with wand, backpack	50 ft (15 m)	Crown diameter
	Ground-based Spray Application; vehicle-mounted tank sprayer	50 ft (15 m)	Crown diameter
	Aerial Spray (ball applicator)	250 ft (76 m)	250 ft (76 m)
	Aerial Application – herbicide ballistic technology (individual plant treatment)	100 ft (30 m)	Crown diameter
	Aerial Spray (boom)	Further consultation required	Further consultation required
Use of Insecticides (pollinators, seed dispersers)		Further consultation required	Further consultation required
Ground/Soil Disturbance/Outplanting/Fencing (Hand tools, e.g. shovel, \`ō\`ō; Small mechanized tools, e.g., auger)		20 ft (6 m)	2x crown diameter
Ground/Soil Disturbance (Heavy Equipment)		328 ft (100 m)	820 ft (250 m)
Surface Hardening/Soil compaction	**Trails (e.g., human, ungulates)**	20 ft (6 m)	2x crown diameter
	Roads/Utility Corridors, Buildings/Structures	328 ft (100 m)	820 ft (250 m)
Prescribed Burns		Further consultation required	Further consultation required
Farming/Ranching/Silviculture		820 ft (250 m)	820 ft (250 m)

Definitions (Wagner *et al*. 1999)

Crown: The leafy top of a tree.
Herb: A plant, either annual, biennial, or perennial, with the non-woody stems dying back to the ground at the end of the growing season.
Shrub: A perennial woody plant with usually several to numerous primary stems arising from or relatively near the ground.
Tree: A woody perennial that usually has a single trunk

References Cited

USFWS. 2010. Endangered and threatened wildlife and plants; determination of endangered status for 48 species on Kauai and designation of critical habitat. Federal Register 75: 18960–19165.

. 2012. Endangered and threatened wildlife and plants; endangered status for 23 species on Oahu and designation of critical habitat for 124 species; final rule. Federal Register 77: 57648–57862.

. 2013a Endangered and threatened wildlife and plants; determination of endangered status for 38 species from Molokai, Lanai, and Maui. Federal Register 78: 32014–32065.

. 2013b. Endangered and threatened wildlife and plants; determination of endangered species status for 15 species on Hawaii Island. Federal Register 78: 64638–64690.

. 2016. Endangered and threatened wildlife and plants; determination of endangered status for 49 species from the Hawaiian Islands. Federal Register 81: 67786–67860.

. 2016. USFWS Rare plant database. Unpublished.

Wagner, W.L., Sohmer, S., and D.R. Herbst. 1999. Manual of the flowering plants of Hawaii, revised edition. Honolulu, Hawaii. University of Hawaii and Bishop Museum Press. 1,919 pp.

<u>PIFWO Invasive Species Biosecurity Protocols</u>

Do not include red text in letters! This is only background information for biologists. (Updated February 2022)

NOTES FOR BIOLOGISTS: This biosecurity protocol should be incorporated when project activities occur within any area containing predominantly native habitat including but not limited to National Parks, National Wildlife Refuges, and State of Hawaiʻi "Natural Areas" (e.g., Natural Area Reserves, State Forest Reserves, etc.). Activities involving transportation of a substantial amount (as determined by your discretion) of materials (i.e., construction materials or aggregate, etc.), vehicles, machinery, equipment, or personnel between sites should also incorporate the protocols. Additional consultation is recommended if the project involves transportation of materials, equipment, vehicles, etc. between islands or transpacific movement. Additionally, check to see if any species-specific protocols (see below) are applicable to your project. Please feel free to reach out to Ryan or Mike with the invasive species team if you have any questions or concerns.

Project activities may introduce or spread invasive species, causing negative ecological consequences to new areas or islands, resulting in potential impacts to fish, wildlife, and their habitat. For example, seeds of invasive plant species (e.g., *Chromolaena odorata*, *Senecio madagascariensis*, *Cyathea cooperi*, or *Miconia calvescens*) can be inadvertently transported on equipment from a previous work site to a new site where the species are not present. Likewise, equipment used in an area infected with a pathogen or insect pest that can have ecological consequences (e.g., rapid ʻōhiʻa death (*Ceratocystis spp.*), black twig borer (*Xylosandrus compactus*), or naio thrips (*Klambothrips myopori*), if not properly decontaminated, can act as a vector to introduce the pathogen into a new area. Additionally, vehicles must be properly inspected and cleaned to ensure vertebrate or invertebrate pests do not stowaway and spread to other areas. These are just a few examples of how even well-intended project activities may inadvertently introduce or spread invasive species.

To avoid and minimize invasive species potential impacts to fish, wildlife, and their habitat we recommend incorporating general biosecurity protocols into your project planning (reference general invasive species biosecurity protocol below or provide link to website here). Additionally, your project occurs in a geographic area and/or involves activities that risk spreading the following specific invasive species: [add species common and scientific names here]. Therefore, we recommend including additional protocols specific to those invasive species (reference relevant species-specific biosecurity protocol(s) below or provide link to website here) [NOTE: if no additional species are applicable, remove the previous two sentences]. Additional consultation is recommended if project activities involve transportation of materials, equipment, vehicles, etc. between islands or transpacific movement of materials or equipment.

Invasive Species Biosecurity Protocol

NOTE: Please review the protocol to ensure you are only including measures applicable to your project. Pay close attention to the "Additional Considerations" section, as some (or all) may not be applicable.

The following biosecurity protocol is recommended to be incorporated into planning for your project to avoid or minimize transportation of invasive species with potential to impact to fish, wildlife, and their habitat. Cleaning, treatment, and/or inspection activities are the responsibility of the equipment or vehicle owner and operator. However, it is ultimately the responsibility of the action agency to ensure that all project materials, vehicles, machinery, equipment, and personnel are free of invasive species before entry into a project site. Please refer to the resources listed below for current removal/treatment recommendations that may be relevant to your project.

1. Cleaning and treatment:
   Project applicants should assume that all project materials (i.e., construction materials, or aggregate such as dirt, sand, gravel, etc.), vehicles, machinery, and equipment contain dirt and mud, debris, plant seeds, and other invasive species, and therefore require thorough cleaning. Treatment for specific pests, for example, trapping and poison baiting for rodents, or baiting and fumigation for insects, should be considered when applicable. For effective cleaning we offer the following recommendations prior to entry into a project site:
   a. Project materials, vehicles, machinery, and equipment must be pressure washed thoroughly (preferably with hot water) in a designated cleaning area. Project materials, vehicles, machinery, and equipment should be visibly free of mud/dirt (excluding aggregate), seeds, plant debris, insects, spiders, frogs (including frog eggs), other vertebrate species (e.g., rodents, mongoose, feral cats, reptiles, etc.), and rubbish. Areas of particular concern include bumpers, grills, hood compartments, wheel wells, undercarriage, cabs, and truck beds. Truck beds with accumulated material are prime sites for hitchhiking invasive species.
   b. The interior and exterior of vehicles, machinery, and equipment must be free of rubbish and food, which can attract pests (i.e., rodents and insects). The interiors of vehicles and the cabs of machinery should be vacuumed clean particularly for any plant material or seeds.

2. Inspection:
   a. Following cleaning and/or treatment, project materials, vehicles, machinery, and equipment, must be visually inspected by its user, and be free of mud/dirt (excluding aggregate), debris, and invasive species prior to entry into a project site. For example, careful visual inspection of a vehicle's tires and undercarriage is recommended for any remaining mud that could contain invasive plant seeds.
   b. Any project materials, vehicles, machinery, or equipment found to contain invasive species (e.g., plant seeds, invertebrates, rodents, mongoose, cats, reptiles, etc.) must not enter the project site until those invasive species are properly removed/treated.

3. For all project site personnel:
    a. Prior to entry into the project site, visually inspect and clean your clothes, boots or other footwear, backpack, radio harness, tools and other personal gear and equipment for insects, seeds, soil, plant parts, or other debris. We recommend the use of a cleaning brush with sturdy bristles. Seeds found on clothing, footwear, backpacks, etc., should be placed in a secure bag or similar container and discarded in the trash rather than being dropped to ground at the project site or elsewhere.

4. Additional considerations (if applicable):
    a. Consider implementing a Hazard Analysis and Critical Control Point (HACCP) plan (https://www.fws.gov/policy/A1750fw1.html) to improve project planning around reducing the risk of introducing or spreading invasive species.
    b. When applicable, use pest-free or low-risk sources of plants, mulch, wood, animal feed or other materials to be transported to a project site.
    c. For projects involving plants from nurseries (e.g., outplanting activities, etc.), all plants should be inspected, and if necessary, appropriately cleaned or treated for invasive species prior to being transported to the project site.
    d. Avoid unnecessary exposure to invasive species at a particular site (to the extent practical) to reduce contamination and spread. For example, if your project involves people or equipment moving between multiple locations, plan and organize timelines so that work is completed in native habitat prior to working in a disturbed location to reduce the likelihood of introducing a pest into the native habitat.
    e. Maintain good communication about invasive species risks between project managers and personnel working on the project site (e.g., conduct briefings and training about invasive species). Ensure prevention measures are communicated to the entire project team. Also consider adding language on biosecurity into contracts or permitting mechanisms to provide clarity to all involved in the project. Report any species of concern or possible introduction of invasive species to appropriate land managers.

For current removal/treatment recommendations please refer to the following:
Hawaiian Islands:

- Hawaiʻi Island – https://www.biisc.org/
- Maui – https://mauiinvasive.org/
- Molokaʻi - https://www.molokaiisc.org/
- Lānaʻi - https://pulamalanai.com/
- Oʻahu – https://www.oahuisc.org/
- Kauaʻi – https://www.kauaiisc.org/

Mariana Islands:

- Guam – https://biosecurity.guam.gov/
- CNMI – http://www.dfwcnmi.com/

Species-Specific Biosecurity Protocols

NOTE: The following section contains specific protocols for a few select invasive species of concern in the Pacific Islands highlighted because of their potential to easily spread and cause great harm to native species and habitats. Other invasive species may not have existing specific protocols or may already be minimized by implementing the general invasive species protocols above (e.g., invasive plants, invertebrates, larger vertebrates). Information on other invasive species can be found here (link to PIFWO website). As new threats emerge that require development of species-specific protocols, those may be added to this list.

Table 1. Current island distribution of invasive species with specific biosecurity protocols in the Pacific Islands (PIFWO jurisdiction).

Island	**Invasive Species with Specific Protocols**			
	Rapid ‘Ōhi‘a Death	**Little Fire Ant**	**Coconut Rhinoceros Beetle**	**Brown Treesnake**
Island of Hawai‘i	widespread	widespread	not present	not present
Maui	not present	incipient	not present	not present
O‘ahu	incipient	incipient	widespread	not present
Kaua‘i	widespread	not present	not present	not present
Guam	NA	widespread	widespread	widespread
CNMI	NA	not present	Rota only	not present
American Samoa	NA	incipient	widespread	not present

Rapid ‘Ōhi‘a Death (ROD)

NOTE: Include for projects occurring in any native habitat where ‘ōhi‘a is present, on islands where ROD is currently found. If working directly with ‘ōhi‘a trees (e.g., sampling suspected trees, clearing an area of ‘ōhi‘a, etc.) or in an area(s) known to be highly infested with ROD, additional consultation is recommended.

- Current Distribution of ROD: island of Hawai‘i, O‘ahu, Kaua‘i

Rapid ‘Ōhi‘a Death (ROD) is a caused by a fungal pathogen (*Ceratocystis* spp.) that attacks and kills ‘ōhi‘a trees (*Metrosideros polymorpha*). ‘Ōhi‘a is endemic to the Hawaiian Islands and is the most abundant native tree species, comprising approximately 80 percent of Hawai‘i’s remaining native forests.

For more information about ROD including its current distribution, ROD science updates, and the latest on ROD protocol, please visit www.rapidohiadeath.org.

To reduce the risk of spreading ROD, the following best management practices and decontamination protocol are recommended:

Best Management Practices for ROD

1. Never transport any part of an ‘ōhi‘a tree between different areas of an island or to a different island.

2. Do not use equipment from ROD infected islands on another island unless it is very specialized equipment and follows the decontamination protocol described below.

3. Avoid wounding ‘ōhi‘a trees and roots with mowers, chainsaws, weed eaters, and other tools. If an ‘ōhi‘a receives a minor injury like a small broken branch, then give the injury a clean, pruning-type cut (close to the main part of the trunk or branch) to promote healing, and then spray the entire wounded area with a pruning seal.

4. Always report suspect ROD ‘ōhi‘a trees observed within you project area. ROD is a wilt disease that cuts off the supply of water and nutrients to the tree. The primary symptom to look for is an entire canopy or a large branch with dying leaves or red discolored leaves. Please record the GPS coordinates and location and take a picture of the tree if possible. Please report suspected ROD ‘ōhi‘a trees to the following agencies:
    a. Island of Hawai‘i – BIISC: 808-969-8268 (ohialove@hawaii.edu)
    b. Maui – MISC: 808-573-6472 (miscpr@hawaii.edu)
    c. Moloka‘i – TNC: 808-553-5236 ext. 6585 (lbuchanan@tnc.org)
    d. O‘ahu – OISC: 808-266-7994 (oisc@hawaii.edu)
    e. Kaua‘i – KISC: 808-821-1490 (kisc@hawaii.edu)

ROD Decontamination Protocol

1. Clothes, footwear, backpacks, and other personal equipment
    a. Before leaving the project site, remove as much mud and other contaminants as possible. Use of a brush with soap and water to clean gear is preferred. Footwear, backpacks, and other gear must be sanitized by spraying with a solution of >70 percent isopropyl alcohol or a freshly mixed 10 percent bleach solution.

2. Vehicles, machinery, and other equipment
    a. Vehicles, machinery, and other equipment must be thoroughly hosed down with water (pressure washing preferred) and visibly free of mud and debris, then sprayed with a solution of >70 percent isopropyl alcohol or a freshly mixed 10 percent bleach solution. Use of a “pump-pot” sprayer is recommended for the solution and a hot water wash is preferred. Be sure to thoroughly clean the undercarriage, truck bed, bumpers, and wheel wells.
    b. If non-decontaminated personnel or items enter a vehicle, then the inside of the vehicle (i.e., floor mats, etc.) must be subsequently decontaminated by removing mud and other contaminants and sprayed with the one of the same aforementioned sanitizing solutions.

3. Cutting tools
    a. All cutting tools, including machetes, chainsaws, and loppers must be sanitized to remove visible mud and other contaminants. Tools must be sanitized using a solution of >70 percent isopropyl alcohol or a freshly mixed 10 percent bleach solution. One minute after sanitizing, one may apply an oil-based lubricant to chainsaw chains or other metallic parts to prevent corrosion as bleach is corrosive to metal.

NOTE: When using a 10 percent bleach solution, surfaces should be cleaned with a minimum contact time of 30 seconds. Bleach must be mixed daily and used within 24 hours, as once mixed it degrades. Bleach will not work to disinfect surfaces that have high levels of organic matter such as sawdust or soil. Because bleach is also corrosive to metal, a water rinse after proper sanitization is recommended to avoid corrosion.

Little Fire Ant (LFA)

NOTE: Include the following information for projects that occur in native habitat on islands where LFA is currently recorded and in areas known to be infested with LFA (check http://stoptheant.org/lfa-in-hawaii/ for status on each island). If other ant species (i.e., yellow crazy ants) may be a concern for your project, please contact the invasive species team.

- Current Distribution of LFA: Island-wide on Guam and island of Hawai‘i; incipient infestation sites on Maui, O‘ahu, and American Samoa; CNMI is also vulnerable and projects there should require that project-related materials, equipment, and vehicles be checked before shipping to the CNMI from Guam and prior to use.

The little fire ant (*Wasmannia auropunctata*), or LFA, is an invasive species with a painful sting that can inhabit many different environments. In Hawai‘i, it often infests agricultural fields and farms, damaging crops and stinging unsuspecting workers. Little fire ants are also highly disruptive to native tropical ecosystems and harmful to wildlife. Slow moving, but tiny and capable of foraging 24 hours a day with multiple queens per colony, LFA is a formidable threat to biodiversity, agriculture, and quality of life on tropical islands in the Pacific.

For more information about LFA including helpful guides and workshops for treating or detecting LFA, please visit www.littlefireants.com.

To reduce the risk of spreading LFA, the following biosecurity protocol is recommended:

Biosecurity Protocol for LFA

1. For projects involving plants from nurseries (e.g., outplanting activities, etc.), all plants should be inspected for little fire ants and other pests prior to being transported to the project site. If plants are found to be infested by ants of any species, plants should be sourced from an alternative nursery and the infested nursery should follow treatment protocols recommended by the Hawai‘i Ant Lab (https://littlefireants.com/wp-content/uploads/2020-Management-of-Pest-Ants-in-Nurseries-min.pdf).

2. All work vehicles, machinery, and equipment should follow steps 1 and 2 in the “Invasive Species Biosecurity Protocol” for (1) cleaning and treatment and (2) inspection for invasive ants prior to entering a project site.

3. Any machinery, vehicles, equipment, or other supplies found to be infested with ants (or other invasive species) must not enter the project site until it is properly treated

(https://littlefireants.com/how-to-treat-for-little-fire-ants-for-homeowners/#recommended-bait-products) and re-tested. Infested vehicles must be treated following recommendations by the Hawaiʻi Ant Lab (https://littlefireants.com/resource-center/) or another ant control expert and in accordance with all State and Federal laws. Treatment is the responsibility of the equipment or vehicle owner. Ultimately however, it is the responsibility of the action agency to ensure that all project materials, vehicles, machinery, and equipment follow the appropriate protocol(s).

4. General Vehicle Ant Hygiene: Even the cleanest vehicle can pick up and spread little fire ant. Place MaxForce Complete Brand Granular Insect Bait (1.0 percent Hydramethylnon; https://labelsds.com/images/user_uploads/Maxforce%20Complete%20Label%201-5-18.pdf) into refillable tamper resistant bait stations. An example of a commercially available refillable tamper resistant bait station is the Ant Café Pro (https://www.antcafe.com/). Place a bait station (or stations) in the vehicle and note that larger vehicles, such as trucks, may require multiple stations. Monitor bait stations frequently (every week at a minimum) and replace bait as needed. If the bait station does not have a sticker to identify the contents, apply a sticker listing contents to the station.

5. Gravel, building materials, or other equipment such as portable buildings should be baited using MaxForce Complete Brand Granular Insect Bait (1.0 percent Hydramethylnon; https://labelsds.com/images/user_uploads/Maxforce%20Complete%20Label%201-5-18.pdf) or AmdroPro (0.73 percent Hydramethylnon; https://connpest.com/labels/AMDROPRO.pdf) following label guidance.

6. Storage areas that hold field tools, especially tents, tarps, and clothing should be baited using MaxForce Complete Brand Granular Insect Bait (1.0 percent Hydramethylnon; https://labelsds.com/images/user_uploads/Maxforce%20Complete%20Label%201-5-18.pdf) or AmdroPro (0.73 percent Hydramethylnon; https://connpest.com/labels/AMDROPRO.pdf) following label guidance.

7. Vehicles that have entered a project site known or thought to overlap with areas infested with LFA should subsequently be tested for LFA with baiting in accordance with protocol recommended by the Hawaiʻi Ant Lab (https://littlefireants.com/survey-your-home-for-lfa/).

8. If LFA are detected, please report it to 808-643-PEST (Hawaiʻi), 671-475-PEST (Guam), or 684-699-1575 (American Samoa). Please visit https://littlefireants.com/identification-of-little-fire-ants/ for assistance in identifying LFA.

Coconut Rhinoceros Beetle (CRB)

NOTE: Include for projects that involve movement or creation of green waste and occur on islands where CRB is currently found. Only include the biosecurity protocol pertaining to the geographic area of the project (Oʻahu or Marianas). For projects in American Samoa contact the invasive species team.

- Current Distribution of CRB: Oʻahu, Guam, Rota, and American Samoa

The coconut rhinoceros beetle (*Oryctes rhinoceros*), or CRB, is a large, horned scarab beetle native to Southeast Asia. An invasive pest where it occurs outside of its native range, the adult beetles primarily attack coconut palms by boring into the crowns to feed on developing leaves. It is also known to feed on bananas, sugarcane, pineapples, oil palms, and pandanus trees. The larval grub stage burrow into and feed upon decomposing mulch and vegetation. On most Pacific Islands it lacks natural predators, leading to severe declines and extirpations of palm species where it has become established. On Guam, researchers have recently documented a shift of CRB to the island's native and threatened cycad tree (*Cycas micronesica*) (Marler et al. 2020). In the Hawaiian Islands, CRB is a documented threat to archipelago's native *Pritchardia* palm species.

For more information about CRB including the current situation in Guam and high/low-risk areas on Oʻahu, please visit http://cnas-re.uog.edu/crb/ or https://www.crbhawaii.org/.

To reduce the risk of spreading CRB, the following biosecurity protocol is recommended:

Biosecurity Protocol for CRB (Oʻahu)

1. Never transport green waste between islands and minimize the creation, storage, and transport of green waste within Oʻahu, this also includes:
    a. Mulch, bark, compost
    b. Soil of any kind
    c. Potted plants of any kind

    Additional consultation is recommended if the project involves transportation of materials, soil, equipment, vehicles, etc. between islands.

2. If felling or trimming palms, contact CRB Response for a free inspection ((808) 679-5244 or email at info@crbhawaii.org)

3. Keep green waste whole until it is ready to be treated and removed.
    a. Chip green waste on site and transport it on the same day to a secure and managed green waste disposal site/facility.
    b. For chipped green waste in high-risk areas, re-chip prior to movement outside the infested area, treat with pesticide (when applicable), heat treatment (>130 degrees F), spread and dry, or store in sealed durable containers.

4. Minimize accumulations of green waste by regularly treating mulch piles or depositing it in sealed green waste bins. In low-risk areas, we also recommend thinly spreading mulch (less than 2 inches deep) and allowing it to dry (no irrigation).

5. If injured or dying coconut palm trees are observed or if CRB are detected, contact CRB Response at (808) 679-5244 or email at info@crbhawaii.org or online at https://www.crbhawaii.org/report

Biosecurity Protocol for CRB (Marianas)

1. Never transport green waste between islands in the Marianas, this also includes:
    a. Mulch, bark, compost
    b. Soil of any kind
    c. Potted plants of any kind

   Additional consultation is recommended if the project involves transportation of materials, equipment, vehicles, etc. between islands.

2. Designate secure and managed green waste disposal sites to reduce the number of potential oviposition (laying of eggs) sites and larval food.

3. Green waste disposal sites should be monitored with CRB traps. The following control measures should be utilized at green waste sites.
    a. Netting - A gill net with a 1 inch mesh measured knot to knot, made from 0.25 mm nylon monofilament, should be laid over piles of green waste such as palm tree cuttings or decaying organic matter. The netting is helpful for trapping adult beetles emerging from the mulch.
    b. If the green waste site is found within or adjacent to chain link fencing, we recommend use of the DeFence trap. These are simply constructed with a 12 ft piece of tekken netting, folded in half, and secured onto a fence line using zip ties. In the middle of the net, attach a solar powered uvLED light, and a CRB pheromone lure protected in a red Solo cup. This trap design is currently among the most effective methods because it does not require many materials and uses the least amount of space on the property.
        i. For more information on trapping methods, please visit https://cnas-re.uog.edu/wp-content/uploads/2015/09/CRB-Trapping.pdf

4. If CRB are detected contact CNMI Forestry at (670) 256-3321 or Department of Lands and Natural Resources at (670) 322-9834 or Guam's Department of Agriculture Biosecurity Division (671) 477-7822 or email at guament@teleguam.net.

Brown Treesnake (BTS)

NOTE: Required for project activities that involve cargo, baggage, materials, etc. shipped from or through Guam prior to departure and upon arrival to the CNMI.

The Brown Treesnake (*Boiga irregularis*), or BTS, was accidentally introduced to Guam likely as a stowaway in military cargo shortly after WWII. On Guam, BTS has caused the extinction or extirpation of many native and endemic species of birds and lizards. The loss of native species has furthermore triggered cascading ecological impacts affecting Guam forest regeneration and ecology (Rogers et al. 2017). Preventing the spread of BTS from Guam to other Pacific Islands is the primary goal of the BTS Technical Working Group (TWG) formed by the 2004 BTS Control and Eradication Act. The BTS TWG developed a BTS interdiction program with the goal of 100-percent inspections of outbound cargo using canine inspection teams.

For more information about BTS including links to partnerships and ongoing research, please visit: https://www.fws.gov/pacificislands/articles.cfm?id=%20149489576

The USDA Wildlife Services are responsible for interdiction on Guam and collaborates with the Department of Defense, the Government of Guam, and private industry to remove snakes from outbound aircraft, sea vessels, and cargo. In the CNMI, the Department of Fish and Wildlife conducts redundant canine or visual inspections of inbound air/seacraft and cargo.

The following protocol is required for project activities that involve cargo, baggage, materials, etc. shipped from or through Guam prior to departure and upon arrival to the CNMI:

BTS Inspection Instructions for Guam and the CNMI:

1. Schedule cargo, aircraft, vehicle, and vessel inspections on Guam with the US Department of Agriculture (USDA) Wildlife Services (WS) for any and all vessels, vehicles, aircraft, and cargo that has been stationed or staged on Guam (for contact info, see Contact list on page 2). Inspections are available 24/7.
    a. All cargo staged on Guam must be inspected before transport to other Pacific Islands.
    b. Examples of cargo include vehicles, pallets of goods, loose boxes, containers filled with goods, bundles of construction materials (rolls of metal sheeting, stacks of plywood/boards, PVC pipes, or any material that provides an abundance of small, dark crevices).

2. Before your Guam-outbound cargo arrives on Saipan, Tinian, or Rota, schedule cargo inspections by Commonwealth of the Northern Mariana Islands (CNMI) Department of Fish and Wildlife (DFW).
    a. Rota – contact Jon Mesgnon (670-287-7683) and Manny Pangelinan (670-483-6261)
    b. Tinian – contact Ton Castro (670-287-9453) and Manny Pangelinan (670-483-6261)
    c. Saipan – contact Joe Cruz (670-285-7877) and Manny Pangelinan (670-483-6261)

3. If you see a snake while in cargo staging areas on Guam or anywhere on other islands:
    a. Report it immediately. Take note of where you are, what the snake looked like, and any notable behaviors. Attempt to kill, apprehend, or injure the snake, and take photos if possible. Keep visual contact with the snake until BTS program personnel arrive.
    b. To report a snake in the CNMI (e.g., Saipan, Tinian, Rota) call 670-28-SNAKE (670-287-6253)
    c. To report a snake on any island to the BTS Rapid Response Team on Guam, call 671-777-HISS (671-777-4477)

d. If the snake is killed, save the carcass, and give to a CNMI, USDA, FWS, or USGS representative.

References Cited

Marler, T.E., Marler, F.C. Matanane, and L.I. Terry. 2020. Burrowing activity of coconut rhinoceros beetle on Guam cycads. Communicative & Integrative Biology, 13:1, 74-83. (https://www.tandfonline.com/doi/full/10.1080/19420889.2020.1774310)

Rogers, H.S., E.R. Buhle, J. Hille Ris Lambers, E.C. Fricke, R.H. Miller, and J.J. Tewksbury. 2017. Effects of an invasive predator cascade to plants via mutualism disruption. Nature Communications. 8:14557. https://www.nature.com/articles/ncomms14557/

United States Department of the Interior

FISH AND WILDLIFE SERVICE
Pacific Islands Fish and Wildlife Office
300 Ala Moana Boulevard, Room 3-122
Honolulu, Hawaiʻi 96850

In Reply Refer To:
01EPIF00-2022-SL-0122

January 20, 2022

Memorandum

To: Joy Tamayose, Wildlife Biologist, Haleakalā National Park

From: Lindsy Asman, Island Team Manager of Maui Nui and Hawaiʻi Islands, Pacific Islands Fish and Wildlife Office

Subject: Species List for the Suppression of Non-native Mosquito Populations to Reduce Transmission of Avian Malaria, Haleakalā National Park, Island and County of Maui

Dear Joy Tamayose:

The U.S. Fish and Wildlife Service (Service) received your email request on December 7, 2021, for a species list and conservation measures to inform the development of an Environmental Assessment (EA) for the suppression of non-native mosquito populations at Haleakalā National Park (Park) and nearby conservation areas. The purpose of the project is to ameliorate the impacts of avian malaria on federally listed Hawaiian forest birds by suppressing or eliminating non-native mosquitoes by releasing incompatible *Wolbachia* bacteria-carrying male southern house mosquitoes within an approximately 262-square-kilometer (64,660-acre) project area on East Maui, Hawaiʻi. When male mosquitoes carrying an incompatible strain of naturally occurring *Wolbachia* bacteria and a wild female mate, the fertilized eggs will not hatch. This proposed technique can result in landscape-scale population suppression of mosquitoes, thereby suppressing avian malaria infection rates, and preventing the ongoing decline of native Hawaiian forest bird communities.

There are three potential methods of release depending on the available technology and other factors: helicopter-assisted pedestrian release, helicopter-assisted long line aerial release, and helicopter-assisted drone aerial release. These actions depend on Haleakalā National Park and State of Hawaiʻi statutory missions and responsibilities, environmental factors, existing travel infrastructure, and input from agency personnel, technical experts, and the public. Haleakalā

National Park notes that potential impacts from increased noise and activities due to use of mechanical equipment and access to wilderness areas.

The Service acknowledges that this project is an extremely important recovery implementation project for Hawaiian forest birds. Addressing the spread of avian malaria is essential to the continued survival of these species (USFWS 2006, p. 57004). The Service is committed to assisting the National Park Service and the State of Hawai‘i Department of Land and Natural Resources in their planning and implementation process. This letter, which contains a species list and recommended conservation measures, is intended to support the development of the EA, project planning, and future Section 7 consultation. Our comments are provided under the authorities of the Endangered Species Act of 1973 (ESA), as amended (16 U.S.C 1531 *et seq.*).

We have reviewed the information you provided and pertinent information in our files, including data compiled by the Hawai‘i Biodiversity and Mapping Program as it pertains to listed species and designated critical habitat. Our data indicate the following federally listed species may occur or transit through the vicinity of the proposed project area: the threatened nēnē or Hawaiian goose (*Branta* [*Nesochen*] *sandvicensis*), the endangered ‘i‘iwi (*Drepanis coccinea*), the endangered ‘ōpe‘ape‘a or Hawaiian hoary bat (*Lasiurus cinereus semotus*), the endangered ‘ua‘u or Hawaiian petrel (*Pterodroma sandwichensis*), the endangered ‘akē‘akē or the Hawai‘i distinct population segment (DPS) of the band-rumped storm-petrel (*Oceanodroma castro*), the endangered ‘ākohekohe (*Palmeria dolei*), the endangered kiwikiu or Maui parrotbill (*Pseudonestor xanthophrys*), the threatened ‘a‘o or Newell’s shearwater (*Puffinus auricularis newelli*), the endangered Blackburn’s sphinx moth (*Manduca blackburni*), and two endangered damselflies (*Megalagrion nesiotes* and *Megalagrion pacificum*). The Hawaiian petrel, band-rumped storm-petrel, and Newell’s shearwater will hereafter collectively be referred to as “Hawaiian seabirds”. The ‘i‘iwi, the Maui Parrotbill and the ‘ākohekohe will hereafter collectively be referred to as “Hawaiian forest birds”. There are approximately 60 federally listed plants within the action area; their recommended avoidance and minimization measures are in Table 1 and Table 2, respectively. There are 16 designated critical habitats for listed species within the project impact area (see enclosure).

Hawaiian goose
Hawaiian geese are found on the islands of Hawai‘i, Maui, Moloka‘i, and Kaua‘i. They are observed in a variety of habitats, but prefer open areas, such as pastures, golf courses, wetlands, natural grasslands and shrublands, and lava flows. Threats to the species include introduced mammalian and avian predators, wind facilities, and vehicle strikes.

To avoid and minimize adverse effects to the nēnē we recommend you incorporate the following measures into your project description:

- Do not approach, feed, or disturb nēnē.
- If nēnē are observed loafing or foraging within the project area during the breeding season (September through April), have a biologist familiar with nēnē nesting behavior survey for nests in and around the project area prior to the resumption of any work. Repeat surveys after any subsequent delay of work of 3 or more days (during which the birds may attempt to nest).

- Cease all work immediately and contact the Service for further guidance if a nest is discovered within a radius of 150 feet (ft) of proposed project, or a previously undiscovered nest is found within the 150-foot radius after work begins.
- In areas where nēnē are known to be present, post and implement reduced speed limits, and inform project personnel and contractors about the presence of endangered species on-site.
- Avoid launching drones when a listed animal is observed in the project area. Recover drones by the safest means possible when a listed animal is observed in proximity to the drone during the mission.
- We recommend any reaction to a drone by a listed animal be reported immediately to the Pacific Islands Fish and Wildlife Office.

Hawaiian forest birds
The current ranges of Hawaiian forest birds are predominately restricted to montane forests above 3,500 ft in elevation due to habitat loss and threats at lower elevations. Hawaiian forest bird habitat has been lost due to development, agriculture, grazing, wildfire, and spread of invasive habitat-altering species. Forest birds are also affected by mosquito-borne diseases. Mosquitoes are not native to Hawaiʻi; their occurrence increases in areas where ungulate presence results in small pools of standing water. Actions such as road construction and development increase human access and result in increased wildfire and invasive species threats. Grazing reduces woody vegetation and increases grass cover, which reduces forest habitat quality and increases risk of wildfire on the landscape.

Avoid conducting activities within forest bird habitat that:
- Promotes the spread or survival of invasive species.
- Increases mosquito populations or stagnant water habitat.
- Increases wildfire threat to montane forest habitats.
- Removes tree cover during the peak breeding season between January 1 and June 30.

Noise Levels
Drones and helicopters may produce far-projecting sound, which could disturb listed forest birds during their breeding season. Helicopter rotor wash can significantly disturb nesting forest birds when helicopters fly very low over the canopy. Therefore, we recommend that *Wolbachia* release be timed to occur outside the breeding season of Hawaiian forest birds. However, we understand that this may not be feasible due to overlapping breeding seasons of some of the species present. Therefore, where breeding seasons cannot be avoided, we recommend that drone operations occur only above tree height level and minimize hovering in one place to limit the likelihood that breeding birds will flush from active nests. We also recommend helicopters avoid flying low near forest bird habitats to avoid rotor wash and significantly disturbing nesting forest birds.

Hawaiian hoary bat
The Hawaiian hoary bat roosts in woody vegetation across all islands and will leave their young unattended in trees and shrubs when they forage. If trees or shrubs 15 ft or taller are cleared during the pupping season, June 1 through September 15, there is a risk that young bats could inadvertently be harmed or killed, since they are too young to fly or move away from

disturbance. Hawaiian hoary bats forage for insects from as low as 3 ft to higher than 500 ft above the ground.

To avoid and minimize impacts to the endangered Hawaiian hoary bat we recommend you incorporate the following applicable measures into your project description:

- Do not disturb, remove, or trim woody plants greater than 15 ft tall during the bat birthing and pup rearing season (June 1 through September 15).
- Do not use barbed wire for fencing.
- We recommend drone and helicopter flights be avoided between dusk and dawn to protect flying bats.

Blackburn's sphinx moth
The adult Blackburn's sphinx moth feeds on nectar from native plants, including *Ipomoea pes-caprae* (beach morning glory), *Plumbago zeylanica* (‘ilie‘e), and *Capparis sandwichiana* (maiapilo). Blackburn's sphinx moth larvae feed on non-native *Nicotiana glauca* (tree tobacco) and native *Nothocestrum* sp. (‘aiea). To pupate, the larvae burrow into the soil and can remain in a state of torpor for a year or more before emerging from the soil. Soil disturbance can result in death of the pupae.

We offer the following survey recommendations to assess whether the Blackburn's sphinx moth occurs within the project area:

- A biologist familiar with the species should survey areas of proposed activities for Blackburn's sphinx moth and its larval host plants prior to work initiation.
  - Surveys should be conducted during the wettest portion of the year (usually November-April or several weeks after a significant rain) and within 4 to 6 weeks prior to project activities.
  - Surveys should include searches for adults, eggs, larvae, and signs of larval feeding (i.e., chewed stems, frass, or leaf damage).
  - If moths, eggs, or larvae, or native ‘aiea or tree tobacco over 3 ft tall, are found during the survey, please contact the Service for additional guidance to avoid impacts to this species.

Hawaiian seabirds
Hawaiian seabirds may traverse the project area at night during the breeding, nesting, and fledging seasons (March 1 to December 15). Operators should not purposely approach bird species or specific locations where listed birds are known to occur (i.e., known nests).

To avoid and minimize potential interactions with seabirds we recommend the following:

- Should a bird approach the drone within 15 ft, drone operations be halted, and the incident reported to the Pacific Islands Fish and Wildlife Office.
- During the seabird breeding season (March 1 to December 15), avoiding flights between dusk and dawn to protect night flying seabirds.

Hawaiian damselflies
Hawaiian damselflies are found in aquatic habitats across the islands, with high species endemism within islands. Breeding habitat includes anchialine pools, perennial streams, marshes,

ponds, and even artificial pools and seeps. Major threats include introduced fish, amphibians, and invertebrates in streams, reduced stream flow from drought and water diversion, small isolated populations, reduced habitat quality from ungulates and nonnative plants, and possibly over-collection. Aquatic habitats may be accessed when creating trails to release sites or landing areas.

To avoid and minimize potential interactions with Hawaiian damselflies we recommend the following:

- Applicable best management practices regarding work in aquatic environments (see enclosure) should be incorporated into the project description to minimize the degradation of water quality and impacts to fish and wildlife resources.
- Permits are required for accurate surveys of this species, so consult with the Service if work will be done in proximity to stream areas or within water bodies.

Federally Listed Plants

Project activities may affect listed plant species by causing physical damage to plant parts (i.e., roots, stems, flowers, fruits, seeds, etc.) as well as impacts to other life requisite features of their habitat which may result in reduction of germination, growth and/or reproduction (Table 1). Cutting and removal of vegetation surrounding listed plants has the potential to alter microsite conditions (e.g., light, moisture, temperature), damaging or destroying the listed plants and also increasing the risk of invasion by nonnative plants which can result in higher incidence or intensity of fire. Activities such as grazing, use of construction equipment and vehicles, and increased human traffic (i.e., trails, visitation, monitoring) can cause ground disturbance, erosion, and/or soil compaction which decrease absorption of water and nutrients and damage plant root systems and may result in reduced growth and/or mortality of listed plants. Soil disturbance or removal has the potential to negatively impact the soil seed bank of listed plant species if such species are present or historically occurred in the project area.

In order to avoid or minimize potential adverse effects to listed plants that may occur on the proposed project site, we recommend minimizing disturbance outside of existing developed or otherwise modified areas. When disturbance outside existing developed or modified sites is proposed, conduct a botanical survey for listed plant species within the project action area, defined as the area where direct and indirect effects are likely to occur. Surveys should be conducted by a knowledgeable botanist with documented experience in identifying native Hawaiian and Pacific Islands plants, including federally listed plant species. Botanical surveys should optimally be conducted during the wettest part of the year (typically October to April) when plants and identifying features are more likely to be visible, especially in drier areas.

If surveys are conducted outside of the wet season, the Service may assume plant presence. The boundary of the area occupied by listed plants should be marked with flagging by the surveyor. To avoid or minimize potential adverse effects to listed plants, we recommend adherence to buffer distances for the activities in the Table 2. Where disturbed areas do not need to be maintained as an open area, restore disturbed areas using native plants as appropriate for the location; refer to the Landscape Industry Council of Hawaiʻi Native Plant Poster (http://hawaiiscape.wpengine.com/publications/), Native Hawaiian Plants for Landscaping, Conservation, and Reforestation (https://www.ctahr.hawaii.edu/oc/freepubs/pdf/of-30.pdf), and Best Native Plants for Landscapes (https://www.ctahr.hawaii.edu/oc/freepubs/pdf/OF-40.pdf).

If listed plants occur in a project area, the avoidance buffers are recommended to reduce direct and indirect impacts to listed plants from project activities. However, where project activities will occur within the recommended buffer distances, additional consultation is required. The impacts to the plants of concern within the buffer area may be reduced by placing temporary fencing or other barriers at the boundary of the disturbance, as far from the affected plants as practicable.

The above guidelines apply to areas outside of designated critical habitat. If project activities occur within designated critical habitat unit boundaries, additional consultation is required.

All activities, including site surveys, risk introducing nonnative species into project areas. Specific attention needs to be made to ensure that all equipment, personnel, and supplies are properly checked and are free of contamination (i.e., weed seeds, organic matter, or other contaminants) before entering project areas. Quarantines and or management activities occurring on specific priority invasive species proximal to project areas need to be considered or adequately addressed. This information can be acquired by contacting local experts such as those on local invasive species committees (Maui Nui: https://mauiinvasive.org/).

Table 1. Federally listed plants occurring within the action area

Species Name	**Common Name**
Alectryon macrococcus var. *macrococcus*	Māhoe
Argyroxiphium sandwicense subsp. *macrocephalum*	ʻĀhinahina
Asplenium dielerectum	No Common Name
Asplenium peruvianum var. *insulare*	No Common Name
Bidens campylotheca subsp. *pentamera*	Koʻokoʻolau
Bidens campylotheca subsp. *waihoiensis*	Koʻokoʻolau
Calamagrostis expansa	No Common Name
Clermontia oblongifolia subsp. *mauiensis*	ʻŌhā wai
Clermontia samuelii subsp. *hanaensis*	ʻŌhā wai
Clermontia samuelii subsp. *samuelii*	ʻŌhā wai
Ctenitis squamigera	Pauoa
Cyanea asplenifolia	Hāhā
Cyanea copelandii subsp. *haleakalaensis*	Hāhā
Cyanea duvalliorum	Hāhā
Cyanea glabra	Hāhā
Cyanea hamatiflora subsp. *hamatiflora*	Hāhā
Cyanea horrida	Hāhā nui
Cyanea kunthiana	Hāhā
Cyanea maritae	Hāhā
Cyanea mceldowneyi	Hāhā
Cyanea obtusa	Hāhā

Cyclosorus boydiae	Kupukupu makaliʻi
Cyperus pennatiformis var. *pennatiformis*	No Common Name
Cyrtandra ferripilosa	Haʻiwale
Deparia kaalaana	No Common Name
Diplazium molokaiense	No Common Name
Gardenia remyi	nānū
Geranium hanaense	Nohoanu
Geranium multiflorum	Nohoanu
Huperzia mannii	Wāwaeʻiole
Huperzia stemmermanniae	No Common Name
Hypolepis hawaiiensis var. *mauiensis*	Olua
Ischaemum byrone	Hilo ischaemum
Joinvillea ascendens subsp. *ascendens*	ʻOhe
Kadua laxiflora	Pilo
Melicope balloui	Alani
Melicope ovalis	Alani
Microlepia strigosa var. *mauiensis*	No Common Name
Nothocestrum latifolium	'Aiea
Ochrosia haleakalae	Hōlei
Peperomia subpetiolata	ʻAla ʻala wai nui
Phyllostegia bracteata	No Common Name
Phyllostegia brevidens	No Common Name
Phyllostegia haliakalae	No Common Name
Phyllostegia pilosa	No Common Name
Plantago princeps var. *laxiflora*	Laukahi kuahiwi
Platanthera holochila	No Common Name
Portulaca villosa	ʻIhi
Ranunculus mauiensis	Makou
Sanicula sandwicensis	No Common Name
Schiedea diffusa subsp. *diffusa*	No Common Name
Schiedea jacobii	No Common Name
Sicyos macrophyllus	ʻAnunu
Wikstroemia villosa	No Common Name
Zanthoxylum hawaiiense	Aʻe

Table 2. Recommended buffer distances to minimize and avoid potential adverse impacts to listed plants from activities listed below.

Action	**Buffer Distance (feet (meters)) - Keep Project Activity This Far Away from Listed Plant**

		Grasses/Herbs/Shrubs and Terrestrial Orchids	**Trees and Arboreal Orchids**
Walking, hiking, surveys		3 ft (1 m)	3 ft (1 m)
Cutting and Removing Vegetation By Hand or Hand Tools (e.g., weeding)		3 ft (1 m)	3 ft (1 m)
Mechanical Removal of Individual Plants or Woody Vegetation (e.g., chainsaw, weed eater)		3 ft up to height of removed vegetation (whichever greater)	3 ft up to height of removed vegetation (whichever greater)
Removal of Vegetation with Heavy Equipment (e.g., bulldozer, tractor, "bush hog")		2x width equipment + height of vegetation	820 ft (250 m)
Use of Approved Herbicides (following label)	Ground-based Spray Application; hand application (no wand applicator; spot treatment)	10 ft (3 m)	Crown diameter
	Ground-based Spray Application; manual pump with wand, backpack	50 ft (15 m)	Crown diameter
	Ground-based Spray Application; vehicle-mounted tank sprayer	50 ft (15 m)	Crown diameter
	Aerial Spray (ball applicator)	250 ft (76 m)	250 ft (76 m)
	Aerial Application – herbicide ballistic technology (individual plant treatment)	100 ft (30 m)	Crown diameter
	Aerial Spray (boom)	Further consultation required	Further consultation required
Use of Insecticides (pollinators, seed dispersers)		Further consultation required	Further consultation required
Ground/Soil Disturbance/Outplanting/Fencing (Hand tools, e.g., shovel, ‘ō‘ō; Small mechanized tools, e.g., auger)		20 ft (6 m)	2x crown diameter
Ground/Soil Disturbance (Heavy Equipment)		328 ft (100 m)	820 ft (250 m)
	Trails (e.g., human, ungulates)	20 ft (6 m)	2x crown diameter

Surface Hardening/Soil compaction	Roads/Utility Corridors, Buildings/Structures	328 ft (100 m)	820 ft (250 m)
Prescribed Burns		Further consultation required	Further consultation required
Farming/Ranching/Silviculture		820 ft (250 m)	820 ft (250 m)

Critical Habitat
There are 16 critical habitat units designated for the above listed plant and animal species (Designated Critical Habitat Units – see enclosure). The Service understands that this project will ultimately benefit Maui forest birds. However, temporary disturbances such as trail-building and ground disturbance may affect the primary constituent elements of these designated critical habitat units. All field, or on-the-ground, activities should have strong biosecurity plans to prevent introducing or spreading harmful invasive species and should employ best management practices to minimize impacts to the primary constituent elements of designated critical habitat units. When analyzing the potential effects from the proposed project, please consider how the primary constituent elements of the designated critical habitat units will be affected.

We appreciate your efforts to conserve protected species. If there are any questions, or if we can be of any assistance, please contact Christina Richards at christina_richards@fws.gov or by telephone at 808-792-9450. When referring to this project, please include this reference number: 01EPIF00-2022-SL-0122.

Sincerely,

Lindsy Asman
Maui Nui & Hawaiʻi Island Team Manager
Pacific Islands Fish and Wildlife Office

Unit	UnitID	Bird_Unit	Acres	Hectares	Category	Status	LeadOffic	CoopOffic	FedReg	PubDate
Dry Cliff 04	DC 04	DC 28	314.8311	127.4076094	Ecosystem	Final	12200	None	81FR17791	20160330
Lowland Dry 01	LD 01		205.8748	83.31458252	Ecosystem	Final	12200	None	81FR17791	20160330
Lowland Mesic 01	LM 01		1881.96	761.6021017	Ecosystem	Final	12200	None	81FR17791	20160330
Lowland Wet 01	LW 01	LW 02	16078.84	6506.873956	Ecosystem	Final	12200	None	81FR17791	20160330
Montane Dry 01	MD 01		429.3643	173.7575785	Ecosystem	Final	12200	None	81FR17791	20160330
Montane Mesic 01	MM 01	MM 18	4056.029	1641.416526	Ecosystem	Final	12200	None	81FR17791	20160330
Montane Wet 01	MW 01	MW 10	82.01556	33.1905199	Ecosystem	Final	12200	None	81FR17791	20160330
Montane Wet 01	MW 01	MW 10	2028.178	820.7747026	Ecosystem	Final	12200	None	81FR17791	20160330
Montane Wet 02	MW 02	MW 11	14582.8	5901.449335	Ecosystem	Final	12200	None	81FR17791	20160330
Montane Wet 03	MW 03	MW 12	2227.641	901.4945026	Ecosystem	Final	12200	None	81FR17791	20160330
Montane Wet 04	MW 04	MW 13	1833.323	741.9195236	Ecosystem	Final	12200	None	81FR17791	20160330
Montane Wet 05	MW 05	MW 14	386.5628	156.436429	Ecosystem	Final	12200	None	81FR17791	20160330
Subalpine 02	SA 02	SA 25	9886.285	4000.837653	Ecosystem	Final	12200	None	81FR17791	20160330
Wet Cliff 01	WC 01	WC 30	289.4984	117.1558335	Ecosystem	Final	12200	None	81FR17791	20160330
Wet Cliff 02	WC 02	WC 31	1407.116	569.4395213	Ecosystem	Final	12200	None	81FR17791	20160330
Wet Cliff 03	WC 03	WC 32	437.7712	177.1597388	Ecosystem	Final	12200	None	81FR17791	20160330
Wet Cliff 04	WC 04	WC 33	184.2478	74.56241945	Ecosystem	Final	12200	None	81FR17791	20160330

U.S. Fish and Wildlife Service
Recommended Standard Best Management Practices

The U.S. Fish and Wildlife Service (USFWS) recommends the following measures to be incorporated into project planning to avoid or minimize impacts to fish and wildlife resources. Best Management Practices (BMPs) include the incorporation of procedures or materials that may be used to reduce either direct or indirect negative impacts to aquatic habitats that result from project construction-related activities. These BMPs are recommended in addition to, and do not over-ride any terms, conditions, or other recommendations prepared by the USFWS, other federal, state or local agencies. If you have questions concerning these BMPs, please contact the USFWS Aquatic Ecosystems Conservation Program at 808-792-9400.

1. Authorized dredging and filling-related activities that may result in the temporary or permanent loss of aquatic habitats should be designed to avoid indirect, negative impacts to aquatic habitats beyond the planned project area.

2. Dredging/filling in the marine environment should be scheduled to avoid coral spawning and recruitment periods, and sea turtle nesting and hatching periods. Because these periods are variable throughout the Pacific islands, we recommend contacting the relevant local, state, or federal fish and wildlife resource agency for site specific guidance.

3. Turbidity and siltation from project-related work should be minimized and contained within the project area by silt containment devices and curtailing work during flooding or adverse tidal and weather conditions. BMPs should be maintained for the life of the construction period until turbidity and siltation within the project area is stabilized. All project construction-related debris and sediment containment devices should be removed and disposed of at an approved site.

4. All project construction-related materials and equipment (dredges, vessels, backhoes, silt curtains, etc.) to be placed in an aquatic environment should be inspected for pollutants including, but not limited to; marine fouling organisms, grease, oil, etc., and cleaned to remove pollutants prior to use. Project related activities should not result in any debris disposal, non-native species introductions, or attraction of non-native pests to the affected or adjacent aquatic or terrestrial habitats. Implementing both a litter-control plan and a Hazard Analysis and Critical Control Point plan (HACCP – see https://www.fws.gov/policy/A1750fw1.html) can help to prevent attraction and introduction of non-native species.

5. Project construction-related materials (fill, revetment rock, pipe, etc.) should not be stockpiled in, or in close proximity to aquatic habitats and should be protected from erosion (*e.g.*, with filter fabric, etc.), to prevent materials from being carried into waters by wind, rain, or high surf.

6. Fueling of project-related vehicles and equipment should take place away from the aquatic environment and a contingency plan to control petroleum products accidentally spilled during the project should be developed. The plan should be retained on site with the person responsible for compliance with the plan. Absorbent pads and containment booms should be stored on-site to facilitate the clean-up of accidental petroleum releases.

7. All deliberately exposed soil or under-layer materials used in the project near water should be protected from erosion and stabilized as soon as possible with geotextile, filter fabric or native or non-invasive vegetation matting, hydro-seeding, etc.

APPENDIX E:
Past, Present, and Reasonably Foreseeable Future Actions on National Park Service, Department of Land and Natural Resources, and The Nature Conservancy Lands

Appendix E: Past, Present, and Reasonably Foreseeable Future Actions on National Park Service, Department of Land and Natural Resources, and The Nature Conservancy Lands

For the purposes of establishing the affected environment and assessing cumulative impacts of the alternatives, the National Park Service, Department of Lands and Natural Resources, and The Nature Conservancy identified the following projects, plans, or actions that have, are currently, or may in the future potentially affect the resources analyzed in the EA.

Haleakalā National Park

Natural Resource Preservation Program Mosquito Monitoring in Kīpahulu Valley. This is a planned action where two sites would be visited every 3 months (primarily at 2 locations, Palikea and Delta). Sites would be occupied for 6–7 days in a row. A helicopter would be required to drop off crews. Kīpahulu sites would be visited June through February. This project is expected to scale up in the next few years. Project activities do occur within designated wilderness.

Air Tour Management Plan. This is a planned and ongoing action where the park is currently developing an Air Tour Management Plan working with the Federal Aviation Administration. Three potential alternatives describing conditions for the conduct of commercial air tour operations over the park, including routes, altitudes, time-of-day restrictions, restrictions for particular events, and maximum numbers of flights, have been developed. The average annual number of commercial air tours conducted over the park from 2017–2019 for all operators is 4,824. The 2017–2019 three-year average is considered the existing baseline for the purposes of understanding the existing number of commercial air tour flights over the park. Flights do currently occur above designated wilderness.

Hydrological Monitoring Systems in Kīpahulu Valley. This is an ongoing action. Hydrological monitoring has been implemented particularly as visitors swim in pools downstream in Kīpahulu Valley. The project requires helicopter use and 5 –6 monitoring sites are within designated wilderness.

Songbird Occupancy Project. This is an ongoing action. Audio Recording Units have been positioned within designated wilderness in the Manawainui, Northeast Rift, and Kīpahulu Valley regions of the park that are visited 1–2 times per year. Project activities do occur within designated wilderness.

Pīpīwai Trail Viewing Platform. This future planned action is approximately 2 years from implementation and outside of wilderness at Waimoku Falls.

Invasive Plant Herbicide Spraying. This ongoing project entails remote helicopter longline herbicide spraying to treat invasive plants. There are follow-up kills for pine resprouts outside of Kīpahulu Valley. Pine spraying conducted in Haleakalā Crater applies the same methodology. The Waimoku Falls area targets a different plant using this same methodology. Project activities do occur within designated wilderness.

Inventory & Monitoring Vegetation Monitoring Plots. This planned action would involve establishing and monitoring plots in Haleakalā Crater and Kīpahulu Valley starting 2023–2024. A ground crew of 4–5 people would be used, with possibly more at camps visiting areas in subalpine shrublands. The crew would be visiting fixed plots and establishing new plots all within a designated area. Three to four people and three hours is needed to complete a plot in the Crater and crews would camp out for the week visiting plots. Crews would also be completing weed transects along fixed trails. Project activities including helicopter use do occur within designated wilderness.

Fire Management Plan Update. Ongoing fuel reduction activities occur in portions of the park including wilderness. Activities include forest thinning, hand removal and herbicide spraying of non-native pine species (Monterey pine, Mexican weeping pine, and maritime pine) that increase the potential for wildfire in habitats as they are not adapted to fire, and prescribed burns.

Ungulate Control. This is an ongoing project for ungulate animal control. The primary and most effective feral animal management method in Haleakalā is an extensive fence system designed to prevent entry of non-native ungulates into the park. These non-natives are removed through either seasonally placed snares or other aerial and ground removal methods. Project activities do occur within designated wilderness.

Trail Maintenance. Extensive networks of primitive administrative trails run throughout the Biological Reserve area of the Haleakalā Wilderness. The only purpose of this trailand route system is to support administrative and research activities. Most trails and routes connect from landing zones and/or research shelters to areas of fence line, transects, monitoring stations, or invasive species treatment areas. This ongoing project would involve periodic trail maintenance including gas-powered trimmers and chainsaws for clearing.

Invasive Plant Control. Ongoing use of herbicides and cutting of non-native plants is conducted to reduce their competition with native plant communities and prevent spread, providing native species the opportunity to compete and repopulate areas where invasives have been removed. A suite of herbicides has been used over the years to accomplish invasive species management goals. Different variations of 'glyphosate' (Round Up, Honcho, Ranger Pro, and Rodeo) as well as variations of 'triclopyr' (Garlon 3A and Garlon 4) are primarily used. Manual techniques of cutting, digging, and pulling are used where appropriate. Project activities do occur within wilderness.

Fence Construction and Maintenance. Ongoing use of fencing in conjunction with other feral animal management measures has proven to be an effective method for controlling the spread of harmful species such as axis deer, feral pigs, feral goats, and feral cattle with little impact to movement of native wildlife species. Helicopter flights into wilderness do occur and power tools for large tree removal can be used. Future activities will likely be more extensive than the normal maintenance that has occurred over the last 10 to 20 years.

Landing Zone and Shelter Maintenance. Improved LZs are areas that have been cleared of trees or debris and/or filled in with natural materials. Landing zones are only improved if repeated use is planned. All LZs are cleared/developed as an ongoing action in accordance with the Interagency Helicopter Operations Guide (110-foot radius minimum). In planning LZs, attempts are made to eliminate the need for establishment of new zones and areas that have the most accommodating naturalsurfaces are preferred. Gas-powered tools and generators are used to maintain and improve shelters within designated wilderness within the Kīpahulu Valley.

Forest Bird Predator Control. An ongoing pilot project to control non-native small mammal species is occurring in the upper section of Kīpahulu Valley. The project includes a grid of traplines, bait stations, and camera traps. Future plans include implementation of a predator management plan for the area and expansion to Manawainui. Project activities occur within wilderness.

Endangered Plant Surveys and Planting. This is an ongoing management action to monitor, maintain, and supplement endangered plants within Haleakalā National Park, including within wilderness. This work is also in coordination with the Plant Extinction Prevention Project under DLNR. Project activities occur within wilderness.

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Ungulate Control. Ongoing animal management activities under the East Maui Watershed Partnership Management Plan and Hanawī Natural Area Reserve Management Plan, as applicable, include regular fence inspections, intensive animal control, transect monitoring, and aerial and on-the-ground scouting. DLNR would continue aerial shooting and support ungulate control at Pu‘u Pahu Reserve, Hanawī Natural Area Reserve, Hāna Forest Reserve, and Ko‘olau Forest Reserve (Loulu, Kūhiwa, and Waiho‘i fenced units). DLNR would also continue the East Maui Irrigation Halehaku feral cattle project to reduce cattle populations. DLNR would continue ungulate and vegetation transect monitoring to assess the effectiveness of public hunting to reduce ungulate populations and impacts and identify and expand public hunting access into Hāna Forest Reserve and Kīpahulu Forest Reserve.

Invasive Plant Control. As a future planned action, DLNR, under the East Maui Watershed Partnership Management Plan and Hanawī Natural Area Reserve Management Plan, as applicable, would develop, implement, and regularly update comprehensive Weed Control Plans that identify priority weeds within management units and outline management strategies and timelines for their control; eliminate populations of top priority weeds in management units; prevent expansion of top priority weeds; and increase weed management capacity by hiring additional staff and utilizing volunteers, the Maui Invasive Species Committee, and other agencies.

Forest Bird Recovery Actions. As an ongoing action, Maui Forest Bird Recovery Project conducts forest bird population monitoring, predator control, and mosquito and avian malaria surveys on DLNR lands within the project area. There is also a proposal to introduce ‘alala (Hawaiian Crow, *Corvus hawaiiensis*) on Maui (location TBD); this would include temporary release aviaries, feeding stations, predator control, and on-ground monitoring.

Watershed Resource Monitoring. As a future planned action, DLNR, under the East Maui Watershed Partnership Management Plan and Hānawi Natural Area Reserve Management Plan, as applicable, would measure rainfall at U.S. Geological Survey rain gauges; measure stream flow from State Division of Aquatic Resources and Commission on Water Resource Management; assess the need to install rain gauges/Remote Automated Weather Stations in East Maui Watershed Partnership areas; measure sedimentation and erosion with information from the State Department of Health and U.S. Geological Survey; and measure biotic stream components obtaining information from the Maui Division Aquatic Resources and other agencies.

Landing Zone Maintenance. Improved LZs are areas that have been cleared of trees or debris and/or filled in with natural materials. As an ongoing action, landing zones are only improved if repeated use is planned. All LZs are cleared/developed in accordance with the Interagency Helicopter Operations Guide (110-foot radius minimum). In planning LZs, attempts are made to eliminate the need for establishment of new zones and areas that have the most accommodating natural surfaces are preferred.

Water Diversion Infrastructure and Maintenance. Activities occur both on DLNR lands that are leased to East Maui Irrigation and private lands. Activities include stabilizing banks downstream of diversion boxes, replacing pipes, and repairing walls using grouted rip rap or similar means.

Endangered plant surveys and outplantings. This is an ongoing management action to implement recovery actions for species-at-risk, including surveys, threat abatement, collection, reintroduction, and monitoring within the state reserves. Work focusses primarily in areas above the ungulate-control fences. This work is coordinated throughout the project area with state and private conservation partners.

Endangered invertebrate surveys and outplantings. This is an ongoing management action to implement recovery actions for species-at-risk, including surveys, threat abatement, collection, reintroduction, and monitoring within the state reserves. Work focusses primarily in areas above the ungulate-control fences. This work is coordinated throughout the project area with state and private conservation partners.

Habitat Restoration Efforts. The efforts include reforestation and outplanting of more than 250,000 trees throughout agency and partner lands in east Maui, the purchase of Kamehamenui lands, and lands at Nuʻu by Haleakalā National Park with habitat restoration efforts underway, continued support for the Division of Forestry and Wildlife Forestry Reserve System and Natural Area Reserve System, the East Maui Watershed Partnership, the Mauna Kahālāwai Watershed Partnership, and the Leeward Haleakalā Watershed Partnership, all of which support habitat restoration and protection in critical habitat areas.

The Nature Conservancy

Western Waikamoi Boundary Fence Retrofit. As a future planned action, TNC will retrofit the western Waikamoi boundary fence for ungulate control.

Cell Tower Infrastructure from Puʻu Nianiau to Koʻolau. As a future planned action, a series of 30-foot poles with infrastructure would be installed to provide cell service all the way to Hāna approximately in late 2023 or early 2024.

Aerial Pine Spraying. As an ongoing action, helicopters are periodically used to treat non-native pines.

Maui Forest Bird Recovery Project. As an ongoing action, Maui Forest Bird Recovery Project conducts forest bird surveys, predator control, and mosquito and avian malaria research within the TNC-managed lands within the project area.

Forest Health and Weed Treatment Efficacy Monitoring. As an ongoing action, drone flights occur 1 day per month for assessment.

Ungulate Monitoring. As an ongoing action, drone flights occur one day per month for assessment working collaboratively with hunters.

Herbicide Ballistic Technology. As an ongoing action, drones are periodically used to apply herbicide to invasive plants within Waikamoi Preserve.

Guided Hikes and Volunteer Trips. As an ongoing action, TNC conducts one monthly guided hike and one bi-monthly volunteer trip.

Weather Station Installation. As a future planned action, a station would be installed in subalpine shrubland and another in eastern Waikamoi. The stations are 10–15 feet tall and have 2 x 2 footprint.

Mozzie Monitoring in Waikamoi. Mozzie Monitors is a citizen science mosquito surveillance program. As an ongoing action, the project is occurring now through December 2023 and involves trapping mosquitoes and both helicopter-assisted and non-helicopter-assisted ground ork.

APPENDIX F:
Threatened and Endangered Plant Species and Plant Species at Risk

Table F-1: Threatened and Endangered Plants Known to Occur in the Analysis Area

Scientific Name	Common Name	Federal Status[1]	Habitat	Known Locations within Analysis Area
Alectryon micrococcus var. *macrococcus*	mahoe	LE	On east Maui, known from the montane mesic ecosystem (USFWS 2012)	State
Asplenium peruvianum var. *insulare* (syn. *A. fragile* var. *insulare*)	diamond spleenwort	LE	Moist, dark microhabitats in various forest and shrubland ecosystems including montane wet, montane mesic, and subalpine ecosystems (USFWS 1998, 2016a).	TNC
Bidens campylotheca ssp. *pentamera*	ko'oko'olau viper beggarticks	LE	Lowland dry, lowland mesic, montane mesic, montane wet, dry cliff and wet cliff ecosystems (USFWS 2019).	Park
Bidens campylotheca ssp. *waihoiensis*	ko'oko'olau, waihoi beggarticks	LE	Lowland wet, montane wet and wet cliff ecosystems (USFWS 2019).	Park
Calamagrostis expansa	Maui reedgrass	LE	Along ridges or on raised hummocks in wet forest and bogs in the montane wet ecosystem. Most of the east Maui occurrences are in exclosures (USFWS 2016b).	TNC
Cyanea copelandii ssp. *haleakalaensis*	hāhā treetrunk cyanea	LE	Lowland mesic, lowland wet, montane wet, and wet cliff ecosystems (USFWS 2016a).	Park, State
Cyanea duvalliorum	hāhā	LE	Lowland wet and montane wet ecosystems (USFWS 2013).	State
Cyanea hamatiflora ssp. *hamatiflora*	hāhā wet forest cyanea	LE	Lowland wet and montane wet ecosystems (USFWS 2016a).	Park
Cyanea horrida	hāhā nui prickly cyanea	LE	Montane mesic and montane wet ecosystems (USFWS 2019).	TNC
Cyanea kunthiana	hāhā Kunth's cyanea	LE	Lowland wet, montane mesic, and montane wet ecosystems (USFWS 2019).	Park, State, TNC
Cyanea maritae	hāhā	LE	Lowland wet and mesic wet ecosystems (USFWS 2019).	Park, State
Cyanea mceldowneyi	hāhā	LE	Montane wet forest at elevations between 3,034 and 4,200 feet (USFWS 1997).	State

Scientific Name	Common Name	Federal Status[1]	Habitat	Known Locations within Analysis Area
Diplazium moloka`iense	no common name	LE	Lowland to montane forests in mesic to wet ecosystems (USFWS 1998)	State
Huperzia mannii (syn. *Phlegmariurus mannii*)	wawae'iole Mann's clubmoss	LE	Typically grows on native tree species including ʻōhiʻa, koa, and ʻaʻaliʻi in mesic to wet montane forests (USFWS 1997).	Park
Joinvillea ascendens subsp. *ascendens*	`ohe	LE	Wet to mesic ʻōhiʻa -koa lowland and montane forest and along intermittent streams (USFWS 2016b).	Park
Melicope balloui	alani Ballou's melicope	LE	Wet forest in Kīpahulu Valley and the slopes of Haleakalā (USFWS 1997).	TNC
Melicope ovalis	alani Hāna melicope	LE	Closed ʻōhiʻa forest in Koʻolau Gap and ʻōhiʻa -koa forest, especially on stable banks of water courses in Kīpahulu Valley (USFWS 1997, USFWS 2011).	Park
Microlepia strigosa var. *mauiensis*	palapalai	LE	On Maui, typical habitat is the montane wet forest ecosystem (USFWS 2016b).	Park
Ochrosia haleakalae	holei	LE	Dry to mesic forest, sometimes wet forest, and often lava, from 2,300 to 4,000 ft (700 to 1,200 m), in the dry cliff, lowland mesic, and montane mesic ecosystems (USFWS 2016b).	State, TNC
Peperomia subpetiolata	`ala `ala wai nui	LE	Montane wet ecosystems (USFWS 2019).	State
Phyllostegia bracteata	bracted phyllostegia	LE	Lowland wet, montane mesic, montane wet and subalpine ecosystems (USFWS 2019).	Park, TNC
Phyllostegia brevidens	no common name	LE	Wet forest in the lowland wet and wet cliff ecosystems from 2,900 to 3,200 feet (USFWS 2016b).	Park
Phyllostegia haliakalae	no common name	LE	Lowland mesic, montane mesic and dry cliff ecosystems (USFWS 2019).	Park, State
Phyllostegia pilosa	no common name	LE	On east Maui, known from the montane wet ecosystem (USFWS 2016a).	State, TNC

Scientific Name	Common Name	Federal Status[1]	Habitat	Known Locations within Analysis Area
Plantago princeps var. *laxifolia* (syn. *P. princeps var. laxiflora*)	ale kuahiwil laukahi	LE	Dry cliff and wet cliff ecosystems (USFWS 2016a).	Park, TNC
Schiedea diffusa ssp. *diffusa*	no common name	LE	Lowland wet and montane wet ecosystems (USFWS 2019).	TNC
Wikstroemia villosa	no common name	LE	Lowland wet, montane mesic, and montane wet ecosystems (USFWS 2019).	TNC

Sources for species listed in table: C. Warren, personal communication, October 27, 2022; M. Keir, personal communication, November 15, 2022; K. Fay, personal communication, October 28, 2022.
1 LE = federally listed endangered

Table F-2: Federally and State Listed Species with Designated Critical Habitat that Occurs in the Analysis Area

Scientific Name	Common Name	Federal and State Status[1]	Known Occurrences of Species within Analysis Area	Locations of Designated Critical Habitat within Analysis Area
Adenophorus periens	pendant kihi fern	LE	No	Park, State
Argyroxiphium sandwicense subsp. *macrocephalum*	\`ahinahina	LT	No	Park
Asplenium peruvianum var. *insulare* (syn. *A. fragile* var. *insulare*)	diamond spleenwort	LE	Yes	Park, State
Bidens campylotheca ssp. *pentamera*	ko'oko‘olau viper beggarticks	LE	Yes	Park, State
Bidens campylotheca ssp. *waihoiensis*	ko'oko'olau waihoi beggarticks	LE	Yes	Park, State
Bidens micrantha subsp. *kalealaha*	ko'oko'olau	LE	No	Park
Clermontia oblongifolia subsp. *mauiensis*	‘ōhā wai	LE	No	Park, State
Clermontia peleana	‘ōhā wai	LE	No	Park, State

Scientific Name	Common Name	Federal and State Status[1]	Known Occurrences of Species within Analysis Area	Locations of Designated Critical Habitat within Analysis Area
Clermontia samuelii	ʻōhā wai	LE	No	Park, State
Cyanea aspleniifolia	hāhā	LE	No	Park, State
Cyanea copelandii ssp. *haleakalaensis*	hāhā treetrunk cyanea	LE	Yes	Park, State
Cyanea duvalliorum	hāhā	LE	Yes	Park, State
Cyanea glabra	hāhā	LE	No	Park, State
Cyanea hamatiflora ssp. *hamatiflora*	hāhā wet forest cyanea	LE	Yes	Park, State
Cyanea horrida	hāhā nui prickly cyanea	LE	Yes	Park, State
Cyanea kunthiana	hāhā Kunth's cyanea	LE	Yes	Park, State
Cyanea maritae	hāhā	LE	Yes	Park, State
Cyanea mceldowneyi	hāhā	LE	Yes	Park, State
Cyrtandra ferripilosa	ha`iwale	LE	No	Park, State
Diplazium moloka`iense	no common name	LE	Yes	Park, State
Geranium arboreum	noho-anu Hawai'i red cranesbill	LE	No	Park
Geranium hanaense	nohoanu	LE	No	Park, State
Geranium multiflorum	noho-anu manyflower geranium	LE	No	Park, State
Huperzia mannii (syn. *Phlegmariurus mannii*)	wawae'iole Mann's clubmoss	LE	Yes	Park, State
Melicope balloui	alani Ballou's melicope	LE	Yes	Park, State

Scientific Name	Common Name	Federal and State Status[1]	Known Occurrences of Species within Analysis Area	Locations of Designated Critical Habitat within Analysis Area
Melicope ovalis	alani Hāna melicope	LE	Yes	Park, State
Mucuna sloanei var. persericea	sea bean	LE	No	Park, State
Peperomia subpetiolata	\`ala \`ala wai nui	LE	Yes	Park, State
Phyllostegia bracteata	bracted phyllostegia	LE	Yes	Park, State
Phyllostegia haliakalae	no common name	LE	Yes	Park, State
Phyllostegia mannii	no common name	LE	No	Park, State
Phyllostegia pilosa	no common name	LE	Yes	Park, State
Platanthera holochila	no common name	LE	No	Park, State
Schiedea haleakalensis	no common name	LE	No	Park
Schiedea jacobii	no common name	LE	No	Park, State
Wikstroemia villosa	no common name	LE	Yes	Park, State
Zanthoxylum hawaiiense	a\`e	LE	No	Park

Sources: USFWS 2022b; C. Warren, personal communication, October 27, 2022; M. Keir, personal communication, November 15, 2022; K. Fay, personal communication, October 28, 2022.
1 LE = federally listed endangered; LT = federally listed threatened

Table F-3: State Plant Species at Risk that Occur in the Analysis Area

Scientific Name	Common Name	NatureServe Status[1]	Habitat	Known Locations within Analysis Area
Anoectochilus sandvicensis	Hawai'i jewel-orchid	S3	Dense, dark, continuously saturated forests, sometimes growing in mosses at base of trees usually in wet muck below (NatureServe 2022).	Park, TNC
Asplenium haleakalense	Haleakalā spleenwort	S1	Epiphyte on mossy tree branches in wet forests, usually 3-5 feet above the ground, less often on fallen logs (NatureServe 2022).	TNC
Asplenium monanthes	single-sorus spleenwort	SNR[2]	Terrestrial or occasionally epiphytic fern found in the understory of wet forest and along stream banks at 3,280 to 7,550 feet meters elevation on the islands of Maui and Hawai'i (Palmer 2003).	TNC
Clermontia grandiflora	'ōhā 'ōhā wai	S3	Grows in upper elevation wet forest under canopy dominated with 'ōhi'a. In Park usually found between 4,000-6,000 feet (J. Mallison, pers. comm. December 14, 2021).	TNC
Clermontia grandiflora subsp. *maxima*	'ōhā 'ōhā wai Large-flower clermontia	S1	Wet forests and bog margins (LBJWC 2022)	TNC
Clermontia tuberculata	'ōhā hāhā 'ōhā wai	S1	Grows on the ground in wet forests, primarily along streambeds (NatureServe 2022).	TNC
Cyanea aculeatiflora	Haleakalā cyanea prickly-flower cyanea	S3	Typically restricted to drainages and gulches in upper elevation wet forest dominated by 'ōhi'a (J. Mallison, pers. comm. December 14, 2021).	TNC
Cyanea macrostegia	hāhā purple cyanea	S3	Typically found in wet hardwood, shrubland/chaparral, forest/woodlands in gulch bottoms or on gulch sides (NatureServe 2022).	TNC

Scientific Name	Common Name	NatureServe Status[1]	Habitat	Known Locations within Analysis Area
Dryopteris fuscoatra var. *lamoureuxii*	i`i crowned woodfern	SNR[3]	Endemic to the island of Maui. Has only been observed in small area of the understory of diverse mesic to wet forest at 3,020 to 3,940 feet in elevation on the northern slopes of east Maui in and around the Makawao and Ko'olau Forest Reserves (Palmer 2003).	TNC
Dryopteris tetrapinnata	i'o nui Waikamoi woodfern	S1	Endemic to East Maui. Known from wet forests in the Waikamoi Preserve (NatureServe 2022).	TNC
Fragaria chiloensis subsp. *sandwicensis*	ohelo papa Chilean strawberry	SNR[2]	Dry, disturbed areas, wet forest, and subalpine shrubland from 3,800 to 10,075 feet (NatureServe 2022).	TNC
Huperzia filiformis	stringleaf clubmoss	SNR[2]	Wet forests (LBJWC 2022).	TNC
Liparis hawaiensis	awapuhiakanaloa Hawaiian twayblade	S3	Found from about 1,600 to over 5,000 feet on bryophyte-covered trees, under bushes, and on wet or sometimes seasonally wet, bare ground, in bogs, and mesic to wet forest as epiphytic or terrestrial plants (Native Plants Hawai'i 2009).	Park, TNC
Melicope haleakalae	alani Stone's pelea	S2	Wet forests (NatureServe 2022).	TNC
Melicope hawaiensis	manena	S2	Moist or, less often, dry forests. On ridges and in gulches and, on Hawaii and parts of East Maui, on old lava flows and on old ash deposits (NatureServe 2022)	TNC
Melicope pseudoanisata (syn. *M. cauliflora*)	Kohala Summit Melicope alani	S3	Mesic to wet forests (LBJWC 2022).	TNC
Phyllostegia ambigua	mountain phyllostegia	S3	Wet forest and perhaps lower parts of subalpine forest at elevations between 3,000 to 6,500 feet (NatureServe 2022).	TNC
Phytolacca sandwicensis	Hawai`i pokeweed	S2	Mesic to wet forest, in open areas or streambeds, straggling or climbing (NatureServe 2022).	Park

Scientific Name	Common Name	NatureServe Status[1]	Habitat	Known Locations within Analysis Area
Rubus macraei	‘ākala ‘ākalakala	S2	Steep rocky slopes and open rocky areas in wet forest and margins of bogs. Sometimes in sheltered thickets of other plants in pastures or subalpine shrubland. Communities where present include 'ohi'a montane wet forest, and 'ama'u-'ohelo subalpine mesic shrubland (NatureServe 2022).	TNC
Sicyos cucumerinus	‘ānunu kūpala Gray’s bur-cucumber	S1	Moist and wet forests on gulch slopes and in gulch bottoms. Mostly at middle elevations, but on East Maui, at least one occurrence is subalpine (NatureServe 2022).	TNC
Strongylodon ruber	Hawai`i jadevine	S2	Mesic to wet forest; a liana species found climbing and hanging from trees. Forest communities where present include Diverse Lowland Mesic Forest, which has a 4-15 m tall canopy and an open understory of shrubs, ferns, and herbs (NatureServe 2022).	Park
Trematolobelia macrostachys	koli`i	S3	Typically found on ridges or other open areas in upper elevation ‘ōhi‘a -dominated wet forest (J. Mallison, pers. comm. December 14, 2021).	TNC

Sources for species listed in table: K. Fay, personal communication, October 28, 2022; C. Warren, personal communication, October 27, 2022; M. Keir, personal communication, November 15, 2022.
1 Status based on NatureServe 2022: S1 = critically imperiled in the state, S2= imperiled in the state; S3 = vulnerable in the state; SNR – no state rank
2 Although these species are not listed or not given a state rank (i.e., SNR) in NatureServe 2022, they are considered species of concern in the State.

APPENDIX G:
DLNR HEPA Significance Criteria Analysis

Appendix G: DLNR HEPA Significance Criteria Analysis

This appendix sets out the State of Hawaii, Department of Land and Natural Resources (DLNR) anticipated determination that the proposed action will *not* have a significant effect on the environment, in accordance with HEPA HAR Chapter 11-200.1 and the applicable “significance criteria” (listed below). This determination will be made pursuant to the requirement of HEPA and is separate from a finding of no significant impact (FONSI), if appropriate, that will be made by the NPS pursuant to NEPA, following review of public comments on the EA.

Based on the analysis in the EA, the DLNR anticipates that the proposed action will not result in significant effects on the environment for the following reasons:

1. **Irrevocably commit a natural, cultural, or historic resource**
   The proposed action involves the release of incompatible mosquitoes and will not irrevocably commit a natural, cultural, or historic resource. There will be no new ground disturbance associated with the proposed action.

2. **Curtail the range of beneficial uses of the environment**
   The proposed action will actually result in long-term beneficial impacts to the east Maui ecosystem and would not curtail the range of beneficial uses of the environment.

3. **Conflicts with the State’s environmental policies or long-term environmental goals established by law**
   The proposed action will not conflict with the State's environmental policies or long-term environmental goals established by law.

4. **Have a substantial adverse effect on the economic welfare, social welfare, or cultural practices of the community or State**
   The proposed action should result in the protection of native forest birds and will therefore not have a substantial adverse effect on the economic welfare or social welfare of the community and State. DLNR and NPS have worked closely with the SHPD and have developed a Cultural Impact Assessment, consistent with HEPA regulations. According to the analysis in the Cultural Impact Assessment and Appendix B in the EA, the proposed action would not result in adverse effects to cultural practices on Maui.

5. **Have a substantial adverse effect on public health**
   The proposed action would involve the release of male mosquitoes that would not bite humans and would reduce the existing mosquito population on east Maui, which could benefit human health by suppressing disease-spreading mosquitoes. Therefore, the proposed action would not have a substantial adverse effect on public health.

6. **Involve adverse secondary impacts, such as population changes or effects on public facilities**
   Release of incompatible mosquitoes under the proposed action would have no adverse secondary impacts such as population changes or effects on public facilities.

7. **Involves a substantial degradation of environmental quality**
   The proposed action would have only minimal impacts to the acoustic environment, wildlife, and visitor experience during mosquito release operations and would result in long-term beneficial impacts to the environment through the suppression of mosquito populations which should result in the conservation of threatened and endangered forest birds. Therefore, the proposed action would not result in a substantial degradation of environmental quality.

8. **Be individually limited but cumulatively has substantial adverse effect upon the environment or involves a commitment for larger actions**
   According to the impact analysis in the EA, the proposed action wound not result in substantial cumulative adverse effects on the environment and would not involve a commitment for larger actions. The proposed action would involve the use of drones and helicopters, which are already being used within the project area. The increase in drone and helicopter use would result in adverse cumulative effects, but these effects would be fairly minor and would only occur during mosquito operations during daylight hours on weekdays.

9. **Have a substantial effect on rare, threatened, or endangered species, or its habitat**
   Aerial release of mosquitoes under the proposed action could have temporary impacts to threatened and endangered species due to noise and presence of drones, and to a lesser extent, helicopters. However, these effects would be temporary and should not result in any meaningful impacts to species. Over the long term, there would be beneficial impacts on threatened or endangered forest birds from the suppression of mosquitoes that spread avian malaria.

10. **Have a substantial adverse effect on air or water quality or ambient noise levels**
    The proposed action would have no perceptible impacts on air or water quality. The primary mosquito release method under the proposed action would be through the use of drones, which don’t impact air or water quality. Helicopter support would be needed occasionally for mosquito release and monitoring, but the level of use would not result in more than negligible impacts to air quality. Ambient noise levels would be affected during mosquito release operations, but because drones would be the primary mosquito release method, impacts to ambient noise levels would not be substantial due to the low noise levels associated with drones. Also, there would be no noise associated with release operations at night or on weekends.

11. **Have a substantial adverse effect or is likely to suffer damage by being located in an environmentally sensitive area such as a flood plain, tsunami zone, beach, erosion-prone area, geologically hazardous land, estuary, fresh water, or coastal waters**
    The project area is located over inland areas only, and therefore the proposed action would not have a substantial adverse effect on or be likely to suffer damage by being located in an environmentally sensitive area such as a flood plain, tsunami zone, sea level rise exposure area, beach, erosion-prone area, geologically hazardous land, estuary, fresh water, or coastal waters.

12. **Have a substantial adverse effect on scenic vistas and view planes identified in county or state plans or studies; or**
    The proposed action involves the use of drones and helicopters, which would be visible during release operations. However, aerial operations would primarily use drones which would fly close to the canopy up to two times a week, which would only be visible for a short period of time in any one location. Therefore, the proposed action would not have a substantial adverse effect on scenic vistas and view planes, during day or night, identified in county or state plans or studies.

13. **Require substantial energy consumption or emit substantial greenhouse gas**
    Under the proposed action, drones would be the primary method for mosquito releases. Drones used under the proposed action would be battery-powered and would not emit greenhouse gases. The minimal amount of helicopter use under the proposed action would involve some greenhouse gas emissions, but it would not rise to the level of being substantial.

APPENDIX H:
Responses to Substantive Public Comments on Environmental Assessment

APPENDIX H: Responses to Substantive Public Comments on Environmental Assessment

CONCERN 1: Commentors were concerned that the level of analysis presented in the Environmental Assessment (EA) was insufficient, and that an Environmental Impact Statement (EIS) should be prepared.

Response: Both the Environmental Assessment (EA) and Environmental Impact Statement (EIS) processes involve rigorous analysis of potential environmental and cultural impacts of proposed agency actions, as required by federal (NEPA) and state (HEPA) regulations. The NEPA and HEPA regulations state, however, that an agency shall prepare an EA for a proposed action that is not likely to have significant effects or when the significance of the effects is unknown. Prior to and during the preparation of this mosquito suppression EA, the project team spent a considerable amount of time analyzing numerous potential effects of the proposed action. Ultimately, none of those potential impacts were determined to be significant, and effects resulting from the selected alternative are known, as indicated in the FONSI, which is supported by the impact analysis in the EA. Therefore, an EIS was not prepared.

CONCERN 2: Commentors were concerned that potential impacts to public health and safety, largely from a concern of perceived increased risk of disease transmission particularly over the long term, were not sufficiently addressed.

Response: The released mosquitoes would pose no risk to human health. Only male mosquitoes would be released. Male mosquitoes do not bite humans or animals and do not transmit diseases. Only female mosquitoes bite humans or animals. The risk of females being accidentally released is estimated to be 1 in 900 million (Crawford et al. 2020). Even if a female mosquito is released, a bite from it would pose no greater risk to humans or wildlife than the wild female mosquitoes currently present in the environment.

The *Wolbachia* bacteria used to generate the incompatible male mosquitoes is already present in Hawaiʻi in the Asian tiger mosquito (*Aedes albopictus*). *Wolbachia* cannot live within vertebrate cells and cannot be transferred to humans even through the bite of a mosquito that carries it (Popovic et al. 2010). Residents of Hawaiʻi are commonly bitten by the Asian tiger mosquito, which is distributed statewide and has remained one of the most abundant mosquitoes at lower elevations since its introduction in 1896. Residents of Hawaiʻi are also commonly bitten by the southern house mosquito (*Culex quinquefasciatus*), the target species in the proposed action, which was introduced to Hawaiʻi in 1826 and occupies both lower elevation and upper elevation habitats across the state. The southern house mosquito is also already naturally infected with *Wolbachia*. Humans in Hawaiʻi therefore are regularly bitten by mosquitoes containing *Wolbachia*, including the strain that would be used in the proposed action (wAlb). No adverse effects have ever been reported in humans, nor is there a biological mechanism allowing adverse effects to occur.

As stated above, the southern house mosquito and the *Wolbachia* bacteria are already present in Hawaiʻi. No new organisms would therefore be introduced to Hawaiʻi by the proposed action. Further, there is no indication that the released mosquitoes would be any better at transmitting disease to humans or wildlife (Popovici et al. 2010). The southern house mosquito does not transmit any human diseases in Hawaiʻi. In contrast, the southern house mosquito is already a remarkably efficient vector of the avian malaria parasite, with an estimated 85–97% of southern house mosquitoes being susceptible to infection and transmission (LaPointe et al. 2005). Increasing the vector competence (ability to transmit disease) of the southern house mosquito is therefore highly unlikely and ecologically insignificant when compared to the known risk of allowing these mosquitoes to proliferate on the landscape. The proposed action has been vetted and remains supported by all state, federal, and private conservation organizations that have management responsibilities towards the recovery of endangered forest birds on East Maui.

The incompatible insect technique (IIT) using *Wolbachia* is an approach that was researched, developed, and first used over 50 years ago for the express purpose of human public health (Laven 1967). Over the following half-century, the approach has continued to be studied, patented, and applied specifically for the benefit of improving public health outcomes for humans where mosquito-borne diseases are a threat. New text was added to Appendix B (Page B-9) of the EA to better describe why there would be no impacts to human health from releasing incompatible male mosquitoes.

CONCERN 3: Commentors were concerned that previous attempts to introduce biological control mechanisms in the past in Hawai'i have had unforeseen and adverse impacts (e.g., mongoose introduction to control rats) and that this will occur with the proposed mosquito releases.

Response: No new organisms would be introduced to Hawaiʻi by the proposed action. The southern house mosquito (*Culex quinquefasciatus*) and the *Wolbachia* bacteria are already present in Hawaiʻi. The *Wolbachia* bacteria used to generate incompatible male mosquitoes occurs in Hawaiʻi in the Asian tiger mosquito (*Aedes albopictus*), introduced to Hawaiʻi in 1896. The southern house mosquito has been widely established in Hawaiʻi since its introduction in 1826 and already naturally carries a strain of *Wolbachia* bacteria.

Researchers and resource managers possess long-term data that aptly demonstrate that the worst-case scenario for native wildlife is currently well underway (Pratt et al. 2009; Paxton et al. 2022). The southern house mosquito continues to vector the parasite to native honeycreepers that causes avian malaria, driving these irreplaceable biocultural resources to extinction. The proposed project aims to control the southern house mosquito in forest habitat, where male and female mosquitoes are already present and causing widespread mortality to endangered forest birds. If released, incompatible male mosquitoes are expected to survive for less than a week before mating and then dying. If releases of incompatible male mosquitoes are halted, there will be no lasting effect on the environment.

The history of biological control in Hawaiʻi is complicated, with success stories largely overshadowed by misinformation. The same lack of regulations and biosecurity measures that enabled the southern house mosquito to first be introduced to Hawaiʻi in 1826 also enabled private plantation owners on Hawaiʻi Island to import the Small Indian Mongoose (*Urva auropunctata*) from Jamaica in 1883 with no official review or oversight. Many other regrettable and ill-planned species introductions were completed prior to the Kingdom of Hawaiʻi publishing the first "Laws of the Hawaiian Islands" in 1890, which sought to regulate pest species introductions and spread. It was not until the 1960's when the now State of Hawaiʻi began to comply with federal laws, including the National Environmental Policy Act (1970) and Endangered Species Act (1973), and established State laws (HRS 150A and HRS 343) to ensure any new species introductions of plants or animals were carefully studied and reviewed. The proposed management action is subject to each of these State and Federal laws, regulations, and review.

CONCERN 4: Commentors were concerned that the introduced mosquitoes would be "genetically modified," "bioengineered," or be considered an unsafe "pesticide."

Response: The proposed use of incompatible male mosquitoes is a non-GMO approach. The U.S. Environmental Protection Agency (EPA) is not regulating this approach as a GMO or a genetically engineered product. According to the EPA, a genetically modified organism (GMO) is "a plant, animal, or microorganism that has had its genetic material (DNA) changed using technology that generally involves the specific modification of DNA, including the transfer of specific DNA from one organism to another. Scientists often refer to this process as genetic engineering."

The proposed technique does not modify any or part of the genome of either mosquitoes or *Wolbachia* bacteria. The incompatible male mosquitoes this project proposes for release are incapable of successfully reproducing and therefore cannot pass on their genes to successive generations. If releases are stopped, the population of mosquitoes already present in the forest within the proposed project area will gradually return to pre-release levels.

The EPA has reviewed the use of incompatible male mosquitoes with *Wolbachia* as a biopesticide. The agency defines biopesticides as "naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants) or PIPs." Many biopesticides registered by the EPA can be used in and around lands cultivated for certified organic food production if ingredients also meet U.S. Department of Agriculture standards.

CONCERN 5: Commentors were concerned that the proposed action is not a long-term solution.

Response: There is no single solution to the extinction crisis endangered Hawaiian forest birds currently face. However, the release of incompatible male mosquitoes with *Wolbachia* is the most promising new approach that resource managers can implement in the near-term to control the primary threat to native forest birds in remote natural areas.

While it is true that the IIT method requires consistent releases of incompatible male mosquitoes to maintain suppression of mosquito populations, this is a method that can be used on a landscape-scale over long periods. It is common for management projects to require repeated actions to maintain the success of the project. For example, fencing to keep out problematic mammals (e.g., rats, pigs, and deer) from sensitive habitats requires regular maintenance. Similarly, controlling weeds or invasive insects usually requires repeated visits to affected sites, sometimes for many decades after an infestation is discovered.

The proposed IIT mosquito suppression project was identified as a priority for Hawai‘i at local and international planning meetings in 2016 and 2017. Over the last six years, federal and state agencies and non-governmental organizations (NGOs) have participated in exhaustive research, development, and planning, and have initiated permitting and environmental compliance. The program would be part of a suite of management actions that are currently in place, or are being considered, designed to protect native forest birds from extinction. These include captive propagation of forest birds, potential translocations of birds to Hawai‘i Island, and future mosquito suppression techniques (USDOI, 2022). These tools, however, are not permanent solutions either. There is a considerable urgency to control mosquito populations to save these birds from extinction. Although IIT is not a permanent solution, the birds cannot afford to wait until new tools are developed, perhaps many years in the future. Should a more long-lasting technique be developed to the point where it could be applied to the landscape, it could be considered in the future, with appropriate environmental compliance. It is also possible that future mosquito suppression techniques will benefit from the procedures developed for the proposed action.

CONCERN 6: Commentors were concerned that the proposed action may be inefficient, ineffective, and costly.

Response: Conservation and resource management in Hawaiʻi can be costly. Programs that aim to preserve Hawaiʻi's watershed forests, protect near-shore beaches and reefs, stabilize and recover endangered species, control destructive invasive species, and support commercial and recreational fishing and hunting programs all require significant recurring state and federal funding. Sometimes funds are used to study and develop new management tools and approaches, while other funds are directed towards specific on-the-ground actions. As mentioned in the response to Concern 5, the proposed action is the most promising tool currently available to protect native forest birds in their present habitat.

The National Park Service (NPS) directs resources, funds, and personnel to preserve the natural and cultural resources and values of the national park system for the enjoyment, education, and inspiration of this and future generations. Likewise, the Hawaiʻi Department of Land and Natural Resources (DLNR) is charged with the task of enhancing, protecting, conserving and managing Hawaiʻi's unique and limited natural, cultural and historic resources that are held in public trust for current and future generations of the people of Hawaiʻi nei, and its visitors, in partnership with others from the public and private sectors.

The NPS and the DLNR have not only the legal mandate, but the kuleana (privilege and responsibility) to protect biocultural resources. Hawaiʻi's unique biodiversity is deeply interlaced with Hawaiian culture. Both NPS and DLNR stewardship aim to perpetuate the unique and continuing connections between Hawaiian culture and this sacred and evolving land. Honeycreepers such as the ʻākohekohe and kiwikiu are ʻaumakua (familial guardians or ancestors), and their endurance in the native forest is an embodiment of Hawaiian culture. As noted in the Cultural Impact Assessment, pg. 48: "Hawaiian culture views natural and cultural resources as being one and the same: without the resources provided by nature, cultural resources could and would not be procured. From a Hawaiian perspective, all natural and cultural resources are interrelated, and all natural and cultural resources are culturally significant. Kepā Maly, ethnographer and Hawaiian language scholar, points out, "In any culturally sensitive discussion of land use in Hawaiʻi, one must understand that Hawaiian culture evolved in close partnership with its natural environment. Thus, Hawaiian culture does not have a clear dividing line of where culture ends and nature begins" (Maly 2001:1).

The Rapid ʻŌhiʻa Death project is an example of another program that requires extensive federal and state funding to preserve ʻōhiʻa (*Metrosideros polymorpha*), a species that is the backbone of the native forest and a significant biocultural resource.

CONCERN 7: Commentors were concerned that the entire range of alternatives was not fully assessed, including alternatives such as reforestation, gene drive in mosquitoes, radiation to sterilize the mosquitoes, or the use of a *Cordyceps* fungus.

Response: With respect to gene drive and radiation, those alternatives were considered but dismissed and are discussed in detail in Appendix B of the EA.

Regarding the use of *Cordyceps* fungus, in 2017 a group of biologists, entomologists, biotechnology experts, and public health specialists discussed the possible solutions to the problem of mosquito-borne diseases (https://reviverestore.org/the-plan-to-restore-a-mosquito-free-hawaii/). The group identified the sterile insect technique, the incompatible insect technique using the *Wolbachia* bacteria, and self-limiting insect approaches using next generation gene tools. *Cordyceps* or other fungus species were not identified as tools for suppressing mosquito populations, and there is not a fungus that is effective at suppressing populations of the southern house mosquito (*Culex quinquefasciatus*). New technology as it becomes available will be explored as potential options in the future.

Reforestation and habitat restoration have occurred in the past and are ongoing actions in and around the project area and are expected to continue. While these efforts contribute significantly to the long-term restoration of suitable habitat throughout endangered forest bird critical habitat, these efforts alone will not prevent the extinction of the species.

Loss of suitable habitat has been extensive in the Hawaiian Islands and is an important threat to forest birds generally. However, introduced mosquitoes are also a threat because forest birds on Maui are highly susceptible to mosquito-borne diseases and are not expected to persist in areas where mosquitoes are present. Therefore, restoration of suitable habitat through reforestation of areas in which mosquitoes are present is not expected to be an effective alternative strategy to prevent the extinction of those species. Restoration of suitable habitat in high elevation areas where mosquitoes are not present, or not expected to be present as global temperatures rise, is an important part of recovery efforts. However, it does not constitute an effective alternative to mosquito control at this time because, 1) the acreage of potential suitable habitat at those high elevations is vanishingly small, and 2) restoration of suitable habitat in those areas takes decades and cannot be completed before the projected extinction timeline of the affected species.

As previously mentioned, the proposed action would be part of a suite of management actions designed, at least in part, for the preservation of native forest birds. The US Fish and Wildlife Service (USFWS) detailed a long-term conservation and recovery plan for several taxa of endangered Hawaiian forest birds, including the remaining populations of ‘ākohekohe and kiwikiu on East Maui (USFWS 2006). The plan prioritized measures to improve and restore degraded habitat through invasive species control and reforestation. The Maui Forest Bird Recovery Working Group (MFBWG) created a comprehensive Kiwikiu Conservation Translocation Plan (MFBWG 2018), which detailed strategies for establishing a kiwikiu population, via conservation translocation, in the Nakula Natural Area Reserve (NAR). The reserve was identified by the USFWS as a forest that held great potential for providing habitat for kiwikiu. The Maui Forest Bird Recovery Project (MFBRP) and the State of Hawai‘i Department of Land and Natural Resources (DLNR) – Division of Forestry and Wildlife (DOFAW) Native Ecosystem Protection and Management (NEPM) program began reforestation efforts in the reserve and conducted experimental restoration trials to explore techniques that may be employed to increase density and diversity of native vegetation within the reserve (Warren et al. 2019). MFBRP and NEPM planted over 170,000 native seedlings from 2012 to 2019, which transformed non-native grasslands to native forest suitable for sustaining a population of kiwikiu. In 2019, after many years of preparation, 14 kiwikiu individuals were transferred to Nakula NAR. After release, birds were monitored using radio telemetry and most birds showed encouraging behavior in the new habitat, foraging independently, and remaining near the release site. Unfortunately, every bird was exposed to avian malaria and 12 of them had either died or disappeared by late November 2019. The failure to establish another population in restored forest further demonstrated the dangers imposed by avian malaria in a changing climate. Population viability models predicted time to extinction of kiwikiu as soon as 2027 (Mounce et al. 2018, Paxton et al. 2022), which further demonstrates the urgency for implementing mosquito suppression techniques in both current and previously occupied ranges where reforestation, habitat restoration, and invasive species control is ongoing. Information has been added to Appendix E of the EA to provide details regarding ongoing habitat restoration efforts, particularly at the state level.

CONCERN 8: A commentor was concerned that insufficient time was provided to review the EA and respond.

Response: The NPS and DLNR prepared a full environmental assessment and provided more than the legally required time for the public to review and comment. The State HEPA regulations require a 30-day public review period for an EA and the NEPA regulations have no minimum requirement for public review periods for an EA, although the NPS NEPA Handbook recommends a 30-day review period. In this case, the EA was open for public review and comment from December 6, 2022 through January 23, 2023, for a total of 48 days. Please see the response to Concern 1 for an explanation of why the NPS and DLNR did not prepare an Environmental Impact Statement (EIS).

CONCERN 9: Commentors were concerned that there has been insufficient study of the proposed action, that more studies should be completed, and that the proposed action is a “rash” decision.

Response: The southern house mosquito has been present in Hawaiʻi for nearly 200 years and already naturally carries the *Wolbachia* bacteria within its cells. This species of mosquito has invaded native forest habitat, which is the last refuge for critically endangered forest birds, and also occupies suburban and urban areas - even taking advantage of breeding indoors in air conditioner condensation/drip pans/drain pans in high rise buildings. As a result, residents of Hawaiʻi have been interacting with and bitten by the southern house mosquito (carrying *Wolbachia*) for generations.

While this project is the first proposed use of incompatible male mosquitoes with *Wolbachia* for conservation purposes, and the first time the approach would be used in Hawaiʻi, there is a substantial body of data that demonstrate the approach is safe, targeted, and results in no adverse effects to humans or the environment (Laven 1967; Moreira et al. 2009; Atyame et al. 2011; Atayme et al. 2015; Kittayapong et al. 2019; Zheng et al. 2019; Crawford et al. 2020; Beebe et al. 2021).

The proposed mosquito suppression project using incompatible male mosquitoes was identified as a priority for Hawaiʻi at local and international planning meetings in 2016 and 2017. Over the following six years, Federal and State agencies and NGOs have participated in exhaustive research, development and planning to facilitate project implementation, and initiated permitting and environmental compliance. Outreach related to the use of incompatible male mosquitoes has been ongoing since 2018, and the use of this approach has been recommended by both executive and legislative branch leadership across the state.

In 2017, the Hawaiʻi Invasive Species Council adopted Resolution 17-2, supporting research and evaluation of landscape-scale control technologies for mosquitoes, and encouraging researchers to review and evaluate approaches that could potentially benefit both native wildlife and human health in Hawai’i. In 2019, House Resolution (HR) 297 passed the Hawaiʻi State House and directed the “[Department of Agriculture] to review the *Aedes aegypti* mosquito with *Wolbachia* bacteria, including *Aedes aegypti* mosquitoes originating from Hawai‘i stock that could be imported for landscape scale mosquito control, and render a determination to place it on the appropriate animal import list.” The resolution required the Departments of Health (DOH), Agriculture (DOA), and Land and Natural Resources (DLNR) to collaborate on a report to the Legislature with recommendations for appropriate vector control programs. In 2021, House Resolution (HR) 95 subsequently passed the Hawai‘i State House urging DLNR, DOA, DOH and the University of Hawai‘i to implement a mosquito control program using *Wolbachia* to reduce mosquito population levels throughout the state. In 2022, the Hawai‘i Board of Agriculture voted to approve the administrative rule change and issuance of an import permit that would enable the proposed project to be implemented.

The period during which these resolutions were introduced and approved, highlights the timeline over which this approach has been under public review and subject to public comment

CONCERN 10: Commentors were concerned that the *Wolbachia* bacteria in the mosquitoes to be released is “foreign” or would be “introduced” to an environment on Maui where it currently does not occur.

Response: The proposed action will not involve introducing any new or foreign organisms to Hawai‘i (see response to Concern 3). Any releases of organisms of this kind are rightfully scrutinized, well studied, and regulated. The incompatible male mosquitoes reared in the lab would be derived from mosquitoes initially collected in Hawai‘i. These are the same species of mosquito, the southern house mosquito (*Culex quinquefasciatus*), that are present in Hawai‘i and responsible for spreading avian malaria. Similarly, the strain of *Wolbachia* in the released male mosquitoes is also present in Hawai‘i in the bodies of another mosquito common in the state, the Asian tiger mosquito (*Aedes albopictus*).

The southern house mosquitoes that exist in Hawai‘i today carry a strain of *Wolbachia* call wPip. The Asian tiger mosquito carries a different strain of *Wolbachia* called wAlb. To create the incompatible southern house mosquitoes, scientists would create a laboratory line of Hawai‘i mosquitoes with the wAlb *Wolbachia* strain. This is done through a multi-step process involving rearing mosquitoes in the lab and removing the wPip *Wolbachia* from their bodies with common antibiotics. The new strain (wAlb) of *Wolbachia* is then injected into the eggs of the *Wolbachia*-free mosquitoes. The resulting mosquitoes are southern house mosquitoes with a stable infection of wAlb *Wolbachia*. These are reared for several generations and carefully tested. All this work is done in sterile laboratory conditions.

The success of the suppression program is predicated on only releasing male southern house mosquitoes. As *Wolbachia* is maternally inherited, no local establishment of wAlb southern house mosquitoes is expected or likely to occur (see response to Concern 12 for more on the issues of female contamination and local establishment). However, as no organisms (mosquito or *Wolbachia*) used in this proposed project are novel to Hawai‘i, local establishment would not constitute introduction of any foreign species. Text has been added to page 6 of the EA to provide this clarification.

CONCERN 11: Commentors were concerned that the proposed project would be an “experiment” that has not been implemented prior.

Response: As mentioned in the response to Concern 9, the proposed action is an application of an established method for controlling insect populations. The IIT method has been used for decades in over ten countries including elsewhere in the United States. This is neither an experiment nor a novel technique being tested in Hawaiʻi. The IIT method is a highly effective and safe technique with a strong record of peer-reviewed studies and successful applications around the world. What is new about this proposed action is that it has not been employed in Hawaiʻi nor for wildlife conservation. As such, protocols will need to be developed for its use in Maui's native forest and other local conditions.

CONCERN 12: Commentors were concerned that female mosquitoes would be released that could ultimately breed and perpetuate or increase rather than suppress the mosquito population.

Response: Several commentors correctly identified that the release of females, "female contamination", would negatively impact the ability of the proposed action to suppress mosquito populations. Potentially released females, however, present no more risk to humans or animals than the mosquitoes that currently occur on Maui. Nor would releases of females increase the population of mosquitoes on Maui.

Given the importance of only releasing male mosquitoes, sorting out females is a vital part of the process. In previous IIT programs similar to the proposed action, sex sorting was accomplished in several ways, with varying rates of success. One of the primary methods used to separate and eliminate females uses sieves, or another similar physical separation method, taking advantage of the fact the female pupae are larger than male pupae. This method alone is estimated to remove >95% of all females, and various additional methods have been used to eliminate remaining females or render them sterile (e.g., exposure to radiation). Using the methods likely to be employed in the proposed action, it is estimated that the risk of releasing a female is 1 out of 900 million released mosquitoes (Crawford et al. 2020). This highly technical process uses physical separation of pupae, followed by imaging and sorting of emerged adults via artificial intelligence (AI) programs to remove remaining females. Following this, an iterative process of vetting AI scanned images is used to further reduce the risk of females being present in any given batch of mosquitoes bound for release. Following the methods described by Crawford et al. (2020), Beebe et al. (2021) did not detect any released females (or larvae containing control *Wolbachia*) throughout the life of their project in Australia. Using a different method, Zeng et al. (2022) estimated a female contamination rate of <1% and saw no local establishment of *Wolbachia*-infected mosquitoes in their study site. The Crawford et al. (2020) sex sorting would result in a female contamination rate that is several orders of magnitude smaller than reported in Zeng et al. (2022).

The released southern house mosquitoes would be transinfected with the wAlb *Wolbachia* strain and the wild mosquitoes in Hawaiʻi currently are naturally infected by the wPip *Wolbachia* strain (see response to Concern 10 for more explanation). Should a wAlb female be released, she would be compatible with the released wAlb male mosquitoes and could produce viable offspring. As such, every effort would be made to reduce or eliminate female contamination in released male mosquitoes. For local establishment of a wAlb population of southern house mosquitoes to form, females would first need to be released and survive long enough to reproduce (mate, find a blood meal, and lay eggs). If overflooding rates of released males are correctly calculated, it is possible that a released female could find a compatible male with which to mate. Scientists have confirmed bidirectional incompatibility between the wAlb and wPip southern house mosquitoes. This means that pairings of wAlb males and wPip females are incompatible, as are pairings of wPip males and wAlb females. Should a released female mate with a wild type wPip male, no offspring would be produced. If a released female successfully produces offspring with a released male, all those offspring would be infected with the wAlb *Wolbachia* strain. These offspring would then need to mate with other wAlb southern house mosquitoes to continue the reproductive cycle, as would all successive generations. Meanwhile, any mating events with wPip wild type mosquitoes would suppress any developing wAlb population. Successful establishment of a wAlb population would thus be the product of a series of extremely unlikely events. Should local establishment be detected, halting releases of wAlb males would allow the wild type wPip mosquitoes to reinvade a portion of treatment area and eliminate the wAlb population. Deliberately releasing wild type wPip male mosquitoes could similarly accomplish the same objective.

Attempting to establish a population of mosquitoes with a *Wolbachia* strain other than that which is already present in an environment is an extremely challenging and resource intensive exercise. In contrast to the releases proposed in this EA, other IIT programs are specifically designed with the goal of replacing a population of mosquitoes with others infected with *Wolbachia* to reduce the transmission of disease. In that type of program both males and females are released. Examining the success of those programs gives some insight into the number of females that may need to be released to successfully establish a population. For example, Hoffman et al. (2011) released between 5,000 and 11,000 females per week (assuming a 1:1 sex ratio). Even at that rate, it took multiple releases over several months to increase the *Wolbachia* frequency in the mosquito population above 50% (indicating they had replaced half the population). Hoffman et al. (2011) also continued to document suppression of their *Wolbachia* mosquitoes through ingress of females from outside the release area. The methods expected to be used in the proposed action estimate that one female may be inadvertently released out of 900 million released mosquitoes (Crawford et al. 2020). Thus, very few females are likely to be released; likely too few to result in local establishment.

CONCERN 13: Commentors were concerned that there is a risk that the release of *Wolbachia*-infected mosquitoes could increase, rather than diminish, disease transmission within the ecosystem and to humans (e.g., malaria, dengue fever, yellow fever, Zika virus, and West Nile Virus).

Response: There is no indication that the released incompatible male mosquitoes will increase disease transmission in humans or wildlife. The general trend seen in the peer-reviewed literature is that *Wolbachia* infection leads to lower rates of disease transmission including that of dengue, chikungunya, Zika, West Nile Virus, and malaria (e.g., Moreira et al. 2009, Hussain et al. 2012, Dutra et al. 2016). The ability of *Wolbachia* to suppress disease transmission is the basis for several applications of IIT. Prime examples are projects aimed at replacing populations of the yellow fever mosquito (*Aedes aegypti*), which is naturally *Wolbachia*-free, with those infected with *Wolbachia*, thereby reducing the spread of dengue and other diseases (e.g., Eliminate Dengue [Eliminate Dengue | FHI 360]).

As several commentors mentioned, there are a few select studies that show the opposite pattern, i.e., increased disease transmission in *Wolbachia*-infected mosquitoes. However, there are significant differences between the proposed action and the methods employed by these studies and the study systems involved. In all the studies highlighted by commentors, the *Wolbachia* infection involved was either natural or achieved by inoculating adult mosquitoes, resulting in transient (unstable) infections (Zele et al. 2013, Dodson et al. 2014, Hughes et al. 2014). As Dodson et al. (2014) stated, “It should be noted that these experiments were performed with mosquitoes transiently infected in the somatic tissues with *Wolbachia*, rather than a stable maternally inherited infection. It remains to be seen whether a stable wAlbB infection will enhance WNV [West Nile Virus] in a similar way.” The released mosquitoes in the proposed action would inherit their *Wolbachia* maternally and the infection would be stable and concentrated in sex cells. It should be noted that local transmission of West Nile Virus, chikungunya, Zika, and malaria (any other form besides avian) has not been documented in Hawaiʻi.

Over 200 species of *Plasmodium*, the malaria parasite, have been identified and each species is host specific, meaning it can only infect certain kinds of animals. Further, most *Plasmodium* species are spread by specific mosquito species or a closely related group of species. Hughes et al. (2014) reviewed the effects of *Wolbachia* infection on transmission of various malaria parasite species. These authors showed that while most *Wolbachia* infections led to a reduction in malaria transmission, some *Wolbachia* infections led to an increase in transmission of rat malaria (*Plasmodium berghei* and *P. yoelli*; limited to Africa), chicken malaria (*P. gallinaceum*; not present in Hawaiʻi), and one case of avian malaria (*P. relictum*). As noted in the response to Concern 2, the southern house mosquito is already a highly efficient vector of the avian malaria parasite, with 85–97% of mosquitoes being susceptible to infection and transmission (LaPointe et al. 2005) and it is improbable that susceptibility could increase beyond what is currently seen in the wild. Notably, Hughes et al. (2014) also showed that *Wolbachia* infection consistently led to a decrease in transmission of human malaria (*P. falciparum*). Regardless, neither the species of mosquito that carries human malaria, nor human malaria itself, are present in Hawaiʻi.

Another important difference between the studies that found increases in disease transmission in *Wolbachia*-infected mosquitoes and the proposed action is that these studies compared *Wolbachia*-uninfected and *Wolbachia*-infected mosquitoes. Zele et al. (2013), referenced in Hughes et al. (2014), found an increase in avian malaria infection between *Wolbachia*-uninfected southern house mosquitoes versus *Wolbachia*-infected southern house mosquitoes. In Hawaiʻi, nearly 100% of southern house mosquitoes are naturally infected with *Wolbachia* (Atkinson et al. 2016) as would be the released incompatible males. A comparison with Zele et al. (2013) is therefore inappropriate.

The text in the Human Health and Safety section of Appendix B (Page B-9) has been updated to include information from this response.

CONCERN 14: Commentors were concerned that transinfected *Wolbachia* will make its way into other mosquito or other insect species non-maternally, i.e. via "horizontal transfer."

Response: *Wolbachia* (wPipV) is already present in the southern house mosquito (C*ulex quinquefasciatus*) in Hawaiʻi, and *Wolbachia* (wAlbA and wAlbB) strains are already found in the Asian tiger mosquito (*Aedes albopictus)* in Hawaiʻi as well. These mosquito species have been in Hawaiʻi since 1826 and 1896, respectively. It is highly improbable that incompatible male mosquitoes, which cannot reproduce and will die out in the environment less than a week after release, are more likely to undergo horizontal transmission of *Wolbachia* than the existing populations of mosquitoes which have been reproducing on the landscape for the last 125–200 years. Further, *Wolbachia* is common among native Hawaiian insects (Bennett et al. 2012).

Wolbachia is an endosymbiotic organism (living within the cells of another organism) that is maternally inherited, i.e., passed down from a mother to her offspring; also known as "vertical transfer". "Horizontal transfer" in this case would be the transmission of *Wolbachia* from one organism to another non-maternally. The mechanism for such a transfer in *Wolbachia* is not known, would only occur following a series of extremely unlikely events, and would require the *Wolbachia* to live outside of their host cells for some period of time. In a laboratory setting, keeping *Wolbachia* alive outside of host cells requires precise conditions to preserve them in a cell-free medium for even short periods (Rasgon et al 2006). In fact, this is required in the process of creating the incompatible mosquitoes in the proposed action. However, some have asserted or implied that the ability to preserve *Wolbachia* outside of cells in a laboratory setting (Rasgon et al. 2006) represents evidence that *Wolbachia* can live extracellularly in the wild (Tolley et al. 2019). But there has yet to be any evidence of free-living *Wolbachia* in the wild and there are numerous environmental factors that would severely limit the lifespan of *Wolbachia* outside of their host cells (e.g., pH, UV radiation). The mechanism for horizontal transmission of *Wolbachia* remains unknown, but the theories for how this has occurred in the past have little relevance to the system in the proposed action. Tolley et al. (2019) suggested that horizontal transfer in ants could have occurred through social interactions or predation, but there remains no direct evidence of this, and this theory is purely speculative.

There is good evidence that, over millions of years, horizontal transfer of *Wolbachia* has occurred numerous times (Tolley et al. 2019, Ding et al. 2020). However, *Wolbachia* shows a high degree of host endemism (only lives within one host species or closely related species) especially the strains involved here, wPip and wAlb (Ding et al. 2020). This high rate of endemism itself is evidence of the rarity of horizontal transfer. Just as several commentors suggested, Loreto and Wallau (2016) theorized that horizontal transfer between mosquito species (or other insects) may cause some unknown impacts in an IIT program. O'Neill (2016) directly addresses the concerns of Loreto and Wallau (2016) and makes several relevant points regarding horizontal transfer including, 1) horizontal transfer is very rare in nature (e.g., Hamm et al. 2014), and 2) natural experiments indicate a low rate of horizontal transfer including in closely related sympatric (living in the same place) mosquitoes. To the second point, both the Asian tiger mosquito (*Aedes albopictus*) and the yellow fever mosquito (*Aedes aegypti*) live in the same environments in many parts of the world, including on Hawai‘i Island. The Asian tiger mosquito is nearly always infected with *Wolbachia* naturally (the same strain that would be used in the proposed action), while the yellow fever mosquito is naturally uninfected by *Wolbachia,* and yet there has never been evidence of horizontal transfer of *Wolbachia* between these species. There also is no evidence that the strain of *Wolbachia* found in southern house mosquitoes has been transmitted to the Asian tiger mosquito (or any other mosquito), or vice versa, in Hawai‘i (or anywhere else) despite co-occurrence for the past >130 years (Atkinson et al. 2016). Further, there is no evidence of transfer of any mosquito *Wolbachia* to other arthropods, including native Hawaiian insects. The low rate of horizontal transfer among related species, such as *A. albopictus* and *A. aegypti*, would suggest that the rate of transfer among unrelated arthropods would be even lower.

CONCERN 15: Commentors were concerned that horizontal gene transfer may occur within the transinfected mosquitoes and unknown evolutionary events may occur as a result.

Response: Commentors listed concerns regarding horizontal gene transfer between the *Wolbachia* endosymbiont and the mosquito. To clarify, this is different from the concerns of horizontal *Wolbachia* transfer involving non-heritable movement of the *Wolbachia* organism between insect species (see response to Concern 14). Horizontal gene transfer in this context would be the theoretical movement of genetic material (DNA) from *Wolbachia* into the southern house mosquito genome. Horizontal gene transfer is a natural process that has occurred innumerable times throughout evolutionary history. Scientists have found segments of DNA within numerous eukaryotic (e.g., animal) organisms that can be traced back to a prokaryotic (i.e., bacteria) organism, often in parasite-host interactions. This may in fact be an important evolutionary process that is just now being realized. However, the process of horizontal gene transfer itself is not a concern. Rather if such a transfer includes transcriptional phenotypic traits that could be acted upon by selective pressures that allows for beneficial traits to be developed. A segment of DNA does not necessarily contain all the required information to be transcribed (read) and conferred into new traits or functions. Much of a genome in fact contains sequences of non-coding DNA, often referred to as “junk DNA.” Thus, the likelihood that such an event could somehow alter the genome of the mosquito in a meaningful way is exceptionally low. Further, horizontal transfer of genes between *Wolbachia* and a mosquito would not constitute the creation of a new species of mosquito as some commentors suggested.

Some commentors singled out a study by Klassen et al. (2009) that purported to show evidence of horizontal gene transfer between *Wolbachia* (wPip) and the yellow fever mosquito (*Aedes aegypti*). These authors found several sequences of DNA within the (typically *Wolbachia*-free) yellow fever mosquito’s genome that had previously been identified from the *Wolbachia* genome. These authors do acknowledge, however, that while the most likely direction of transfer was from the *Wolbachia* to the mosquito, it cannot be determined for certain the transfer did not occur in the opposite direction. Most importantly, these examples of gene transfer occurred as a result of a natural evolutionary event(s), not as a result of any human-caused process, such as in the proposed action, therefore the timescale required for these transfer events is unknown. Further, given that the wPip strain of *Wolbachia* has co-evolved with the southern house mosquito likely for millions of years, it is considerably more likely that horizontal gene transfer may have naturally occurred between these species than between the transinfected wAlb and the southern house mosquito.

Finally, concerns such as horizontal gene transfer are predicated on establishment of a reproducing population of southern house mosquitoes infected with wAlb strain of *Wolbachia.* The purpose of the proposed action is to suppress the population of southern house mosquitoes within the project area on East Maui. Local establishment of wAlb southern house mosquitoes would work against that goal and extreme care would be taken to avoid that scenario. For more information, please see response to Concern 12.

CONCERN 16: Commentors were concerned that Native Hawaiian concerns, including Environmental Justice, were not appropriately addressed and that they would be disproportionately affected by the project.

Response: With respect to Environmental Justice, there is no evidence that the release of incompatible male mosquitoes on east Maui will have any human health impacts. Therefore, there would be no disproportionately high and adverse human health impacts to Native Hawaiians that would result in Environmental Justice concerns. Please refer to Appendix B of the EA for a discussion of Environmental Justice and how it was considered but dismissed from further analysis.

Impacts to Ethnographic Resources and Traditional Cultural Practices are addressed in Appendix B of the EA. The proposed action will result in limited visual and noise impacts to the feeling and setting of ethnographic resources, including the Haleakalā Summit, Kīpahulu Valley, and Kaupō Gap Traditional Cultural Property. Noise associated with helicopter or drone flights and their visual intrusion could potentially be a disturbance to the traditional users of park or state areas and could potentially detract from their enjoyment and use. However, these noise and visual impacts have been minimized in order to limit negative impacts to ethnographic resources. Park operations, e.g., flight times and flight paths, would be planned to balance efficiency and any potential impacts. The proposed action will minimize the use of helicopters and focus on the use of drones, which are smaller and quieter than helicopters. Any necessary helicopter flights would be planned to avoid the park’s annual commercial-free days. As specified in the park's

Commercial Services Plan, commercial-free days are opportunities for Kānaka Maoli (Native Hawaiians) to conduct traditional cultural practices in the park without commercial tours present. In 2023, the commercial-free days will occur on January 6 (end of Makahiki); May 24 (Zenith Noon); June 21 (Summer Solstice); July 18 (Zenith Noon); October 27 (start of Makahiki); and December 21 (Winter Solstice). The commercial-free days are designated prior to the start of the calendar year and change slightly each year. They are determined in consultation with the Native Hawaiian Community.

The NPS consulted with the Native Hawaiian Community, including 11 individuals and 17 Native Hawaiian Organizations, to identify any impacts from the proposed action and no substantial comments have been received to date. Additionally, DLNR prepared a Cultural Impact Assessment (CIA) as part of compliance with the Hawaiʻi, Environmental Policy Act (HEPA). Based on the research and ethnographic data within the CIA report, it was found that it would be unlikely that the proposed action would adversely impact traditional or customary practices. Yet, the interviews completed as part of the CIA make it clear that additional education and outreach is needed, particularly to the practitioner community. There was concern expressed by interviewees that the project could potentially and adversely impact native flora and fauna. The CIA recommended education and outreach to the East Maui community, particularly hunters and other practitioners, as a critical component of the project (Watson 2022: 85).

Thus far the NPS has conducted two virtual public meetings to collect initial comments in the development of the. Information may be found here: ParkPlanning - Suppression of Invasive Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui (nps.gov) and here: About | Birds Not Mosquitoes. The state DLNR and Birds not Mosquitoes, a public-private partnership, plans to do additional outreach to East Maui communities, and statewide, to educate about this project.

Additionally, to mitigate potential public concerns regarding *Wolbachia*-incompatible mosquito releases, the IIT project team consulted with the DLNR Maui Branch Manager to identify areas on state lands commonly used by hunters or cultural practitioners. Most public hunting areas within the East Maui project area are only open on weekends when it's unlikely that mosquito release operations will take place. Further, most treatment area points on public hunting lands are in remote upland areas rarely visited by hunters. The one exception is the Makawao Forest Reserve, where there are approximately 60 release points, which would take 1–2 hours per release to treat by aerial methods. The reserve is open for hunting and other recreational activities daily. Those activities may include plant and flower gathering for lei making and other traditional Hawaiian practices. The project team met with the DLNR Na Ala Hele trail advisory committee on July 27, 2022, to discuss potential concerns and how best to communicate IIT implementation plans in that popular recreational area. The project team will work with DLNR to post signage on trails communicating release plans, and to participate in public outreach events. On DLNR lands, Native Hawaiian organizations would be notified prior to any planned release efforts.

The CIA also found that native birds could be considered a cultural resource as they are entwined in both Hawaiian culture and tradition across the islands. The history of the birds in Hawaiʻi is one of tremendous adaptive radiation due to geographic isolation resulting in numerous species of birds found nowhere else on earth. The use of helicopters and drones under the proposed action could temporarily disturb native forest birds, but over the long term there would be substantial benefits by minimizing the spread of avian malaria and reducing bird mortality. Any minimal impacts to ethnographic resources and traditional cultural practices would likely be temporary at any given location, though releases would likely occur over the long term. Reduction of avian malaria as proposed would conserve numerous rare birds important to Native Hawaiian culture providing a beneficial impact, outweighing the adverse impacts.

CONCERN 17: During the public comment period, commenters submitted additional literature for review.

Response: The NPS and DLNR reviewed all literature that was submitted during the public comment period on the EA and incorporated relevant information into the EA or comment responses as necessary.

CONCERN 18: Commenters were concerned that wildland fires would be ignited by drones and helicopters.

Response: Wildland fire mitigation measures for helicopters are included in Table 6 of the EA.

All uncrewed aircraft systems (UAS), also known as "drones", will be closely monitored by the operator and field teams while adhering to guidance developed by the NPS Natural Resource Stewardship and Science Directorate and policies established by Federal Aviation Administration. The DLNR Division of Forestry and Wildlife (DOFAW) is mandated under the Land Fire Protection Law, Chapter 185, Hawaiʻi Revised Statute to take measures for the prevention, control, and extinguishment of wildland fires within all forest reserves and natural area reserves on East Maui (DLNR, DOFAW 2018). DOFAW is statutorily required to cooperate with county and federal government fire control agencies to develop plans for wildfire prevention. UAS operators under NPS or DOFAW operational control will be required to have an up-to-date FAA 14 CFR Part 107 Remote Pilot Certificate and FAA Certificate of Waiver or Authorization. UAS operations will follow best practice protocols established by the National Wildfire Coordinating Group which provides guidance detailed in the Interagency Helicopter Operation Guide. NPS law enforcement will monitor UAS operations and approve flight plans and thus will be able to respond immediately to UAS mishaps. The Hawaiʻi Fire Department, in coordination with NPS Fire Management officers and the DOFAW Fire Management Program, will respond to any on-site emergency, including downed UAS vehicles to ensure that there is no risk of wildfire. Text was added to Table 6 in the EA to include these practices as mitigation measures under the proposed action.

CONCERN 19: Commenters expressed concern about impacts to bats and dragonflies that would eat the transinfected male mosquitoes released under the proposed action.

Response: Native taxa such as damselflies and bats have been consuming multiple mosquito species containing *Wolbachia* (including *Aedes albopictus* and *Culex quinquefasciatus*) since the introduction of mosquitoes intermittently with no adverse effects. *Wolbachia* cannot live in vertebrates and thus cannot affect bats (Popovici et al. 2010). See the response to Concern 14 for examination of "horizontal transfer" of *Wolbachia.* There is no indication that consumption of transinfected mosquitoes would present a risk to native damselflies. In Hawaiʻi, native wildlife do not rely on mosquitoes as a prey base. Hawaiʻi's native fauna evolved over millions of years as constituents in a diverse community assemblage. In contrast, mosquitoes are comparatively recent introductions, having invaded Hawaiʻi less than 200 years ago.

CONCERN 20: Commenters suggested that the EA did not analyze the environmental effects of dropping mosquito packaging in the project area.

Response: Although the final design has not been decided upon, agency and private partners are committed to designing release packaging that is suitably biodegradable and will maintain biosecurity protocols. However, until a final product is designed, specific decay rates or other relevant variables are not known. As strict biosecurity protocols will be followed, the release packets present no risk to the environment. Although many thousands of release packets would be dropped across the project area throughout the duration of the project, the small packets would be spread diffusely and the biodegradable material would decompose quickly; thus, the impact to the environment would be negligible.

From a visitor experience standpoint, the release packets are unlikely to be observed by members of the public. The appearance of the release packets is not yet known and would depend on how the packets are designed to fall and land (e.g., on the ground or in trees). However, to fit into a release mechanism of a drone, the release packets are likely to only be a few inches wide and be very light. The visibility of the packets to members of the public will depend on two primary factors, 1) public access to the project area, and 2) spacing of releases. The vast majority of the project area is not publicly accessible and thus, the public would not have an opportunity to come across any release packets prior to the packets degrading throughout most of the project area. In the areas that the public can access, the large spacing between release points would make encountering a release packet very unlikely. The distance between release locations would be determined by initial trials but are likely to be several hundred meters apart. A spacing of 400 meters (1,312 feet), as presented in the EA, would mean that for a member of the public to see a release packet, they would be finding an object only a few inches wide within an equivalent area of approximately 30 football fields of dense forest. The rate of decay of the packets will dictate how many packets within an area one could observe at any given moment, but this decay rate is likely very high given typical rainfall patterns, making the chance of observing multiple packets unlikely.

CONCERN 21: Commenters suggested the restoration of natural water flow on Maui would be a possible solution to the abundance of mosquitoes on Maui.

Response: It is true that human infrastructure in streams in Hawaiʻi can create additional larval habitat for the southern house mosquito. However, the abundance of mosquitoes on Maui is not caused by stream diversions or other human-caused water flow disturbances. Mosquitoes breed in all kinds of natural water sources including, but not limited to, tree cavities, pig wallows, natural depressions, and streamside pools. Kīpahulu Valley's Palikea Stream is a prime example as it has no human infrastructure or streamflow interruptions in core area of the project, where mosquito larval habitat is found in natural features along the stream.

CONCERN 22: Commenters wanted clarification on the number of bird species protected by the Migratory Bird Treaty Act (MBTA) that are within the project area because there are two different numbers stated in the EA.

Response: There are eight (8) bird species protected by the MBTA in the project area. The EA text has been revised to reflect the presence of those 8 bird species.

CONCERN 23: One commenter suggested that under the no-action alternative there would be adverse impacts to visitors trying to experience wilderness solitude due to the presence of biting mosquitoes in wilderness areas and that suppression of mosquitoes would be a benefit to this wilderness quality.

> **Response:** Currently, there is no public access to designated wilderness within the project area, so there would be no impacts to visitors' ability to experience solitude or primitive and unconfined recreation associated with the presence or suppression of mosquitoes. Effects on visitors' ability to experience threatened and endangered bird species (outside of wilderness) is described in the Visitor Use and Experience section of the EA. Also, the impact of the preservation or loss of forest bird species is described under the natural quality of wilderness character.

CONCERN 24: One commenter suggested that the EA acknowledge the concerns around unanticipated outcomes and that a monitoring and response plan will be implemented.

> **Response:** Although the EA implies that a monitoring plan will be developed, we have added text on page 13 of the EA to specifically indicate that a monitoring plan will be developed. The monitoring plan will likely include measures of success as well as certain provisions looking for unanticipated outcomes, such as female contamination.

CONCERN 25: One commenter suggested that there was a discrepancy in the EA regarding the number of monitoring sites that would be used. More specifically, page 14 of the EA indicates that eight sites would be used where the table only lists five monitoring sites.

> **Response:** The EA text and Table 5 indicate that five monitoring sites will be accessed by helicopter and the remaining three will not require the use of helicopters. However, the text on page 14 of the EA has been revised to state this more clearly.

CONCERN 26: One commenter noted that page 20 of the EA states that "personnel would not disturb, remove or trim woody plants greater than 15 feet tall during the bat birthing and pup rearing season of June 1 through September 15" and that the EA also states that any tree cover would not be removed during the forest bird breeding season of November 1 through June 30. This would mean there are only 6 weeks a year of allowable trail clearing time. If ground releases become necessary and it falls outside of that limited window, crews may not be able to access key areas.

> **Response:** It is possible that the commenter is confusing trail clearing with tree clearing. Trail clearing and maintenance could occur throughout the year without disturbance to birds and bats.

CONCERN 27: One commenter was concerned about limiting mosquito releases to two months out of the year by helicopter could limit the effectiveness of the project. A commenter was also concerned with limiting drone releases to two times per week in the entire project area.

> **Response:** As the commenter suggested, a primary goal of the EA was to limit negative impacts to the environment from the proposed action. This required limiting the number of helicopter flights and, thus, the impacts of those flights (e.g., noise). It was determined that the stated helicopter use frequency of two months per year would allow for an acceptable level of impacts. To estimate impacts for the use of helicopters to release mosquitoes, it was necessary to specify the number of flights projected for use. As such the language suggested by the commentors to remove specific limits on helicopter flights could not be incorporated into the EA. However, the impact analysis would apply to any use of helicopters for 56–78 flight hours per year, which

could be applied at a different schedule than two months. These flights would also not necessarily have to take place within a single calendar year. Similarly, the helicopter impacts analysis would apply to flights for helicopter-assisted drone flights up to 78 flight hours per year provided they are not in addition to direct helicopter releases.

One commentor suggested clarifying language regarding the frequency of releases of incompatible male mosquitoes. The frequency of release is estimated to be up to two times per week per release location (as indicated on page 8 of the EA). Figure 2 shows potential release locations throughout the core of the project area. Each of these locations could receive incompatible male mosquitoes up to twice per week. It is anticipated that a subset of the project area will receive mosquito releases in the initial phase of the project. This would reduce the total number of release locations accessed during a planned treatment, but each release location may still receive mosquitoes at a frequency of up to two times per week.

CONCERN 28: One commenter was concerned about limiting drone launch sites to “front country” areas only.

Response: Operating drones from backcountry locations would typically necessitate the use of helicopters to transport operators to launch sites. The frequency of releases, estimated up to twice per week per location, would require a much greater impact from helicopters than is included in the impacts analysis of this EA and would present additional logistical challenges. The front country launch sites shown in Figure 3 are examples of sites that may be used, but do not represent all possible launch sites. Drone models exist that can access the vast majority of the project area from road-accessible locations. However, there may be some as-yet-determined limitations to the drone releases that require launch sites closer to release sites. Should helicopter-assisted drone operations be required on a regular basis, such as to access a portion of the project area, the additional impacts may need to be analyzed. Furthermore, should the required release frequency be determined to be less than twice per week, helicopter-assisted drone operation may not unduly increase the impacts of additional helicopter flights. The subheading for Figure 3 on page 11 of the EA was revised to clarify that that the drone launch locations are examples of sites that might be used.

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HAWAIʻI

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CONSERVATION AND RESOURCES ENFORCEMENT
ENGINEERING
FORESTRY AND WILDLIFE
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LAND
STATE PARKS

STATE OF HAWAIʻI
DEPARTMENT OF LAND AND NATURAL RESOURCES

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EXEMPTION NOTICE

Regarding the preparation of an environmental assessment under the authority of
Chapter 343, HRS and Section 11-200.1-17, HAR

Project Title:	Mosquito Control Research Using *Wolbachia*-based Incompatible Insect Technique
Project Location:	Maui (2) 2-3-005:004: Waikamoi Preserve (2) 2-4-016:004: Waikamoi Preserve (2) 1-2-004:013: Hanawi Natural Area Reserve (2) 2-3-005:001: Haleakala National Park (2) 1-8-001:007: Haleakala National Park (2) 1-3-001:003: Haleakala National Park (2) 1-7-004:016: Haleakala National Park (2) 1-6-001:001: Haleakala National Park (2) 1-6-001:002: Haleakala National Park (2) 1-2-010:001: Haleakala National Park Kauai (4) 1-4-001:003: Alakai Wilderness Preserve (4) 1-4-001:013: Kokee State Park
Chapter 343 Trigger(s):	Use of State Funds and Lands
Project Description:	The main objective of this project is to initiate research to inform incompatible insect technique applications for the control of invasive *Culex quinquefasciatus* mosquitoes which are the primary vector of avian malaria. The disease threatens the survival of remaining endangered forest bird species where they persist in high elevation montane forest habitat on Maui and Kauai. Male mosquitoes which have been given an incompatible strain of *Wolbachia* bacteria are to be released on the landscape, and upon release those males will breed with wild female mosquitoes. As a result of those pairings, the wild female mosquitoes will lay eggs which will not hatch, and no offspring will be produced. When releases of incompatible male mosquitoes are completed consecutively, the approach results in the suppression of mosquito populations at a landscape-scale. If releases are halted, mosquito

EXHIBIT 2

	populations will gradually return to pre-release levels as wild female and male mosquitoes migrate back into the treated area from surrounding forest habitat. Initial research will contribute to EPA registration of male *Culex quinquefasciatus* mosquitoes with *Wolbachia* as a biopesticide, as well as determine the minimum number of male mosquitoes that must be released in each area to ensure population suppression. This project may be funded by Federal sources.
Consulted Parties:	U.S. Fish and Wildlife Service
Authorization:	November 13, 2015, Land Board submittal (C-6). Delegation of Authority to the Chairperson or their authorized representative to declare exempt from the preparation of an Environmental Assessment those Department actions which are included in the Department-wide exemption list when the Board of Land and Natural Resources has delegated the authority to conduct those actions.
Exemption Class & Description:	**Exemption Classes:** **General Exemption Type 5** *Basic data collection, research, experimental management, and resource and infrastructure testing and evaluation activities that do not result in a serious or major disturbance to an environmental resource.* PART 1 13. Research that the Department declares is designed specifically to monitor, conserve, or enhance native species or native species' habitat. 16. Research to identify, monitor, control, or eradicate introduced species. Date of Agency Exemption List: November 10, 2020.
Determination:	The Department of Land and Natural Resources declares that this project will likely have minimal or no significant impact on the environment and is therefore exempt from the preparation of an environmental assessment under the above exemption classes.

DGS Suzanne D. Case Jun 17, 2022

Suzanne D. Case, Chairperson
Board of Land and Natural Resources

Date

Signature:

Email: david.g.smith@hawaii.gov

From:	Tina Lia
To:	DLNR.BLNR.Testimony
Subject:	[EXTERNAL] Testimony: 8/26/22 BLNR Meeting
Date:	Wednesday, August 24, 2022 6:59:22 PM

Testimony: Tina Lia
Board of Land and Natural Resources
Meeting 8/26/22 9:00am
Agenda Item: C. DIVISION OF FORESTRY AND WILDLIFE 1. Request for delegation of authority to the Chairperson to enter into a Memorandum of Agreement with American Bird Conservancy for Video Production Services related to the Birds Not Mosquitoes partnership.

Wolbachia Mosquitoes in Hawaii: Unsettled Science (Part 2)

On June 28, 2022, the Hawaii Board of Agriculture approved the addition of three mosquitoes to the List of Restricted Animals in anticipation of the planned biopesticide IIT experiment in Hawaii's native bird habitats. The initial process for bringing these mosquitoes into the state has also begun, with the board approving the first species – the Southern House Mosquito (Culex quinquefasciatus), inoculated with strains of Wolbachia bacteria – for importation and immediate field release by permit.[1]

The project is called "Mosquito Control Research Using Wolbachia-based Incompatible Insect Technique" and is promoted as a population control effort to save Hawaii's endangered native birds. The multi-agency partnership *Birds, Not Mosquitoes*, a steering committee formed in 2017 and comprised of state, federal, and non-governmental organizations, is coordinating the plan. Prior to the June 28th meeting, an exemption notice was signed by the Hawaii Board of Land and Natural Resources Chairperson Suzanne D. Case, stating that the Department of Land and Natural Resources (one of the lead agencies in the partnership) declares that this project will likely have minimum or no significant impact on the environment and is therefore exempt from the preparation of an environmental assessment.

With strong evidence of serious risks to public health and to the native birds targeted by this project, island residents are challenging the DLNR's assertion and demanding a full scope Environmental Impact Study. Recent testimony shows that over 75% of the public is opposed to rushing forward with this experimental plan. Additional questions regarding invasive species control and conflicts of interest have been left unanswered by the state, and the task of researching and evaluating safety concerns now falls on the people. Our findings are alarming.

Of primary concern is the potential for increased pathogen infection due to non-sexual horizontal transmission of the introduced Wolbachia strains between the introduced biopesticide mosquitoes and the existing "wild" mosquitoes. Multiple factors are involved here, but first let's look at the three mosquito species planned for import into Hawaii and just a few of the diseases they are known to transmit:

• The Southern House Mosquito (Culex quinquefasciatus) transmits avian malaria parasitic disease to birds and West Nile virus to both birds and humans

• The Asian Tiger Mosquito (Aedes albopictus) transmits both dengue fever and Zika virus to humans

EXHIBIT 3

• The Yellow Fever Mosquito (Aedes aegypti) transmits dengue fever and yellow fever to humans

Horizontal transmission is defined as the spread of an infectious agent from one group or individual to another, directly or indirectly. In the case of the host arthropods (insects) and the infectious agent of Wolbachia bacteria, the horizontal transmission referenced here would be non-sexual. Imported Wolbachia bacterium strains involved in this project include wAlbA, wAlbB, and wPip4. These newly introduced strains (referred to here as "X") are not currently present within the corresponding Culex quinquefasciatus species of Hawaii's established mosquito population.

Tropical disease expert Dr. Lorrin Pang agreed to speak with me as a private citizen to explain more about horizontal spread of disease and the risks involved with this project. Pang has authored over 75 publications in peer-reviewed medical journals covering a broad range of studies such as malaria, dengue, rabies, rat lungworm, and COVID. He's been an advisor and voting member of the U.S. Congress Medical Research Program for the past several years, serving on committees for infectious diseases – many of which are mosquito-borne. From 1985-2005, he worked with the WHO and Walter Reed Institute's Malaria Program, focusing on global malaria control efforts through interventions combining diagnostics, chemotherapeutics, vector control, and vaccine development. As a public health leader on the islands, he has mitigated mosquito-borne illnesses – including dengue and Zika – for over two decades. Pang was honored for his life-saving intervention in Hawaii's dengue fever outbreak.

Dr. Pang has been compiling studies documenting horizontal Wolbachia bacterial spread, and he's concerned about the potential for significant adverse outcomes of the state's proposal:

> "The intent to save rare birds is sound. If the project goes as planned, this would be a valuable tool for future interventions. However, with new life forms coming to the islands, there is too much potential for unexpected, dangerous, irreversible 'evolutionary' events. This is especially true when the new organisms cannot be contained to their target ecosystem. Already there are published papers pointing out the real threat of horizontal spread of the novel Wolbachia beyond the male Culex mosquito. The papers cover two general areas – the widespread detection of Wolbachia across so many diverse types of insects, and more recently, the growing number of reports of mechanisms of how this might occur. First, we all must agree that unintended horizontal spread of Wpip4 (imported strain) to, say, female Culex, Aedes mosquitoes (human disease vectors), or other insect vectors of diseases would be a catastrophe, and probably irreversible. Hawaii has a bad history of invasive species entering and spreading unabated, including their spread of infectious diseases.
>
> Proponents may be right that this intervention will save the native birds in the short-term, but long-term consequences to other island ecologies and to these same native birds may ultimately be detrimental. When one realizes the latter, the damage may be impossible to recall or repair, like the effect we've seen with so many other invasive species in Hawaii."

The safety assurances of the state's biopesticide project are based heavily on the premise that only male mosquitoes will be released. Because the males are infected with an incompatible bacteria strain, when they mate with existing wild females, the offspring are not viable.

However, Dr. Pang points to a more recent study out of Singapore describing Wolbachia bacteria strain "evolutionary associations" between mosquito hosts. The results of this mechanism widespread into diverse insect populations are not known. It may start with a few horizontal transfers to female mosquitoes. After that, the mating Wolbachia-X-compatible pair will quickly produce viable X offspring and spread the X bacteria strain (the term for this is "sweep"). If that were to happen here, the full capacity of those offspring to transmit disease would be unknown. This type of spread and sweep could also affect other insects, not just the targeted mosquito.

The combination of horizontal and vertical spread dramatically contradicts the state's safety narrative. While the potential for accidental misidentification and release of lab-reared X-infected females (who bite and breed) has already been downplayed, the possibility of unintentionally producing these females in the wild has not been addressed at all. As Pang puts it,

> "It is enough to say that the new Wolbachia strain can spread horizontally as a life form to other mosquitos (say Aedes, the vectors of human disease) and perhaps create that Wolbachia female Culex which everyone is bending over backwards to avoid via lab contamination."

Dr. Pang further points out that there is a big difference between the standard Sterile Insect Technique (SIT) strategies used in the past that were based on radiation or chemicals, and the relatively new Incompatible Insect Technique (IIT). The mathematical models may be similar for estimating threshold criteria to affect mosquito population dynamics, but standard methods of sterility are not bacterial life forms that might escape horizontally and amplify in other ecological niches. According to Pang,

> "While sterility models can predict the thresholds needed to exterminate a species (in this case insects), the radiation sterility factor (standard SIT) does not behave the same as a life form (i.e., Wpip4 Wolbachia bacteria). There may be different modeling between radiation and Wolbachia 'sterility' for the male mosquitoes, depending on male mosquito fitness – but more importantly, for the unintended female Culex to which the Wolbachia X spreads horizontally. How is this supposed to be self-contained? Horizontal spread has the potential to be a disaster that cannot be recalled. The bacterium is a life form, and you might not be able to turn back the clock by simply shutting off the male mosquito 'fountains.' "

The evidence of horizontal spread of Wolbachia shows that the bacteria go not only to sexual cells, but also to somatic cells (non-sexual cells of the body). Wolbachia can also live outside of intra-cellular systems for several months.[3] Dr. Pang emphasizes two additional studies documenting widespread horizontal transmission of Wolbachia. The first focuses on predatory wasps spreading the bacteria through contaminated mouth parts when feeding serially on target insects such as aphids[4]. Pang calls for more research into which predators, like the damselfly and dragonfly, sequentially feed on both male and female mosquitoes. This scenario might play out in either the predator of adults feeding on adult mosquitoes (X-infected and wild), or the X-infected predator of larva feeding on wild mosquito larva in common breeding sites. The second study looks at ant colonies spreading Wolbachia through the gastrointestinal (GI) tract when the ants feed on their fungus gardens.[3] Pang asks an important question, "What about shared sugar feeding sites for X-infected male and wild adult male and female mosquitoes?" The sparser the sugar sites, the more communal interaction they will have. Dr.

Pang finds these studies of horizontal transfer across species of insects worrisome, and says, "Even if this project achieved miraculous blocking of avian malaria to the native birds, what else would it do?"

To complicate matters more, the Wolbachia bacteria itself is parasitic, manipulating the reproductive biology of the host to increase its own transmission. Parasitic organisms can also alter the behavior of the hosts they live inside, and we just don't know how this might play out in our native bird habitats. Will the X-infected mosquitoes or their offspring be capable of moving up to even higher elevations? Would they be more aggressive? How would increased pathogen infection and elevated capacity for disease transmission factor into these scenarios?

Consider the example posed by the article "Parasites brainwash grasshoppers into death dive,"[5] where a parasitic worm brainwashes the grasshopper host it invades to jump into water and commit suicide. The parasite accomplishes this by chemically influencing the grasshopper's brain, producing proteins which directly and indirectly affect the host's central nervous system. This causes an altering of the grasshopper's behavior so that it acts in a way it never usually would. Other parasites are noted as manipulators of their hosts' behavior, including "enslaver" fungi that make their insect hosts die perched in a position that favors the dispersal of spores by the wind. It is widely believed that Wolbachia bacteria is such a parasite (intracellular) that modifies the mosquito host's behaviors in ways we are only now beginning to understand.

There are far too many unknowns here, and this research project has the very real potential of further endangering Hawaii's native bird populations – possibly even leading to their extinction and the extinction of native birds not currently threatened. Why the state would choose to proceed with an experimental population control technique with limited background information when there are alternatives available with decades of study behind them is unclear at this point. Supporters of this proposal seem to have good intentions with their focus on mosquito control to save the birds, but there appears to be a lack of awareness about the serious risks posed by horizontal transmission of the Wolbachia X-strain bacteria and the resulting consequences of that spread.

Hawaii is united in our support for conservation of the native birds that are vital to honoring and preserving the culture, history, and natural environment of the islands. This biopesticide IIT experiment is not the answer. With the understanding of the multiple dangers posed to our ecosystems, native birds, and public health, it is now time for all of the agencies involved in this plan to agree that it cannot be rushed forward. As one public testifier so clearly stated, "Hawaii is not a petri dish." The public demands a full scope Environmental Impact Study. We will not allow the state to recklessly sidestep that crucial process.

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(End of Part 2)

References:

1. Board of Agriculture Meeting Agenda: Items IV.C.1 and IV.C.2 (06/28/2022) https://hdoa.hawaii.gov/wp-content/uploads/2022/06/06-28-22-AGENDA.pdf
2. "Wolbachia infection in wild mosquitoes (Diptera: Culicidae): implications for

transmission modes and host-endosymbiont associations in Singapore" – Huicong Ding, Huiqing Yeo, Nalini Puniamoorthy (BMC, 12/09/2020) https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-020-04466-8

3. "Wolbachia Horizontal Transmission Events in Ants: What Do We Know and What Can We Learn?" – Sarah J. A. Tolley, Peter Nonacs, Panagiotis Sapountzis (Frontiers in Microbiology, 03/06/2019) https://www.frontiersin.org/articles/10.3389/fmicb.2019.00296/full
4. "The Intracellular Bacterium Wolbachia Uses Parasitoid Wasps as Phoretic Vectors for Efficient Horizontal Transmission" – Muhammad Z. Ahmed, Shao-Jian Li, Xia Xue, Xiang-Jie Yin, Shun-Xiang Ren, Francis M. Jiggins, Jaco M. Greeff, Bao-Li Qiu (National Center for Biotechnology Information, National Library of Medicine, 02/12/2015) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4347858/
5. "Parasites brainwash grasshoppers into death dive" – Shaoni Bhattacharya (New Scientist, 08/31/2005) https://www.newscientist.com/article/dn7927-parasites-brainwash-grasshoppers-into-death-dive/

Additional Reading:

"GENETIC DIVERSITY OF WOLBACHIA ENDOSYMBIONTS IN CULEX QUINQUEFASCIATUS FROM HAWAI'I, MIDWAY ATOLL AND AMERICAN SAMOA" – Carter T. Atkinson, William Watcher-Weatherwax, Dennis A. LaPointe (Hawaii Cooperative Studies Unit, UH at Hilo, 02/2016) https://dspace.lib.hawaii.edu/bitstream/10790/2671/TR074CarterWolbachia.pdf

"Infection of New- and Old-World Aedes albopictus (Diptera: Culicidae) by the Intracellular Parasite Wolbachia: Implications for Host Mitochondrial DNA Evolution" – Peter Armbruster, William E. Damsky, Jr., Rosanna Giordano, Josephine Birungi, Leonard E. Munstermann, Jan E. Conn (Journal of Medical Entomology, 05/01/2003) https://academic.oup.com/jme/article/40/3/356/877101

"Wolbachia" – Karine Prevot (The Embryo Project Encyclopedia, 01/29/2015) https://embryo.asu.edu/pages/wolbachia

"Sugar feeding patterns of New York Aedes albopictus mosquitoes are affected by saturation deficit, flowers, and host seeking" – Kara Fikrig, Sonile Peck, Peter Deckerman, Sharon Dang, Kimberly St Fleur, Henry Goldsmith, Sophia Qu, Hannah Rosenthal, Laura C. Harrington (PLOS Neglected Tropical Diseases, 10/26/2020) https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0008244#:~:text=Sugar%20also%20may%20enhance%20male,%2Dseeking%20behavior%20%5B20%5D

"Plant-mediated interspecific horizontal transmission of an intracellular symbiont in insects" – Elena Gonella, Massimo Pajoro, Massimo Marzorati, Elena Crotti, Mauro Mandrioli, Marianna Pontini, Daniela Bulgari, Ilaria Negri, Luciano Sacchi, Bessem Chouaia, Daniele Daffonchio, Alberto Alma (Nature, Scientific Reports, 11/13/2015) https://www.nature.com/articles/srep15811

"Wolbachia Associations with Insects: Winning or Losing Against a Master Manipulator" – Claudia C. Correa, J. W. O. Ballard (Frontiers in Ecology and Evolution, 01/19/2016) https://www.frontiersin.org/articles/10.3389/fevo.2015.00153/full

Your comments were successfully submitted at Jan 21, 2023 10:27 PM Mountain Time

EXHIBIT 4

Park:	Haleakala National Park
Project:	Suppression of Invasive Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui
Document:	Mosquito Suppression Environmental Assessment - December 2022

Name:	Tina Lia
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Organization:	Hawaii Unites
Keep My Info Private:	No

Comments: I am OPPOSED to this "Mosquito Suppression Environmental Assessment - December 2022" for the island of Maui. I demand that the State of Hawaii and its multi-agency partnership Birds, Not Mosquitoes complete a detailed, full scope Environmental Impact Statement (EIS) documenting the impacts to our native birds, wildlife, environment, and public health.

The State of Hawaii's planned "Mosquito Control Research Using Wolbachia-based Incompatible Insect Technique" project is promoted as a population control effort to save Hawaii's endangered native birds. The state has notified the public that the mosquito control strategy being implemented has decades of research behind it and is therefore safe. The reality is that the Sterile Insect Technique (SIT) that these decades of research are based on uses irradiation-induced sterility, and this is not the technique planned for use in this project. The Incompatible Insect Technique (IIT), based on Wolbachia-induced cytoplasmic incompatibility (a kind of male sterility), planned for use here in the islands is something entirely different. It is also a relatively new approach, and certainly has not been studied in Hawaii's unique ecosystems. Further, the biopesticide planned for use, "DQB Males," has not gone through the EPA registration process of scientific, legal, and administrative procedures through which the biopesticide would be examined for ingredients; site use; amount, frequency, and timing of use; and storage and disposal practices. Risk assessments to evaluate harms to humans, wildlife, fish, plants, endangered species, and non-target organisms have not been conducted. Potential contamination of surface water or ground water, leaching, runoff, and spray drift have not been evaluated.

This planned project comes with many risks to the land, birds, wildlife, and people of the Hawaiian Islands. While state agencies and wildlife officials are hoping this new strategy will prevent extinction of the native birds, it may very well cause their extinction. Some of the possible dangers include horizontal transmission of the introduced bacteria strain(1), increased pathogen infection in mosquitoes(2), irreversible evolutionary events(1), population replacement(3) (lab-bred mosquitoes replacing existing wild mosquitoes), accidental release of lab-reared females(3), creation of lab-strain females in the wild(1), horizontal gene transfer(4), biopesticide drift, and mosquitoes becoming a better vector of avian malaria(2) and/or West Nile Virus(5) (human and bird). Peer-reviewed studies document precedents for these concerns.

The Maui Environmental Assessment (EA) lists numerous potential impacts that require mitigation measures: wildland fire ignition by helicopters; spread of invasive weeds; noise-producing activities adversely affecting native wildlife; disturbances to native and special status plants and acceleration of erosion; noise disturbances and other impacts to special status wildlife species (including disturbances to nesting and roosting); adverse impacts within critical special status species habitats; transport and establishment of introduced invasive weeds and diseases/pathogens; disturbances of traditional cultural practices; threats to human health and safety; noise impacts on landowners, communities, wilderness, and sensitive environmental resources; noise and viewscape impacts on the visitor experience; impacts to wilderness character; threats to endangered nene and Hawaiian waterbirds; risks to Hawaiian forest birds and Hawaiian hoary bats from drone hovering and helicopter rotor wash; risks of breeding birds being flushed from active nests; disturbances of day roosting Hawaiian hoary bats; and risks of disturbing bat pup rearing. In the "Drone Release" section of the EA, it's noted that, "mosquitoes would likely be released in small biodegradable packages designed to open upon contact with the canopy or forest floor." The EA also states that, "these mosquito packages (dropped via aerial means) would result in an impact to the undeveloped quality of wilderness for as long as they remain in the environment (until they biodegrade)." The effects of this packaging on the environment and wildlife are not addressed.

The EA notes that the sound produced by each drone "is similar to loud highway noise," that "drone noise could possibly be loud enough to disrupt conversations," and that aircraft wildlife collisions could happen. The document states that "it is possible that a drone could inadvertently fly into a flock of birds." Helicopters planned for use in this project would be even louder, larger, more powerful, and more dangerous to birds and wildlife.

Environmental Justice is also a clear concern with this project. Per EPA documentation(6), the "EPA seeks to achieve environmental justice, the fair treatment and meaningful involvement of any group, including minority and/or low- income populations, in the development, implementation, and enforcement of environmental laws, regulations, and policies. To help address potential environmental justice issues, the Agency seeks information on any groups or segments of the population who, as a result of their location, cultural practices, or other factors, may have atypical or disproportionately high and adverse human health impacts or environmental effects from exposure to the pesticide(s) discussed in this document, compared to the general population."

In the EA's "Cultural Impact Assessment" section, seven Native Hawaiian lineal descendants and recognized cultural experts were interviewed. All expressed concerns about the impacts of the project, focused on the effects it could have on cultural resources and traditions, native birds, public health, wildlife, and our fragile ecosystems. Additional concerns include the experimental aspect of the project; the state's history of creating new problems by bringing in invasive species such as the mongoose; the sensitivity of the project area, with people depending on native flora and fauna for their livelihoods; impacts on other animals like 'opae (shrimp) and 'o'opu (goby fish) that live in streams; whether or not adequate studies or research have been done; residual effects on other insects; impacts on native plants used for lei making, weaving, and other cultural practices; impacts on water sources; impacts on other islands from water sources connected through tides and currents; and the need to keep the public informed. The state's assessment concludes, "If the project and concerns about the use of this biocontrol discourage practitioners from conducting their traditional or customary practices, it would be an adverse effect to these cultural activities." As a result of their location, cultural practices, and other factors, Native Hawaiians may have atypical or disproportionately high and adverse human health impacts and environmental effects from exposure to the biopesticide.

A primary concern of this project is the potential for increased pathogen infection due to non-sexual horizontal transmission of the introduced Wolbachia strains between the introduced biopesticide mosquitoes and the existing "wild" mosquitoes. Adding to that concern are the diseases that the mosquito species planned for import into Hawaii transmit. The Southern House Mosquito (Culex quinquefasciatus) can transmit avian malaria parasitic disease to birds and West Nile virus to both birds and humans.

Horizontal transmission is defined as the spread of an infectious agent from one group or individual to another, directly or indirectly. In the case of the host arthropods (insects) and the infectious agent of Wolbachia bacteria, the horizontal transmission referenced here would be non-sexual. Imported Wolbachia bacterium strains involved in this project include wAlbA, wAlbB, and wPip4. These newly introduced strains (referred to here as "X") are not currently present within the corresponding Culex quinquefasciatus species of Hawaii's established mosquito population.

Tropical disease and vector expert Dr. Lorrin Pang has spoken as a private citizen about horizontal transmission, or "horizontal spread," of disease and the risks involved with this project. Pang has authored over 75 publications in peer-reviewed medical journals covering a broad range of studies such as malaria, dengue, rabies, rat lungworm, and COVID. He's been an advisor and voting member of the U.S. Congress Medical Research Program for the past several years, serving on committees for infectious diseases – many of which are mosquito-borne. From 1985-2005, he worked with the WHO and Walter Reed Institute's Malaria Program, focusing on global malaria control efforts through interventions combining diagnostics, chemotherapeutics, vector control, and vaccine development. As a public health leader on the islands, he has mitigated mosquito-borne illnesses – including dengue and Zika – for over two decades. Pang was honored for his life-saving intervention in Hawaii's dengue fever outbreak.

Dr. Pang has been compiling studies documenting horizontal Wolbachia bacterial spread, and he's concerned about the potential for significant adverse outcomes of the state's proposal:

"The intent to save rare birds is sound. If the project goes as planned, this would be a valuable tool for future interventions. However, with new life forms coming to the islands, there is too much potential for unexpected, dangerous, irreversible 'evolutionary' events. This is especially true when the new organisms cannot be contained to their target ecosystem. Already there are published papers pointing out the real threat of horizontal spread of the novel Wolbachia beyond the male Culex mosquito. The papers cover two general areas – the widespread detection of Wolbachia across so many diverse types of insects, and more recently, the growing number of reports of mechanisms of how this might occur. First, we all must agree that unintended horizontal spread of Wpip4 (imported strain) to, say, female Culex, Aedes mosquitoes (human disease vectors), or other insect vectors of diseases would be a catastrophe, and probably irreversible. Hawaii has a bad history of invasive species entering and spreading unabated, including their spread of infectious diseases.

Proponents may be right that this intervention will save the native birds in the short-term, but long-term consequences to other island ecologies and to these same native birds may ultimately be detrimental. When one realizes the latter, the damage may be impossible to recall or repair, like the effect we've seen with so many other invasive species in Hawaii."

The safety assurances of the state's biopesticide project are based heavily on the premise that only male mosquitoes will be released. Because the males are infected with an incompatible bacteria strain, when they mate with existing wild females, the offspring are not viable. However, Dr. Pang points to a more recent study out of Singapore(1) describing Wolbachia bacteria strain "evolutionary associations" between mosquito hosts. The results of this mechanism widespread into diverse insect populations are not known. It may start with a few horizontal transfers to female mosquitoes. After that, the mating Wolbachia-X-compatible pair will quickly produce viable X offspring and spread the X bacteria strain (the term for this is "sweep"). If that were to happen here, the full capacity of those offspring to transmit disease would be unknown. This type of spread and sweep could also affect other insects, not just the targeted mosquito.

The combination of horizontal and vertical transmission dramatically contradicts the state's safety narrative. While the potential for accidental misidentification and release of lab-reared X-infected females (who bite and breed) has already been downplayed, the possibility of unintentionally producing these females in the wild has not been addressed at all. As Pang puts it,

"It is enough to say that the new Wolbachia strain can spread horizontally as a life form to other mosquitos (say Aedes, the vectors of human disease) and perhaps create that Wolbachia female Culex which everyone is bending over backwards to avoid via lab contamination."

Dr. Pang further points out that there is a big difference between the standard Sterile Insect Technique (SIT) strategies used in the past that were based on radiation or chemicals, and the relatively new Incompatible Insect Technique (IIT). The mathematical models may be similar for estimating threshold criteria to affect mosquito population dynamics, but standard methods of sterility are not bacterial life forms that might escape horizontally and amplify in other ecological niches. According to Pang,

"While sterility models can predict the thresholds needed to exterminate a species (in this case insects), the radiation sterility factor (standard SIT) does not behave the same as a life form (i.e., Wpip4 Wolbachia bacteria). There is very different modeling for the target insect – but more importantly, for the unintended groups to which the bacteria horizontally spread. How is this supposed to be self-contained? Horizontal spread has the potential to be a disaster that cannot be recalled. The bacterium is a life form, and you might not be able to turn back the clock by simply shutting off the male mosquito 'fountains.' "

The evidence of horizontal spread of Wolbachia shows that the bacteria go not only to sexual cells, but also to somatic cells (non-sexual cells of the body). Wolbachia can also live outside of intra-cellular systems for several months.(7) Dr. Pang emphasizes two additional studies documenting widespread horizontal transmission of Wolbachia. The first focuses on predatory wasps spreading the bacteria through contaminated mouth parts when feeding serially on target insects such as aphids(8). Pang calls for more research into which predators, like the damselfly and dragonfly, sequentially feed on both male and female mosquitoes. This scenario might play out in either the predator of adults feeding on adult mosquitoes (X-infected and wild), or the X-infected predator of larva feeding on wild mosquito larva in common breeding sites. The second study looks at ant colonies spreading Wolbachia through the gastrointestinal (GI) tract when the ants feed on their fungus gardens.(7) Pang asks an important question, "What about shared sugar feeding sites for X-infected male and wild adult male and female mosquitoes?" The sparser the sugar sites, the more communal interaction they will have. Dr. Pang finds these studies of horizontal transfer across species of insects worrisome, and says, "Even if this project achieved miraculous blocking of avian malaria to the native birds, what else would it do?"

To complicate matters more, the Wolbachia bacteria itself is parasitic, manipulating the reproductive biology of the host to increase its own transmission. Parasitic organisms can also alter the behavior of the hosts they live inside, and we just don't know how this might play out in our native bird habitats. Will the X-infected mosquitoes or their offspring be capable of moving up to even higher elevations? Would they be more aggressive? How would increased pathogen infection and elevated capacity for disease transmission factor in these scenarios?

Consider the example posed by the article "Parasites brainwash grasshoppers into death dive,"(9) where a parasitic worm brainwashes the grasshopper host it invades to jump into water and commit suicide. The parasite accomplishes this by chemically influencing the grasshopper's brain, producing proteins which directly and indirectly affect the host's central nervous system. This causes an altering of the grasshopper's behavior so that it acts in a way it never usually would. Other parasites are noted as manipulators of their hosts' behavior, including "enslaver" fungi that make their insect hosts die perched in a position that favors the dispersal of spores by the wind.

It is widely believed that Wolbachia bacteria is such a parasite (intracellular) that modifies the mosquito host's behaviors in ways we are only now beginning to understand.

In addition to the possibility of creating lab-strain females in the wild through horizontal transmission, there is also the issue of accidental release of misidentified females. Per the Department of Land and Natural Resources "Permit Application for Restricted Commodities into Hawaii" for the Southern House Mosquito Culex quinquefasciatus(10):

"MosquitoMate and Verily will regularly sample release containers by releasing the contents into lab cages and then examining mosquito sex and number. There is an EPA reviewed value of 1 female release per 250,000 males with the MosquitoMate product. A similar value is likely to be estimated for Culex quinquefasciatus given that similar automation, engineering, and machine learning technology is being applied to sex sorting."

The EPA's "Emerging Mosquito Control Technologies" webpage(11) confirms these figures in the section titled, "Will this technology adversely affect human health or the environment?":

"Like all pesticides, MosquitoMate's technology is regulated under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)…The expected accidental release rate of one Wolbachia infected female for every 250,000 infected males is considered negligible exposure to humans resulting in a negligible human health risk."

With posting of the EA, we've come to learn that the Maui project area covers 64,666 acres, and that the state intends to release between 50 and 6,000 lab-bred mosquitoes per acre per treatment. Treatments would occur up to twice per week, amounting to up to 775,992,000 mosquitoes per week. That is over 40 BILLION invasive biopesticide mosquitoes released per year just on the island of Maui!

With a release rate of one female for every 250,000 males, there could be up to 3,103 lab-strain-infected females released on the island per week. Just one accidental female mosquito released can produce 160,000 females in her eight-week lifespan. This is a conservative estimate that takes into account a 50% mortality rate for offspring, a low-end of 100 eggs laid (the range is 100-300), an 80% emergence rate, an even sex ratio, and a first egg lay only (studies have found Culex females with two sperm packets, indicating two matings). One female Culex quinquefasciatus female can produce 20 living daughters in two weeks. Those daughters can produce 400 living granddaughters by four weeks. Those 400 living granddaughters can produce 8,000 living great-granddaughters by six weeks. Those great-granddaughters can produce 160,000 living great-great-granddaughters by the eighth week of the first female released.

These are not incompatible females. They will produce viable young when mated with the lab-reared males, and with any wild males that are not infected with the Wolbachia bacteria (not all Culex in the wild are infected with Wolbachia). If 3,103 lab-reared females are accidentally released per week, and each female can produce 160,000 more females, that amounts to potentially 496,480,000 (close to half a billion) accidental introduced-strain female mosquitoes within each eight-week life span of the initial accidental release scourge. These females bite and can spread disease. This is not addressed at all in the EA.

Population replacement can occur through mating of accidental lab-reared females and mating of wild mosquitoes who have become infected with the introduced Wolbachia bacteria strain through horizontal transmission. If the wild mosquitoes are replaced by the lab-strain-infected mosquitoes, the outcomes for our endangered native birds, public health, and our fragile ecosystems are unknown. What if the entire mosquito population becomes more capable of transmitting disease to birds, humans, and other wildlife?

These mosquito releases are planned indefinitely until the state finds another solution. This project intends to expand to Kauai next, and then throughout the islands. The EA notes an interest in establishing at least one biopesticide lab here in Hawaii. Federal level information(12) describes long-term plans for the islands, including lab research and development, "gene drives," "next generation tools," "synthetic biology control tools," "novel technology deployment," and "precision-guided Sterile Insect Technique (pgSIT)" (CRISPR technology). "Genetic Modification of Forest Birds" using CRISPR-Cas9 gene editing is also discussed in the EA. While "technology for this approach is not available for near-term implementation," development and deployment of this "tool" does appear to be a goal at the federal level. One of the labs that the state would be importing the biopesticide mosquitoes from, Verily Life Sciences, is a subsidiary of Google.

Per the "Understanding the Risks" section of the U.S. Department of the Interior Strategy document(12), "although used world-wide for human health, Wolbachia IIT is a novel tool for conservation purposes and its degree of efficacy in remote forest landscapes is unknown." This project is an experiment on Hawaii's people, wildlife, and 'aina. Not only is the extent of the risks involved undetermined, but the outcome itself of the planned goal is admittedly unknown. Because this is an experiment involving human disease vectors, the informed consent of the public is required. We do not consent.

Public testimony has shown that the people of Hawaii are overwhelmingly (over 75%) opposed to the state moving forward with this plan. Recent public comments on the HDOA's EPA Application for Emergency Exemption6 are over 95% against the use of this biopesticide. We are calling for a halt to this project. The scope, risks, and experimental nature of the state's plan require a detailed, comprehensive Environmental Impact Statement documenting the impacts to our native birds, wildlife, environment, and public health.

Who will take responsibility if something goes wrong – the federal government, the State of Hawaii, partners in the multi-agency steering committee Birds, Not Mosquitoes, private landowners? Adequate studies and research have not been conducted; and safer, less experimental alternatives have not been considered. Conflicts of interest have not been disclosed or addressed, and the state is rushing forward with this risky, experimental project without the consent of the people of these islands.

Per Article XI of the Constitution of the State of Hawaii(13) – Conservation, Control and Development of Resources:

CONSERVATION AND DEVELOPMENT OF RESOURCES Section 1. For the benefit of present and future generations, the State and its political subdivisions shall conserve and protect Hawaii's natural beauty and all natural resources, including land, water, air, minerals and energy sources, and shall promote the development and utilization of these resources in a manner consistent with their conservation and in furtherance of the self-sufficiency of the State.

ENVIRONMENTAL RIGHTS Section 9. Each person has the right to a clean and healthful environment, as defined by laws relating to environmental quality, including control of pollution and conservation, protection and enhancement of natural resources. Any person may enforce this right against any party, public or private, through appropriate legal proceedings, subject to reasonable limitations and regulation as provided by law.

I do not accept the Anticipated Finding of No Significant Impact (DEA-AFONSI) for the "Suppression of Non-native Wild Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui" Environmental Assessment. I demand an Environmental Impact Statement.

Mahalo, Tina Lia Founder & President Hawaii Unites www.HawaiiUnites.org Kihei, Maui, HI

References:

1. "Wolbachia infection in wild mosquitoes (Diptera: Culicidae): implications for transmission modes and host-endosymbiont associations in Singapore" – Huicong Ding, Huiqing Yeo, Nalini Puniamoorthy (BMC, 12/09/2020) https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-020-04466-8

2. "Wolbachia Can Enhance Plasmodium Infection in Mosquitoes: Implications for Malaria Control?" – Grant L. Hughes, Ana Rivero, Jason L. Rasgon (PLOS Pathogens, 09/04/2014) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4154766/

3. "Wolbachia-mediated sterility suppresses Aedes aegypti populations in the urban tropics" (preprint) – Singapore Consortium, Ng Lee Ching (medRxiv, 06/17/2021) https://www.medrxiv.org/content/10.1101/2021.06.16.21257922v1.full

4. "Horizontal gene transfer between Wolbachia and the mosquito Aedes aegypti" – Lisa Klasson, Zakaria Kambris, Peter E Cook, Thomas Walker, Steven P Sinkins (BMC Genomics, 01/20/2009) https://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-10-33

5. "Wolbachia Enhances West Nile Virus (WNV) Infection in the Mosquito Culex tarsalis" – Brittany L. Dodson, Grant L. Hughes, Oluwatobi Paul, Amy C. Matacchiero, Laura D. Kramer, Jason L. Rasgon (PLOS Neglected Tropical Diseases, 07/10/2014) https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0002965

6. "DQB Males (Wolbachia pipientis, DQB Strain, Contained in Live Adult Culex quinquefasciatus Males); Receipt of Application for Emergency Exemption, Solicitation of Public Comment" – Environmental Protection Agency (Federal Register, 12/15/22) https://www.federalregister.gov/documents/2022/12/15/2022-27220/dqb-males-wolbachia-pipientis-dqb-strain-contained-in-live-adult-culex-quinquefasciatus-males

7. "Wolbachia Horizontal Transmission Events in Ants: What Do We Know and What Can We Learn?" – Sarah J. A. Tolley, Peter Nonacs, Panagiotis Sapountzis (Frontiers in Microbiology, 03/06/2019) https://www.frontiersin.org/articles/10.3389/fmicb.2019.00296/full

8. "The Intracellular Bacterium Wolbachia Uses Parasitoid Wasps as Phoretic Vectors for Efficient Horizontal Transmission" – Muhammad Z. Ahmed, Shao-Jian Li, Xia Xue, Xiang-Jie Yin, Shun-Xiang Ren, Francis M. Jiggins, Jaco M. Greeff, Bao-Li Qiu (National Center for Biotechnology Information, National Library of Medicine, 02/12/2015) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4347858/

9. "Parasites brainwash grasshoppers into death dive" – Shaoni Bhattacharya (New Scientist, 08/31/2005) https://www.newscientist.com/article/dn7927-parasites-brainwash-grasshoppers-into-death-dive/

10. "Permit Application for Restricted Commodities into Hawaii" – Department of Land and Natural Resources (HDOA, 04/01/22) https://hdoa.hawaii.gov/wp-content/uploads/2022/06/Submittals-6-28-22.pdf

11. "Emerging Mosquito Control Technologies" (EPA, 07/19/22) https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/emerging-mosquito-control-technologies

12. "U.S. Department of the Interior Strategy for Preventing the Extinction of Hawaiian Forest Birds" – National Park Service, U.S. Fish and Wildlife Service, U.S. Geological Survey, Office of Native Hawaiian Relations (U.S. Fish and Wildlife Service, 12/15/22) https://www.fws.gov/sites/default/files/documents/DOI Strategy for Preventing the Extinction of Hawaiian Forest Birds (508).pdf

13. Constitution of the State of Hawaii, Article XI
https://lrb.hawaii.gov/constitution/#articlexi

Comment ID: 2295724-125202/512

From:	Tina Lia
To:	DLNR.BLNR.Testimony
Cc:	DLNR.CO.PublicDLNR
Subject:	[EXTERNAL] BLNR Meeting 3/24/23 9:15am Testimony Agenda Item C-2: Oppose
Date:	Wednesday, March 22, 2023 11:21:23 PM
Attachments:	2023_0324_Testimony_Tina_Lia_Attachments.pdf

RE: C-2 Request Approval of Final Environmental Assessment and Authorization for the Chairperson to Issue a Finding of No Significant Impact for the "Suppression of Invasive Mosquito populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui"

We're opposed to the request for approval of the Final Environmental Assessment for the planned biopesticide mosquito releases on Maui. This project is an experiment on our island home, and the outcome is admittedly unknown. The Final Environmental Assessment[1] does not adequately address the serious risks of this plan or the concerns of the public.

Sufficient research has not been conducted to assess the risks of horizontal transmission[2,3,4], increased pathogen infection[5], evolutionary events[2], population replacement[6], or accidental release of females[6]. The Final Environmental Assessment attempts to minimize the possibility of *Wolbachia* bacteria causing mosquitoes to become more capable of spreading diseases like avian malaria[5] and West Nile virus[7]. Scientific studies document these risks.

An Environmental Risk Assessment for this biopesticide has not been conducted by the EPA to determine the environmental, ecological, and human health risks; and the significant environmental consequences of the project have not been adequately studied. **This plan may actually cause the extinction of endangered native birds, and it could impact human health**.

Landscape level control of *Culex quinquefasciatus* mosquitoes using this Incompatible Insect Technique (IIT) has never been done before. Even with *Aedes* mosquitoes, the largest project area was 724 acres[8]. Federal documentation connected to this project confirms that "although used world-wide for human health, *Wolbachia* IIT is a novel tool for conservation purposes and its degree of efficacy in remote forest landscapes is unknown."[9] Additionally, the species planned for use in this project, *Culex quinquefasciatus*, has never been used for a stand-alone IIT field release.[8] It is inaccurate to state that *Wolbachia* IIT is being used for mosquito suppression globally. The majority of countries using *Wolbachia* mosquitoes through the World Mosquito Program[10] are using the method of population replacement, not suppression[11]. These are two entirely different techniques.

This project may have also been improperly segmented per HAR § 11-200-7[12] (replaced 2019). The revised rule, HAR § 11-200.1-10[13] – Multiple or phased actions, provides:

EXHIBIT 5

A group of actions shall be treated as a single action when:

(1) The component actions are phases or increments of a larger total program;

(2) An individual action is a necessary precedent to a larger action;

(3) An individual action represents a commitment to a larger action; or

(4) The actions in question are essentially identical and a single EA or EIS will adequately address the impacts of each individual action and those of the group of actions as a whole.

On June 17, 2022, Board of Land and Natural Resources Chairperson Suzanne D. Case signed an exemption notice for "Mosquito Control Research Using *Wolbachia*-based Incompatible Insect Technique."[14] The Final Environmental Assessment, dated March 24, 2023, states that the Department of Land and Natural Resources filed the exemption notice "to conduct limited import of male mosquitoes for preliminary transport trials and mark release recapture studies."[1]

The Hawaii Environmental Policy Act (HEPA) Citizen's Guide (2014)[15] states: "A proposed action must be described in its entirety and cannot be broken up into component parts, which if each is taken separately, may have minimal impact on the environment. Segmenting a project generally is forbidden." Because the project has been improperly segmented in this way, there have been no details or analysis of the preliminary trials or the mark release recapture studies. There has been no disclosure as to what type of mosquito is being transported, where the mosquitoes are being transported from, and whether or not the mosquitoes are being tested for pathogens prior to transport. We demand that all actions of the mosquito project – including trial imports, mark release recapture studies, and field releases – be addressed in one Environmental Impact Statement.

The Advisory Committee on Plants and Animals' recommendation to approve import and release of *Culex quinquefasciatus* mosquitoes[16] should be null and void due to the conflicts of interest of committee members pursuant to HRS 84-14[17]. The Ethics Guide for State Board and Commission Members[18] states that members must not take official action affecting a business in which they have "financial interest." "Financial interest" in a business includes "employment." Whether a business can be a government agency is unstated. The following members of the Advisory Committee on Plants and Animals unanimously voted (7/0) on June 9, 2022 to recommend approval of the import permit[16]:

- Darcy Oishi, Committee Chairperson, Hawaii Department of Agriculture (HDOA)
- Dr. Maria Haws, Professor of Aquaculture, Pacific Aquaculture & Coastal Research Center, University of Hawaii at Hilo
- Cynthia King, Entomologist, Division of Forestry & Wildlife, Department of Land & Natural Resources (DLNR), Ex Officio Member Designated Representative

- Gracelda Simmons, Environmental Management Program Manager, Hawaii Department of Health, Ex Officio Member Designated Representative

- Thomas Eisen, Planner, Environmental Review Program, Department of Business, Economic Development and Tourism, Ex Officio Member Designated Representative

- Joshua Fisher, Wildlife Biologist, U. S. Fish and Wildlife Service (USFWS)

- Dr. Samuel Ohu Gon III, Senior Scientist and Cultural Advisor, The Nature Conversancy – Hawaii (TNC)

Of the seven voting members' agencies, only those of Thomas Eisen and Darcy Oishi are not partner agencies in *Birds, Not Mosquitoes*. As employees of partner agencies, Dr. Maria Haws (University of Hawaii), Cynthia King (DLNR), Gracelda Simmons (Hawaii Department of Health), Joshua Fisher (USFWS), and Dr. Samuel Ohu Gon III (TNC) all have conflicts of interest.

Both Dr. Samuel Ohu Gon III[19] and Cynthia King[20] are also members of the *Birds, Not Mosquitoes* steering committee. The purpose of the steering committee, as stated in the National Fish and Wildlife Foundation Hawaii Conservation Business Plan[21], includes coordinating permits for this project. These are additional conflicts of interest, particularly for Dr. Samuel Ohu Gon III, who, with his vote, has taken official action affecting a business in which he has financial interest.

The Final Environmental Assessment (EA) does not address the concern of accidental pathogen introduction. The U.S. Department of the Interior Strategy for Preventing the Extinction of Hawaiian Forest Birds[9] confirms that The Nature Conservancy has contracted with mosquito lab Verily Life Sciences. There is no mention of this contract in the EA. No documented assurances have been made that Verily Life Sciences will be testing mosquitoes for human diseases or avian diseases to ensure that they are pathogen-free prior to shipping to Hawaii.

As this project involves the interstate transport of *Culex* mosquitoes, a known vector of poultry diseases, we are concerned about impacts to local poultry farms and egg production in Hawaii. Has the USDA inspected the Verily Life Sciences insectary? There is no mention in the EA of a USDA permit (e.g., OV VS 16-6 permit from APHIS) for the interstate transport of poultry pathogen vectors by a California shipper. The USDA Animal and Plant Health Inspection Service (APHIS)[22] states:

> "The Veterinary Services, Organisms and Vectors (OV) Permitting Unit regulates the importation into the United States, and interstate transportation, of organisms and vectors of **pathogenic diseases** of livestock and **poultry**.
>
> The Code of Federal Regulations, in 9 CFR, §122.2[23], mandates that '**no organisms or vectors shall be** imported into the United States or **transported from one State** or Territory or the District of Columbia **to another State** or

> Territory or the District of Columbia **without a permit**.' "

Given that interstate transport of the vector (live *Culex)* is occurring from Maui to Verily Life Sciences' lab in South San Francisco, California[24], and those *Culex* may contain a highly contagious poultry pathogen, namely avian pox virus[25], this movement needs a federal permit. Additionally, the return trip from California to Hawaii[24] would require a federal permit. Lab mosquitoes are blood-fed from sources that are not identified in the EA, potentially including bird blood. These mosquitoes could be transporting avian pathogens back to Hawaii.

Even though male mosquitoes don't bite, male *Culex* mosquitoes are known to spread viruses to female mosquitoes through mating (e.g., St. Louis encephalitis virus[26]), as has been shown for dengue virus in *Aedes albopictus*[27].

The EA's assertion that released mosquitoes pose no risk to human health is based on unsound science. The 2010 article by Popovici et al.[28] cited in the EA has been discredited by the EPA. The EPA Human Studies Review Board met in 2018[29], and the following question was posed:

> "Is the research described in the published article 'Assessing key safety concerns of a *Wolbachia*-based strategy to control dengue transmission by Aedes mosquitoes' scientifically sound, providing reliable data for the purpose of contributing to a weight of evidence determination in EPA's assessment of the risks to human health associated with releasing *Wolbachia*-infected mosquitoes?"[30]

The Board's response states: "The Board concluded that the research described in the article by Popovici et al. was not scientifically sound and does not provide reliable data to contribute to a weight of evidence determination for assessment of human health risks due to release of *Wolbachia*-infected mosquitoes."[30]

The Hawaii Department of Agriculture has applied for an EPA Emergency Exemption[8] for use of the mosquitoes without going through regulatory safety processes. The EPA application is still under review, and the biopesticide mosquitoes have not been approved for emergency release. The Board of Land and Natural Resources cannot approve this Final Environmental Assessment and declare before the public that there is a Finding of No Significant Impact (FONSI) when there is still a possibility that the EPA will deny the Emergency Exemption due to safety concerns. This biopesticide cannot be approved for release when its safety is still under review by the EPA.

Additional concerns not adequately addressed in the Final Environmental Assessment: lack of adequate detail as required by HEPA[15]; failure to identify the *Wolbachia* strain planned for use in this project; failure to identify and describe the mark release recapture study as a proposed action; failure to adequately identify the mosquito packages planned for release into the environment; failure to adequately address the effects on the environment from the release of biodegradable packages with an unknown decay rate; failure to identify biosecurity protocols; failure to adequately address viewscape impacts, noise disturbances to forest bird breeding and nesting, and significant environmental consequences, including impacts to the untrammeled, natural qualities of the wilderness character; failure to adequately address the potential negative impacts of introducing an invasive species to the islands; failure to identify the origin of biopesticide mosquitoes for this project as Palmyra Atoll[8]; failure to identify the origin of *Wolbachia* bacteria for the project as Kuala Lumpur in Malaysia[8]; failure to identify the strain of *Wolbachia* bacteria planned for import in connection with this project that does not exist on these islands[31,32]; failure to address the concerns of tropical disease and vector expert Dr. Lorrin Pang (private citizen) regarding the serious risks of this project[33]; failure to adequately study or address the impacts to endangered native Hawaiian hoary bats, native dragonflies, and endangered native damselflies; failure to study and address biopesticide wind drift; failure to adequately address Environmental Justice (human health impacts of this project have not been adequately studied, and the proposed action would impact ethnographic resources and traditional cultural practices); failure to conduct a feasibility study to provide a detailed analysis that considers all of the critical aspects of the proposed project in order to determine the likelihood of it succeeding; and failure to establish, under the precautionary principle, that the proposed activity will not result in significant harm.

Further, per HRS §171-4 (d)[34], BLNR Chair Dawn N.S. Chang and Board Member Vernon Char must recuse themselves from participating in any discussion or voting in this matter, given that they have clear conflicts of interest. Chang is employed by the DLNR[35], a lead agency in the mosquito project. Char is employed by a law firm[36] whose clients include The Nature Conservancy[37], another lead partner in the project.

Hawaii Unites has launched a petition to "Demand an Environmental Impact Statement for the Experimental Mosquito Release on Maui"[38] which has received more than 2,500 signatures. We have yet to receive a response from any of the decision makers.

We're opposed to the authorization for the Chairperson to issue a Finding of No Significant Impact (FONSI). The scope, risks, and experimental nature of the plan require detailed, comprehensive studies and documentation of the impacts to our native birds, wildlife, environment, and public health. The subject action will have a significant effect. **We demand an Environmental Impact Statement (EIS).**

Mahalo,
Tina Lia
Founder and President
Hawaii Unites
HawaiiUnites.org

REFERENCES:

1. "Suppression of Invasive Mosquito Populations to Reduce Transmission of Avian Malaria to Threatened and Endangered Forest Birds on East Maui" (State of Hawaii Department of Land and Natural Resources, 3/24/23) https://dlnr.hawaii.gov/wp-content/uploads/2023/03/C-2-1.pdf

2. "*Wolbachia* infection in wild mosquitoes (Diptera: Culicidae): implications for transmission modes and host-endosymbiont associations in Singapore" – Huicong Ding, Huiqing Yeo, Nalini Puniamoorthy (BMC, 12/9/20) https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-020-04466-8

3. "*Wolbachia* Horizontal Transmission Events in Ants: What Do We Know and What Can We Learn?" – Sarah J. A. Tolley, Peter Nonacs, Panagiotis Sapountzis (Frontiers in Microbiology, 3/6/19) https://www.frontiersin.org/articles/10.3389/fmicb.2019.00296/full

4. "The Intracellular Bacterium *Wolbachia* Uses Parasitoid Wasps as Phoretic Vectors for Efficient Horizontal Transmission" – Muhammad Z. Ahmed, Shao-Jian Li, Xia Xue, Xiang-Jie Yin, Shun-Xiang Ren, Francis M. Jiggins, Jaco M. Greeff, Bao-Li Qiu (National Center for Biotechnology Information, National Library of Medicine, 2/12/15) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4347858/

5. "*Wolbachia* Can Enhance *Plasmodium* Infection in Mosquitoes: Implications for Malaria Control?" – Grant L. Hughes, Ana Rivero, Jason L. Rasgon (PLOS Pathogens, 9/4/14) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4154766/

6. "*Wolbachia*-mediated sterility suppresses *Aedes aegypti* populations in the urban tropics" – The Project Wolbachia – Singapore Consortium, Ng Lee Ching (medRxiv, 6/17/21) https://www.medrxiv.org/content/10.1101/2021.06.16.21257922v1.full

7. "*Wolbachia* Enhances West Nile Virus (WNV) Infection in the Mosquito *Culex tarsalis*" – Brittany L. Dodson, Grant L. Hughes, Oluwatobi Paul, Amy C. Matacchiero, Laura D. Kramer, Jason L. Rasgon (PLOS Neglected Tropical Diseases, 7/10/14) https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0002965

8. HDOA EPA Application for Emergency Exemption https://hawaiiunites.org/wp-content/uploads/2023/02/EPA-HQ-OPP-2022-0896-0002_content.pdf https://www.regulations.gov/document/EPA-HQ-OPP-2022-0896-0002

9. U.S. Department of the Interior Strategy for Preventing the Extinction of Hawaiian Forest Birds
https://www.fws.gov/sites/default/files/documents/DOI%20Strategy%20for%20Preventing%20the%20Extinction%20of%20Hawaiian%20Forest%20Birds%20%28508%29.pdf

10. World Mosquito Program: Global progress
https://www.worldmosquitoprogram.org/en/global-progress

11. World Mosquito Program: How our method compares
https://www.worldmosquitoprogram.org/en/learn/how-our-method-compares

12. HAR § 11-200-7
https://casetext.com/regulation/hawaii-administrative-rules/title-11-department-of-health/subtitle-1-general-departmental-provisions/chapter-200-environmental-impact-statement-rules-repealed/subchapter-5-applicability-repealed/section-11-200-7-multiple-or-phased-applicant-or-agency-actions-repealed

13. HAR § 11-200.1-10
https://casetext.com/regulation/hawaii-administrative-rules/title-11-department-of-health/subtitle-1-general-departmental-provisions/chapter-2001-environmental-impact-statement-rules/subchapter-6-applicability/section-11-2001-10-multiple-or-phased-actions

14. HDOA Request to Import and Establish Permit Conditions for Southern House Mosquito (6/28/22)
https://hawaiiunites.org/wp-content/uploads/2023/02/2022_0628_HDOA_Request_to_Import_and_Establish_Permit_Conditions_for_Southern_House_Mosquito.pdf

15. Hawaii Environmental Policy Act (HEPA) Citizen's Guide (2014)
https://files.hawaii.gov/dbedt/erp/OEQC_Guidance/2014-GUIDE-HEPA-Citizens-Guide.pdf

16. Advisory Committee on Plants & Animals Meeting - June 9, 2022
https://www.youtube.com/watch?v=Wt_Jbygvek4

17. HRS 84-14
https://www.capitol.hawaii.gov/hrscurrent/vol02_ch0046-0115/HRS0084/HRS_0084-0014.htm

18. Ethics Guide for State Board and Commission Members
https://ethics.hawaii.gov/wp-content/uploads/BCEthicsGuide.pdf

19. The Constructed Environment: 2023 Conference: Plenary Speakers: Sam Gon
https://constructedenvironment.com/2023-conference/program/sam-gon

20. Facebook: Birds, Not Mosquitoes: Get to know Birds, Not Mosquitoes (4/22/22) https://www.facebook.com/BirdsNotMosquitoes/photos/a.106335221571876/315991167272946/

21. National Fish and Wildlife Foundation Hawaii Conservation Business Plan (3/2021) https://www.nfwf.org/sites/default/files/2021-09/HI%20Business%20Plan%20%28August%202021%29.pdf

22. USDA Animal and Plant Health Inspection Service (APHIS): Organisms and Vectors Guidance & Permitting https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-and-animal-product-import-information/organisms-vectors/ct_organisms_and_vectors

23. 9 CFR, §122.2 https://www.ecfr.gov/current/title-9/chapter-I/subchapter-E/part-122

24. Board of Land and Natural Resources Meeting 3/10/23 https://www.youtube.com/watch?v=u8oyjKaDTGg

25. Detection and molecular characterization of *Avipoxvirus* in *Culex* spp. (Culicidae) captured in domestic areas in Rio de Janeiro, Brazil – Carolina Soares van der Meer et al. (Nature, Scientific Reports, 8/5/22) https://www.nature.com/articles/s41598-022-17745-4

26. Venereal Transmission of St. Louis Encephalitis Virus by *Culex quinquefasciatus* Males (Diptera: Culicidae) – Donald A. Shroyer (Journal of Medical Entomology, 5/1990) https://academic.oup.com/jme/article-abstract/27/3/334/2220754?login=false

27. Sexual transmission of dengue viruses by Aedes albopictus – L. Rosen (NIH National Library of Medicine, 9/1987) https://pubmed.ncbi.nlm.nih.gov/3661831/

28. Assessing key safety concerns of a Wolbachia-based strategy to control dengue transmission by Aedes mosquitoes – Jean Popovici et al. (2010) https://www.epa.gov/sites/default/files/2018-04/documents/4g._popovici_article.pdf

29. April 24-26, 2018 Meeting of the Human Studies Review Board https://www.epa.gov/osa/april-24-26-2018-meeting-human-studies-review-board

30. April 24-26, 2018 EPA Human Studies Review Board Meeting Report https://www.epa.gov/sites/default/files/2018-07/documents/final_hsrb_report_from_april_2018.pdf

31. HDOA Request to Import Southern House Mosquitoes for Immediate Field Release (6/9/22)
https://hdoa.hawaii.gov/wp-content/uploads/2018/05/DLNR-Culex-quinquefasciatus-PA-All-Docs.pdf

32. University of Hawaii at Mānoa Request to: (1) Determine if the Establishment of the Southern House Mosquito, Culex quinquefasciatus, a Vector of Avian Influenza in Hawaii, Constitutes an Ecological Disaster;…(4) Determine the Probable Impact on the Environment if the Southern House Mosquito, Culex quinquefasciatus, an Unlisted Insect, Inoculated with a Foreign Wolbachia Bacteria Species, is Accidently Released;… (6/8/21)
https://hdoa.hawaii.gov/wp-content/uploads/2018/05/HDOA-Mosquito-Request-PA_Final-6.8.21.pdf

33. Wolbachia Mosquitoes in Hawaii: Unsettled Science (Part 2) (7/21/22)
https://mailchi.mp/12fb7ffe5f31/saturday-song-circle-in-paia-12pm-2pm-15015381

34. Hawaii Revised Statutes HRS §171-4
https://www.capitol.hawaii.gov/hrscurrent/vol03_ch0121-0200d/HRS0171/HRS_0171-0004.htm

35. Dawn N.S. Chang Financial Disclosure filed 1/2/23
https://hawaiiethics.my.site.com/public/s/hsecm-fd-public/a0i6R00000Y0Yv4QAF/fd2023010909

36. Vernon Char Financial Disclosure filed 6/1/22
https://hawaiiethics.my.site.com/public/s/hsecm-fd-public/a0i6R00000TQdsNQAT/fd2022010431

37. Char Sakamoto Ishii Lum & Ching Attorneys at Law (Present and Former Clients: The Nature Conservancy)
http://lawcsilc.com/Clients.html

38. Petition: Demand an Environmental Impact Statement for the Experimental Mosquito Release on Maui
https://www.change.org/Maui_Mosquito_Experiment_EIS

SECTION 166.20(a)(2): DESCRIPTION OF PESTICIDE REQUESTED

- **Common Chemical Name (Active Ingredients):** *Wolbachia pipientis*, wAlbB (DQB strain)

- **Trade Name:** DQB Males
  EPA Reg. No.: Unregistered

- **Confidential Statement of Formula:** Attached to this submission

- **Formulation:**
  wAlbB contained in live adult male *Culex quinquefasciatus* mosquitoes (DQB strain)
  active ingredient < 0.3%*
  *percent (w/w) of adult male mosquitoes

- **Mosquito and Wolbachia source:**

The DQB line of mosquitoes was developed through transfection of *Wolbachia pipientis* wAlb*B* isolated from *Ae. albopictus* KLP strain mosquitoes originating from Kuala Lumpur, Malaysia into *Culex quinquefasciatus* Palmyra strain mosquitoes originating from Palmyra Atoll. Prior to transfection, the naturally occurring wPip infection was removed from the Palmyra strain through antibiotic treatment using tetracycline and rifampicin as described in Pike & Kingcombe 2009 following the feeding protocol outlined in Dobson and Rattanadechakul 2001. Methods for DQB line generation are substantively similar to those outlined in MRID 51788911 with non-significant changes to account for Culex egg morphology. The DQB line was not created using genetic modification and the mosquitoes are not genetically modified organisms.

Table 1. Taxonomic designation of the *Wolbachia* present in the DAB line of *Ae. aegypti.*

Kingdom	Bacteria
Phylum	Proteobacteria
Class	Alphaproteobacteria
Order	Rickettsiales
Family	Rickettsiaceae
Genus	*Wolbachia*
Species	Pipientis
Clade	Supergroup: B
Strain	DQB: (**D**ebug) (*Culex* ***q****uinquefasciatus*) (wAlb**B**) DQB contains

Within *Culex quinquefasciatus*, the strain of incompatible bacterium will be *Wolbachia wAlbA, Wolbachia wAlbB,* or *Wolbachia wPip4.* These *Wolbachia* bacterium are not present within the corresponding species of Hawaii's established mosquito population. The presence of this bacterium will make these males sexually incompatible with the wild, established female mosquitoes. Once imported, the male, sexually incompatible males will be released according to EPA and HDOA label directions to suppress the population of the established mosquito populations. Based on the prior use of this technology in California, Florida, and Kentucky, there are no data to suggest releases of these male mosquitoes to have a negative impact on agriculture, the environment, or public health and safety. Existing wild-type bacteria strain that may be imported is wPipV, which is already found on all of the main Hawaiian islands.

DISCUSSION:

1. **Persons Responsible:**

DLNR Chairperson, Suzanne Case
DOFAW Administrator, David Smith
DOFAW Entomologist, Cynthia King
Department of Land and Natural Resources – Oahu
1151 Punchbowl Street, Honolulu, HI 96813

DLNR-DOFAW, Hawaii Invertebrate Program Captive Propagation Facility - Oahu
779 Ulukahiki Street, Kailua, Honolulu, HI 96813, (808) 266-7989

DLNR Waimano Baseyard – Oahu
2680 Waimano Home Road, Pearl City, HI 96782, (808) 266-7989

Kaua'i Branch Manager, Sheri Mann, Division of Forestry & Wildlife, 3060 Eiwa Street Rm. 306, Lihue, HI 96766. (808) 274-3433

O'ahu Branch, Division of Forestry & Wildlife, 2135 Makiki Heights Drive, Honolulu, HI 96822. (808) 973-9778

Maui (& Moloka'i) Branch, Division of Forestry & Wildlife, 1955 Main Street, Room 301, Wailuku, HI 96793. (808) 984-8100

Hawai'i Branch, Division of Forestry & Wildlife, 19 E. Kawili Street, Hilo, HI 96720. (808) 974-4221

2. **Locations and Safeguards:**

All mosquitoes for import will originate from Hawaii biotypes collected from

- *Wolbachia albopictus A* (*wAlbA*) imported in *C. quinquefasciatus*. In Hawaii, this strain already exists in *Aedes albopictus*.
- *Wolbachia albopictus B* (*wAlbB*) imported in *C. quinquefasciatus*. In Hawaii, this strain already exists in *Aedes albopictus*.
- *Wolbachia wPip4* imported in *C. quinquefasciatus*. This strain does not currently exist in Hawaii. It naturally exists in parts of Europe, Asia, the Middle East, and Africa, and is bidirectionally incompatible with strain *wPip5*. Strain *wPip5* is the most common strain in *C. quinquefasciatus* in Hawaii (Atkinson, C. T., W. Watcher-Weatherwax, and D. A. LaPointe. (2016) Genetic diversity of *Wolbachia* endosymbionts in *C. quinquefasciatus* from Hawaii, Midway Atoll and American Samoa. Technical Report HCSU-074).

Once imported, we will rear the imported mosquitoes to the maximum capacity of our facilities. Male mosquitoes with one or more of the imported strains (*wAlbA / wAlbB / wPip4*) could then be used for incompatible crosses to females that carry *wPip5.* The attached letter from the DLNR describes how there is an ecological disaster occurring (*i.e.* Hawaii's native birds going extinct). The imported mosquito[e]s are intended for release (only males are intended for release) to mitigate this disaster. Based on the prior use of this technology in California, Florida, and Kentucky, we do not expect releases of these male mosquitoes to have a negative impact on agriculture, the environment, or public health and safety.

PQB NOTES: *In addition to this request, the applicants have submitted a request to import the aforementioned species of unlisted Wolbachia bacteria. The import request for the Wolbachia species was submitted to the PQB Advisory Subcommittee on Bacteria for review and recommendation. The Advisory Subcommittee on Bacteria unanimously deemed these Wolbachia species to be low risk, and recommended approval of the import request via a letter of authorization. Hawaii Administrative Rules §4-71A-25(b) states: "An unlisted microorganism that is determined by the department to be a low risk microorganism may be allowed import by a letter of authorization issued by the Chief without advisory committee review or board approval."*

DISCUSSION:

1. <u>Persons Responsible</u>:

1) Floyd A. Reed, UHM, 2538 McCarthy Mall, Edmondson Hall 216, Honolulu, Hawaii 96822, (808) 956-6489.

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DEPARTMENT OF LAND AND NATURAL RESOURCES

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EXEMPTION NOTICE

Regarding the preparation of an environmental assessment under the authority of Chapter 343, HRS and Section 11-200.1-17, HAR

Project Title:	Mosquito Control Research Using *Wolbachia*-based Incompatible Insect Technique
Project Location:	Maui (2) 2-3-005:004: Waikamoi Preserve (2) 2-4-016:004: Waikamoi Preserve (2) 1-2-004:013: Hanawi Natural Area Reserve (2) 2-3-005:001: Haleakala National Park (2) 1-8-001:007: Haleakala National Park (2) 1-3-001:003: Haleakala National Park (2) 1-7-004:016: Haleakala National Park (2) 1-6-001:001: Haleakala National Park (2) 1-6-001:002: Haleakala National Park (2) 1-2-010:001: Haleakala National Park Kauai (4) 1-4-001:003: Alakai Wilderness Preserve (4) 1-4-001:013: Kokee State Park
Chapter 343 Trigger(s):	Use of State Funds and Lands
Project Description:	The main objective of this project is to initiate research to inform incompatible insect technique applications for the control of invasive *Culex quinquefasciatus* mosquitoes which are the primary vector of avian malaria. The disease threatens the survival of remaining endangered forest bird species where they persist in high elevation montane forest habitat on Maui and Kauai. Male mosquitoes which have been given an incompatible strain of *Wolbachia* bacteria are to be released on the landscape, and upon release those males will breed with wild female mosquitoes. As a result of those pairings, the wild female mosquitoes will lay eggs which will not hatch, and no offspring will be produced. When releases of incompatible male mosquitoes are completed consecutively, the approach results in the suppression of mosquito populations at a landscape-scale. If releases are halted, mosquito

	populations will gradually return to pre-release levels as wild female and male mosquitoes migrate back into the treated area from surrounding forest habitat. Initial research will contribute to EPA registration of male *Culex quinquefasciatus* mosquitoes with *Wolbachia* as a biopesticide, as well as determine the minimum number of male mosquitoes that must be released in each area to ensure population suppression. This project may be funded by Federal sources.
Consulted Parties:	U.S. Fish and Wildlife Service
Authorization:	November 13, 2015, Land Board submittal (C-6). Delegation of Authority to the Chairperson or their authorized representative to declare exempt from the preparation of an Environmental Assessment those Department actions which are included in the Department-wide exemption list when the Board of Land and Natural Resources has delegated the authority to conduct those actions.
Exemption Class & Description:	**Exemption Classes:** **General Exemption Type 5** *Basic data collection, research, experimental management, and resource and infrastructure testing and evaluation activities that do not result in a serious or major disturbance to an environmental resource.* PART 1 13. Research that the Department declares is designed specifically to monitor, conserve, or enhance native species or native species' habitat. 16. Research to identify, monitor, control, or eradicate introduced species. Date of Agency Exemption List: November 10, 2020.
Determination:	The Department of Land and Natural Resources declares that this project will likely have minimal or no significant impact on the environment and is therefore exempt from the preparation of an environmental assessment under the above exemption classes.

DGS Suzanne D. Case Jun 17, 2022

Suzanne D. Case, Chairperson Date
Board of Land and Natural Resources

Signature: DGS

Email: david.g.smith@hawaii.gov

From: **Fretz, Scott** scott.fretz@hawaii.gov
Subject: RE: MRR Study: Makawao Forest Reserve
Date: February 9, 2023 at 2:30 PM
To: Tina Lia tinalia@live.com

Aloha Ms. Lia:

Thank you for your follow up inquiry. You are correct that an exemption was filed for the MRR study. However, after further review and scheduling, it is our intention to carry out the MRR study as part of the actions described and analyzed in the EA. The MRR study will be done using IIT mosquitoes, as described in the EA.

Scott

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From: Tina Lia <tinalia@live.com>
Sent: Thursday, February 2, 2023 3:04 PM
To: Fretz, Scott <scott.fretz@hawaii.gov>
Subject: [EXTERNAL] MRR Study: Makawao Forest Reserve

Aloha Dr. Fretz,

Thank you for your message explaining that the DLNR does not intend to initiate the mark-release-recapture (MRR) study until the EA has received final approval. It had been my understanding that the MRR study was not part of the proposed action in the EA. It was not mentioned nor described as part of the proposed action. Rather, the EA states that "DLNR filed an **exemption notice** regarding the preparation of an environmental assessment under the authority of Chapter 343, Hawaii Revised Statutes (HRS) and Section 11-200.1-17. HAR, to conduct limited import of male mosquitoes for preliminary transport trials and **mark release recapture studies**."

When I asked about the MRR study at the virtual public meeting for the EA on January 5, 2023, Chris Warren said that the study would happen in the western project area. The project area map shows Makawao Forest Reserve to be the westernmost parcel.

Following is the question I posed and the response (26:25 marker):

Q: (Tina Lia) “Regarding the mark-release-recapture study mentioned in the environmental assessment, why is the study necessary, and when and where will it be occurring? Will incompatible mosquitoes be released as a part of that study?”

A: (Chris Warren) "Yeah, that's great. You know, the mark-release-recapture study is part of the initial field trials, and we would learn really critical things during those trials that would make sure that this method is as efficient as it possibly can be. And at the moment, **we are discussing not using IIT mosquitoes for this at all**. It would be, you know, again only male mosquitoes released in a small area, likely in the **western portion of the project area** that is more readily accessible but still away from places that people access on a regular basis."

I found his answer concerning because the release of compatible male mosquitoes, rather than the incompatible ones, is something that is not mentioned or evaluated in the EA. Providing potential male mates could increase the mosquito population, which could have adverse impacts to forest birds. This is at odds with the EA which specifically states, "This project would release only male mosquitoes with a different strain of *Wolbachia* bacteria to that occurring in southern house mosquitoes in East Maui."

Could you please clarify which is the environmental review document that covers the mark-release-recapture study? Is it the EA exemption notice or the draft EA? The draft EA makes it seem that the exemption notice covers the MRR study, but your answer implies that the MRR study is covered by the EA. Also, the EA is only for the release of incompatible mosquitoes, whereas compatible mosquitoes are being discussed for release in the western project area as part of the MMR.

Thank you for taking the time to respond to these concerns.

Aloha,
Tina Lia
tinalia@live.com
(808) 298-6335

On Feb 2, 2023, at 11:06 AM, Fretz, Scott <scott.fretz@hawaii.gov> wrote:

Aloha Ms. Lia:

Thank you for your inquiry. The actions proposed for the mark-release-recapture study are covered in the Environmental Assessment (EA) that was published on December 23, 2022. We do not intend to initiate the study until the EA has received final approval. Therefore, no decisions have been made regarding the Makawao Forest Reserve as a study site.

Scott

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From: Tina Lia <tinalia@live.com>
Sent: Monday, January 30, 2023 1:39 PM
To: Fretz, Scott <scott.fretz@hawaii.gov>
Subject: [EXTERNAL] MRR Study: Makawao Forest Reserve

Aloha Mr. Fretz,

I'm writing inquire about the Mark-Release-Recapture (MRR) study for the State of Hawaii's multi-agency *Birds, Not Mosquitoes* project "Mosquito Control Research Using Wolbachia-based Incompatible Insect Technique." Can you confirm that the Makawao Forest Reserve is a release site for the MRR study? If so, have signs been posted notifying the public of the MRR study being conducted?

Mahalo,
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Parasites & Vectors

RESEARCH

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Wolbachia infection in wild mosquitoes (Diptera: Culicidae): implications for transmission modes and host-endosymbiont associations in Singapore

Huicong Ding[†], Huiqing Yeo[†] and Nalini Puniamoorthy[*]

Abstract

Background: *Wolbachia* are intracellular bacterial endosymbionts found in most insect lineages. In mosquitoes, the influence of these endosymbionts on host reproduction and arboviral transmission has spurred numerous studies aimed at using *Wolbachia* infection as a vector control technique. However, there are several knowledge gaps in the literature and little is known about natural *Wolbachia* infection across species, their transmission modes, or associations between various *Wolbachia* lineages and their hosts. This study aims to address these gaps by exploring mosquito-*Wolbachia* associations and their evolutionary implications.

Methods: We conducted tissue-specific polymerase chain reaction screening for *Wolbachia* infection in the leg, gut and reproductive tissues of wild mosquitoes from Singapore using the *Wolbachia* surface protein gene (*wsp)* molecular marker. Mosquito-*Wolbachia* associations were explored using three methods—tanglegram, distance-based, and event-based methods—and by inferred instances of vertical transmission and host shifts.

Results: Adult mosquitoes (271 specimens) representing 14 genera and 40 species were screened for *Wolbachia*. Overall, 21 species (51.2%) were found positive for *Wolbachia*, including five in the genus *Aedes* and five in the genus *Culex*. To our knowledge, *Wolbachia* infections have not been previously reported in seven of these 21 species: *Aedes* nr. *fumidus*, *Aedes annandalei*, *Uranotaenia obscura*, *Uranotaenia trilineata*, *Verrallina butleri*, *Verrallina* sp. and *Zeugnomyia gracilis*. *Wolbachia* were predominantly detected in the reproductive tissues, which is an indication of vertical transmission. However, *Wolbachia* infection rates varied widely within a mosquito host species. There was no clear signal of cophylogeny between the mosquito hosts and the 12 putative *Wolbachia* strains observed in this study. Host shift events were also observed.

Conclusions: Our results suggest that the mosquito-*Wolbachia* relationship is complex and that combinations of transmission modes and multiple evolutionary events likely explain the observed distribution of *Wolbachia* diversity across mosquito hosts. These findings have implications for a better understanding of the diversity and ecology of *Wolbachia* and for their utility as biocontrol agents.

Keywords: *Wolbachia*, *Wolbachia* surface protein gene, Reproductive endosymbiont, Tissue-specific polymerase chain reaction, Transmission modes, Host-endosymbiont association

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Background

Wolbachia are intracellular endosymbiotic bacteria that alter host reproduction [1]. They are widespread in arthropods, infecting a wide range of insect, crustacean, and nematode species [2, 3]. In some cases, *Wolbachia* exist in a mutualistic relationship with their hosts [4–6]. However, *Wolbachia* are most often recognised as reproductive manipulators that bias the sex ratio of the host offspring towards the production of more infected females [7, 8]. This reproductive manipulation is commonly achieved through four phenotypes—male killing [9], feminisation [10, 11], parthenogenesis [12, 13], and cytoplasmic incompatibility [14, 15]—which increase the endosymbiont's reproductive success [16]. Owing to their strong influence on host reproduction, an increasing amount of research is being dedicated to exploring the impacts of reproductive endosymbionts on host population dynamics and evolution [17, 18], especially in medically important insects such as mosquitoes. The promising use of *Wolbachia* to alter both mosquito reproduction [19] and arboviral transmission [20] has prompted the deployment of novel *Wolbachia*-infected mosquitoes for population replacement and suppression [21].

Several countries, including Singapore, have started to employ *Wolbachia* as biocontrol agents of mosquitoes by releasing infected mosquitoes [22–24]. However, the presence of naturally occurring endosymbionts in wild mosquito populations has not been adequately assessed. The release of mosquitoes artificially infected with *Wolbachia* might have a profound impact on closely interacting wild mosquito populations through various transmission modes. For instance, horizontal transmission of an introduced *Wolbachia* strain may result in manipulation of the reproductive biology of non-target species, which could potentially result in an unintentional population crash, opening up niches for other vector species [25]. Another possible effect of this type of biocontrol method is the increased likelihood of co-infections with other naturally occurring *Wolbachia* strains or other endosymbionts, such as *Cardinium*, *Rickettsia*, and *Spiroplasma.* These co-infections may result in a synergistic effect on mosquito host fitness and future transmission of endosymbionts [26–29]. Without a detailed characterisation of *Wolbachia* prevalence and diversity among wild mosquitoes, the ecological risk of releasing artificially infected mosquitoes might be overlooked. Therefore, bearing the precautionary principle in mind, it is important to investigate the natural occurrences of *Wolbachia.*

There is also a need to discern the main mode of infection transmission among mosquitoes. Although *Wolbachia* are mainly thought to be vertically transmitted [15, 30], there have been accounts of horizontal transmissions into wild populations through parasitism [31, 32], or through proximity to infected individuals [33]. *Wolbachia* may not be strictly localised in germline tissues, as they have also been detected in somatic tissues such as the gastrointestinal tract and haemolymph [34–36]. The detection of *Wolbachia* in the gastrointestinal tract suggests that they could be horizontally transmitted through uptake from the environment or host sharing [34, 37, 38], whereas their detection in non-gastrointestinal somatic tissues, such as those of jointed appendages, could indicate horizontal bacterial genome integration into the host genome [36]. Currently, detection of *Wolbachia* in mosquitoes is mostly achieved through conventional polymerase chain reaction (PCR) methods using DNA extracted from an entire individual or its abdomen [39–47]. This limits our ability to identify the site of endosymbiont infection within an individual (tissue tropism). Tissue-specific screening of *Wolbachia* is necessary to provide insights and infer the extent of vertical and horizontal transmission.

It has been proposed that host mitochondrial DNA (mtDNA) and *Wolbachia* are maternally co-transmitted within the cytoplasm [17, 48], which suggests a congruency between host mtDNA and *Wolbachia* phylogenies—a consequence of cytoplasmic hitchhiking driven by endosymbiont transmission [17]. In insect systems such as bedbugs where vertical transmission has been established to be the main mode of transmission, *Wolbachia* exhibit clear patterns of cophylogeny with their hosts, with few instances of host shifting or multiple infections within a single host species [49, 50]. In contrast, cophylogeny is not apparent among nematodes and bees, and numerous acquisitions of *Wolbachia* infections through horizontal transmission as well as losses have been shown in these diversified host lineages [51, 52]. The modes of *Wolbachia* transmission among mosquitoes have not been well established, nor has the extent of multiple infections within mosquito hosts or host shifting of these bacteria.

There is presently no comprehensive analysis of the evolutionary associations between *Wolbachia* and their mosquito host species. An understanding of host-endosymbiont associations will not only further our ability to discern the mode of transmission which influences *Wolbachia* diversity, but will also allow for an evaluation of *Wolbachia* host specificity, speciation, and their ability to establish in new hosts. All of this is key to understanding the diversity and ecology of *Wolbachia*, and their utility in biocontrol methods.

This study has three major research objectives. First, to examine the prevalence and diversity of *Wolbachia* among wild mosquitoes from Singapore. Second, to determine the tissue tropism of *Wolbachia* infection in mosquitoes using a tissue-specific PCR screening method. Finally, to reconstruct the evolutionary associations between *Wolbachia* and their mosquito hosts to provide a basis for an understanding of host-endosymbiont evolution.

Methods

Adult mosquito collection and identification

Mosquito samples were collected from 12 localities across Singapore between March 2018 and November 2019 (Fig. 1a). Three methods were employed to collect the samples: CO_2-baited Centers for Disease Control and Prevention traps, sweep-netting using hand-held fan traps, and larval sampling [53]. For the latter, dipping was carried out at streams and ponds and pipettes were used to collect larvae from various microhabitats, including tree holes, plant axils, and artificial containers. Thereafter, the field-collected larvae were reared to adults in an incubator maintained at 26 °C and 70% relative humidity, under a 12:12-h (day:night) photoperiod. Larvae were fed with pulverised fish food (TetraMin Granules) daily. Mosquitoes were identified using relevant taxonomic keys and descriptions [54–59]. A subset of individuals from commonly sampled species was selected and preserved in phosphate-buffered saline solution at – 80 °C for subsequent dissection step.

Tissue-specific dissection

Tissue-specific dissection was carried out on each adult mosquito sample to isolate the leg, gut, and reproductive tissues (Fig. 1b–d). To prevent the contamination of tissues with bacteria on the external surface of the mosquito, the leg was removed first before isolating the gut and reproductive tissues. All dissection equipment and microscope slides were thoroughly wiped with 70% ethanol before commencing dissection of the next sample. Dissected tissues were individually placed into a 96-well plate on ice to prevent DNA degradation.

DNA extraction, PCR amplification, and sequencing

DNA extraction of each dissected tissue was performed using 7 µl of QuickExtract DNA Extraction Solution (Lucigen, Madison, USA) in a thermocycler (Eppendorf, Hamburg, Germany) with the following protocol: 65 °C for 18 min, followed by 98 °C for 2 min, ending with cooling on ice for at least 10 min. All dissected tissues were screened for *Wolbachia* infections following single-primer PCR protocols described by Martin et al. [26] with slight modifications to the cycle conditions. The *Wolbachia* surface protein gene (*wsp*) general primers, wsp81F (5′-TGGTCCAATAAGTGATGAAGAAACTAGCT-3′) and wsp691R (5′-AAAAATTAAACGCTACTCCAGCTTCTGCAC-3′), were used in this study [60]. In addition, a fragment of the cytochrome c oxidase subunit I (*cox*1) gene of the mosquito hosts was also amplified using primers LCO1498 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) [61]. This served to confirm host identity and acted as an internal control. We used DNA from known *Wolbachia*-infected *Nasonia* specimens as positive controls for this study.

All PCR procedures were performed in reaction mixtures consisting of 12.5 µl of GoTaq G2 Green Mastermix (Promega, Madison, USA), 1 µl of 1 mg ml^{-1} bovine

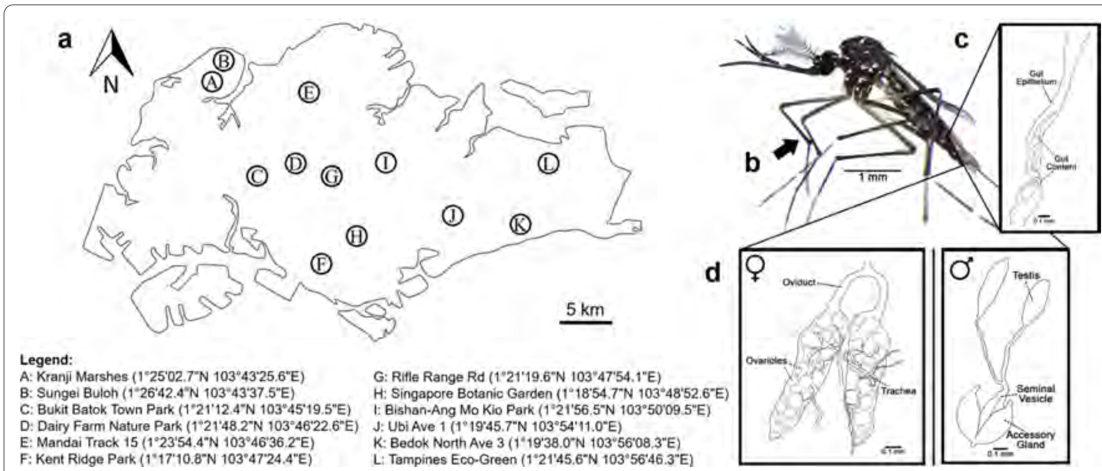

Fig. 1a–d Map of sampling sites and diagrammatic images of *Aedes aegypti* and its dissected tissues. **a** Various mosquito collection localities across Singapore and their respective coordinates, **b** mosquito leg, **c** gut, **d** female reproductive tissue (*left*) and male reproductive tissue (*right*)

serum albumin, 0.184 µl of 25 mM magnesium chloride, 1.5 µl of extracted DNA, and 1.5 µl each of 5 µM *wsp* forward and reverse primers for *Wolbachia* PCR screens or 1.0 µl each of 5 µM LCO1498 and HCO2198 primers for *cox*1 PCRs. Double-distilled water was used to top up the reaction mixture to a final volume of 25 µl. PCR amplification of positive and negative controls was also conducted simultaneously.

PCR conditions were as follow: 94 °C for 5 min, followed by 35 cycles of 95 °C for 30s, 55 °C for 45s, and 72 °C for 1 min, with a final elongation step of 72 °C for 10 min. Amplicons were separated by gel electrophoresis on 2% agarose gel stained with GelRed (Biotium, Fremont, USA) and visualised under a ultraviolet transilluminator (Syngene, Cambridge, UK). PCR products were purified using SureClean Plus (Bioline, London, UK) following the manufacturer's protocol. Samples were sequenced by First Base Laboratories (Axil Scientific, Singapore), using a 3730XL DNA Analyzer (Applied Biosystems, Waltham, USA). Obtained sequences were edited and aligned using Geneious Prime (version 2019.2.3) (https://geneious.com). Similarities with publicly available sequences were assessed using the Basic Local Alignment Search Tool (BLAST) [62].

Statistical analyses

To test if there were significant differences in *Wolbachia* infection across the different mosquito tissues, Cochran's Q-test was carried out. As a follow-up, McNemar's post hoc test was employed to identify the tissue pairs that differed significantly in infection. Individuals for which the internal control (*cox*1 gene) was not amplified successfully for any of the three dissected tissues were excluded from this statistical analysis. The effect of sex on host infection was also tested using binary logistics regression with sex as a categorical dependent variable and infection outcome as a binary independent variable. Logistic regression was conducted on a subset that only included species that had a roughly similar representation of both sexes, i.e. for every species included, the number of individuals of the less common sex was proportionally at least 60% of the number of individuals of the more common sex. This was to prevent a biased analysis due to a dataset with unequal representation of the sexes. Statistical significance was determined as $P < 0.05$. All statistical analyses were performed in R version 3.6.2 [63] with packages nonpar [64], rcompanion [65], and ISLR [66].

Sequence analyses

Multiple alignment of consensus sequences was carried out using the ClustalW algorithm with default settings (gap penalty = 15, gap extension penalty = 6.66) [67], in software MEGA X [68]. Mosquito *cox*1 sequences generated in this study were aligned with 61 reference *cox*1 barcodes of identified local mosquitoes from Chan et al. [53]. For *wsp* sequences, the generated sequences were aligned with 54 available *wsp* sequences of known *Wolbachia* strains obtained from GenBank [69]. Short sequence reads (< 500 base pairs) were excluded.

Neighbour-joining (NJ) phylogenetic trees for mosquito hosts and *Wolbachia* were reconstructed using the sequenced *cox*1 gene fragment and the *wsp* gene, respectively. *cox*1 sequences from previous publications were not included because a comparison of the genetic relationships between the hosts was not the aim of this research. Instead, 54 *wsp* sequences from GenBank were included in the construction of the *Wolbachia* NJ tree. The NJ tree reconstruction was performed with the Kimura two-parameter model as the nucleotide substitution model in MEGA X [68]. Internal gaps were treated as indels and terminal gaps as missing for *wsp* sequences. Bootstrap probabilities were estimated by generating 1000 bootstrap replicates. We designated two biting midge species, *Culicoides asiana* (KJ162955.1) and *Culicoides wadai* (KT352425.1), as outgroups for the host NJ tree construction. Due to the lack of an appropriate endosymbiont outgroup [51], the *Wolbachia* NJ tree was midpoint rooted.

When possible, *Wolbachia* strains were classified into supergroups and putative strains using 97% bootstrap probability as a threshold [60]. *Wolbachia* surface protein sequences that did not have 97% bootstrap support were evaluated on a case-by-case basis. For example, sequences which clustered closely together and had a relatively high support value (> 90%) were deemed as originating from the same putative strain.

Putative strains which were infectious to only one host species were categorized as 'specialists' and those which infected two or more hosts as 'generalists'. Then, the standardised phylogenetic host specificity (SPS) score of each generalist strain was calculated by adapting the method outlined by Poulin et al. [70] and Kembel et al. [71]. SPS measures the degree of phylogenetic relatedness among host species infected by the same endosymbiont strain. It also tests for significance by comparing it with null models generated with 999 replicates of random host-endosymbiont associations. A positive SPS value with a high *P*-value ($P > 0.95$) indicates a high degree of host flexibility where *Wolbachia* infect hosts which are phylogenetically even. A negative SPS value with low *P*-value ($P < 0.05$) suggests a low degree of host flexibility where the infected hosts are phylogenetically clustered together. SPS scores were calculated using R package picante [71].

Evolutionary analyses of the mosquito-*Wolbachia* relationship

Three distinct methods were used to explore the evolutionary associations between mosquito hosts and their *Wolbachia* endosymbionts. The analyses were carried out using pruned phylogenies where each species is represented by a single individual.

First, using the software TreeMap 3.0 [72], a tanglegram was created between host and endosymbiont NJ trees to visualise mosquito-*Wolbachia* associations. A tanglegram is useful as a pictorial representation of the interactions between two phylogenies [73]. TreeMap also seeks to minimise the entanglement between the two trees to provide a clearer visualisation of the phylogenetic relationship between host and endosymbiont [72].

Second, ParaFit Global test, a distance-based method, was employed to quantitatively estimate congruence between the host and endosymbiont phylogenetic trees by comparing genetic distances among infected host species and the *Wolbachia* strains [74]. The null hypothesis for this test states that the associations between host and endosymbiont trees are random, whereas the alternative hypothesis suggests that there are strong associations between hosts and parasites, which are indicated by phylogenetic distances. Significance was tested by comparing the observed associations between host and endosymbiont with randomised associations generated with 5000 permutations. The respective host-endosymbiont associations which contributed significantly to the ParaFit Global statistics were also identified by performing a Parafit Link test. ParaFit tests were performed with the Cailliez correction to correct for negative eigenvalues generated [75] using R package ape [76].

Third, an event-based analysis was performed in Jane 4.0 [77] to map out potential evolutionary events of the endosymbiont in relation to the host phylogeny [78]. Five evolutionary events were considered: co-speciation (host and endosymbiont speciate simultaneously), duplication (intra-host speciation), duplication with host shift (endosymbiont host shifts), loss (host speciates but endosymbiont fails to establish in one of the new lineages), failure to diverge (host speciates and endosymbiont remains in both lineages). As each event is expected to have differing likelihoods, default cost values were attached to each of the events. Jane 4.0 determined the best reconstruction of evolutionary events by minimising the overall cost. The following cost-scheme regime was used with 100 generations and a population size of 300: co-speciation = 0, duplication = 1, duplication with host shift = 2, loss = 1, and failure to diverge = 1 [79]. As a follow-up, random tip mapping (randomisation of host-endosymbiont associations) was carried out for 50 iterations, to determine if the overall cost of reconstruction was significantly lower than expected by chance. If 5% or fewer of the random solutions have costs lower than the reconstructed coevolution phylogeny, there is support for the coevolution of the hosts and endosymbionts through co-speciation.

Results

Prevalence of *Wolbachia* in wild-caught mosquitoes

A total of 271 adult mosquitoes, representing 40 species and 14 genera, were collected from 12 localities in Singapore (Fig. 1a). Overall, infection prevalence was moderate with 119 out of 271 (43.9%) individuals screening positive for *Wolbachia* (Table 1). In total, 21 (51.2%) species were positive for *Wolbachia*. According to our knowledge, *Wolbachia* infection in seven of these species is reported here for the first time (Table 1). *Wolbachia* were detected in all genera except for *Aedeomyia*, *Anopheles* and *Mimomyia* (i.e. 11 out of 14 genera; 78.6%). Five out of the seven *Aedes* species collected (71.4%) were positive for *Wolbachia*, while in the genus *Culex*, five out of 16 species (31.3%) were positive. Some of the screened species in the genera *Aedes* and *Culex* that were positive for *Wolbachia*, such as *Aedes albopictus* and *Culex quinquefasciatus*, are medically important vector species.

The infection rates varied across the mosquito species. Notably, there was variation in the percentage of infection between species that are epidemiologically related. For instance, *Wolbachia* infection was not detected in *Aedes aegypti*. However, infection was moderately high (56.8%) for *Aedes albopictus*. There was also a difference in the infection rate of two closely related species, *Culex pseudovishnui* (86.4%) and *Culex vishnui* (0%) [53].

Locality did not seem to play a role in the *Wolbachia* infection of mosquito hosts. Among species that have a wide range across Singapore, the percentage of infection was consistent in populations across different habitats. For example, the infection percentage was consistently high for *Cx. pseudovishnui*, while consistently low for *Malaya genurostris*. Based on our results, species identity was a better predictor of infection status than locality.

Based on a data subset containing 153 individuals (45.8% males) representing 12 mosquito species, sex was a significant explanatory variable, and there was a significantly lower infection prevalence in males than females (odds ratio 0.434; binary logistics regression: $Z = -2.48$, $df = 151$, $P = 0.013$).

Tissue tropism of *Wolbachia* infection in mosquitoes

Among the 159 successfully amplified *cox*1 sequences, *Wolbachia* infection was mainly observed in the reproductive tissues. Among the reproductive tissues of 159 dissected individuals, 42.1% (n = 67) were infected. Percentage infection was lower in the gut (5.7%, n = 9) and leg (3.1%, n = 5) tissues. The difference in

Table 1 Percentage infection of *Wolbachia* in 40 mosquito species collected from 12 Singapore localities

Mosquito species	Localities												Total	Infection (%)	Supergroup
	BN	BA	BB	DF	KR	KJ	M	RR	SBG	SBL	T	U			
Aedeomyia catastica	–	0/1	–	–	–	–	–	–	–	–	–	–	0/1	0.0	–
Aedes aegypti	0/1	–	–	–	–	–	–	–	–	–	–	0/13	0/14	0.0	–
Aedes albolineatus	–	–	–	–	–	–	0/3	–	–	–	–	–	0/3	0.0	–
Aedes albopictus	–	–	–	6/10	6/10	3/6	6/11	–	–	–	–	–	21/37	56.8	A, B
Aedes annandalei[a]	–	–	–	–	3/4	–	8/9	–	–	–	–	–	11/13	84.6	A
Aedes nr. *fumidus*[a]	–	–	–	–	–	–	–	–	–	6/10	–	–	6/10	60.0	A
Aedes gardnerii	–	–	–	–	–	–	1/1	–	–	–	–	–	1/1	100.0	A
Aedes malayensis	–	–	–	1/2	13/16	0/2	–	–	–	–	–	–	14/20	70.0	A
Anopheles barbirostris complex	–	–	–	0/2	–	–	0/2	–	–	–	–	–	0/4	0.0	–
Anopheles lesteri	–	–	–	–	–	0/2	–	–	–	–	–	–	0/2	0.0	–
Anopheles sinensis	–	0/12	–	–	–	–	–	–	–	–	–	–	0/12	0.0	–
Armigeres kesseli	–	–	–	–	3/3	–	–	–	–	–	–	–	3/3	100.0	B
Coquillettidia crassipes	–	–	–	2/2	6/7	4/4	–	–	–	–	–	–	12/13	92.3	B
Culex (*Lophoceramyia*) spp.[c]	–	–	–	–	0/1	0/2	1/9	–	–	–	0/2	–	1/14	7.1	B
Culex bitaeniorhynchus	–	–	–	–	0/1	–	–	–	–	–	–	–	0/1	0.0	–
Culex brevipalpis	–	–	–	0/1	–	–	0/2	–	–	–	–	–	0/3	0.0	–
Culex nigropunctatus	–	–	–	–	–	0/1	0/2	–	–	–	–	–	0/3	0.0	–
Culex pseudovishnui	–	–	–	–	11/12	–	4/4	–	3/5	1/1	–	–	19/22	86.4	B
Culex quinquefasciatus	–	5/8	–	–	–	–	–	–	–	–	–	–	5/8	62.5	B
Culex sitiens	–	–	–	–	–	–	–	–	–	2/4	–	–	2/4	50.0	B
Culex sp.	–	–	–	–	–	–	0/2	–	–	–	–	–	0/2	0.0	–
Culex tritaeniorhynchus	–	–	–	–	–	2/5	–	–	–	0/1	0/1	–	2/7	28.6	UC[b]
Culex vishnui	–	–	–	–	–	–	0/2	–	–	–	0/3	–	0/5	0.0	–
Malaya genurostris	–	–	2/4	–	0/1	4/13	–	–	0/1	–	–	–	6/19	31.6	B
Mansonia dives	–	–	–	–	–	–	0/2	–	–	–	–	–	0/2	0.0	–
Mansonia indiana	–	–	–	–	–	3/3	–	–	–	–	–	–	3/3	100.0	B
Mimomyia luzonensis	–	–	–	–	–	0/1	–	–	–	–	–	–	0/1	0.0	–
Tripteroides sp.	–	–	–	–	0/7	–	½	–	–	–	–	–	1/9	11.1	UC[b]
Uranotaenia obscura[a]	–	–	–	2/4	–	–	2/2	1/1	–	–	–	–	5/7	71.4	A
Uranotaenia sp.	–	–	–	1/2	–	–	–	–	–	–	–	–	1/2	50.0	A
Uranotaenia trilineata[a]	–	–	–	–	–	–	1/1	–	–	–	–	–	1/1	100.0	B
Verrallina butleri[a]	–	–	–	–	–	1/1	–	–	–	–	–	–	1/1	100.0	UC[b]
Verrallina sp.[a]	–	–	–	–	–	–	–	1/5	–	–	–	–	1/5	20.0	UC[b]
Zeugnomyia gracilis[a]	–	–	–	1/2	–	–	1/13	1/4	–	–	–	–	3/19	15.8	B
Total	0/1	5/21	2/4	13/25	42/62	17/40	25/67	3/10	3/6	9/16	0/6	0/13	119/271	43.9	

BN Bedok North Avenue 3, *BA* Bishan-Ang Mo Kio Park, *BB* Bukit Batok Town Park, *DF* Dairy Farm Nature Park, *KR* Kent Ridge Park, *KJ* Kranji Marshes, *M* Mandai Track 15, *RR* Rifle Range Road, *SBG* Singapore Botanic Garden, *SBL* Sungei-Buloh, *T* Tampines Eco-Green, *U* Ubi Avenue 1

[a] Species in which, according to our knowledge, *Wolbachia* infection has not been previously reported

[b] *Wolbachia* infections that were unclassified (*UC*) with respect to supergroup [60] because their DNA sequences were either too short (< 400 base pairs), or there were alignment issues during the phylogenetic analyses

[c] *Culex* (*Lophoceramyia*) comprises seven unique species, which were not identified here

percentage infection across the three dissected tissues was statistically significant (Cochran's Q-test: $Q = 109.5$, $df = 2$, $P < 0.0001$). The percentage of infection in the reproductive tissues was significantly higher than in the gut (McNemar's post hoc test: $P < 0.0001$) and leg tissues (McNemar's post hoc test: $P < 0.0001$), but the difference in percentage of infection between the gut and leg tissues was not significant (McNemar's post hoc test: $P = 1.0$). Notably, the amplicon size of *wsp* in the gut and leg tissues tended to be shorter than 400 base pairs.

Wolbachia diversity among mosquito fauna from Singapore

Following Zhou et al. [60], all *wsp* sequences obtained in this study can be broadly classified into A and B *Wolbachia* supergroups. Out of 21 infected species, six were infected with supergroup A, ten with supergroup B, and one species, *Ae. albopictus*, was infected with both supergroups (Fig. 2). Infection of the remaining four species (*Culex tritaeniorhynchus*, *Tripteroides* sp., *Verrallina butleri*, and *Verrallina* sp.) was unclassified due to short sequences (< 400 base pairs) or sequence alignment issues during sequences analyses. The analysed *wsp* sequences were also clustered into 12 putative strains: 'Wol 1' to 'Wol 12'. Four (Wol 1, Wol 2, Wol 3, and Wol 8) out of the 12 putative strains could be matched to previously typed strains [60, 80]. *Wolbachia* strains from this study are also closely related to those isolated from other insect groups (Fig. 2). For instance, Wol 9 and Wol 10 are closely related to the *Wolbachia* strains harboured by *Drosophila* spp. (bootstrap value > 99%).

Host specificity of *Wolbachia* strains

The degree of host specificity varied across the 12 putative strains. Seven out of the 12 strains (Wol 2, Wol 4, Wol 5, Wol 6, Wol 8, Wol 10, and Wol 12) were considered as specialists. These strains were host specific and were only detected in one host species each (Fig. 3). The remaining five strains were considered as generalists as they were found in more than one host. Amongst the generalists, Wol 3 was found in the highest number of host species, i.e. three, *Coquillettidia crassipes*, *Mansonia indiana*, and *Culex sitiens*. The SPS scores revealed that Wol 1 had the lowest degree of host flexibility (SPS test: $Z = -1.41$, $P = 0.049$). Wol 7 had the highest degree of host flexibility, but this was not statistically significant (SPS test: $Z = 0.07$, $P = 0.779$) (Table 2).

Evolutionary relationship between mosquitoes and *Wolbachia*

We recorded 18 counts of mosquito-*Wolbachia* associations in wild-caught mosquitoes from Singapore. A visualisation of these associations using a tanglegram showed patterns of broad associations (Fig. 3). For instance, the clade which consists of *Aedes* species was observed to be mostly associated with *Wolbachia* supergroup A. In contrast, other species, especially the clade representing various *Culex* species, had numerous associations with *Wolbachia* supergroup B.

The distance-based quantitative test showed that mosquito and *Wolbachia* phylogenies were weakly congruent at the global level (ParaFit Global test: ParaFit Global = 0.006, P = 0.048). Among the numerous

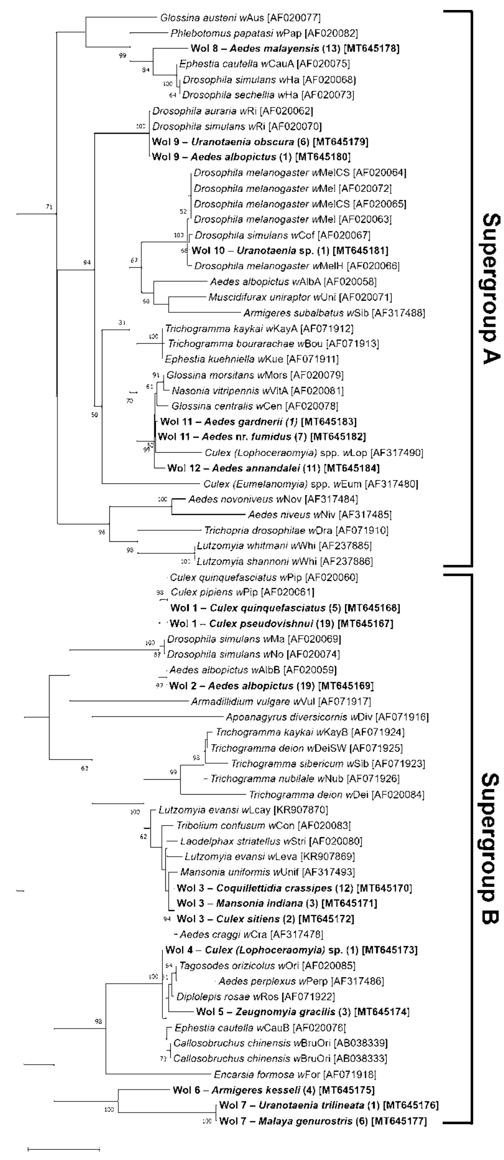

Fig. 2 *Wolbachia* neighbour-joining (NJ) tree constructed with the *Wolbachia* surface protein gene (*wsp*). All analysed sequences generated from this study (*bold*) were broadly classified into *Wolbachia* supergroups A or B and clustered into 12 putative strains ('Wol 1'–'Wol 12'). The number of sequences of each putative strain is indicated *within parentheses*. Also included are 54 sequences obtained from GenBank. Taxa are labelled as the host from which the *Wolbachia* strain was isolated, followed by the strain name. The NJ tree was mid rooted due to a lack of appropriate outgroups [45]. Bootstrap probability (generated with 1000 replicates) higher than 50% is indicated on the tree. Genbank accession number of each sequence is indicated *within brackets*

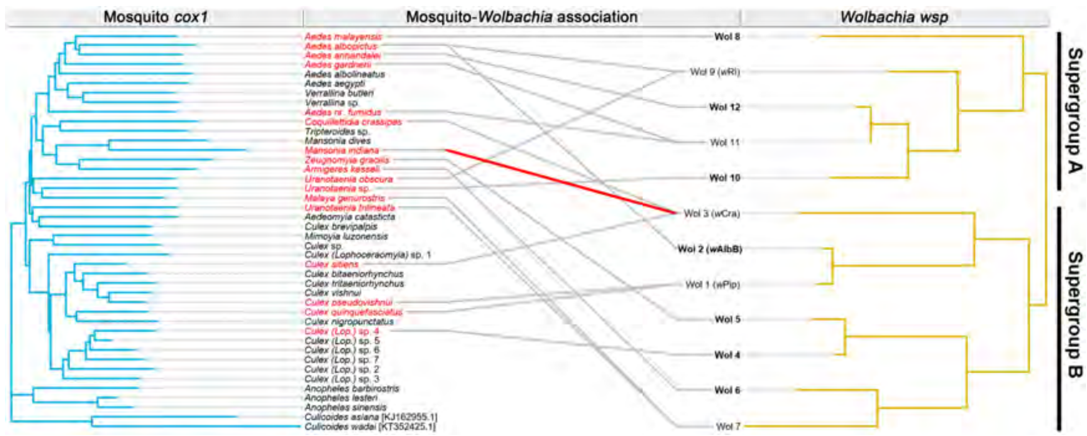

Fig. 3 Tanglegram of mosquito *cox*1 NJ tree compared to the *Wolbachia* endosymbiont NJ tree. Mosquito host species that harboured *Wolbachia* infection are indicated in *red*. Specialist *Wolbachia* strains are in *bold*. *Grey lines* represent the associations between hosts and endosymbionts. A *red line* indicates the host-endosymbiont association that was significant in the Global ParaFit test of congruence between host and endosymbiont phylogenies (Parafit Link test: ParaFit Link = 0.045, $P = 0.029$)

Table 2 Standardised phylogenetic host-specificity (*SPS*) scores of putative *Wolbachia* generalists

Putative *Wolbachia* strain	No. of infected hosts	Phylogenetic host-specificity score	SPS score	*P*-value
Wol 1	2	0.281	− 1.41	0.049*
Wol 3	3	0.391	− 0.162	0.421
Wol 7	2	0.281	0.068	0.779
Wol 9	2	0.281	− 0.234	0.249
Wol 11	2	0.281	− 0.817	0.157

* $P < 0.05$

host-endosymbiont links, only the association between *Mansonia indiana* and Wol 3 was statistically significant (ParaFit Link test: ParaFit Link = 0.045, $P = 0.029$) (Fig. 3).

The event-based analysis between mosquito and *Wolbachia* phylogenies resulted in a reconstructed output of one co-speciation event, three counts of duplication, seven counts of duplication with host shift, 29 losses, and six counts of failure to diverge, amounting to a total cost of 52 (Fig. 4). Interestingly, the number of duplications with a host shift and losses was much greater than co-speciation events. Notably, multiple host shift events tend to follow after loss events occurring earlier in the evolutionary history of the endosymbiont. For example, we see instances of consecutive host shifts to new hosts that were not previously infected (Fig. 4, red arrows). Additionally, based on random tip mapping, 14% of the random solutions had lower costs than the reconstructed output. Overall, there was support for multiple host shift events and losses of *Wolbachia* among the mosquitoes, and no clear signal for mosquito-*Wolbachia* cophylogeny.

Discussion

Detection of *Wolbachia* infection and distribution in wild mosquitoes

In this study, the PCR-based *Wolbachia* screening method had a high positive detection rate with 86.3% of all sequenced amplicons having successful BLAST matches to *Wolbachia*. This suggests that the conventional PCR method used is adequate for *Wolbachia* detection. Even if the study had been carried out without the additional DNA sequencing step, observed amplicon bands would likely have indicated true positives.

Our results indicate that *Wolbachia* are widespread across members of the family Culicidae. To our knowledge, *Wolbachia* infections have not been previously reported in seven of the mosquito species collected in this study. Overall, the percentage infection of screened individuals was 43.9%, which was largely congruent with percentages reported in past studies from the Oriental region, i.e. 31% infection in Malaysia [81], 26.4% in Sri Lanka [39], and 61.6% in Thailand [82]. At the species level, previous studies reported *Wolbachia* infection in 40% of all tested mosquito species in India [83], 18.2% in Sri Lanka [39], 51.7% in Taiwan [84], and between 28.1% and 37.8% in Thailand [82, 85]. Our study showed that 51.2% of all tested species were infected with *Wolbachia*,

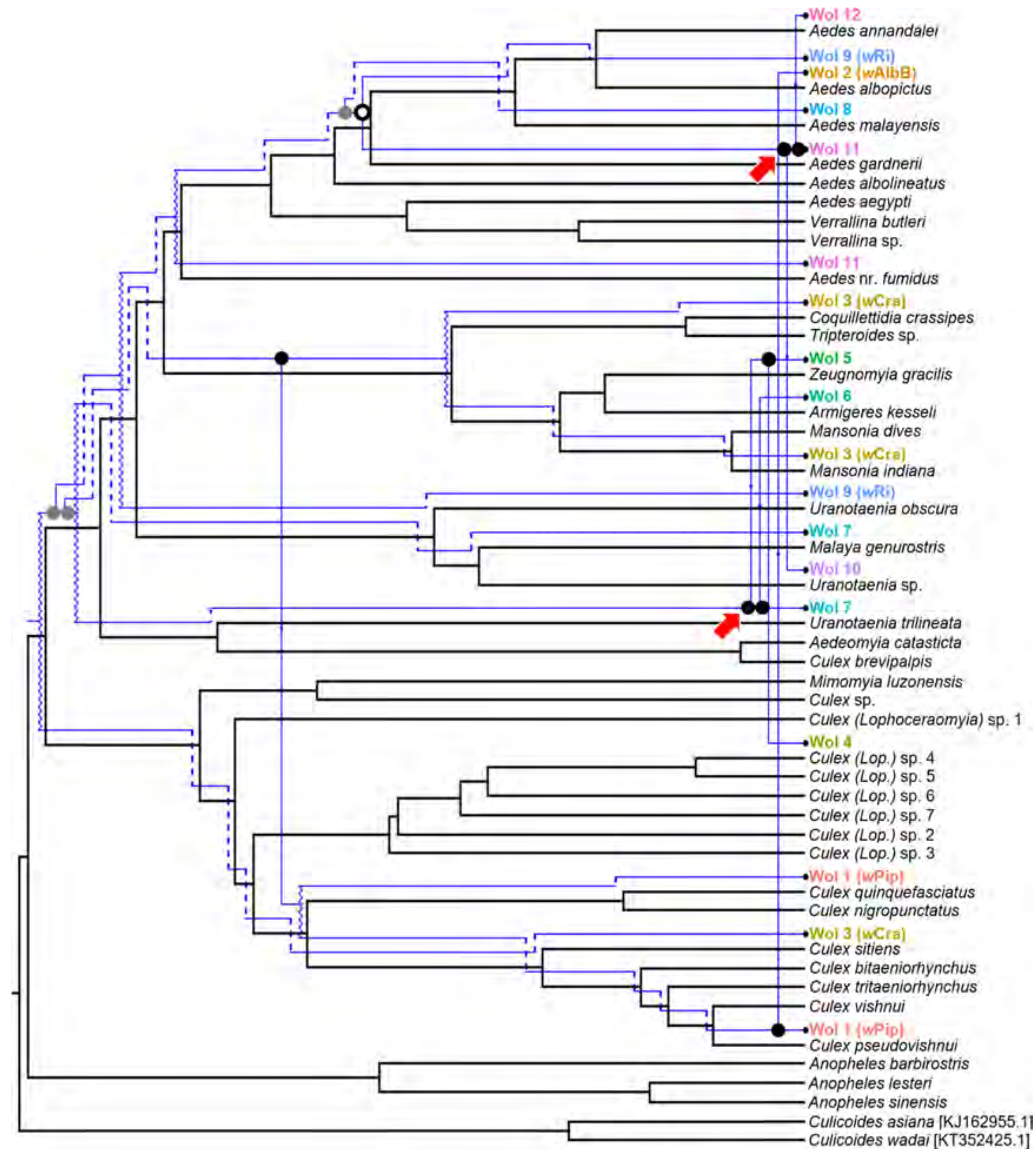

Fig. 4 Least-cost evolutionary reconstruction between mosquito (*black*) and *Wolbachia* (*blue*) phylogenies achieved using Jane 4.0. In total, one co-speciation event (*open circle*), three counts of duplication (*grey dot*), seven counts of duplication with host shift (*black dot* with an *arrow* pointing outwards), 29 losses (*dotted line*), and six counts of failure to diverge (*squiggly line*) were mapped out. *Red arrows* indicate periods where multiple host shifts occurred in succession

which is generally higher than the percentage reported in most studies. This was likely due to the broad range of species tested, including those from the genera *Malaya*, *Verrallina*, and *Zeugnomyia* [85]. It is also possible that infection prevalence may vary across geographical regions.

Wolbachia detection in three medically important mosquito genera, *Culex*, *Anopheles*, and *Aedes*, was highly consistent with that of past studies. These genera are responsible for the transmission of vector-borne diseases such as filariasis, malaria and arboviral diseases [86]. Within the genus *Culex*, *Wolbachia* infection has been reported to be variable across its member species [39, 46, 82, 84]. In this study, infections were observed only in five out of 16 *Culex* species. We noticed moderately high *Wolbachia* infection in *Cx. quinquefasciatus* (62.5%), which is a member of the *Culex pipiens* complex responsible for the transmission of filariasis in Singapore [86, 87]. Surprisingly, no *Wolbachia* infection was observed in *Cx. vishnui*–which has been found to harbour Japanese encephalitis virus in Southeast Asia [89]–although it is closely related to *Cx. pseudovishnui* [88] in which the rate of *Wolbachia* infection was high. However, studies in India and Thailand showed a reverse pattern, with *Wolbachia* infection present in *Cx. vishnui* but not in *Cx. pseudovishnui* [39, 85]. As the two species are morphologically similar [53], DNA barcoding was conducted to aid morphological identification, and thus avoid any misidentification. The results lend further support to possible variation in infection prevalence between geographically distant populations.

We did not detect *Wolbachia* in any of the wild-caught *Anopheles* species (18 individuals representing three species), many of which are potential malaria vectors [86]. This is largely consistent with previous reports from different countries [39, 90, 91]. The absence of *Wolbachia* in *Anopheles* mosquitoes is thought to be due to the unsuitability of *Anopheles* reproductive tissues for *Wolbachia* establishment [84, 85]. However, there have been recent reports of *Wolbachia* detected in wild *Anopheles* mosquitoes from West Africa [42, 92, 93] and Malaysia [94]. Knowledge of natural *Wolbachia* infections in *Anopheles* mosquitoes is important for malaria control strategies [93], hence more wild-caught *Anopheles* samples should be screened in Singapore to determine more accurately their infection status.

Wolbachia were not detected in *Ae. aegypti*, the primary vector of dengue in Southeast Asia [87]. Conversely, *Wolbachia* infection was moderately high in the secondary vector *Ae. albopictus*. These results are highly consistent with those of past studies, which reported an absence of infection in wild *Ae. aegypti* [21, 95], but found stable infection in wild *Ae. albopictus* [96]. Although *Ae. aegypti* and *Ae. albopictus* belong to the same subgenus, *Stegomyia*, and occupy similar ecological niches [97], they are rarely found in the same locality, [43, 98, 99], as also observed in this study. This could imply a certain degree of competitive exclusion between the two species, preventing them from occupying the same space. There is evidence that symbionts may influence a host's resource acquisition and specificity, which may ultimately lead to competitive exclusion between closely related host species with differing symbiont infections [100, 101]. However, research on *Wolbachia*-induced competitive exclusion is scarce except for a few studies on heterogonic gall wasps [102], grasshoppers [103], and gall-inducing aphids [104]. Given the widespread influence of *Wolbachia*, future research should explore potential cases of *Wolbachia*-induced competitive exclusion between closely related species of mosquitoes as this may have major implications for an understanding of their symbioses and speciation.

Additionally, although *Ae. aegypti* is frequently artificially infected with *Wolbachia* for biocontrol purposes [105–109], our findings suggest that infected *Ae. aegypti* might not be stably maintained in the wild. This may be advantageous for vector population suppression as the cytoplasmic-incompatibility effect of any artificially introduced *Wolbachia* strain will likely be fully manifested in the uninfected native population [21]. However, this also implies that this type of biocontrol method may have low long-term effectiveness if the infection cannot be naturally sustained in the wild population. The detection of natural *Wolbachia* infection in wild *Ae. aegypti*, therefore, has huge implications for vector control programmes [21]. Not only does it inform the selection of a suitable *Wolbachia* strain prior to its field release, but it can also be used to gauge the long-term effectiveness of a specific vector control programme.

Interestingly, the sex of the mosquitoes had an effect on their *Wolbachia* infection status. This could be an artefact of various *Wolbachia*-induced reproductive phenotypes, such as parthenogenetic and male-killing ones, resulting in offspring that are largely female [15]. If this were true, over multiple generations with vertical *Wolbachia* transmission, one should observe an increasing proportion of females that are infected. Hence, the phenomenon observed here could be a consequence of reproductive manipulation by *Wolbachia* and vertical transmission.

While we were unable to statistically test for the effects of locality on infection status due to uneven and small sample sizes of the respective species across different localities, our results suggest that mosquitoes found in localities across Singapore have roughly equal chances of harbouring *Wolbachia*. This also suggests that underlying physiological factors and phylogenetic relatedness in mosquitoes contribute more to their infection by *Wolbachia* than the habitat in which they are found.

The reproductive effect of *Wolbachia* can be masked or enhanced by other reproductive endosymbionts such as *Cardinium*, *Rickettsia*, and *Spiroplasma* [7, 26–29]. Unfortunately, we were unable to detect these endosymbionts due to a high degree of false positives with the PCR-based screening methods used here (Additional file 1). This was likely due to using primers that are not optimised for screening mosquito-specific endosymbionts [110–112]. As a result, co-infections with various reproductive endosymbionts, which would have provided greater insights into the synergistic effects of co-infections on mosquito evolution, could not be identified among the wild mosquitoes examined here. There is, hence, a need to develop and optimise alternative screening methods, such as multilocus sequence typing (MLST) techniques, especially for the detection of *Cardinium*, *Rickettsia*, and *Spiroplasma* in mosquitoes.

Tissue tropism of *Wolbachia* infection in mosquitoes

Wolbachia were detected mainly in the reproductive tissues, which agrees with results from studies across multiple insect groups [15, 84, 113], and suggests that *Wolbachia* are mainly vertically transmitted. Interestingly, through the course of this study, there was significant variation in reproductive traits (testis and ovary length) across and within species. These reproductive traits did not vary significantly with *Wolbachia* infection status, even after accounting for phylogenetic relatedness (see Additional file 2).

Infection in the gut and leg tissues was detected, albeit infrequently. This is not surprising, as previous studies have also detected *Wolbachia* in those tissues [34–36, 114]. Interestingly, the nucleotide sequences from gut and leg infections tend to be shorter in length. Considering that *Wolbachia* are unlikely to survive extracellularly for a long period of time [35], the small amplicon size suggests potential horizontal integration of the *Wolbachia* genome into the host genome for a few species. This phenomenon has been observed in several *Wolbachia* hosts [115, 116], and mosquito species such as *Ae. aegypti* and *Cx. quinquefasciatus* [117, 118]. A recent study showed that horizontal integration of the *Wolbachia* genome into the host genome can have implications for sex determination and evolution. This is evident in the common pillbug *Armadillidium vulgare*, and results in the formation of a new sex chromosome [119]. Researchers have also proposed that horizontal gene transfer between an endosymbiont and host can result in evolutionary innovation where new functional genes arise in both host and bacteria [117, 118].

Future research should explore the relative importance of each transmission method with relation to host-endosymbiont ecology and evolution. Tissue-specific screening methods such as those used here can be used in other arthropods, especially when the mode of transmission is not clear. Currently, most *Wolbachia* screening is conducted on ground specimens or specimens in their entirety [39–41]. In these cases, researchers are unable to determine tissue tropism of *Wolbachia* infection, which could provide clues to its mode of transmission. Thus, adopting tissue-specific screening methods would enable researchers to verify or refute the commonly reported assumption that *Wolbachia* is transmitted vertically [15, 30].

Diversity and host-specificity of *Wolbachia* strains

Not only does the *wsp* molecular marker allow successful detection of *Wolbachia* infection across numerous taxa, it also enables strain genotyping and evolutionary comparison between detected *Wolbachia* strains [60]. In this study, *Wolbachia wsp* sequences were clustered into 12 putative *Wolbachia* strains falling within supergroup A or B. This is consistent with the results of previous studies that looked at *Wolbachia* infections in mosquitoes [39, 80, 85]. Each mosquito host species was only infected by strains belonging to supergroups A or B, with the exception of *Ae. albopictus*, which harboured both. Infection with more than one strain (superinfection of wild *Ae. albopictus* with *Wolbachia* supergroups A and B) has been previously reported, and this phenomenon was commonly observed to be fixed in the examined populations due to strong cytoplasmic incompatibility effects [120, 121]. This suggests stable vertical transmission of both strains in *Ae. albopictus*. Additionally, only four out of 12 putative strains were identified as previously typed *Wolbachia* strains reported by Zhou et al. [60] and Ruang-Areerate et al. [80]—Wol 1, Wol 2, Wol 3, and Wol 8 were identified as *w*Pip, *w*AlbB, *w*Cra, and *w*Ri strain, respectively.

Host specificity is thought to be a characteristic of the ancestral *Wolbachia* strain, with host flexibility reported mainly in *Wolbachia* supergroups A and B [122]. In our study, we found a combination of specialists and generalists, with more of the former. A study of mosquitoes from Taiwan showed a similar pattern [84]. In beetles, a mixture of *Wolbachia* supergroup A host-specific and host-flexible strains within a population has also been reported [49]. While our estimates of specialists and generalists might vary with greater sampling effort, the higher numbers of specialists observed can be explained by the process of reciprocal selection between host and endosymbiont over evolutionary time [123]. This is also known as Red Queen dynamics, where the endosymbiont constantly adapts to its host to ensure continued establishment in the same host [124]. An alternative, generalist strategy can also be maintained in a population. It ensures survival in an environment where resources (i.e. hosts) are rarely found [123]. However, there are generally more instances of host specialists than generalists across numerous parasitic and endosymbiotic taxa [125–127].

The SPS scores revealed that host flexibility among the generalists varied greatly. Understanding *Wolbachia* host specificity has huge implications, especially for the optimisation of *Wolbachia* biocontrol strategies. Not only should researchers select strains that can effectively limit pathogen replication [128], they should also select strains for their host specificity. This is not possible without the screening of a wide variety of species or closely related species, which was achieved in this study. Using a host-specific strain will decrease the likelihood of host shift to non-target species, and thereby minimise the overall ecological risk of a strategy.

Evolutionary relationships between mosquitoes and *Wolbachia*

Host-*Wolbachia* relationships are often understudied and limited to a few taxa [52]. Studies have shown that the evolutionary associations between *Wolbachia* and their insect hosts do vary across taxa [49–52, 129]. Likewise, our exploratory analyses of mosquito hosts and their *Wolbachia* infection support such a complex relationship, with neither co-speciation nor host shifting fully accounting for evolutionary association in these lineages.

Based on the tanglegram, a broad association pattern between mosquitoes and *Wolbachia* strains was observed (Fig. 3). *Aedes* mosquitoes tended to be associated with *Wolbachia* supergroup A, while other mosquito species, particularly of the genus *Culex*, were largely associated with *Wolbachia* supergroup B. This showed that closely related *Wolbachia* strains are likely to establish themselves in related hosts. There might have been radiation of *Wolbachia* in these clades after their respective initial establishment. Nevertheless, the observed variations in host-endosymbiont associations make us question the mosquito-*Wolbachia* association pattern.

The ParaFit analysis showed weak support for congruency between host and endosymbiont phylogenies. Among the 18 host-*Wolbachia* associations, only the link between *Mansonia indiana* and Wol 3 showed a significant association (Fig. 3). This was interesting considering that Wol 3 was largely host flexible. Given that this was the only significant association, it is worth carrying out further genus-specific study on *Mansonia* spp. to elucidate coevolutionary patterns within a group of closely related mosquito species. It is possible that the degree to which *Wolbachia* co-evolve with their mosquito hosts varies across different taxonomic levels [74]. The analyses carried out thus far suggest that mosquito-*Wolbachia* associations are likely random at higher taxonomic levels, and that mosquito-*Wolbachia* co-speciation occurs at finer phylogenetic resolution (i.e. similar to patterns seen in diffuse coevolution).

The event-based analysis performed in Jane 4.0 (Fig. 4) indicated that co-speciation events were infrequent as compared to other evolutionary events. We noticed a greater proportion of host shifts and numerous losses. Interestingly, the least cost coevolutionary reconstruction indicated multiple consecutive host shifts occurring near the tips of the cladogram. This suggests that co-speciation does not fully explain the evolutionary associations between mosquito hosts and *Wolbachia*. Instead, recent host shifting through horizontal transmission seems to promote *Wolbachia* diversification. This lends greater support to the idea that horizontal transmission between distantly related species is possible [32, 33, 130].

Furthermore, losses, which represent endosymbiont extinction events that occurred upon host speciation, seem to dominate the evolutionary history of *Wolbachia*. Extinction events are believed to be frequent in host-endosymbiont systems [123], due to either evolution of resistance in the host or declining host population size, which result in the inability of highly specialised endosymbionts to establish themselves [131, 132]. Additionally, losses could potentially influence endosymbiont evolution through the creation of vacant niches [131]. The observed losses followed by host shifts in the mosquito-*Wolbachia* relationship are possible consequences of vacant niche exploitation by generalists. Perhaps this enabled successful endosymbiont invasion due to minimal intra-strain competition. If this were true, horizontal *Wolbachia* transmission and losses may play a bigger role in accounting for *Wolbachia* diversity than previously thought.

As this was an exploratory study, we were unable to determine the exact mechanism behind the diversity and evolutionary associations of *Wolbachia*. The presence of numerous specialists could be a sign of mosquito-*Wolbachia* coevolution since coevolution is fundamentally reciprocal selection between host and endosymbiont which gives rise to micro-evolutionary changes [133]. The numerous host shifts and losses might have, however, blurred the effects of vertical transmission over a long evolutionary period [52]. Thus, co-speciation might have occurred within smaller clades of *Wolbachia* and mosquitoes, but at higher taxa levels, horizontal transmission and loss events are more likely the prominent force driving *Wolbachia* evolution.

Strengths, limitations, and future directions

The three distinct methods employed here to explore evolutionary associations have both strengths and limitations. The tanglegram allows for clear visualisation of host-endosymbiont association without taking into account any evolutionary relationships, but there have been calls for careful interpretation of the results generated using this method as the degree of entanglement may not necessarily represent phylogenetic congruence [134]. The Global ParaFit test seeks to address this limitation by testing for global congruency with an unbiased, statistical approach [74]. The event-based method enables the evaluation of potential evolutionary events that might have occurred throughout an endosymbiont's evolutionary history such as co-speciation, duplication, and host shifting. This last method, however, cannot fully differentiate a topological congruence from an evolutionary event [135]. Without knowledge of the time of divergence for both symbiont and host, a co-phylogenetic pattern may be better explained by ecological factors (as compared to co-speciation) given that bacterial lineages often evolve faster than their hosts [136, 137], and the high likelihood of host shifts among closely related species [133].

The *Wolbachia wsp* gene has been shown to provide well-resolved phylogenies [60], and this study provides an exploratory snapshot of the evolutionary associations between mosquito hosts and their *Wolbachia* endosymbionts. There is, of course, a potential caveat, since only a single gene was used to construct the respective phylogenetic trees. To obtain a more accurate phylogeny, future studies could adopt MLST [17, 51], or whole-genome shotgun sequencing [52]. The former could potentially characterise putative *Wolbachia* strains that cannot be distinguished with *wsp* gene primers.

Notwithstanding their limitations, the employment of various analytical methods allows for a comprehensive examination of the evolutionary associations between *Wolbachia* and mosquito hosts which are presently lacking in the literature. The scope of future studies that examine the evolution of medically important vector species could be narrowed to the Aedini tribe, as this would provide greater statistical power for the examination of mosquito-endosymbiont associations.

Conclusion

To our knowledge, this is the first study to examine *Wolbachia* infections in wild mosquitoes in Singapore. We detected 12 putative strains of *Wolbachia* among 40 mosquito species, and recorded infections in seven species for which, to our knowledge, *Wolbachia* infections have not been previously reported. By employing a tissue-specific PCR screening method, we were able to observe that the *Wolbachia* infections were preferentially located in the reproductive tissues, which provides support for vertical transmission as the main mode of infection transmission. However, even if *Wolbachia* infection is mainly transmitted vertically, this is unlikely to fully explain the observed diversity of *Wolbachia* and why closely related *Wolbachia* lineages were found in distantly related mosquito species. Hence, this study also served as an exploratory study which examined mosquito-*Wolbachia* evolutionary associations across a wide range of host mosquito species through three evolutionary analyses. Overall, we propose that the evolutionary associations between mosquito hosts and *Wolbachia* are consequences of both vertical and horizontal transmission and various evolutionary events.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13071-020-04466-8.

Additional file 1: Table S1. Polymerase chain reaction (PCR) screening of *Cardinium*, *Rickettsia*, and *Spiroplasma* in wild mosquitoes from Singapore.

Additional file 2: Figure S1. Weighted reproductive tissue length across various mosquito species.

Abbreviations

BLAST: Basic Local Alignment Search Tool; *cox*1: Cytochrome c oxidase subunit I gene; MLST: Multilocus sequence typing; mtDNA: Mitochondrial DNA; NJ: Neighbour joining; PCR: Polymerase chain reaction; SPS: Standardised phylogenetic host specificity; *wsp*: *Wolbachia* surface protein gene.

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Authors' contributions

HY and NP designed the research. HD and HY collected the mosquitoes from the field. HY identified the mosquito samples. HD performed the DNA extraction and PCR. HD and HY carried out the sequence analyses. HD, HY, and NP interpreted the results and wrote the manuscript. All the authors read and approved the final draft of the manuscript.

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Availability of data and materials

The datasets generated and/or analysed during this study are available in the Dryad repository, https://doi.org/10.5061/dryad.zs7h44j63. Sequence data that support the findings of this study have been deposited in Genbank with the accession codes MT645167–MT645184.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Weeks AR, Reynolds KT, Hoffmann AA, Tracy K, Ary R, Hoffmann A. *Wolbachia* dynamics and host effects: what has (and has not) been demonstrated? Trends Ecol Evol. 2002;17:257–62.
2. Duron O, Bouchon D, Boutin S, Bellamy L, Zhou L, Engelstädter J, et al. The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. BMC Biol. 2008;6:1–12.
3. Zug R, Hammerstein P. Still a host of hosts for *Wolbachia*: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. PLoS ONE. 2012;7:7–9.
4. Zchori-Fein E, Borad C, Harari AR. Oogenesis in the date stone beetle, *Coccotrypes dactyliperda*, depends on symbiotic bacteria. Physiol Entomol. 2006;31:164–9.
5. Moran NA, Mccutcheon JP, Nakabachi A. Genomics and evolution of heritable bacterial symbionts. Annu Rev Genet. 2008;42:165–90.
6. Fenn K, Blaxter M. Are filarial nematode *Wolbachia* obligate mutualist symbionts? Trends Ecol Evol. 2004;19:163–6.
7. Zchori-Fein E, Perlman SJ. Distribution of the bacterial symbiont *Cardinium* in arthropods. Mol Ecol. 2004;13:2009–16.
8. Weeks AR, Turelli M, Harcombe WR, Reynolds KT, Hoffmann AA. From parasite to mutualist: rapid evolution of *Wolbachia* in natural populations of *Drosophila*. PLoS Biol. 2007;5:e114.
9. Jiggins FM, Hurst GDD, Majerus MEN. Sex ratio distortion in *Acraea encedon* (Lepidoptera: Nymphalidae) is caused by a male-killing bacterium. Heredity (Edinb). 1998;81:87–91.
10. Rousset F, Bouchon D, Pintureau B, Juchault P, Solignac M. *Wolbachia* endosymbionts responsible for various alterations of sexuality in arthropods. Proc R Soc B Biol Sci. 1992;250:91–8.
11. Richard FJ. Symbiotic bacteria influence the odor and mating preference of their hosts. Front Ecol Evol. 2017;5:143.
12. Weeks AR, Breeuwer JAJ. *Wolbachia*-induced parthenogenesis in a genus of phytophagous mites. Proc R Soc B Biol Sci. 2001;268:2245–51.
13. Ma WJ, Schwander T. Patterns and mechanisms in instances of endosymbiont-induced parthenogenesis. J Evol Biol. 2017;30:868–88.
14. Moretti R, Calvitti M. Male mating performance and cytoplasmic incompatibility in a *w*Pip *Wolbachia* trans-infected line of *Aedes albopictus* (*Stegomyia albopicta*). Med Vet Entomol. 2013;27:377–86.
15. Werren J, Baldo L, Clark ME. *Wolbachia*: master manipulators of invertebrate biology. Nat Rev Microbiol. 2008;6:741–51.
16. Tseng SP, Wetterer JK, Suarez AV, Lee CY, Yoshimura T, Shoemaker DW, et al. Genetic diversity and *Wolbachia* infection patterns in a globally distributed invasive ant. Front Genet. 2019;10:1–15.
17. Atyame CM, Delsuc F, Pasteur N, Weill M, Duron O. Diversification of *Wolbachia* endosymbiont in the *Culex pipiens* mosquito. Mol Biol Evol. 2011;28:2761–72.
18. Kajtoch Ł, Kotásková N. Current state of knowledge on *Wolbachia* infection among Coleoptera: a systematic review. PeerJ. 2018;6:e4471.
19. Bourtzis K, Dobson SL, Xi Z, Rasgon JL, Calvitti M, Moreira LA, et al. Harnessing mosquito–*Wolbachia* symbiosis for vector and disease control. Acta Trop. 2014;132:150–63.
20. Blagrove MSC, Arias-goeta C, Di GC, Failloux A, Sinkins SP. A *Wolbachia* *w*Mel transinfection in *Aedes albopictus* is not detrimental to host fitness and inhibits chikungunya virus. PLoS Negl Trop Dis. 2013;7:e2152.
21. Ross P. An elusive endosymbiont: does *Wolbachia* occur naturally in *Aedes aegypti*? Ecol Evol. 2020;10:1581–91.
22. Nguyen TH, Le NH, Nguyen TY, Vu SN, Tran ND, Le TN, et al. Field evaluation of the establishment potential of *w*melpop *Wolbachia* in Australia and Vietnam for dengue control. Parasites Vectors. 2015;8:1–14.
23. Nazni WA, Hoffmann AA, NoorAfizah A, Cheong YL, Mancini MV, Golding N, et al. Establishment of *Wolbachia* strain *w*AlbB in Malaysian populations of *Aedes aegypti* for dengue control. Curr Biol. 2019;29:4241–8.
24. National Environment Agency. *Wolbachia-Aedes* mosquito suppression strategy. 2018. https://www.nea.gov.sg/corporate-functions/resources/research/wolbachia-aedes-mosquito-suppression-strategy/frequently-asked-questions. Accessed 13 Sept 2020.
25. Iturbe-Ormaetxe I, Walker T, O'Neill SL. *Wolbachia* and the biological control of mosquito-borne disease. EMBO Rep. 2011;12:508–18.
26. Martin OY, Puniamoorthy N, Gubler A, Wimmer C, Bernasconi MV. Infections with *Wolbachia*, *Spiroplasma*, and *Rickettsia* in the Dolichopodidae and other Empidoidea. Infect Genet Evol. 2013;13:317–30.
27. White JA, Kelly SE, Cockburn SN, Perlman SJ, Hunter MS. Endosymbiont costs and benefits in a parasitoid infected with both *Wolbachia* and *Cardinium*. Heredity. 2011;106:585–91.
28. Zhang YK, Chen YT, Yang K, Qiao GX, Hong XY. Screening of spider mites (Acari: Tetranychidae) for reproductive endosymbionts reveals links between co-infection and evolutionary history. Sci Rep. 2016;6:1–9.
29. Engelstädter J, Telschow A, Yamamura N. Coexistence of cytoplasmic incompatibility and male-killing-inducing endosymbionts, and their impact on host gene flow. Theor Popul Biol. 2008;73:125–33.
30. Engelstädter J, Hurst GDD. The ecology and evolution of microbes that manipulate host reproduction. Annu Rev Ecol Evol Syst. 2009;40:127–49.
31. Heath BD, Butcher RDJ, Whitfield WGF, Hubbard SF. Horizontal transfer of *Wolbachia* between phylogenetically distant insect species by a naturally occurring mechanism. Curr Biol. 1999;9:313–6.
32. Ahmed MZ, Li S, Xue X, Yin X, Ren S. The intracellular bacterium *Wolbachia* uses parasitoid wasps as phoretic vectors for efficient horizontal transmission. PLoS Pathog. 2015;11:e1004672.
33. Li S, Ahmed MZ, Lv N, Shi P, Wang X, Huang J-L, et al. Plant-mediated horizontal transmission of *Wolbachia* between whiteflies. ISME J. 2017;11:1019–28.
34. Frost CL, Pollock SW, Smith JE, Hughes WOH. *Wolbachia* in the flesh: symbiont intensities in germ-line and somatic tissues challenge the conventional view of *Wolbachia* transmission routes. PLoS ONE. 2014;9:e95122.
35. Pietri JE, DeBruhl H, Sullivan W. The rich somatic life of *Wolbachia*. Microbiol Open. 2016;5:923–36.
36. Dobson SL, Bourtzis K, Braig HR, Jones BF, Zhou W, Rousset F, et al. *Wolbachia* infections are distributed throughout insect somatic and germ line tissues. Insect Biochem Mol Biol. 1999;29:153–60.
37. Espino CI, Gómez T, González G, Brazil Do Santos MF, Solano J, Sousa O, et al. Detection of *Wolbachia* bacteria in multiple organs and feces of the triatomine insect *Rhodnius pallescens* (Hemiptera, Reduviidae). Appl Environ Microbiol. 2009;75:547–50.
38. Andersen SB, Boye M, Nash DR, Boomsma JJ. Dynamic *Wolbachia* prevalence in *Acromyrmex* leaf-cutting ants: potential for a nutritional symbiosis. J Evol Biol. 2012;25:1340–50.
39. Nugapola NWNP, De Silva WAPP, Karunaratne SHPP. Distribution and phylogeny of *Wolbachia* strains in wild mosquito populations in Sri Lanka. Parasites Vectors. 2017;10:1–8.
40. Sunish IP, Rajendran R, Paramasivan R, Dhananjeyan KJ, Tyagi BK. *Wolbachia* endobacteria in a natural population of *Culex quinquefasciatus* from filariasis endemic villages of south India and its phylogenetic implication. Trop Biomed. 2011;28:569–76.
41. Thongsripong P, Chandler JA, Green AB, Kittayapong P, Wilcox BA, Kapan DD, et al. Mosquito vector-associated microbiota: metabarcoding bacteria and eukaryotic symbionts across habitat types in Thailand endemic for dengue and other arthropod-borne diseases. Ecol Evol. 2018;8:1352–68.
42. Niang EHA, Bassene H, Makoundou P, Fenollar F, Weill M, Mediannikov O. First report of natural *Wolbachia* infection in wild *Anopheles funestus* population in Senegal. Malar J. 2018;17:1–6.
43. Kulkarni A, Yu W, Jiang J, Sanchez C, Karna AK, Martinez KJL, et al. *Wolbachia pipientis* occurs in *Aedes aegypti* populations in New Mexico and Florida, USA. Ecol Evol. 2019;9:6148–56.
44. Leggewie M, Krumkamp R, Badusche M, Heitmann A, Jansen S, Schmidt-Chanasit J, et al. *Culex torrentium* mosquitoes from Germany are negative for *Wolbachia*. Med Vet Entomol. 2018;32:115–20.
45. Bozorg-Omid F, Oshaghi MA, Vahedi M, Karimian F, Seyyed-Zadeh SJ, Chavshin AR. *Wolbachia* infection in West Nile Virus vectors of northwest Iran. Appl Entomol Zool. 2020;55:105–13.
46. Jeffries CL, Tantely LM, Raharimalala FN, Hurn E, Boyer S, Walker T. Diverse novel resident *Wolbachia* strains in culicine mosquitoes from Madagascar. Sci Rep. 2018;8:1–15.
47. Shaikevich E, Bogacheva A, Rakova V, Ganushkina L, Ilinsky Y. *Wolbachia* symbionts in mosquitoes: intra- and intersupergroup recombinations,

horizontal transmission and evolution. Mol Phylogenet Evol. 2019;134:24–34.
48. Hurst GDD, Jiggins FM. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. Proc R Soc B Biol Sci. 2005;272:1525–34.
49. Sontowski R, Bernhard D, Bleidorn C, Schlegel M, Gerth M. *Wolbachia* distribution in selected beetle taxa characterized by PCR screens and MLST data. Ecol Evol. 2015;5:4345–53.
50. Balvín O, Roth S, Talbot B, Reinhardt K. Co-speciation in bedbug *Wolbachia* parallel the pattern in nematode hosts. Sci Rep. 2018;8:1–9.
51. Lefoulon E, Bain O, Makepeace BL, D'Haese C, Uni S, Martin C, et al. Breakdown of coevolution between symbiotic bacteria *Wolbachia* and their filarial hosts. PeerJ. 2016;4:e1840.
52. Gerth M, Röthe J, Bleidorn C. Tracing horizontal *Wolbachia* movements among bees (Anthophila): a combined approach using multilocus sequence typing data and host phylogeny. Mol Ecol. 2013;22:6149–62.
53. Chan A, Chiang L-P, Hapuarachchi HC, Tan C-H, Pang S-C, Lee R, et al. DNA barcoding: complementing morphological identification of mosquito species in Singapore. Parasites Vectors. 2014;7:1–12.
54. Rattanarithikul R, Harbach RE, Harrison BA, Panthusiri P, Coleman RE, Richardson JH. Illustrated keys to the mosquitoes of Thailand. VI. Tribe Aedini. Southeast Asian J Trop Med Public Health. 2010;41:1–225.
55. Rattanarithikul R, Harbach RE, Harrison BA, Panthusiri P, Coleman RE. Illustrated keys to the mosquitoes of Thailand. V. Genera *Orthopodomyia*, *Kimia*, *Malaya*, *Topomyia*, *Tripteroides*, and *Toxorhynchites*. Southeast Asian J Trop Med Public Health. 2007;38:1.
56. Rattanarithikul R, Harrison BA, Panthusiri P, Peyton EL, Coleman RE. Illustrated keys to the mosquitoes of Thailand. III. Genera *Aedeomyia*, *Ficalbia*, *Mimomyia*, *Hodgesia*, *Coquillettidia*, *Mansonia*, and *Uranotaenia*. Southeast Asian J Trop Med Public Health. 2006;37:1–85.
57. Rattanarithikul R, Harrison BA, Panthusiri P, Coleman RE. Illustrated keys to the mosquitoes of Thailand. I. Background; geographic distribution; lists of genera, subgenera, and species; and a key to the genera. Southeast Asian J Trop Med Public Health. 2005;36:1–80.
58. Rattanarithikul R, Harbach RE, Harrison BA, Panthusiri P, Jones JW. Illustrated keys to the mosquitoes of Thailand. II. Genera *Culex* and *Lutzia*. Southeast Asian J Trop Med Public Health. 2005;36:1–97.
59. Rattanarithikul R, Harrison BA, Harbach RE, Panthusiri P, Coleman RE. Illustrated keys to the mosquitoes of Thailand. IV. *Anopheles*. Southeast Asian J Trop Med Public Health. 2006;37:1–128.
60. Zhou W, Rousset F, O'Neill S. Phylogeny and PCR-based classification of *Wolbachia* strains using *wsp* gene sequences. Proc R Soc Lond B. 1998;265:509–15.
61. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol. 1994;3:294–9.
62. Madden T. The BLAST sequence analysis tool. NCBI Handbook. 2nd Ed. Bethesda: National Center for Biotechnology Information (US); 2013.
63. R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. 2019.
64. Sweet L. Nonpar: a collection of nonparametric hypothesis tests. R package version 1.0.1. 2017. https://CRAN.R-project.org/package=nonpar. Accessed 23 Jun 2020.
65. Mangiafico S. Rcompanion: functions to support extension education program evaluation. R package version 2.3.7. 2019. https://CRAN.R-project.org/package=rcompanion. Accessed 23 Jun 2020.
66. James G, Witten D, Hastie T, Tibshirani R. ISLR: data for an introduction to statistical learning with applications in R. R package version 1.2. 2017. https://CRAN.R-project.org/package=ISLR. Accessed 23 Jun 2020.
67. Thompson JD, Higgins DG, Gibson TJ. ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994;22:4673–80.
68. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35:1547–9.
69. NCBI Resource Coordinators. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res. 2016;44:D7-19.
70. Poulin R, Krasnov BR, Mouillot D. Host specificity in phylogenetic and geographic space. Trends Parasitol. 2011;27:355–61.
71. Kembel SW, Cowan PD, Helmus M, Cornwell W, Morlon H, Ackerly D, et al. Picante: R tools for integrating phylogenies and ecology. Bioinformatics. 2010;26:1463–4.
72. Charleston M. TreeMap 3b. 2011. https://sites.google.com/site/cophylogeny. Accessed 23 Jun 2020.
73. Matsen FA, Billey SC, Kas A, Konvalinka M. Tanglegrams: a reduction tool for mathematical phylogenetics. IEEE/ACM Trans Comput Biol Bioinforma. 2018;15:343–9.
74. Legendre P, Desdevises Y, Bazin E. A statistical test for host-parasite coevolution. Syst Biol. 2002;51:217–34.
75. Balbuena JA, Míguez-Lozano R, Blasco-Costa I. PACo: a novel Procrustes application to cophylogenetic analysis. PLoS ONE. 2013;8:e61048.
76. Paradis E, Schliep K. Ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics. 2019;35:526–8.
77. Conow C, Fielder D, Ovadia Y, Libeskind-Hadas R. Jane: a new tool for the cophylogeny reconstruction problem. Algorithms Mol Biol. 2010;5:1–10.
78. Charleston M. Jungles: a new solution to the host/parasite phylogeny reconciliation problem. Math Biosci. 1998;149:191–223.
79. Li YM, Shivas RG, Cai L. Cryptic diversity in *Tranzscheliella* spp. (Ustilaginales) is driven by host switches. Sci Rep. 2017;7:43549.
80. Ruang-Areerate T, Kittayapong P, Baimai V, O'Neill SL. Molecular phylogeny of *Wolbachia* endosymbionts in Southeast Asian mosquitoes (Diptera: Culicidae) based on *wsp* gene sequences. J Med Entomol. 2003;40:1–5.
81. Noor-Shazleen-Husnie MM, Emelia O, Ahmad-Firdaus MS, Zainol-Ariffin P, Aishah-Hani A. Detection of *Wolbachia* in wild mosquito populations from selected areas in Peninsular Malaysia by loop-mediated isothermal amplification (LAMP) technique. Trop Biomed. 2018;35:330–46.
82. Wiwatanaratanabutr I. Geographic distribution of wolbachial infections in mosquitoes from Thailand. J Invertebr Pathol. 2013;114:337–40.
83. Ravikumar H, Ramachandraswamy N, Sampathkumar S, Prakash BM, Huchesh HC, Uday J, et al. A preliminary survey for *Wolbachia* and bacteriophage *WO* infections in Indian mosquitoes (Diptera: Culicidae). Trop Biomed. 2010;27:384–93.
84. Tsai K-H, Lien J-C, Huang C-G, Wu W-J, Chen W-J. Molecular (sub) grouping of endosymbiont *Wolbachia* infection among mosquitoes of Taiwan. J Med Entomol. 2004;41:677–83.
85. Kittayapong P, Baisley KJ, Baimai V, O'Neill SL. Distribution and diversity of *Wolbachia* infections in Southeast Asian mosquitoes (Diptera: Culicidae). J Med Entomol. 2000;37:340–5.
86. Lam-Phua SG, Yeo H, Lee RML, Chong CS, Png AB, Foo SY, et al. Mosquitoes (Diptera: Culicidae) of Singapore: updated checklist and new records. J Med Entomol. 2019;56:103–19.
87. Foster WA, Walker ED. Mosquitoes (Culicidae). In: Mullen G, Durden L, editors. Medical and Veterinary Entomology. New York: Academic Press; 2018. p. 261–325.
88. Yeo G, Wang Y, Chong SM, Humaidi M, Lim XF, Mailepessov D, et al. Characterization of fowlpox virus in chickens and bird-biting mosquitoes: a molecular approach to investigating avipoxvirus transmission. J Gen Virol. 2019;100:838–50.
89. Vythilingam I, Oda K, Chew TK, Mahadevan S, Vijayamalar B, Morita K, et al. Isolation of Japanese encephalitis virus from mosquitoes collected in Sabak Bernam, Selangor, Malaysia in 1992. J Am Mosq Control Assoc. 1995;11:94–8.
90. de Oliveira CD, Gonçalves DS, Baton LA, Shimabukuro PHF, Carvalho FD, Moreira LA. Broader prevalence of *Wolbachia* in insects including potential human disease vectors. Bull Entomol Res. 2015;105:305–15.
91. Shaikevich E, Bogacheva A, Ganushkina L. *Dirofilaria* and *Wolbachia* in mosquitoes (Diptera: Culicidae) in central European Russia and on the Black Sea coast. Parasite. 2019;26:1–12.
92. Baldini F, Segata N, Pompon J, Marcenac P, Robert Shaw W, Dabiré RK, et al. Evidence of natural *Wolbachia* infections in field populations of *Anopheles gambiae*. Nat Commun. 2014;5:1–7.
93. Gomes FM, Hixson BL, Tyner MDW, Ramirez JL, Canepa GE, Alves e Silva TL, et al. Effect of naturally occurring Wolbachia in *Anopheles gambiae* s.l. mosquitoes from Mali on *Plasmodium falciparum* malaria transmission. Proc Natl Acad Sci. 2017;114:12566–71.
94. Wong ML, Liew JWK, Wong WK, Pramasivan S, Mohamed Hassan N, Wan Sulaiman WY, et al. Natural *Wolbachia* infection in field-collected

Anopheles and other mosquito species from Malaysia. Parasites Vectors. 2020;13:1–15.
95. Gloria-Soria A, Chiodo TG, Powell JR. Lack of evidence for natural *Wolbachia* infections in *Aedes aegypti* (Diptera : Culicidae). J Med Entomol. 2018;55:1354–6.
96. Kittayapong P, Baimai V, O'Neill SL. Field prevalence of *Wolbachia* in the mosquito vector *Aedes albopictus*. Am J Trop Med Hyg. 2002;66:108–11.
97. Lounibos LP, Juliano SA. Where vectors collide: the importance of mechanisms shaping the realized niche for modeling ranges of invasive *Aedes* mosquitoes. Biol Invasions. 2018;20:1913–29.
98. Chan KL, Chan YC, Ho BC. *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Singapore city. 4. Competition between species. Bull World Health Organ. 1971;44:643–9.
99. Coon KL, Brown MR, Strand MR. Mosquitoes host communities of bacteria that are essential for development but vary greatly between local habitats. Mol Ecol. 2016;25:5806–26.
100. Brucker RM, Bordenstein SR. Speciation by symbiosis. Trends Ecol Evol. 2012;27:443–51.
101. Janson EM, Stireman JO, Singer MS, Abbot P. Phytophagous insect-microbe mutualisms and adaptive evolutionary diversification. Evol Int J Org Evol. 2008;62:997–1012.
102. Schuler H, Egan SP, Hood GR, Busbee RW, Driscoe AL, Ott JR. Diversity and distribution of *Wolbachia* in relation to geography, host plant affiliation and life cycle of a heterogonic gall wasp. BMC Evol Biol. 2018;18:1–15.
103. Martínez-Rodríguez P, Bella JL. *Chorthippus parallelus* and *Wolbachia*: overlapping orthopteroid and bacterial hybrid zones. Front Genet. 2018;9:604.
104. Amit L, Ben-Shlomo R, Chiel E. Are microbial symbionts involved in the speciation of the gall-inducing aphid, *Slavum wertheimae*? Arthropod Plant Interact. 2017;11:475–84.
105. Hancock PA, White VL, Callahan AG, Godfray CHJ, Hoffmann AA, Ritchie SA. Density-dependent population dynamics in *Aedes aegypti* slow the spread of *w*Mel *Wolbachia*. J Appl Ecol. 2016;53:785–93.
106. Walker T, Johnson PH, Moreira LA, Iturbe-Ormaetxe I, Frentiu FD, McMeniman CJ, et al. The *w*Mel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. Nature. 2011;476:450–5.
107. McMeniman CJ, Lane RV, Cass BN, Fong AWC, Sidhu M, Wang YF, et al. Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. Science. 2009;323:141–4.
108. Ant TH, Herd CS, Geoghegan V, Hoffmann AA, Sinkins SP. The *Wolbachia* strain *w*Au provides highly efficient virus transmission blocking in *Aedes aegypti*. PLoS Pathog. 2018;14:1–19.
109. Fraser JE, De Bruyne JT, Iturbe-Ormaetxe I, Stepnell J, Burns RL, Flores HA, et al. Novel *Wolbachia*-transinfected *Aedes aegypti* mosquitoes possess diverse fitness and vector competence phenotypes. PLoS Pathog. 2017;13:1–19.
110. Davis MJ, Ying Z, Brunner BR, Pantoja A, Ferwerda FH. Rickettsial relative associated with papaya bunchy top disease. Curr Microbiol. 1998;36:80–4.
111. Majerus TMO, Schulenburg JH, Majerus MEN, Hurst GDD. Molecular identification of a male-killing agent in the ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). Insect Mol Biol. 1999;8:551–5.
112. Weeks AR, Velten R, Stouthamer R. Incidence of a new sex-ratio-distorting endosymbiotic bacterium among arthropods. Proc R Soc Lond B. 2003;270:1857–65.
113. Goodacre SL, Martin OY. Modification of insect and arachnid behaviours by vertically transmitted endosymbionts: infections as drivers of behavioural change and evolutionary novelty. Insects. 2012;3:246–61.
114. Minard G, Mavingui P, Moro CV. Diversity and function of bacterial microbiota in the mosquito holobiont. Parasites Vectors. 2013;6:1–12.
115. McNulty SN, Abubucker S, Simon GM, Mitreva M, McNulty NP, Fischer K, et al. Transcriptomic and proteomic analyses of a *Wolbachia*-free filarial parasite provide evidence of trans-kingdom horizontal gene transfer. PLoS ONE. 2012;7:1–12.
116. Kondo N, Nikoh N, Ijichi N, Shimada M, Fukatsu T. Genome fragment of *Wolbachia* endosymbiont transferred to X chromosome of host insect. Proc Natl Acad Sci. 2002;99:14280–5.
117. Klasson L, Kambris Z, Cook PE, Walker T, Sinkins SP. Horizontal gene transfer between *Wolbachia* and the mosquito *Aedes aegypti*. BMC Genomics. 2009;10:1–9.
118. Woolfit M, Iturbe-Ormaetxe I, McGraw EA, O'Neill SL. An ancient horizontal gene transfer between mosquito and the endosymbiotic bacterium *Wolbachia pipientis*. Mol Biol Evol. 2009;26:367–74.
119. Leclercq S, Thézé J, Chebbi MA, Giraud I, Moumen B, Ernenwein L, et al. Birth of a W sex chromosome by horizontal transfer of *Wolbachia* bacterial symbiont genome. Proc Natl Acad Sci. 2016;113:15036–41.
120. Afizah AN, Roziah A, Nazni WA, Lee HL. Detection of *Wolbachia* from field collected *Aedes albopictus* Skuse in Malaysia. Indian J Med Res. 2015;142:205–10.
121. Kittayapong P, Baisley KJ, Sharpe RG, Baimai V, O'Neill SL. Maternal transmission efficiency of *Wolbachia* superinfections in *Aedes albopictus* populations in Thailand. Am J Trop Med Hyg. 2002;66:103–7.
122. Gerth M, Gansauge MT, Weigert A, Bleidorn C. Phylogenomic analyses uncover origin and spread of the *Wolbachia* pandemic. Nat Commun. 2014;5:1–7.
123. de Vienne DM, Refrégier G, López-Villavicencio M, Tellier A, Hood ME, Giraud T. Cospeciation vs. host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. New Phytol. 2013;198:347–85.
124. Price PW, Westoby M, Rice B, Atsatt PR, Fritz RS, Thompson JN, et al. Parasite mediation in ecological interactions. Annu Rev Ecol Syst. 1986;17:487–505.
125. Ikeda-Ohtsubo W, Brune A. Cospeciation of termite gut flagellates and their bacterial endosymbionts: *Trichonympha* species and "*Candidatus* Endomicrobium trichonymphae". Mol Ecol. 2009;18:332–42.
126. Drès M, Mallet J. Host races in plant-feeding insects and their importance in sympatric speciation. Philos Trans R Soc B Biol Sci. 2002;357:471–92.
127. Giraud T, Refrégier G, Le Gac M, de Vienne DM, Hood ME. Speciation in fungi. Fungal Genet Biol. 2008;45:791–802.
128. Bian G, Xu Y, Lu P, Xie Y, Xi Z. The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*. PLoS Pathog. 2010;6:e1000833.
129. Ahmed MZ, Breinholt JW, Kawahara AY. Evidence for common horizontal transmission of *Wolbachia* among butterflies and moths. BMC Evol Biol. 2016;16:1–16.
130. Le Clec'h W, Chevalier FD, Genty L, Bertaux J, Bouchon D, Sicard M. Cannibalism and predation as paths for horizontal passage of *Wolbachia* between terrestrial isopods. PLoS ONE. 2013;8:e60232.
131. Ricklefs RE. Evolutionary diversification, coevolution between populations and their antagonists, and the filling of niche space. Proc Natl Acad Sci. 2010;107:1265–72.
132. de Castro F, Bolker BM. Parasite establishment and host extinction in model communities. Oikos. 2005;111:501–13.
133. de Vienne DM, Giraud T, Shykoff JA. When can host shifts produce congruent host and parasite phylogenies? A simulation approach. J Evol Biol. 2007;20:1428–38.
134. de Vienne DM. Tanglegrams are misleading for visual evaluation of tree congruence. Mol Biol Evol. 2019;36:174–6.
135. Chen R, Wang Z, Chen J, Jiang LY, Qiao GX. Insect-bacteria parallel evolution in multiple-co-obligate-aphid association: a case in Lachninae (Hemiptera: Aphididae). Sci Rep. 2017;7:1–9.
136. Degnan PH, Lazarus AB, Brock CD, Wernegreen JJ. Host-symbiont stability and fast evolutionary rates in an ant-bacterium association: cospeciation of *Camponotus* species and their endosymbionts *Candidatus blochmannia*. Syst Biol. 2004;53:95–110.
137. Moran NA. Accelerated evolution and Muller's rachet in endosymbiotic bacteria. Proc Natl Acad Sci. 1996;93:2873–8.

Publisher's Note

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Opinion

Wolbachia Can Enhance *Plasmodium* Infection in Mosquitoes: Implications for Malaria Control?

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The symbiotic bacterium *Wolbachia* is an attractive agent for vector-borne pathogen control. It has long been studied for its ability to manipulate host reproduction and spread into arthropod populations [1]. These properties, coupled with the recently identified ability to inhibit diverse pathogens [2–6], open avenues for its use in controlling vector-borne disease. Numerous *Wolbachia*-based control strategies are being investigated (reviewed in [7–9]), with some studies having progressed to field trials [10,11]. However, a worrying trend is emerging whereby *Wolbachia* infections have been demonstrated to enhance rather than suppress pathogens in some systems [12–18]. *Plasmodium* parasites, which are the causal agent of malaria, seem particularly prone to *Wolbachia*-mediated pathogen enhancement [13–16].

Wolbachia-based strategies have been proposed to control malaria [19]. *Anopheles* mosquitoes (the vectors of human malaria parasites) are not naturally infected by *Wolbachia* [20,21], but artificial transfer of this bacterium between species can be accomplished in the laboratory (reviewed in [22]). Pathogen interference phenotypes appear to be most prominent when *Wolbachia* is transferred into a novel host [16,23]. Given that *Anopheles* are for the most part naturally uninfected by *Wolbachia* (but see [24]), they can be considered an open niche for infection and a prime mosquito genus for *Wolbachia*-based control strategies. However, the main impediment for developing a control strategy is the difficulty in creating a stable artificial infection in *Anopheles* [19]. While examining *Plasmodium* interference in a stably infected host is the gold standard, a more convenient system is to transiently infect mosquitoes by intrathoracic microinjection. Using this system, the infection persists during the lifetime of the transinfected individual but is not transmitted to its offspring. Transient infection allows the rapid assessment of *Wolbachia*-host interactions without the need for generating stable artificial infections [5]. It is uncertain how representative transient infections are of stable inherited associations; however, similarities in tissues tropism and fitness costs incurred upon the host between stable and transiently infected *Anopheles* mosquitoes are evident [5,14,25]. Furthermore, both types of infection have been shown to inhibit the human malaria parasite *Plasmodium falciparum* [5,25]. However, studies using transient infection models have found that *Wolbachia* can enhance certain *Plasmodium* species [13,14].

The *Plasmodium* interference phenotype is therefore not universal, but context dependent. While *P. falciparum* is suppressed by the *w*AlbB strain of *Wolbachia* from *Aedes albopictus* [5,25], transient infections have shown the opposite effect on rodent malaria parasites. *Anopheles gambiae* transiently infected with *w*AlbB exhibited enhanced *P. berghei* development at the oocyst stage [14]. Similarly, *w*AlbB increased the number of *P. yoelii* oocysts in *An. stephensi*, although the phenotype was modulated by temperature [13]. At a temperature optimal for parasite development, *Wolbachia* increased parasite intensity compared to uninfected controls, but at warmer temperatures, *Wolbachia* inhibited *Plasmodium* development [13].

While *P. falciparum* is a major parasite in sub-Saharan Africa, four other parasites also cause human malaria worldwide: *P. malariae*, *P. ovale*, *P. knowlesi*, and *P. vivax* (the etiological agent of the most prevalent form of relapsing malaria). To our knowledge, the effect of *Wolbachia* on these other human *Plasmodium* parasites is unknown. The question is relevant for two reasons. First, the precedent that a particular *Wolbachia* strain can inhibit one parasite yet enhance another has already been documented [5,14], indicating that effects on parasites can be species-specific. Troublingly, *P. malariae*, *P. ovale*, *P. knowlesi*, and *P. vivax* are phylogenetically more closely related to rodent malaria parasites, which are enhanced by *Wolbachia* infections [13,14], than they are to *P. falciparum* (Figure 1) [26,27]. Second, many human *Plasmodium* parasites occur in sympatry and are transmitted by the same vectors. A case in point is *P. falciparum* and *P. vivax*, both of which occur in sympatry over large stretches of the Asian continent where they are both transmitted by *An. stephensi* [28,29]. Any potential control strategy devised in regions where more than one parasite species occurs needs to thoroughly investigate the effect of *Wolbachia* on all parasite species transmitted by the vector, as well as other pathogens such as filarial worms or arboviruses (both as single infections and in the context of coinfections) to ensure that *Wolbachia*-infected mosquitoes do not inadvertently enhance transmission of secondary pathogens.

While difficult, forecasting the long-term evolutionary response in this tripartite relationship between *Wolbachia*, *Plasmodium*, and *Anopheles* is very important. Natural *Wolbachia*–mosquito associations in which the symbiont and the host have tightly coevolved exist and may provide powerful models for studying the long-term evolutionary effects of *Wolbachia*

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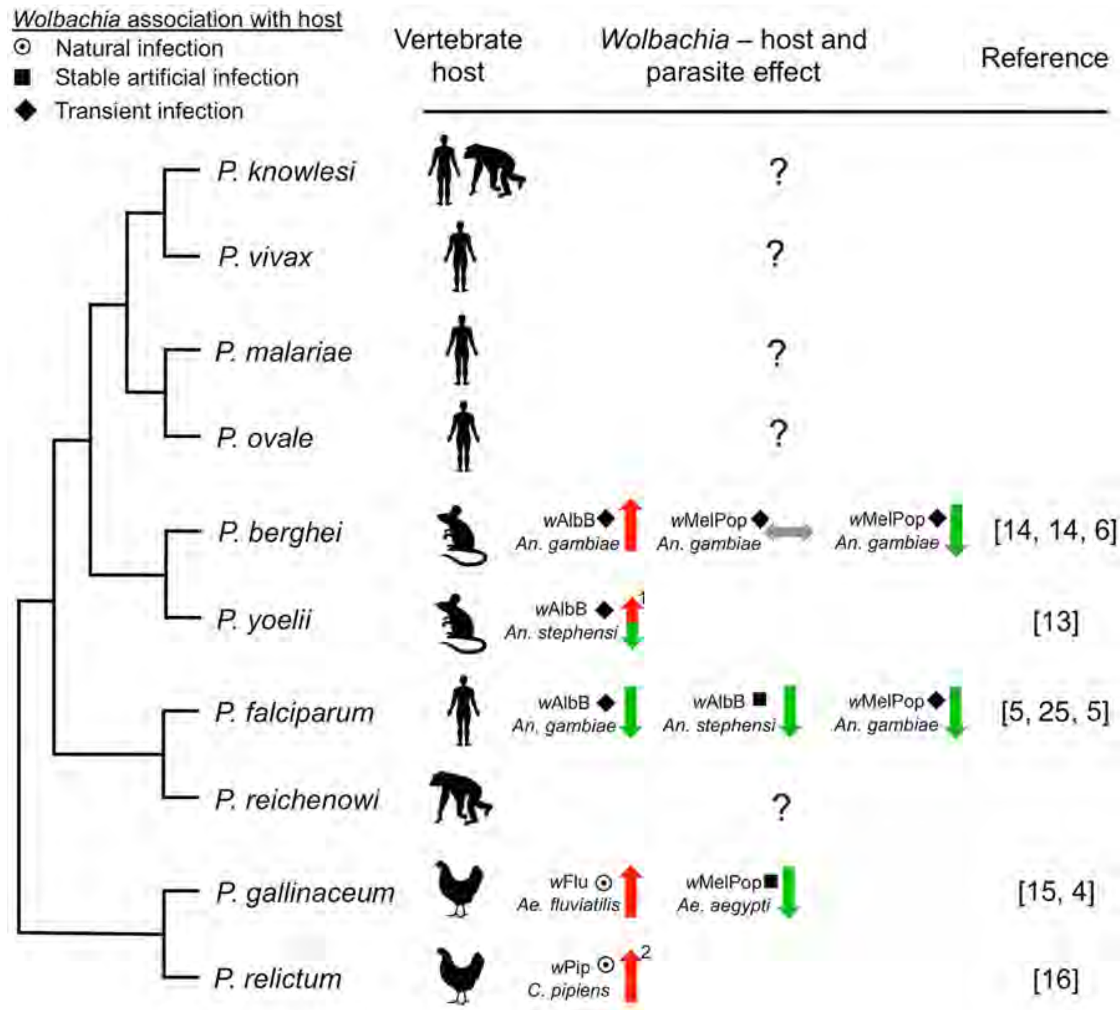

Figure 1. Representative phylogenetic dendrogram of *Plasmodium* parasites, their vertebrate hosts, and the influence of *Wolbachia* infection on parasite development within the mosquito vector. The protective effect of *Wolbachia* is variable and dependent on the *Wolbachia* strain and the insect host background, suggesting that complex tripartite interactions influence the effect on *Plasmodium*. The type of association between *Wolbachia* with the vector may also influence *Plasmodium*. Only one human malaria parasite (*P. falciparum*) has been assessed, while the effect of *Wolbachia* infection on the other four human parasites is unknown. Arrows indicate suppression (green), enhancement (red), or no effect (grey) of *Plasmodium*. The type of association within the host is depicted by symbols (target: natural infection, square: stable artificial infection, diamond: transient artificial infection). Numbers indicate: (1) the phenotype is temperature sensitive, (2) *Wolbachia* infection also increases insect life span [31], which has implications for pathogen transmission. Phylogeny was reconstructed based on work from Carlton et al. [26] and Martinsen et al. [27].
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infections. The evidence currently available suggests that natural *Wolbachia* infections can also enhance malaria parasite development within the mosquito. *Aedes fluviatilis* naturally infected with the *w*Flu *Wolbachia* strain had a significantly higher number of *P. gallinaceum* oocysts compared to an *Ae. fluviatilis* line which had been cleared of the *Wolbachia* infection [15]. *Ae. fluviatilis* is not, however, a natural vector of *P. gallinaceum*, and it is well known that the outcome of experiments using such laboratory models can differ significantly from those of natural mosquito–*Plasmodium* combinations (e.g., Boete [30]). Recent studies carried out in *Culex pipiens* mosquitoes, which are naturally infected with the *w*Pip *Wolbachia* strain and transmit the avian malaria parasite *P. relictum*, have also demonstrated *Plasmodium* enhancement. In this natural system, *Wolbachia* protects the mosquito host against the detrimental fitness effects incurred by *Plasmodium* infection [31] and increases the susceptibility of *C. pipiens* to *P. relictum*, with *w*Pip-infected mosquitoes having a higher prevalence of *Plasmodium* sporozoites in the salivary glands [16]. These studies show that the

Plasmodium-inhibiting properties of *Wolbachia* are far from universal; certain mosquito–*Wolbachia*–*Plasmodium* combinations and experimental conditions transform *Wolbachia*-infected mosquitoes into better vectors of malaria parasites. This is worrisome for the general implementation of *Wolbachia*-based control strategies.

Given that *Wolbachia*-based control strategies will use stable transinfected mosquitoes, the key question is whether stable and natural infections will behave in the same way. The stable transfer of *Wolbachia* into the host likely alters many aspects of host homeostasis, as evidenced by the novel phenotypes induced by infection [32–34], and as such, these associations likely differ from natural associations where *Wolbachia* and its host have coevolved. Another question is whether stable artificial infections will evolve over time. Theory and empirical studies show that these maternally transmitted bacteria will tend to evolve towards mutualistic associations with their host [35–38]. However, the evolutionary outcomes of pathogen interference or enhancement are harder to predict. A more complete mechanistic understanding of how *Wolbachia* infection modulates *Plasmodium* parasites is critical to address these important evolutionary questions and to evaluate if they are likely to occur in timescales relevant for disease control.

To date, two stable artificial *Wolbachia* transinfections have been assessed for their effect on *Plasmodium*. First, an *Aedes aegypti* line infected with *w*MelPop had inhibited *P. gallinaceum* infection [4]; *Ae. aegypti* is not, however, the natural vector of this parasite. Second, and more recently, the *w*AlbB strain was stably transferred into *An. stephensi*, one of the main vectors of human malaria in Asia [25]. This groundbreaking work demonstrated that stable artificial infections in epidemiologically relevant malaria vectors are feasible, and that *P. falciparum* can be inhibited by *Wolbachia* within its natural vector. If the severe fitness effects induced by *Wolbachia* in *Anopheles* can be overcome [25], then this approach is highly promising.

The work by Bian and colleagues [25] dramatically enhances the prospect for the use of *Wolbachia* in a malaria control strategy, but many questions still remain. What are the effects of *Wolbachia* on the other four species of *Plasmodium* parasites that infect humans? How relevant are transient infection models? Do some strains of *Wolbachia* enhance pathogens in a field context? What are the long-term evolutionary consequences of novel *Wolbachia*-host associations on *Plasmodium* development within the insect host? What are the mechanisms behind pathogen interference and enhancement of *Wolbachia* on *Plasmodium* parasites, and are the mechanisms of enhancement seen in rodent and avian model systems relevant to human malaria parasites? How influential are environmental variables on pathogen inhibition phenotypes? While many of these questions may be difficult to answer in the short term, assessing the relevance of transient infections would seem within the grasp of the scientific community. Although challenging, understanding the evolutionary consequences of novel *Wolbachia* associations on pathogen transmission and identifying the mechanisms behind *Wolbachia* modulation of *Plasmodium* is critical for developing effective control strategies and assessing their long-term feasibility. Insights from non-Anopheline systems where *Wolbachia* naturally infects the vector may be useful in this regard [16,31,39].

In conclusion, *Wolbachia*-based control of vector-borne pathogens is a promising novel strategy that has many advantages over other conventional and contemporary control methods. The development of a stable infection in *Anopheles* means the prospect of *Wolbachia*-based control of malaria can now be entertained [25], but many important questions need to be resolved before this idea can become a reality. While the concerns raised here focus on *Plasmodium*, these issues are relevant for *Wolbachia* control of any vector-borne pathogen [18]; we suggest that transinfected mosquitoes intended for release into nature should be assessed for inhibition (or lack thereof) of all relevant pathogens circulating in the system.

References

1. Werren JH, Baldo L, Clark ME (2008) *Wolbachia*: master manipulators of invertebrate biology. Nat Rev Microbiol 6: 741–751.
2. Hedges LM, Brownlie JC, O'Neill SL, Johnson KN (2008) *Wolbachia* and virus protection in insects. Science 322: 702.
3. Kambris Z, Cook PE, Phuc HK, Sinkins SP (2009) Immune activation by life-shortening Wolbachia and reduced filarial competence in mosquitoes. Science 326: 134–136.
4. Moreira LLA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AAT, et al. (2009) A *Wolbachia* symbiont in *Aedes aegypti* limits infection with Dengue, Chikungunya, and *Plasmodium*. Cell 139: 1268–1278.
5. Hughes GL, Koga R, Xue P, Fukastu T, Rasgon JL (2011) *Wolbachia* infections are virulent and inhibit the human malaria parasite *Plasmodium falciparum* in *Anopheles gambiae*. PLoS Pathog 7: e1002043.
6. Kambris Z, Blagborough AM, Pinto SB, Blagrove MSC, Godfray HCJ, et al. (2010) *Wolbachia* stimulates immune gene expression and inhibits *Plasmodium* development in *Anopheles gambiae*. PLoS Pathog 6: e1001143.
7. McGraw EA, O'Neill SL (2013) Beyond insecticides: new thinking on an ancient problem. Nat Rev Microbiol 11: 181–193.
8. Bourtzis K, Dobson SL, Xi Z, Rasgon JL, Calvitti M, et al. (2014) Harnessing mosquito-*Wolbachia* symbiosis for vector and disease control. Acta Trop 132S: S150–S163.
9. Iturbe-Ormaetxe I, Walker T, O' Neill SL (2011) *Wolbachia* and the biological control of mosquito-borne disease. EMBO Rep 12: 508–518.
10. Hoffmann AA, Montgomery BL, Popovici J, Iturbe-Ormaetxe I, Johnson PH, et al. (2011) Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. Nature 476: 454–457.
11. Walker T, Johnson PH, Moreira LA, Iturbe-Ormaetxe I, Frentiu FD, et al. (2011) The *w*Mel Wolbachia strain blocks dengue and invades caged *Aedes aegypti* populations. Nature 476: 450–453.
12. Graham RI, Grzywacz D, Mushobozi WL, Wilson K (2012) *Wolbachia* in a major African crop pest increases susceptibility to viral disease rather than protects. Ecol Lett 15: 993–1000.
13. Murdock CC, Blanford S, Hughes GL, Rasgon JL, Thomas MB (2013) Temperature alters malaria transmission blocking by *Wolbachia*. Sci Rep 4: 3932.
14. Hughes GL, Vega-Rodriguez J, Xue P, Rasgon JL (2012) *Wolbachia* strain *w*AlbB enhances infection by the rodent malaria parasite *Plasmodium* berghei in *Anopheles gambiae* mosquitoes. Appl Environ Microbiol 78: 1491–1495.
15. Baton LA, Pacidônio EC, Gonçalves DDS, Moreira LA (2013) *w*Flu: characterization and evaluation of a native *Wolbachia* from the mosquito *Aedes fluviatilis* as a potential vector control agent. PLoS ONE 8: e59619.
16. Zélé F, Nicot A, Berthomieu A, Weill M, Duron O, et al. (2013) *Wolbachia* increases susceptibility to *Plasmodium* infection in a natural system. Proc Biol Sci 281: 20132837.
17. Hussain M, Lu G, Torres S, Edmonds JH, Kay BH, et al. (2013) Effect of *Wolbachia* on replication of West Nile virus in a mosquito cell line and adult mosquitoes. J Virol 87: 851–858.
18. Dodson BL, Hughes GL, Paul O, Matacchiero AC, Kramer LD, et al. (2014) *Wolbachia* Enhances West Nile Virus (WNV) Infection in the Mosquito *Culex tarsalis*. PLoS Negl Trop Dis 8: e2965.
19. Walker T, Moreira LA (2011) Can *Wolbachia* be used to control malaria? Mem Inst Oswaldo Cruz 106 (Suppl. I): 212–217.
20. Ricci I, Cancrini G, Gabrielli S, D'Amelio S, Favi G (2002) Searching for *Wolbachia* (Rickettsiales: Rickettsiaceae) in mosquitoes (Diptera: Culicidae): large polymerase chain reaction survey and new identifications. J Med Entomol 39: 562–567.
21. Rasgon JL, Scott TW (2004) An initial survey for *Wolbachia* (Rickettsiales: Rickettsiaceae) infections in selected California mosquitoes (Diptera: Culicidae). J Med Entomol 41: 255–257.
22. Hughes GL, Rasgon JL (2014) Transinfection: a method to investigate *Wolbachia*-host interactions and control arthropod-borne disease. Insect Mol Biol 23: 141–151.
23. Bian G, Xu Y, Lu P, Xie Y, Xi Z (2010) The endosymbiotic bacterium *Wolbachia* induces resistance to Dengue virus in *Aedes aegypti*. PLoS Pathog 6: e1000833.
24. Baldini F, Segata N, Pompon J, Marcenac P, Robert Shaw W, et al. (2014) Evidence of natural

Wolbachia infections in field populations of *Anopheles gambiae*. Nat Commun 6: 3985.
25. Bian G, Joshi D, Dong Y, Lu P, Zhou G, et al. (2013) *Wolbachia* invades *Anopheles stephensi* populations and induces refractoriness to *Plasmodium* infection. Science 340: 748–751.
26. Carlton JM, Escalante AA, Neafsey D, Volkman SK (2008) Comparative evolutionary genomics of human malaria parasites. Trends Parasitol 24: 545–550.
27. Martinsen ES, Perkins SL, Schall JJ (2008) A three-genome phylogeny of malaria parasites (*Plasmodium* and closely related genera): evolution of life-history traits and host switches. Mol Phylogenet Evol 47: 261–273.
28. Korgaonkar NS, Kumar A, Yadav RS, Kabadi D, Dash AP (2012) Mosquito biting activity on humans & detection of *Plasmodium falciparum* infection in *Anopheles stephensi* in Goa, India. Indian J Med Res 135: 120–126.
29. Adak T, Singh OP, Das MK, Wattal S, Nanda N (2005) Comparative susceptibility of three important malaria vectors *Anopheles stephensi*, *Anopheles fluviatilis*, and *Anopheles sundaicus* to *Plasmodium vivax*. J Parasitol 91: 79–82.
30. Boëte C (2005) Malaria parasites in mosquitoes: laboratory models, evolutionary temptation and the real world. Trends Parasitol 21: 445–447.
31. Zélé F, Nicot A, Duron O, Rivero A (2012) Infection with *Wolbachia* protects mosquitoes against *Plasmodium*-induced mortality in a natural system. J Evol Biol 25: 1243–1252.
32. Clancy DJ, Hoffmann AA (1997) Behavior of *Wolbachia* endosymbionts from *Drosophila simulans* in *Drosophila serrata*, a novel host. Am Nat 149: 975–988.
33. Suh E, Mercer D, Fu Y, Dobson SL (2009) Pathogenicity of life-shortening *Wolbachia* in *Aedes albopictus* after transfer from *Drosophila melanogaster*. Appl Env Microbiol 75: 7783–7788.
34. Bouchon D, Rigaud T, Juchault P (1998) Evidence for widespread *Wolbachia* infection in isopod crustaceans: molecular identification and host feminization. Proc Biol Sci 265: 1081–1090.
35. McGraw EA, Merritt DJ, Droller JN, O'Neill SL (2002) *Wolbachia* density and virulence attenuation after transfer into a novel host. Proc Natl Acad Sci U S A 99: 2918–2923.
36. Weeks AR, Turelli M, Harcombe WR, Reynolds KT, Hoffmann AA (2007) From parasite to mutualist: rapid evolution of *Wolbachia* in natural populations of *Drosophila*. PLoS Biol 5: E114.
37. Turelli M (1994) Evolution of incompatibility-inducing microbes and their hosts. Evolution 48: 1500–1513.
38. Carrington LB, Hoffmann AA, Weeks AR (2010) Monitoring long-term evolutionary changes following *Wolbachia* introduction into a novel host: the *Wolbachia* popcorn infection in *Drosophila simulans*. Proc Biol Sci. 277: 2059–2068.
39. Hughes GL, Samuels SK, Shaikh K, Rasgon JL, Vardo-Zalik AM (2014) Discrimination of the *Plasmodium mexicanum* vectors *Lutzomyia stewarti* and *Lutzomyia vexator* by a PCR-RFLP assay and *Wolbachia* infection. J Vector Ecol 39: 224–227.

Local News

Hawai‘i Unites sues DLNR over mosquito suppression plan to save native birds

Hawaii Public Radio | By Zoe Dym
Published May 11, 2023 at 12:32 PM HST

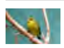
LISTEN • 1:45

Hawai'i Unites

A local environmental nonprofit has sued the state Department of Land and Natural Resources and Board of Land and Natural Resources over its mosquito suppression plan to reduce avian malaria at Haleakalā National Park.

Hawai‘i Unites, the volunteer organization in opposition to the state's biopesticide plan, argues that DLNR did not prepare an environmental impact statement for the project.

However, DLNR officials note that they collaborated with the National Park Service for an environmental assessment in December 2022.


Local News
Board approves final EA needed to start saving endangered birds from mosquitos
LISTEN • 1:35

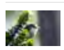
The Park Service found that there would be no significant impact and that an EIS would not be needed. BLNR approved the plan in March.

The project would release male mosquitoes infected with a Wolbachia bacteria strain that would make them infertile. When they mate with a female mosquito, scientists say the eggs will never hatch.

This method has been successful for human health viruses like Dengue and Zika, but has never been used to reduce avian malaria.

Members of Hawai‘i Unites say they are worried the release could increase the spread of avian malaria.

One particular study from 2014 found that mosquitoes with Wolbachia were more likely to transmit avian malaria than those without the bacteria. The study also mentioned that the outcome could be drastically different in the natural world compared to lab-bred mosquitoes.


Local News
Kaua‘i researchers trap mosquitoes to prevent extinction of native honeycreepers
LISTEN • 1:10

“The East Maui project area is over 89 times the size of the largest Wolbachia mosquito release area globally to date, and the southern house mosquito has never even been used before with this technique for stand-alone field release,” said Tina Lia, the founder and president of Hawai‘i Unites.

Forest bird coordinator with Haleakalā National Park Chris Warren explained that mosquitoes already in the park are "highly susceptible to malaria."

"I think it's like 85% to 97%, were susceptible to infection and transmission. So those are the mosquitoes that are out there right now," Warren said. "What we said in our response to this question in the finding of no significant impact from the EA is that it's improbable that susceptibility could even increase above that."

The project to suppress the mosquito population and reduce avian malaria will move forward despite the lawsuit. Infertile mosquitoes could be released as early as this month.

To read the environmental assessment, click here. For the Hawai‘i Unites lawsuit, click here.

Tags: Local News | haleakala national park | Maui | conservation | animals

Zoe Dym
Zoe Dym is a news producer at Hawai‘i Public Radio.
See stories by Zoe Dym

EXHIBIT 6

Related Stories

Untitled design (4).png
Local News
Non-venomous gopher snake captured in Pukalani
HPR News Staff, May 17, 2023
A non-venomous female gopher snake was found in Pukalani Monday afternoon. The snake, which is an illegal and invasive animal in Hawai'i, was taken away from the scene and euthanized by state Department of Agriculture inspectors later that day.
LISTEN • 0:43

green_sea_turtle.jpg
The Conversation
Researcher finds elevated levels of lead in Kailua Bay turtles
Catherine Cruz, May 16, 2023
A study of Hawaiian green sea turtles in Kailua Bay using tissue dating back 10 years ago found traces of lead in their blood and shells. But despite the findings, researcher Katherine Shaw said there's no cause for panic.
LISTEN • 9:19

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4. In 'Julieta and the Romeos,' a teen aims to uncover the identity of her mystery man
5. Opinion: Progress can be a turtle

Birds, Not Mosquitoes
May 17 at 5:30 PM ·

The Environmental Assessment for East Maui was approved…now what??

On Maui, Birds, Not Mosquitoes is starting to pilot small-scale releases of Wolbachia-incompatible male mosquitoes as part of a phased approach to landscape-scale mosquito control (Incompatible Insect Technique). The partnership is excited to take this momentous step toward mosquito control on Maui.

This next phase will be used to fine-tune the release parameters–learning where and how far the incompatible male mosquitoes travel after they are released.

These pilot releases alone are not expected to affect the overall population size of the southern house mosquito.

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ʻiʻiwi © Jim Denny

#BirdsNotMosquitoes #IOlaNaManuNahele #ConservationPartnerships #EndangeredSpecies #BirdsofHawaii #NativeHawaiianBirds #ʻiʻiwi #ResistExtinction #aolemosquitoes

8

Like Comment

EXHIBIT 7

Birds Not Mosquitoes · Jun 2 · 4 min read

Giving Thirst Traps a Whole New Meaning

> Thirst Trap: An alluring or flirty photo meant to capture a viewer's attention.

What's a mosquito trap? It's like a thirst trap but it's out in nature and not on Instagram!

Standard mosquito traps use lures to attract female mosquitoes to what they think is an inviting place to lay their eggs or a thirst-quenching blood meal. Female mosquitoes (the biters) look for a blood meal to help their eggs develop. But to inform future phases of this project, we needed to trap, collect, and monitor *male* mosquitoes, not females. Male mosquitoes do not bite and are not carriers of disease so fewer lures and traps have been developed to attract them. The mosquito researchers and technicians at Maui Forest Bird Recovery Project (MFBRP) have been working to adapt traditionally female-oriented traps to attract male mosquitoes.

Why attract and trap male mosquitoes, not females?

EXHIBIT 8

The mosquito control being implemented on Maui is a type of Incompatible Insect Technique (IIT) that will release *Wolbachia*-incompatible male southern house mosquitoes into wild breeding grounds to suppress the overall southern house mosquito population. (If that seems confusing, read our last article to understand the science behind IIT). To inform the small-scale releases, MFBRP had been working to trap wild male southern house mosquitoes to understand their current population numbers, where they fly, and which lures they are most attracted to. This information is vital to successfully implementing IIT.

Trapping to Understand the Current Population

Understanding the distribution and population sizes of wild male southern house mosquitoes helps determine how many incompatible males would need to be released in order to reduce the number of females at a site and to out-compete wild males for mating with the females. The more *Wolbachia*-incompatible male mosquitoes that are released at a location, the higher their chance of successful mating with wild females, which leads to a higher likelihood of non-viable eggs being laid (non-viable means the eggs won't hatch). Releasing too many males at a location will not have negative ecological impacts, however, releasing them in the most efficient locations helps us make the best use of our time and male mosquito resources.

Trapping to Learn What Lures Attract Male Southern House Mosquitoes

Currently, three different types of lures are being used: CO2, "stinky" lure, and floral. Some traps use only one type of lure and some traps use combinations of different lures. Click to learn more about each type of lure.

Photograph of a mosquito trap connected to its battery source.

CO2 lures release small amounts of CO2 into the air (approximately the same amount that each of us exhales). Initially used to attract female mosquitoes to a potential blood meal, this lure can attract males because where females go...males can follow!
"Stinky" lures use a mixture of organic compounds with a pungent, stinky smell similar to those found in human skin odors. This smell attracts some mosquito species of both

sexes.

Floral lures use a flowery smell to attract mosquitoes. Male mosquitoes only eat nectar so they may be attracted to this lure.

From the research conducted with the different lures, MFBRP has found male mosquitoes in both the CO2 + stinky lure and stinky lure-only traps. However, the catch numbers were too low to accurately reflect male mosquito preferences. Future trapping efforts for the *Wolbachia*-incompatible male mosquitoes will exclude the use of floral lures to only research CO2 and a "stinky" lure vs. just stinky lure effectiveness.

Trapping to Inform Landscape Scale Releases

Research from these traps informed the next phase of this project: small-scale releases of the *Wolbachia*-incompatible male mosquitoes in the high-elevation forests on the slopes of Haleakalā, Maui. Now, the traps will be vital in catching the released male mosquitoes to help guide management and decisions for the IIT project. Small-scale pilot releases of *Wolbachia*-incompatible male mosquitoes started in mid-May after the Finding of No Significant Impact for the East Maui Environmental Assessment. These pilot releases will also inform the larger landscape use of this mosquito control technique on Maui.

It Takes a Crew!

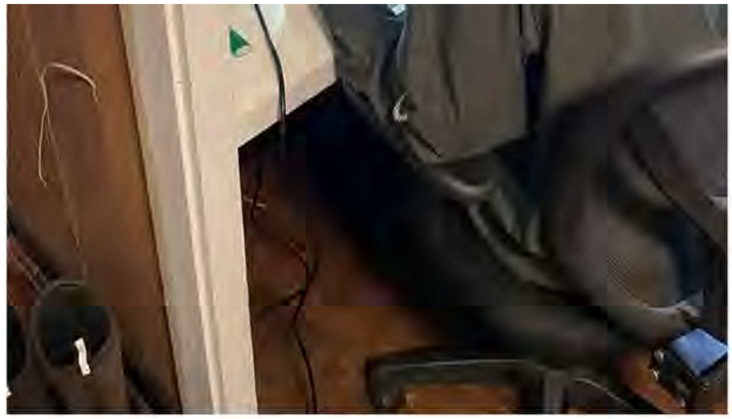
It's no small feat to set and continuously run mosquito traps.

On the first day of a trapping session, crews go out in the evening to turn on the traps and each morning they go back out tocollect each trap's overnight catch and data. Overnight data is the most important because southern house mosquitoes are most active at night, dawn, and dusk.

During the morning shift, field technicians go out to each trap to manually take measurements of the trap function, collect the small netted bags attached to each trap (which may contain mosquitoes), and change the trap battery if needed.

Back at the office, the bags containing suspected mosquitoes are frozen to ensure that the insects are immobilized for identification. Later, a field

technician trained in mosquito identification examines the contents. Ideally, male southern house mosquitoes are captured, but sometimes other insects like moths, midges, flies, and other mosquito species are attracted to the lures and are caught in the trap. They take notes on the bag contents–even if no mosquitoes are present. Not catching the target species is still important data! After some very careful sorting, the technician uploads the bag's data into the project data set.



This routine continues day after day, collecting as much data as possible for the trapping session's duration.

Mosquito control on Maui would not be possible without this dedicated and hard-working mosquito crew! Unfortunately, we don't all have the privilege to work as mosquito crew technicians for MFBRP, but we can be part of a larger "mosquito crew" in our own neighborhoods and communities! Here are a few simple things we can each do to help eliminate mosquito breeding grounds and decrease invasive mosquito populations:

-Dump out standing water in buckets, wheelbarrows, and bins.
-Fix leaky faucets and hoses that are dripping water.
-Flush or dump out water from bromeliads and other leafy plants once a week.
-Clean out gutters so water can run freely.
-Clean up trash and debris; for example, dispose of old tires or anything that can hold water.

For more information about Birds, Not Mosquitoes and the use of IIT mosquito control in Hawaiʻi, visit our website at birdsnotmosquitoes.org.

About Maui Forest Bird Recovery Project: Maui Forest Bird Recovery Project is a project of the Pacific Cooperative Studies Unit under the Research Corporation of the University of Hawaiʻi-Mānoa and is dedicated to developing and implementing techniques that recover Maui's endangered birds. They are a vital Birds, Not Mosquitoes partner providing expertise and technical support on Maui.

CURRICULUM VITAE 2018

Name:	Lorrin Wayie Pang	
Military Rank:	Lt. Colonel, Medical Corp (Retired) Walter Reed Army Institute of Research	
Date/Birthplace:	30 March 1953 Honolulu, Hawaii	
Wife's Name:	Kathleen K. Shida Pang	
Children	Two daughters	
Education/Training:	1971-75	Princeton University, BS Chemistry, Cum Laude
	1975-79	Tulane Medical School, MD
	1976-79	Tulane School of Public Health MPH in Tropical Medicine
	1979-80	Federal University of Brazil; Recife, Pernambuco, Post Graduate Studies in Pathology and Infectious Diseases
	1980-81	Letterman Army Hospital, San San Francisco, CA, Medicine Intern
	1981-82	Walter Reed Army Institute of Research, Washington DC, Preventive Medicine Residency
Positions Held:	1982-87	Epidemiologist, AFRIMS (Walter Reed Inst. Overseas Laboratory) Bangkok, Thailand
	1987-90	Chief, Preventive Medicine Service, Tripler Army Medical Center, Honolulu, Hawaii
	1987-89	Clinical Associate Professor, School of Public Health, University of Hawaii, Honolulu, Hawaii
	1990-92	Medical Officer, Malaria Unit, World Health Organization, Geneva, Switzerland.

EXHIBIT 9

1992-97	Clinician/Epidemiologist, Walter Reed Institute of Research Overseas Laboratory, Brazil.
1994-95	Adviser to Pan American Health Organization (Meningitis Vaccine)
1985-05	Adviser to World Health/UNDP Organization (Tropical Disease Research Unit: Chagas Disease, Leishmaniasis, Malaria, Clinical Trials), 2000 malaria program changed to United Nations Global Fund (for work in Central America)
1997-00	Chief, Department of Bacteriology and Molecular Genetics, AFRIMS, Walter Reed Institute of Research Overseas Laboratory, Bangkok, Thailand.
1997-00	Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.
2000-Present	District Health Officer, Maui County State of Hawaii
2002-04	Consultant Glaxo Smith Kline Pharmaceuticals-
2013	Consultant DNDI (Drugs for Neglected Disease Initiative, affiliate of Doctors without Borders)
2013	Visiting Professor of Medicine, Federal University of Brasilia, Brazil
2013-Present	Reviewer of grants for US Congress (CDMRP, Congress Directed Medical Research Program)

Awards:

Army Achievement Medal, 1982, 1996
Army Research and Development Medal, 1987
Army Meritorious Service Medal, 1990, 1997

Certification:

Medical License State of Louisiana, 1980- 2000
Hawaii State License, 2000-present
Board Certification in Preventive Medicine, 1990

2002 Discovery Channel feature covering dengue outbreak and eradication on Maui

2006-8 listed on Americas Best Doctors list (3% of nation's doctors)

Publications Peer Reviewed Journals:

1. Lemon SM, Miller RN, Pang LW, Prier RE, Bernard KW. Failure to achieve predicted antibody responses with intradermal and intramuscular human diploid cell rabies vaccine. Lancet 1984;19:1098-1100.

2. Webster HK, Boudreau EF, Childs GE, Yongvanitchit, Pang LW. Antimalarial drug susceptibility testing of *P. falciparum* in Thailand using a microdilution radiosotope method. Am J Trop Med Hyg 1985;34(2):228-35.

3. Pang LW, Boudreau EF, Childs GE, Webster HK, Supernantalerk C, Somutsakorn P. The failure of large dose erythromycin in combination with standard doses of chloroquine or quinine to treat human falciparum malaria. Bull WHO 1985;63(4):739-43.

4. Tan SG, Green CA, Andre RG, Baimai V, Pang LW. Genetics of esterases and 6 phosphogluconate dehydrogenase in the anopheles maculatus complex. Acta Tropica 1986;43:113-23.

5. Childs GE, Pang LW, Wimonwattrawatee T, Pooyindee N, Nanakorn A, Limchitee S, Webster HK. *In vitro* mefloquine resistance of *Plasmodium falciparum* isolated from the Burmese border region of Thailand. SEA J Trop Med Publ Hlth 1987;18(4):438-43.

6. Pang LW, Limsomwong N, Boudreau EF, Singharaj P. Doxycycline prophylaxis for falciparum malaria. Lancet 1987;23:1161-4.

7. Webster HK, Boudreau EF, Pang LW, Permpanich B, Sookto P, Wirtz RA. Development of immunity in natural *Plasmodium falciparum* malaria antibodies to the falciparum sporozoite vaccine 1 antigen (R32tet32). J Clin Microbiol 1987;25(6):1002-8.

8. Harbach RE, Gingrich JB, Pang LW. Some entomological observations on malaria transmission in a remote village in northwestern Thailand. J Am Mos Contr Assn 1987;3(2):296-301.

9. Pang LW. Doxycycline prophylaxis for malaria (letter). Lancet 1987;24:970.

10. Boudreau EF, Pang LW, Dixon KE, Webster HK, Pavanand K, Tosingha L, Somutsakorn P, Canfield C. Treatment efficacy of halofantrine (WR171,669) in initial field trials in Thailand. Bull WHO 1988;66(2):227-35.

11. Limsomwong N, Pang LW, Singharaj P. Malaria prophylazis with proguanil in children living in a malaria endemic area. Am J Trop Med Hyg 1988;38(2):231-6.

12. Pang LW, Limsomwong N, Singharaj P. Falciparum and vivax malaria prophylaxis with low dose doxycycline. J Infect Dis 1988;158(5):1124-7.

13. Pang LW, Limsomwong N, Webster HK, Karwacki JJ. Circumsporozoite antibodies and falciparum malaria incidence in children living in a malaria endemic area. Bull WHO 1988;66(3):359-63.

14. Childs GE, Pang LW. Analysis of dose-response curves for the *in vitro* susceptibility of *Plasmodium falciparum* to antimalarials using a pocket computer. Am J Trop Med Hyg 1988;38:15-8.

15. Pang LW, Limsomwong N, Singharaj P, Canfield CJ. Malaria prophylaxis with proguanil and sulfisoxazole in children living in a malaria endemic area. Bull WHO 1989;67(1):51-8.

16. Childs GE, Boudreau EF, Milhous WK, Wimonwattratee T, Pooyindee N, Pang LW, Davidson DE. A comparison of the *in vitro* activities of amodiaquine and desethylamodiaquine against isolates of *Plasmodium falciparum*. Am J Trop Med Hyg 1989;40:7-11.

17. Shida KK, Lewchalermvongse B, Pang LW. *Plasmodium berghei* malaria infection causes increased cardiac output in rats. Experiment Parasitol 1989;68:253-9.

18. Pang LW. Chemoprophylaxis and treatment of malaria (letter). NEJM 1989;320:1561.

19. Boudreau EF, Fleckenstein L, Pang LW, Childs GE, Schroeder AC, Ratnavotorn B, Phintuyothin P. Mefloquine kinetics in cured and recrudescent patients with acute falciparum malaria and in healthy volunteers. Clin Pharm Ther 1990;48(4):399-409.

20. Desowitz R, Shida K, Pang L, Buchbinder G. *Plasmodium berghei* malaria in the rat: a model for malaria in pregnancy. Am J Trop Med Hyg 1990;41(6):630-4.

21. Sanchez JJ, Hoke CC, McCown J, DeFraites RF, Takafuji ET, Diniega BM, Pang LW. Further experience with Japanese encephalitis vaccine. Lancet 1990;21:972-3.

22. Roscelli JD, Bass JW, Pang L. Guillain-Barre syndrome and influenza vaccination in the US Army, 1980-1988. Am J Epidemiol 1991;133:952-5.

23. Boudreau EF, Pang LW, Chaikummao S, Witayraut C, Thiemanum W, Pookasorn M. Comparison of mefloquine, choroquine plus fansidar and chloroquine alone as malarial prophylaxis in eastern Thailand. SEA J Trop Med Publ Hlth 1991;22:183-9.

24. Sasaki D, Pang LW, Minette H, et al. Incidence and risk factors of leptospirosis in Hawaii. Am J Trop Med Hyg 1993;48(1):35-43.

25. Shmuklarsky MJ, Boudreau EF, Pang L, et al. Failure of doxycycline as a causal prophylactic agent against *Plasmodium falciparum* malaria in healthy non-immune volunteers. Ann Intern Med 1994;120(4):294-9.

26. Withers BJ, Kelley PW, Pang LW, et al. Vaccine-Preventable disease susceptibility in a young adult Micronesian population. SEA J Trop Med Publ Hlth 1994;25(3):569-72.

27. Kramer KJ, Pang LW, Minette HP, Perrone JB. Evaluation of the quantitative buffy coat analysis (QBC) system for the detection of Leptospira in human blood. SEA J Trop Med Publ Hlth 1994;25:788-9.

28. Andrade AL, et al. High prevalence of asymptomatic malaria in gold mining areas of Brazil. Clin Infect Dis 1995;20:475.

29. Pang LW, Alencar FEC, Cerutti C, et al. Hepatitis E infection in the Brazilian Amazon. Am J Trop Med Hyg 1995;52(4):347-8.

30. Alencar FEC, Cerutti C Jr, Durlacher RR, et al. Atovaquone and Proguanil for the treatment of malaria in Brazil. J Infect Dis 1997;175:1544-7.

31. Berman JD, Badaro R, Thakur CP, et al. Efficacy and toxicity of liposomal-amphotericin B (AmBisome) for visceral leishmaniasis in developing nations: A review of a TDR clinical development program. Bull WHO 1998;76(1):25-32.

32. Gomes M, Wayling S, Pang LW. Interventions to improve the use o antimalarials in Southeast Asia: an overview. Bull WHO 1998;76(S1).

33. Zalis MG, Pang L, Silveira MS, Milhous WK, Wirth DF, et al. Characterization of *Plasmodium falciparum* isolated from the Amazon region of Brazil: evidence for quinine resistance. Am J Trop Med Hyg 1998;58(5):630-7.

34. Cerutti C Jr, Durlacher RR, de Alencar FEC, Segurado AAC, Pang LW. *In Vivo* Efficacy of Mefloquine for the Treatment of *Falciparum* Malaria in Brazil. J Infect Dis 1999;180:2077-80.

35. Dalsgaard A, Forslund A, Bodhiddatta L, Serichantalergs O, Pitarangsi C, Pang L, Shimada T, Echeverria P. A high proportion of *Vibrio cholerae* strains isolated from children with diarrhoea in Bangkok, Thailand are multiple antibiotic resistant and belong to heterogenous non-O1, non-O139 O-serotypes. Epidemiol Infect 1999;122:217-26.

36. Cerutti Junior C, Marques C, Alencar FE, Durlacher RR, Alween A, Segurado AA, Pang LW, Zalis MG.Antimalarial drug susceptibility testing of Plasmodium falciparum in Brazil using a

radioisotope method. Mem Inst Oswaldo Cruz. 1999 Nov-Dec;94(6):803-9.

37. Fonseca MO, Pang L, de Avila Sdo L, Arruk VG, Tozetto-Mendoza TR, Ferreira AW, Saes-Alquezar A, Boulos M.Cross-reactivity of anti-Plasmodium falciparum antibodies and HIV tests. Trans R Soc Trop Med Hyg. 2000 Mar-Apr;94(2):171-2.

38. Sethabutr O, Venkatesan M, Yam S, Pang LW, Smoak BL, Sang WK, Echeverria P, Taylor DN, Isenbarger DW.Detection of PCR products of the ipaH gene from Shigella and enteroinvasive Escherichia coli by enzyme linked immunosorbent assay.
Diagn Microbiol Infect Dis. 2000 May;37(1):11-6.

39. Sanchez JL, Bendet I, Grogl M, Lima JB, Pang LW, Guimaraes MF, Guedes CM, Milhous WK, Green MD, Todd GD.Malaria in Brazilian military personnel deployed to Angola.
J Travel Med. 2000 Sep-Oct;7(5):275-82.

40. Wongsrichanalai C, Sirichaisinthop J, Karwacki JJ, Congpuong K, Miller RS, Pang L, Thimasarn K.Drug resistant malaria on the Thai-Myanmar and Thai-Cambodian borders. Southeast Asian J Trop Med Public Health. 2001 Mar;32(1):41-9. Review.

41. Houng HS, Sethabutr O, Nirdnoy W, Katz DE, Pang LW.Development of a ceuE-based multiplex polymerase chain reaction (PCR) assay for direct detection and differentiation of Campylobacter jejuni and Campylobacter coli in Thailand.
Diagn Microbiol Infect Dis. 2001 May-Jun;40(1-2):11-9.

42. Isenbarger DW, Hien BT, Ha HT, Ha TT, Bodhidatta L, Pang LW, Cam PD.Prospective study of the incidence of diarrhoea and prevalence of bacterial pathogens in a cohort of Vietnamese children along the Red River. Epidemiol Infect. 2001 Oct;127(2):229-36.

43. Wongsrichanalai C, Lin K, Pang LW, Faiz MA, Noedl H, Wimonwattrawatee T, Laoboonchai A, Kawamoto F.In vitro susceptibility of Plasmodium falciparum isolates from Myanmar to antimalarial drugs.Am J Trop Med Hyg. 2001 Nov;65(5):450-5.

44. Duarte EC, Pang LW, Ribeiro LC, Fontes CJ. Association of subtherapeutic dosages of a standard drug regimen with failures in preventing relapses of vivax malaria. Am J Trop Med Hyg. 2001 Nov;65(5):471-6.

45. Cunha ML, Piovesan-Alves F, Pang LW. Community-based program for malaria case management in the Brazilian Amazon.
Am J Trop Med Hyg. 2001 Dec;65(6):872-6.

46. Pang LW, Piovesan-Alves F. Economic advantage of a community-based malaria management program in the Brazilian Amazon.
Am J Trop Med Hyg. 2001 Dec;65(6):883-6.

47. PV, Pang L, Kitsutani P, et al. Dengue Fever, Hawaii 2001-2002, EID 2005 May 11(5).

48. Faiz MA, Yunus EB, Rahman MR, Hossain MA, Pang LW, Rahman ME, Bhuiyan SN. Failure of national guidelines to diagnose uncomplicated malaria in Bangladesh. Am J Trop Med Hyg 2002 Oct;67(4):396-9.

49. Fontes CJ, Ribeiro LC, Pang LW. Proguanil plus sulfamethoxazole in the treatment of uncomplicated Plasmodium falciparum malaria. Southeast Asian J Trop Med Public Health. 2002 Dec;33(4):685-8.

50. Sanders JW, Isenbarger DW, Walz SE, Pang LW, Scott DA, Tamminga C, Oyofo BA, Hewitson WC, Sanchez JL, Pitarangsi C, Echeverria P, Tribble DR. An observational clinic-based study of diarrheal illness in deployed United States military personnel in Thailand: presentation and outcome of Campylobacter infection. Am J Trop Med Hyg 2002 Nov;67(5):533-8.

51. Noedl H, Faiz MA, Yunus EB, Rahman MR, Hossain MA, Samad R, Miller RS, Pang LW, Wongsrichanalai C. Drug-resistant malaria in Bangladesh: an in vitro assessment. Am J Trop Med Hyg 2003 Feb;68(2):140-2.

52. Murine Typhus – Hawaii 2002, MMWR vol 52/No 50 19 Dec 2003, pp 1224-25.

53. Duarte EC, Pang L, Fontes CJ. [Internal validity of clinical trials for Plasmodium vivax malaria treatment: analysis of evaluation study of in vivo Plasmodium vivax emergence of resistance to standard doses of primaquine]. Rev Soc Bras Med Trop. 2003 May-Jun;36(3):383-6. Epub 2003 Jul 31. Portuguese.

54. Duarte EC, Gyorkos TW, Pang L, Abrahamowicz M. Epidemiology of malaria in a hypoendemic Brazilian Amazon migrant population: a cohort study. Am J Trop Med Hyg. 2004 Mar;70(3):229-37. Erratum in: Am J Trop Med Hyg. Am J Trop Med Hyg. 2004 Apr;70(4):459.

55. Erdem G, Abe L, Kanenaka RY, Pang L, Mills K, Mizumoto C, Yamaga K, Effler PV. Pediatr Infect Dis J Characterization of a community cluster of group a streptococcal invasive disease in Maui, Hawaii. 2004 Jul;23(7):677-9.

56. Kalmar EM, Alencar FE, Alves FP, Pang LW, Del Negro GM, Camargo ZP, Shikanai-Yasuda MA. Paracoccidioidomycosis: an epidemiologic survey in a pediatric population from the Brazilian Amazon using skin tests. Am J Trop Med Hyg. 2004 Jul;71(1):82-6.

57. Rohner AL, Pang LW, Iinuma G, Tavares DK 3rd, Jenkins KA, Geesey YL. Effects of Upcountry Maui water additives on health. Hawaii Med J. 2004 Sep;63(9):264-5.

58. Fonseca MO, Pang LW, de Paula Cavalheiro N, Barone AA, Heloisa Lopes M. Randomized trial of recombinant hepatitis B vaccine in HIV-infected adult patients comparing a standard dose to a double dose. Vaccine. 2005 Apr 22;23(22):2902-8.

59. Hayes JM, Rigau-Pérez JG, Reiter P, Effler PV, Pang L, Vorndam V, Hinten SR, Mark KE, Myers MF, Street K, Bergau L, Meyer C, Amador M, Napier M, Clark GG, Biggerstaff BJ, Gubler DJ. Risk factors for infection during a dengue-1 outbreak in Maui, Hawaii, 2001. Trans R Soc Trop Med Hyg. 2006 Jun;100(6):559-66. Epub 2005 Dec 13.

60. Simpson JA, Agbenyega T, Barnes KI, Di Perri G, Folb P, Gomes M, Krishna S, Krudsood S, Looareesuwan S, Mansor S, McIlleron H, Miller R, Molyneux M, Mwenechanya J, Navaratnam V, Nosten F, Olliaro P, Pang L, Ribeiro I, Tembo M, van Vugt M, Ward S, Weerasuriya K, Win K, White NJ.Population pharmacokinetics of artesunate and dihydroartemisinin following intra-rectal dosing of artesunate in malaria patients. PLoS Med. 2006 Nov;3(11):e444.

61. Tribble DR, Sanders JW, Pang LW, Mason C, Pitarangsi C, Baqar S, Armstrong A, Hshieh P, Fox A, Maley EA, Lebron C, Faix DJ, Lawler JV, Nayak G, Lewis M, Bodhidatta L, Scott DA . Traveler's diarrhea in Thailand: randomized, double-blind trial comparing single-dose and 3-day azithromycin-based regimens with a 3-day levofloxacin regimen. Clin Infect Dis. 2007 Feb 1;44(3):338-46. Epub 2006 Dec 28.

62. Tribble DR, Baqar S, Pang LW, Mason C, Houng HS, Pitarangsi C, Lebron C, Armstrong A, Sethabutr O, Sanders JW. J Clin Microbiol. Diagnostic approach to acute diarrheal illness in a military population on training exercises in Thailand, a region of campylobacter hyperendemicity. 2008 Apr;46(4):1418-25. Epub 2008 Jan 30.

63. Ling C, Henderson S, Henderson R, Henderson M, Pedro T, Pang L. Cost benefit considerations of preventing elderly falls through environmental modifications to homes in Hana, Maui.Hawaii Med J. 2008 Mar;67(3):65-8.

64. Sugihara N, Watanabe M, Tomioka M, Braun KL, Pang L. Saving money through exercise: Estimating the investment-to-return ratio of an elderly exercise program on Kaua'i. *Hawai'i Medical Journal*. 2011:116-120. Erratum correction in following issue.

65. Desure AR, Peterson K, Gianan FV, Pang LW. An Exercise Program to Prevent Falls in Institutionalized Elderly with Cognitive Deficits: A Crossover Pilot Study. Hawaii J Med Public Health. Nov 2013; 72(11): 391–395.

66. Coradi de Freitas D, Gomes LT, Fontes CJ, Tauil PL, Pang LW, Duarte EC. Sensitivity of nested-PCR for plasmodium detection in pooled whole blood samples and its usefulness to blood donor screening in endemic area. Transfusion and Apheresis Science. Published online 10 Feb 2014.

67. Elisabeth Carmen Duarte, Walter Massa Ramalho, Pedro Luiz Tauil, Cor Jésus Fernandes Fontes, Lorrin Pang. The changing distribution of malaria in the Brazilian Amazon, 2003-2004 and 2008-2009. Rev. Soc. Bras. Med. Trop. vol.47 no.6 Uberaba Nov./Dec. 2014. http://dx.doi.org/10.1590/0037-8682-0274-2014.

68. Mnatzaganian CL, Pellegrin KL, Miyamura J, Valencia D, Pang L. Association between sugar cane burning and acute respiratory illness on the island of Maui. Environ Health. 2015 Oct 7;14:81. doi: 10.1186/s12940-015-0067-y.

69. Olivia Jenkins, Sara Routley, MA, Tina Pedro-Gomes, MS, and Lorrin Pang, MD, MPH. A Pilot Dental Survey on Maui. Hawaii J Med Public Health. 2016 Nov; 75(11): 332–336.

70. Mills KM, Sadler S, Peterson K, Pang L. An Economic Evaluation of Preventing Falls Using a New Exercise Program in Institutionalized Elderly. J Phys Act Health. 2018 Jun 1;15(6):397-402.

71. Mills KM1* and Pang LW. Importance of Utilizing Standardized Method of Calculating Cost Benefit of Physical Activity Interventions. J Health Sci Educ Vol 2(5): 1-2.

72. Challenges of Measuring Individuals versus Events as Health Outcomes: Falls in Elderly as an Example Pang LW1 and Mills KM2, Vol 3(5). J Health Sci Educ -1-173. Nov 2019.

73. Pang GC, Calder M, Hauschild EM, et al. (2020) A Reduced Serial Interval Can have a Higher Impact on the Spread of Covid-19 Relative to R0: An Educational Video Demonstrating the Spread of Flu, Covid-19 and Covid-19 with Early Transmission. J Health Sci Educ 4: 195. J Health Sci Educ Vol 4(5): 1-5.

74. Amy T. Hou, Genevieve C. Pang, Kristin M. Mills, Krizhna L. Bayudan, Dayna M. Moore, Luz P. Medina, Lorrin W. Pang (2021). A Rapid Method to Evaluate Pre-Travel Programs for COVID-19: A Study in Hawaii, MedRxiv, doi: https://doi.org/10.1101/2021.03.06.21251482.

75. In Press 2022. RE: AJTMH-21-1053.R2, An Effective Barrier to Prevent Crop Contamination by Slug Vectors of Angiostrongylus cantonensis by Pang, Lorrin; Coppolo, Christy; Hauptman, Sara - published: Am. J. Trop. Med. Hyg., 00(00), 2022, pp. 1–6
doi:10.4269/ajtmh.21-1053
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76. Pre-procedural testing improves estimated COVID-19 prevalence and trends
View ORCID ProfileGenevieve C. Pang, View ORCID ProfileAmy T. Hou, Krizhna L. Bayudan, Ethan A. Frank, Jennifer Pastiglione, Lorrin W. Pang doi:
https://doi.org/10.1101/2022.04.13.22273200https://www.medrxiv.org/content/10.1101/2022.04.13.22273200v1.

77. Pham K, Pang L: JAMA (https://jamanetwork.com/journals/jama/fullarticle/2793357) Comment to Paxlovid failure.

78. Summary article airport testing.

79. Rat Lung barrier valve effect.

Statement: Dr. Lorrin Pang, Private Citizen

BACKGROUND

I am a tropical disease and vector expert speaking as a private citizen on this matter. I've authored over 75 publications in peer-reviewed medical journals covering a broad range of studies such as malaria, dengue, rabies, rat lungworm, and COVID. I've been an advisor and voting member of the U.S. Congress Medical Research Program for the past several years, serving on committees for infectious diseases – many of which are mosquito-borne. From 1985-2005, I worked with the World Health Organization (WHO) and Walter Reed Institute's Malaria Program, focusing on global malaria control efforts through interventions combining diagnostics, chemotherapeutics, vector control, and vaccine development. As a public health leader on the islands, I've mitigated mosquito-borne illnesses – including dengue and Zika – for over two decades. I was honored for my life-saving intervention in Hawaii's dengue fever outbreak.

I've attached a confidential submission for publication which highlights some of the items discussed below: That population changes are often determined by pathways set up in parallel, not just sequentially; that models must be set up by the initial assumptions with the math derivations of the formula to follow; that the models must predict intuitively the changes in populations when extreme limits are reached (steady state and non-steady state); that tracking units of the parameters of the math expression is a very useful practice in complicated models.

(CV attached; confidential research article attached)

CONCERNS

Horizontal Transmission: Non-Sexual

A primary concern is non-sexual horizontal transmission of the introduced *Wolbachia* strains between the introduced biopesticide mosquitoes and the existing wild mosquitoes. Imported *Wolbachia* bacterium strain *w*AlbB has been disclosed as the strain for use in this biopesticide. Additional strains *w*AlbA and *w*Pip4 are also planned for import in connection with this project. These newly introduced strains (referred to here as "X") are not currently present within the corresponding *Culex quinquefasciatus* species of Hawaii's established mosquito population.

I have been compiling studies documenting horizontal *Wolbachia* bacterial spread, and I'm concerned about the potential for significant adverse outcomes of the state's proposal. The intent to save rare birds is sound. If the project goes as planned, this would be a valuable tool for future interventions. However, with new life forms coming to the islands, there is too much potential for unexpected, dangerous, irreversible "evolutionary" events. This is especially true when the new organisms cannot be contained to their target ecosystem. Already there are published papers pointing out the real threat of horizontal spread of the novel *Wolbachia* beyond the male *Culex* mosquito. The papers cover two general areas – the widespread detection of *Wolbachia* across so many diverse types of insects, and more recently, the growing number of reports of mechanisms of how this might occur. First, we all must agree that unintended horizontal spread of the imported strain(s) to, say, female *Culex*, *Aedes* mosquitoes, or other insect vectors of diseases

EXHIBIT 10

would be a catastrophe, and probably irreversible. Hawaii has a bad history of invasive species entering and spreading unabated, including their spread of infectious diseases.

A recent study out of Singapore[1] describes *Wolbachia* bacteria strain "evolutionary associations" between mosquito hosts. The results of this mechanism widespread into diverse insect populations are not known. It may start with a few horizontal transfers to female mosquitoes. After that, the mating *Wolbachia*-X-compatible pair will quickly produce viable X offspring and spread the X bacteria strain (the term for this is "sweep"). If that were to happen here, the full capacity of those offspring to transmit disease would be unknown. This type of spread and sweep could also affect other insects, not just the targeted mosquito.

The possibility of unintentionally producing X-infected females in the wild has not been adequately addressed. The introduced *Wolbachia* strain can spread horizontally as a life form to other mosquitos (including *Aedes* – vectors of human disease) and perhaps create that *Wolbachia* female *Culex,* which everyone is bending over backwards to avoid via lab contamination.

There is a big difference between the standard Sterile Insect Technique (SIT) strategies used in the past that were based on radiation or chemicals, and the relatively new Incompatible Insect Technique (IIT). The mathematical models may be similar for estimating threshold criteria to affect mosquito population dynamics, but standard methods of sterility are not bacterial life forms that might escape horizontally and amplify in other ecological niches. While sterility models can predict the thresholds needed to exterminate a species (in this case insects), the radiation sterility factor (standard SIT) does not behave the same as a life form (*Wolbachia* bacteria). There may be different modeling between radiation and *Wolbachia* "sterility" for the male mosquitoes, depending on male mosquito fitness – but more importantly, for the unintended female *Culex* to which the *Wolbachia* X spreads horizontally. How is this supposed to be self-contained? Horizontal spread has the potential to be a disaster that cannot be recalled. The bacterium is a life form, and you might not be able to turn back the clock by simply shutting off the male mosquito "fountains."

The evidence of horizontal spread of *Wolbachia* shows that the bacteria go not only to sexual cells, but also to somatic cells (non-sexual cells of the body). *Wolbachia* can also live outside of intra-cellular systems for several months.[2] Two additional studies clearly document widespread horizontal transmission of *Wolbachia*. The first focuses on predatory wasps spreading the bacteria through contaminated mouth parts when feeding serially on target insects such as aphids[3]. More research into which predators, like the damselfly and dragonfly, sequentially feed on both male and female mosquitoes is needed to determine how this may affect Maui's ecosystems. This scenario might play out in either the predator of adults feeding on adult mosquitoes (X-infected and wild), or the X-infected predator of larvae feeding on wild mosquito larvae in common breeding sites. The second study looks at ant colonies spreading *Wolbachia* through the gastrointestinal (GI) tract when the ants feed on their fungus gardens.[2] What about shared sugar feeding sites for X-infected male and wild adult male and female mosquitoes? The sparser the sugar sites, the more communal interaction they will have. I find these studies of horizontal transfer across species of insects highly concerning. Even if this project achieved miraculous blocking of avian malaria to the native birds, what else would it do?

Studies that downplay the possibility of horizontal transmission based on *aedes aegypti* mosquitoes are flawed references because *aedes aegypti* are resistant to *Wolbachia*.

Horizontal Transmission: Venereal

An additional concern is the possibility of sexual/venereal horizontal transmission of the introduced bacteria through mosquitoes mating. This pathway is not well published and requires more study[4], specifically with the *Culex q*. mosquitoes planned for use in this project. While the proponents of the project claim that horizontal transmission only occurs on an evolutionary scale with a very slow spread, there are examples showing that it may occur during a short duration of weeks and then months. X-infected male mosquitoes may transmit the introduced strain to wild females through blood, mucous, and semen during mating. Granted, if this occurs via venereal route in the wild female mosquito, the first half of their life their matings will be sterile. However, after this their matings will produce offspring of both sexes and soon will "sweep" the population with the introduced *Wolbachia* strain.

There is a paper in *Nature*[5] by Frydman et al. studying a fruit fly lab model of entry into the germline from somatic tissue in about a week or two. Germ cells just touching somatic cells within the female mosquito's body can pick up the bacteria. Whether or not accidentally released X-infected females and wild males would have sterile mating outcomes would become irrelevant at this stage. Even if the introduction of X-infected males was stopped, the initial mating with females X-infected through horizontal transmission (and compatible through entry of the bacteria into the female germline) would produce viable offspring of both sexes of X-infected mosquitoes.

Vertical Transmission Suppressed: Horizontal Transmission Increased

When vertical transmission of the *Wolbachia* bacteria is suppressed, horizontal transmission of the bacteria can increase.[6] The *Wolbachia* is trying to survive. If vertical transmission is blocked, the bacteria maintain horizontal transmission until the host is fertile again, then they return to vertical transmission. During the time that the bacteria is challenged by the inability to transmit vertically, it may spread horizontally to other mosquitoes, spiders, fruit flies, and other insects (including insect vectors of disease).

A related topic is beginning to be examined in the science community. Human pathogens (i.e., Zika and dengue viruses) used to be thought of as "dead-end" infections in male mosquitoes who may have become infected through horizontal transmission mechanisms. Males don't bite humans to expand the virus in human hosts. However, it is now of concern that these viral pockets in male mosquitoes, though relatively rare, serve as back-up reservoirs to reinoculate females when the female-mosquito/human cycle breaks down. For example, if all humans get a virus and mass immunity puts the cycle on "hold" until enough non-immune humans are born or transient immunity is lost, the female mosquitoes will tap into the viral back-up reservoir via horizontal transmission.

Proponents of the use of this biopesticide argue that horizontal transmission, venereal or otherwise, is so rare that it can be ignored in the math models. They extrapolate from the models where cytoplasmic incompatibility (CI) is not operating for sterilization of matings, for example in the sweep model. Much of the spread of *Wolbachia* is vertical (V). Let us say that for every *Wolbachia* spread, 99.9% are via vertical spread and only 0.1% are horizontal (H). Let us pick a unit population where 100K new mosquitoes become infected with *Wolbachia* via sex. Of this 100K, only 100 would have become infected with the bacteria through H and the rest through V. But in the CI application, none get it through V and still females are inoculated somatically through the H mechanism of mating. This 100 may move quickly to the germline cells, but does the bacteria remain only in 100, or can it expand through lack of competition to fill the niche that the V transfers would have occupied? Was there competition between V and H descending lines; and if there is no longer a V line (due to CI), will H females expand?

Even when there is successful vertical transmission, there is horizontal transmission to non-germ cells such as neural tissue, which changes the behavior of the host to support what is in the germ cells. The bacterium is altruistic and helps sister cells to dominate as a safety backup, sacrificing the individual self for the sake of the larger group. This is useful because the larger group does have some common genes which the individual shares. When the two systems (V and H) are running predominantly vertical transmission with some relatively minor horizontal transmission, the horizontal is a back-up system to reinoculate the vertical system if the vertical system ever fails. This horizontal system is relatively small compared to the vertical system but is rather important in many systems including *Wolbachia*. If the CI process shuts down vertical transmission, the horizontal system is still running, and it may grow because that lineage does not compete with a vertical system which has been blocked by sterility.

With the *Wolbachia*, when the vertical production is plentiful, the horizontal movement will seem inconsequential, often a dead end; but once the vertical system collapses (through CI or through natural sterility of the insect – for instance, through mosquito HIV), the horizontal system in males will still be there and can restart the vertical system again when it moves to the germ cells in females. With the use of this biopesticide, the sterility might not stop completely, but the horizontal system will still act to save the *Wolbachia* line in males by making females that will expand the strain line vertically. All of the sacrificed horizontal transmission when the vertical system operates will be useful to reinoculate the system when CI passes or is "low."

<u>Math Model: Choke Points and Rate Limiting Step</u>

The math model for this project does not seem to account for choke points. If only a certain number of larvae from compatible mosquitoes will survive due to availability of, say, food sources in standing water breeding sites, then any reduction in viable offspring due to incompatibility may not significantly affect the number of surviving larvae. The viable larvae will compete for microbes to eat, and only a specific number of larvae will have enough food to survive (rate determining step). That number may remain relatively constant based on volume of food-source microbes, and the non-viable offspring of incompatible mosquitoes may have no effect, or limited effect, on the survival rate of larvae in the breeding site overall.

Determination of efficacy of the biopesticide might be based on a flawed set up of the math model. The question is, do things affecting a population occur in sequence or in parallel; and if we treat them like resistors on an electrical circuit, isn't the rate limiting step like a capacitor somewhere in the circuitry? A very restrictive rate limiting step such as the paucity of microbial food in breeding water severely limiting the number of larvae reaching the adult stage would cause the reproduction/sterility interventions to be ineffective. Even if the proportions of X-infected male mosquitoes released were increased, there would be very little impact.

Math Model: Biopesticide Wind Drift

Further diluting the math model basic assumptions is the factor of wind drift. Mosquitoes carried on the wind into and out of the release sites of the project area have not been factored into the math model or the overall plan. Lowland male (and female) wild mosquitoes can travel by wind drift up from lowlands to the project area and dilute the intervention mating pool, affecting the efficacy goal of 90% lab-reared male matings. This rapid drift could dilute the proportion of novel *Wolbachia*-infected male mosquitoes.

Considering these factors, the mark-release-recapture study to estimate whether more or less mosquitoes would be released could be open to interpretation. In human trials, empirical data from feasibility analysis precedes formal studies. We go over numbers from human subjects and use the control group to draw conclusions. If this biopesticide mosquito project is to draw on historical controls, the cause-and-effect interpretation will have many ecological confounders and will risk the ecologic fallacy. If this possibility is inevitable, these conditions should be stated now.

Superinfection: Multiple Strains

Mosquitoes and other insects can be infected with more than one strain of *Wolbachia* bacteria at the same time. This is called "superinfection.[7]" *Culex q*. mosquitoes are very susceptible to many strains of *Wolbachia*. Superinfection in *Culex q*. has not been studied for this project. Superinfection could affect cytoplasmic incompatibility, horizontal transmission, evolutionary events, and population replacement.

Wolbachia: Increased Pathogen Infection and Disease-Spreading Capability

Peer-reviewed studies have shown *Wolbachia* bacteria to cause increased pathogen infection in mosquitoes[8] and to cause mosquitoes to become more capable of transmitting both avian malaria[8] and West Nile virus (avian and human)[9]. More study is needed in this area, specifically study of the *Culex quinquefasciatus* mosquito and the *w*AlbB, *w*AlbA, and *w*Pip4 *Wolbachia* strains, along with any combinations (superinfections) of bacteria strains planned for use in this project. Increased pathogen infection and increased disease-spreading capability could be detrimental to the endangered native bird populations, other animals, insects, humans, and subsequently the ecosystems as a whole.

Novel Experiment

This biopesticide mosquito release is an experiment. *Culex q*. has never been used for cytoplasmic incompatibility stand-alone field release. Scientists advising on this project have not studied horizontal transmission or movement of *Wolbachia* from somatic cells to germ cells in

Culex q. *Wolbachia*-infected mosquitoes are more widely released globally for population replacement, not suppression. Efficacy studies are focused on the population replacement method. The population suppression method has not been sufficiently studied. The potential collateral damage from the use of this biopesticide is unknown.

Alternatives: Not Considered

Alternative approaches to mitigating avian malaria have not been considered, including treatment of avian malaria in the mosquito phase through antimalarial drug feeding (i.e., primaquine and ivermectin) in rabbits and/or battery-powered warm artificial blood packs containing the antimalarial drugs. The range of blood-feeding females is a lot wider than extrapolated from sugar feedings of males.

CONCLUSION

While I have chosen to address each mechanism separately, all mechanisms interact with each other. There has been insufficient study in each area of concern and in the combination of mechanisms. The precautionary principle calls for further study of the probability of efficacy and the potential for collateral damage. The use of this novel biopesticide requires a feasibility study, independent of the proposal itself, analyzing and considering all of the critical aspects of the proposed project in order to determine the likelihood of it succeeding. Though I have been presented with the math model, I would like to see the basic assumptions factored in prior to the derivation of the actual expressions/conclusions. I would like to see incorporation of choke points and rate limiting factors, wind drift and expansion of horizontal transfer reservoirs if/when vertical transmission is blocked. Mitigation measures must be established to assure that side-effects would be contained. Detailed study in each area of concern, separately and together, is needed.

Proponents may be right that this intervention will save the native birds in the short-term, but long-term consequences to other island ecologies and to these same native birds may ultimately be detrimental. When one realizes the latter, the damage may be impossible to recall or repair, like the effects we've seen with so many other invasive species in Hawaii.

REFERENCES

1. "Wolbachia infection in wild mosquitoes (Diptera: Culicidae): implications for transmission modes and host-endosymbiont associations in Singapore" – Huicong Ding, Huiqing Yeo, Nalini Puniamoorthy (BMC, 12/09/2020) https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-020-04466-8

2. "Wolbachia Horizontal Transmission Events in Ants: What Do We Know and What Can We Learn?" – Sarah J. A. Tolley, Peter Nonacs, Panagiotis Sapountzis (Frontiers in Microbiology, 03/06/2019) https://www.frontiersin.org/articles/10.3389/fmicb.2019.00296/full

3. "The Intracellular Bacterium Wolbachia Uses Parasitoid Wasps as Phoretic Vectors for Efficient Horizontal Transmission" – Muhammad Z. Ahmed, Shao-Jian Li, Xia Xue, Xiang-Jie Yin, Shun-Xiang Ren, Francis M. Jiggins, Jaco M. Greeff, Bao-Li Qiu (National Center for Biotechnology Information, National Library of Medicine, 02/12/2015)
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4347858/

4. "Infection by *Wolbachia*: from passengers to residents" – Hervé Merçot, Denis Poinsot (Comptes Rendus Biologies, 2009)
https://www.sciencedirect.com/science/article/pii/S1631069108002709

5. "Somatic stem cell niche tropism in *Wolbachia*" – Horacio M. Frydman, Jennifer M. Li, Drew N. Robson, Eric Wieschaus (Nature, 05/25/2006)
https://people.bu.edu/hfrydman/publications/Frydman%202006%20-%20Somatic%20stem%20cell%20niche%20tropism%20in%20Wolbachia.pdf

6. "The rich somatic life of *Wolbachia*" – Jose E. Pietri, Heather DeBruhl, William Sullivan (MicrobiologyOpen, 2016)
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5221451/

7. "*Wolbachia* transinfections in *Culex quinquefasciatus* generate cytoplasmic incompatibility" – T. H. Ant, C. Herd, F. Louis, A. B. Failloux, S. P. Sinkins (Insect Molecular Biology, 06/13/2019)
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7027843/

8. "*Wolbachia* Can Enhance *Plasmodium* Infection in Mosquitoes: Implications for Malaria Control?" – Grant L. Hughes, Ana Rivero, Jason L. Rasgon (PLOS Pathogens, 09/4/14)
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4154766/

9. "*Wolbachia* Enhances West Nile Virus (WNV) Infection in the Mosquito *Culex tarsalis*" – Brittany L. Dodson, Grant L. Hughes, Oluwatobi Paul, Amy C. Matacchiero, Laura D. Kramer, Jason L. Rasgon (PLOS Neglected Tropical Diseases, 07/10/14)
https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0002965

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IN THE CIRCUIT COURT OF THE FIRST CIRCUIT

STATE OF HAWAI‘I

HAWAI‘I UNITES, a 501(c)(3) nonprofit corporation; TINA LIA, an individual, Plaintiffs, v. BOARD OF LAND AND NATURAL RESOURCES, STATE OF HAWAI‘I, and DEPARTMENT OF LAND AND NATURAL RESOURCES, STATE OF HAWAI‘I, Defendants.	Civil No. 1CCV-23-0000594 (JMT) (Environmental Court) **NOTICE OF HEARING** Judge: Hon. John M. Tonaki Hearing Date: July 21, 2023 Hearing Time: 9:00 a.m.

NOTICE OF HEARING

TO: DEPARTMENT OF THE ATTORNEY GENERAL
425 Queen Street
Honolulu, Hawaiʻi 96813

Attorney for Defendants
Board of Land and Natural Resources,
State of Hawaiʻi and
Department of Land and Natural Resources,
State of Hawaiʻi

NOTICE IS HEREBY GIVEN that the Motion for Temporary Restraining Order and Preliminary Injunction shall come for hearing before the Honorable John M. Tonaki, Judge of the above-entitled court, on **July 21, 2023 at 9 a.m.** or as soon thereafter as counsel may be heard. This is an in-person hearing and will be heard at the location below:

Kaʻahumanu Hale
777 Punchbowl Street
4th Floor, Courtroom 17
Honolulu, Hawai`i 96813

DATED: Honolulu, Hawaiʻi, June 20, 2023.

/s/ Timothy Vandeveer
Margaret (Dunham) Wille
Timothy Vandeveer

Attorneys for Plaintiffs
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IN THE CIRCUIT COURT OF THE FIRST CIRCUIT

STATE OF HAWAIʻI

HAWAIʻI UNITES, a 501(c)(3) nonprofit corporation; TINA LIA, an individual,

Plaintiffs,

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BOARD OF LAND AND NATURAL RESOURCES, STATE OF HAWAIʻI, and DEPARTMENT OF LAND AND NATURAL RESOURCES, STATE OF HAWAIʻI,

Defendants.

Civil No. 1CCV-23-0000594 (JMT)
(Environmental Court)

CERTIFICATE OF SERVICE for

PLAINTIFFS' MOTION FOR TEMPORARY RESTRAINING ORDER AND PRELIMINARY INJUNCTION; MEMORANDUM IN SUPPORT OF MOTION; DECLARATION OF COUNSEL; DECLARATION OF TINA LIA; DECLARATION OF DR. LORRIN W. PANG; EXHIBITS 1-11; NOTICE OF HEARING; CERTIFICATE OF SERVICE

CERTIFICATE OF SERVICE

The undersigned hereby certifies that on the date indicated below, a copy of the above-named document along with this Certificate of Service will be duly served upon the following parties in the manner indicated below:

DEPARTMENT OF THE ATTORNEY GENERAL — Certified U.S. Mail
425 Queen Street
Honolulu, Hawaiʻi 96813

Attorney for Defendants
Board of Land and Natural Resources,
State of Hawaiʻi and
Department of Land and Natural Resources,
State of Hawaiʻi

DATED: Honolulu, Hawaiʻi, June 20, 2023.

/s/ Timothy Vandeveer
Margaret (Dunham) Wille
Timothy Vandeveer

Attorneys for Plaintiffs
Hawaiʻi Unites and Tina Lia