# Kawailoa Wind Project Habitat Conservation Plan
# FY 2019 Annual Report



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Incidental Take License ITL 14/ Incidental Take Permit TE59861A-0

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# EXECUTIVE SUMMARY

This report summarizes work performed by Kawailoa Wind, LLC (Kawailoa Wind), owner of Kawailoa Wind Project (Project), during the State of Hawaiʻi fiscal year 2019 (FY 2019; July 1, 2018 – June 30, 2019) under the terms of the approved Habitat Conservation Plan (HCP), dated October 27, 2011, and pursuant to the obligations contained in the Project's state Incidental Take License ITL-14 (ITL) and federal Incidental Take Permit TE-59861A-0 (ITP). The Project was constructed in 2011 and 2012, and was commissioned to begin operating on November 2, 2012. Species covered under the HCP include six federally-listed threatened and endangered species and one state-listed endangered species. Kawailoa Wind is currently developing an HCP Amendment, in collaboration with the U.S. Fish and Wildlife Service (USFWS) and Hawaiʻi Division of Forestry and Wildlife (DOFAW), to 1) support a request to increase the amount of take for the Hawaiian hoary bat beyond the take authorized under the current ITP/ITL, and 2) add the endangered Hawaiian petrel as a Covered Species.

Fatality monitoring at the Project continued throughout FY 2019 at all turbines within the 35-meter radius circular search plots. The mean search interval for turbines and met towers in FY 2019 was 3.5 days and 7.0 days, respectively.

Four 28-day carcass persistence trials were conducted in FY 2019, using 60 bat surrogates and 12 medium-sized bird carcasses. For FY 2019, the probability that a carcass persisted until the next search was 1.00 (95% CI = [0.0.69, 1.00]) for all bat surrogate carcasses, and 1.00 (95% CI = [0.62, 1.00]) for medium-sized bird carcasses.

Searcher efficiency trials were conducted over 24 trial days with 81 trial carcasses in FY 2019. The overall searcher efficiencies in FY 2019 for bat surrogate (N = 69) and medium-sized bird (N = 12 carcass trials were 91.3 percent (95% CI = [0.83, 0.96]) and 100 percent (95% CI = [0.82, 1.00]), respectively.

Kawailoa Wind continued the scavenger control program used to increase the probability that fatalities at the wind facility have the opportunity to be discovered by searchers. Overall, the scavenger control program documented the removal of 67 mongooses in FY 2019 at the Project.

Five Hawaiian hoary bat fatalities were found in FY 2019. The Project's total observed bat take from November 2012 through FY 2019 is 40. The fatality estimate for non-incidental observed bats using the Evidence of Absence estimator (Dalthorp et al. 2017) at the upper 80 percent credibility level is 79 and the total indirect take for this estimate is 9 adult equivalents. Combining these values, there is an approximately 80 percent chance that actual take of Hawaiian hoary bats at the Project was less than or equal to 88 adults.

As a result of this level of take, Kawailoa Wind has incorporated additional minimization and mitigation measures to reduce the risk to the Hawaiian hoary bat. In the beginning of FY 2019, Kawailoa Wind reduced the number of turbine stop/start events per night by extending the rolling average time from 10 to 20 minutes. Additionally, Kawailoa Wind installed acoustic deterrents at all 30 Project turbines in May and June 2019.

One endangered bird fatality was found in FY 2019. In August 2018, a Hawaiian petrel was observed incidentally within the Project Area. The Hawaiian petrel was not originally included as a Covered Species in the HCP and ITP/ITL. Kawailoa Wind is requesting that their HCP Amendment also add the Hawaiian petrel as a Covered Species.

Non-listed bird fatalities found at the Project in FY 2019 included two species protected by the Migratory Bird Treaty Act (MBTA): white-tailed tropicbird (*Phaethon lepturus*), and cattle egret (*Onychoprion fuscatus*). In addition, 33 fatalities of non-native introduced birds without MBTA protection were documented.

During FY 2019, four ultrasonic bat detectors were managed at the Project. These were located at WTG's 1, 10, 21, and 25. Between July 2018-June 2019, Hawaiian hoary bats were detected on 323 of 1,262 detector-nights (25.6 percent of detector-nights) at these locations. In addition to the four permanent detectors, two bat detectors were deployed at WTG 30 to document bat interactions with the ultrasonic acoustic bat deterrent "proof of concept" test.

The 'Uko'a Wetland mitigation program for Tier 1 mitigation continued for waterbirds and bats through FY 2019 including invasive vegetation control, predator control and monitoring, fence maintenance, bat lane construction, and bat acoustic monitoring. Hawaiian hoary bat research projects conducted by USGS and WEST for Tier 2 and 3 bat mitigation continued in FY 2019. Tier 1 mitigation for Newell's shearwater was completed in FY 2015. Tier 1 pueo or Hawaiian short-eared owl (*Asio flammeus sandwichensis*) mitigation was completed in FY 2017.

Kawailoa Wind and Tetra Tech conducted 19 meetings with USFWS and DOFAW staff in FY 2019, primarily to discuss the HCP Amendment. In addition, Kawailoa Wind and Tetra Tech met with the Endangered Species Recovery Committee three times during FY 2019.

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# 1.0   Introduction

The Habitat Conservation Plan (HCP) for the Kawailoa Wind Project (Project) was approved by the Hawaiʻi Division of Forestry and Wildlife (DOFAW) in 2012. On December 8, 2011, the U.S. Fish and Wildlife Service (USFWS) issued Kawailoa Wind, LLC (Kawailoa Wind) a federal incidental take permit (ITP) for the Project, and DOFAW issued a state incidental take license (ITL) on January 6, 2012. The ITP and ITL cover the incidental take of six federally listed threatened and endangered species, as well as one state-listed endangered species (referred to as the Covered Species) over a 20-year permit term. The Covered Species include: the Hawaiian stilt or aeʻo (*Himantopus mexicanus knudseni*), Hawaiian coot or ʻalae keʻokeʻo (*Fulica alai*), Hawaiian duck or koloa maoli (*Anas wyvilliana*), Hawaiian gallinule or ʻalae ʻula (*Gallinula chloropus sandvicensis*), Newell's shearwater or ʻaʻo (*Puffinus newelli*), Hawaiian hoary bat or ʻopeʻapeʻa (*Lasiurus cinereus semotus*), and the state-listed Hawaiian short-eared owl or pueo (*Asio flammeus sandwichensis*).

Project construction occurred in 2011 and 2012, and was commissioned to begin operating on November 2, 2012. It is owned and operated by Kawailoa Wind, a wholly-owned subsidiary of DESRI IV, LLC, which is an investment fund managed by D.E. Shaw Renewable Investments, LLC.

Kawailoa Wind, in collaboration with USFWS and DOFAW, has been developing an HCP Amendment to amend the current ITP and ITL since 2015. The purpose of the HCP Amendment is to 1) support a request to increase the amount of take for the Hawaiian hoary bat beyond the take authorized under the current ITP/ITL, and 2) add the endangered Hawaiian petrel or ʻuaʻu (*Pterodroma sandwichensis*) as a Covered Species. The State Draft HCP Amendment was published in the Office of Environmental Quality Control Environmental Notice on October 23, 2018 (Tetra Tech 2018a), and the comment period ended on February 22, 2019. The Federal Programmatic Environmental Impact Statement addressing the Draft HCP Amendment was published in the *Federal Register* in April 2019.

This report summarizes work performed for the Project during the State of Hawaiʻi 2019 fiscal year (FY 2019; July 1, 2018–June 30, 2019) pursuant to the terms and obligations of the approved HCP, ITL, and ITP.

# 2.0   Fatality Monitoring

In FY 2019, all 30 wind turbine generators (WTGs) were searched for fatalities twice per week, and the two meteorological (met) towers were searched once per week. Search plots consisted of a 35-meter radius circular plot centered on each WTG, and 50-meter radius plot centered on the two unguyed met towers. The FY 2019 mean search interval for WTGs was 3.5 days (standard deviation [SD] = 0.6 days), and the mean search interval for met towers was 7.0 days (SD = 1.1 days).

The search plots were primarily searched by trained dogs accompanied by their handlers. When conditions limited the use of dogs (e.g., weather, injury, availability of canine search team, etc.),

search plots were visually surveyed by Project staff. Overall, canine teams conducted 93.9 percent of the searches in FY 2019. Vegetation within the search plots was managed (e.g., mowed) to maximize searcher efficiency (Sections 4.0 and 5.0).

# 3.0   Carcass Persistence Trials

Four 28-day carcass persistence trials were conducted in FY 2019 using bat surrogates (black rat; *Rattus rattus*) and wedge-tailed shearwater carcasses (*Puffinus pacificus*). Wedge-tailed shearwaters are medium-sized birds that are suitable surrogates for the listed bird species covered in the HCP (see above), as well as the listed Hawaiian petrel. For FY 2019, the probability that a carcass persisted until the next search was 1.00 (95% CI = [0.62, 0.1.00]) for all bat surrogate carcasses (N=60), and was 1.00 (95% CI = [0.69, 1.00]) for medium-sized bird carcasses (N=12).

# 4.0   Searcher Efficiency Trials

Tetra Tech personnel (non-searchers) administered 81 searcher efficiency trials on 24 trial days during FY 2019. Similar to the carcass persistence trials, wedge-tailed shearwaters were used as surrogates for listed bird species, and black rats were used as surrogates for bats. Searcher efficiency trials occurred throughout the year, and 97.5 percent were conducted on canine search teams and 2.5 percent were conducted on visual searchers in FY 2019.

Vegetation category (short vs. medium) of the search plot was documented at the time the carcasses were placed and when they were found. The overall searcher efficiency for canine searchers (i.e., combined vegetation classes) in FY 2019 was 92.5 percent (95% CI = [0.84, 0.97]) for bat surrogates (N = 67) and 100 percent (95% CI = [0.82, 1.00]) for medium-sized bird (N = 12) carcasses. The overall searcher efficiency in FY 2019 for visual searchers was 50 percent (95% CI = [0.06, 0.94]) for bat surrogates (N=2). No medium-sized bird trials were conducted on visual searchers in FY 2019.

The mean searcher efficiencies in FY 2019 for canine searchers for bat surrogate (N = 55) and medium-sized bird (N = 8) carcass trials in short vegetation were 92.7 percent (95% CI = [0.84, 0.98]) and 100.0 percent (95% CI = [0.74, 1.00]), respectively. The mean searcher efficiencies in FY 2019 for canine searchers for bat surrogate (N= 12) and medium-sized bird (N= 4) carcass trials in medium vegetation were 91.7 percent (95% CI = [0.67, 0.99]) and 100 percent (95% CI = [0.56, 1.00]), respectively. The mean searcher efficiencies for visual searchers for bat surrogate (N=1) in short vegetation was 100 percent (95% CI = [0.15, 1.00]) and in medium vegetation (N=1) was zero percent.

# 5.0   Vegetation Management

Vegetation in the search plots consists mainly of Guinea grass (*Megathyrsus maximus*), Bermuda grass (*Cynodon dactylon*), and sensitive plant (*Mimosa pudica*). All search plots around the WTGs and met towers were mowed regularly to increase visibility during fatality searches. All plots were mowed to a height of 3 to 4 inches, depending on the type of mower used, and were cut every 3 to 4 weeks. There were no unsearchable areas or rock-lined swales within the 35-meter radius search plots.

# 6.0   Scavenger Trapping

Active trap locations in FY 2019 covered the same general area where traps were deployed in FY 2018. Traps deployed at the Project in FY 2019 include 22 Doc-250 and 21 GoodNature A24 traps; the same number and types of traps were deployed in FY 2018.  The scavenger control program documented the removal of 67 mongooses in FY 2019. No rats or cats were trapped.

# 7.0   Documented Fatalities and Take Estimates

All fatalities observed at the Project during FY 2019 are listed in Appendix 1. All observed, downed wildlife were handled and reported in accordance with the Downed Wildlife Protocol provided by USFWS and DOFAW. No injured (live) downed wildlife were observed at the Project in FY 2019.

Six listed species fatalities were found in FY 2019 (including one Hawaiian petrel fatality and five Hawaiian hoary bat fatalities), as described below. Based on these observed fatalities, Kawailoa Wind estimated the total take estimate for listed species. An upper credible limit (UCL) of take is estimated from three components: (1) observed direct take (ODT) during protocol (standardized) surveys, (2) unobserved direct take (UDT), and (3) indirect take. The Evidence of Absence software program (EoA; Dalthorp et al. 2017), the agency-approved analysis tool for analyzing direct take, uses results from bias correction trials and ODT to generate UCL of direct take (i.e., ODT + UDT). The USFWS and DOFAW have requested that these calculations be reported at the 80 percent UCL. Values from this analysis can be interpreted as: there is an 80 percent probability that actual direct take at the Project over the analysis period was less than or equal to the 80 percent UCL. Associated indirect take is estimated based on observations of the temporal distribution of Covered Species fatalities at the Project and life history characteristics of, or assumed to be representative of, the associated Covered Species.

## 7.1   Hawaiian Petrel

On August 20, 2018, a single medium-sized seabird (procellarid) carcass was observed incidentally within the Project Area, and later identified through genetic analysis as a Hawaiian petrel. The carcass was observed approximately 37 meters from the base of WTG 30. The Hawaiian petrel was

not included as a Covered Species in the approved HCP and ITP/ITL. When the approved HCP was developed, the Hawaiian petrel was not known to occur regularly on Oʻahu. Very few individuals have been recorded on the island in the last several decades, and when found were often grounded and assumed to have been disoriented vagrants from other islands (Young et al. 2019). The most recent evidence of a population on Oʻahu was limited to sub-fossil remains primarily on the ʻEwa plains, which precede European contact (Pyle and Pyle 2017). The species was considered very unlikely to transit the Project Area; therefore, take was thought to be highly unlikely. The decision to exclude the Hawaiian petrel from the approved HCP and ITP/ITL was consistent with technical advice received from USFWS and DOFAW at the time of permitting.

Based on the two observed fatalities at the Project and recent surveys documenting Hawaiian petrel occurrence on Oʻahu (Young et al. 2019), Kawailoa Wind is requesting authorization for incidental take of the Hawaiian petrel in the HCP Amendment.

## 7.2   Hawaiian Hoary Bat

Five Hawaiian hoary bat fatalities were documented during FY 2019 (Table 1, Appendix 1). These fatalities were all found within the 35-meter fatality search plots. All bat carcasses were transferred to U.S. Geological Survey (USGS) for genetic testing.

A total of 40 Hawaiian hoary bat fatalities have been observed at the Project since operations began on November 2, 2012. These include fatalities in each year since the initiation of commercial operations: FY 2013 (5), FY 2014 (9), FY 2015 (9), FY 2016 (5), FY 2017 (2), FY 2018 (5), and FY 2019 (5) (Table 2). Two of these 40 bats were found outside of fatality search plots and classified as incidental observations.

**Table 1. Observed Fatalities of Hawaiian Hoary Bats at the Project in FY 2019**

| Age | Sex | Date Documented | WTG | Distance to WTG (meters) | Bearing from WTG (degrees) |
|-----|-----|-----------------|-----|--------------------------|----------------------------|
| Adult | Unknown | July 19, 2018 | 6 | 11 | 244 |
| Adult | Unknown | August 3, 2018 | 17 | 24 | 298 |
| Adult | Unknown | August 6, 2018 | 11 | 26 | 228 |
| Adult | Unknown | August 17, 2018 | 24 | 15 | 268 |
| Adult | Unknown | October 26, 2018 | 29 | 27 | 193 |

**Table 2. Hawaiian Hoary Bat Fatalities Observed Since Operations Began and Cumulative Take Estimates**

| Fiscal Year | Number of Observed Fatalities[1] | Cumulative Take Estimate[2] |
|:---:|:---:|:---:|
| 2013 | 4 | 11 |
| 2014 | 9 | 26 |
| 2015 | 9 | 38 |
| 2016 | 4 | 49 |
| 2017 | 2 | 60 |
| 2018 | 5 | 73 |
| 2019 | 5 | 88 |
| **Total** | **38** | **88** |

1 Does not include bat fatalities found outside of the search areas (i.e., incidental observations).

2 Cumulative take represents the 80 percent UCL of cumulative direct take estimated from Evidence of Absence estimator (Dalthorp et al. 2017) plus the associated indirect take calculated using USFWS (USFWS 2016) guidance.

The estimated direct take (ODT + UDT) for the 40 Hawaiian hoary bat fatalities found between the start of operation (November 2, 2012) and end of FY 2019 is less than or equal to 79 bats (80 percent UCL; Appendix 2). Because two of the 40 observed bat fatalities were found outside of the search areas (i.e., were incidental observations), 38 fatalities were used in the analysis, and the two incidental observations are accounted for in the estimated value of UDT. The two incidental observations were not found during FY 2019.

Indirect take is estimated to account for the potential loss of individuals that may occur indirectly as the result of the loss of an adult female through direct take during the period that females may be pregnant or supporting dependent young. Indirect take for the Project is calculated using the October 2016 USFWS guidance as follows:

- The average number of pups attributed to a female that survive to weaning is assumed to be 1.8.

- The sex ratio of bats taken through UDT is assumed to be 50 percent female, unless there is substantial evidence (10 or more bats) to indicate a different sex ratio.

- The assessment of indirect take to a modeled UDT accounts for the fact that it is not known when the unobserved fatality may have occurred. The period of time from pregnancy to end of pup dependency for any individual bat is estimated to be 3 months. Thus, the probability of taking a female bat that is pregnant or has dependent young is 25 percent.

- The conversion of juveniles to adults is one juvenile to 0.3 adults.

Based on the USFWS methodology (2016), the estimate of cumulative indirect take in FY 2018 is calculated as:

- **Total juvenile take calculated from observed female take (April 1 – September 15)**

  o 2 (observed females) * 1.8 (pups per female) = 3.6 juveniles

- **Total juvenile take calculated from observed unknown sex take (April 1 – September 15)**

  o 15 (observed unknown sex) * 0.5 (assumed sex ratio) * 1.8 (pups per female) = 13.5 juveniles

- **Total juvenile take calculated from unobserved take**

  o 44 (unobserved direct take) * 0.5 (assumed sex ratio) * 0.25 (proportion of calendar year females could be pregnant or have dependent pups) * 1.8 (pups per female) = 9.9 juveniles

- **Total Calculated Juvenile Indirect Take** = 27.0 (3.6 + 13.5 + 9.9)

- **Total Adult Equivalent Indirect Take =** 0.3 (juvenile to adult conversion factor) * 27.0 = 8.1

Therefore, the estimated indirect take based on the UCL of Hawaiian hoary bat direct take at the Project is nine adults (rounded up from 8.1).

The UCL for Project take of the Hawaiian hoary bat at the 80 percent credibility level is 88 adult bats (79 estimated direct take + 9 estimated indirect take). That is, there is an approximately 80 percent probability that actual take at the Project at the end of FY 2019 is less than or equal to 88. This estimate falls within the Tier 4 bat take request detailed in the Draft HCP Amendment. The Draft HCP Amendment addresses the exceedance of the currently authorized bat take limit in the approved HCP through the identification of additional avoidance and minimization measures, as well as additional compensatory mitigation for the Hawaiian hoary bat (Tetra Tech 2018a).

Table 2 presents the cumulative take estimate (direct take + indirect take) by FY since operations began. Direct take is estimated using the Evidence of Absence estimator at the 80 percent UCL (Dalthorp et al. 2017). Indirect take is calculated using USFWS (2016) guidance.

## 7.3   Non-listed Species

Thirty-six bird fatalities representing 10 species were documented at WTGs at the Project in FY 2019. No fatalities have been observed at either of the two met towers. Two of the species observed in FY 2019 are protected by the Migratory Bird Treaty Act (MBTA): white-tailed tropicbird (one bird; *Phaethon lepturus*), and cattle egret (two birds; *Onychoprion fuscatus*). In addition, 33 fatalities of non-native introduced birds without MBTA protection were documented: common waxbill (10 birds; *Estrilda astrild*), common myna (7 birds; *Acridotheres tristis*), spotted dove (5 birds; *Spilopelia chinensis*), zebra dove (3 birds; *Geopelia striata*), nutmeg mannikin (5 birds; *Lonchura punctulata*), Japanese white-eye (one bird; *Zosterops japonicus*), gray francolin (one bird;

*Francolinus pondicerianus*), and black francolin (one bird; *Francolinus francolinus*). For a complete list of fatalities for FY 2019 see Appendix 1.

# 8.0   Wildlife Education and Observation Program

Wildlife Education and Observation Program (WEOP) trainings continue to be conducted on an as-needed basis to provide on-site personnel with the information they need to be able to respond appropriately in the event they observe a listed species or encounter a fatality while on-site. Tetra Tech biologists conducted 27 WEOP trainings in FY 2019.

# 9.0   Mitigation

The Project's current mitigation requirements are described in Section 7.6 of the approved HCP (SWCA 2010). Additional mitigation is described in Section 7 of the Draft HCP Amendment (Tetra Tech 2018a).

## 9.1   Hawaiian Hoary Bats

For the Hawaiian hoary bat, mitigation is required based on where the estimated Project take falls with respect to tiers identified in the approved HCP. Management activities for Tier 1 mitigation continued at ʻUkoʻa Wetland during FY 2019 (see Section 9.1.2). USFWS and DOFAW approved bat research projects for Tiers 2/3 mitigation published or continued in FY 2019 (see Section 9.1.3). Kawailoa Wind contributed funds toward the purchase and long-term protection of the Helemano Wilderness Area for Tier 4 mitigation under the proposed HCP Amendment, as described in Section 9.1.5 below.

### 9.1.1   Onsite Acoustic Surveys

Based on commitments in the approved HCP, bat activity was intensively monitored throughout the Project during the first three years of systematic fatality monitoring (beginning in late 2012). Having identified no significant findings during the required intensive acoustic monitoring period, in the second quarter of FY 2017, Kawailoa Wind reduced the acoustic monitoring effort at the Project to four permanent ground-based units distributed throughout the Project at WTGs 1, 10, 21, and 25 (see Figure 1). These locations were randomly chosen after eliminating detectors with high or low detection rates. Each detector site contains one Wildlife Acoustics SM2BAT+ ultrasonic detector (SM2) with one SM3-U1 ultrasonic microphone located 6.5 meters above ground.

Hawaiian hoary bats were detected on 323 of 1,262 detector-nights (25.6 percent of detector-nights) during FY 2019 at the four sampling locations. This represents the most detections and greatest proportion of nights with detections compared to the previous sampling years (Table 3).
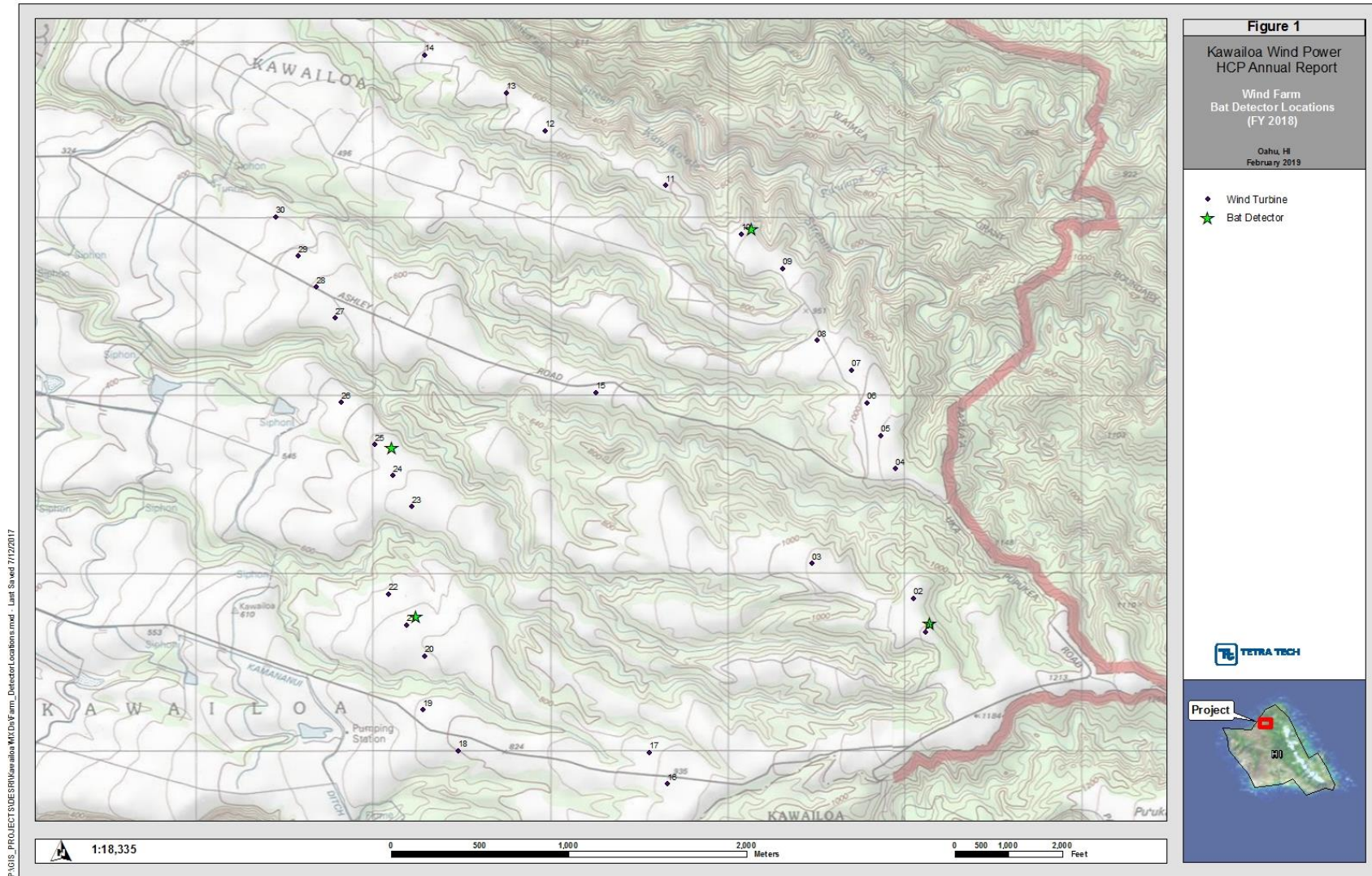
**Figure 1. Four Bat Acoustic Detector Locations at the Project in FY 2019**

Note: Location of the two temporary detectors at WTG 30 are not shown.

**Table 3. Detector-Night Data at Four Detector Sites for FY 2014 to FY 2019**

| Sampling Period | No. of Detector-Nights | No. of Nights with Detections | Proportion of Detector-Nights with Detections |
|---|---|---|---|
| FY 2014 (July 2013 - June 2014) | 1,271 | 91 | 0.072 |
| FY 2015 (July 2014 - June 2015) | 1,021 | 155 | 0.152 |
| FY 2016 (July 2015 - June 2016) | 1,298 | 208 | 0.160 |
| FY 2017 (July 2016 - June 2017) | 1,378 | 173 | 0.126 |
| FY 2018 (July 2017 - June 2018) | 1,421 | 275 | 0.194 |
| FY 2019 (July 2018 - June 2019) | 1,262 | 323 | 0.256 |
| Note: FY 2013 not included due to minimal number of detector-nights compared to other years. | | | |

The temporal patterns of ground-based detection rates for the FY 2019 sampling period were relatively similar to the detections rates observed in previous sampling years (Figure 2). Higher detection rates were observed during the lactation (July to August), late pre-pregnancy (March), and pregnancy (April to June) reproductive periods, with declining detections rates beginning at the onset of the post-lactation (September) reproductive period[1]. The lowest detection rates were observed during the late post-lactation and early pre-pregnancy (November to January) reproductive periods.
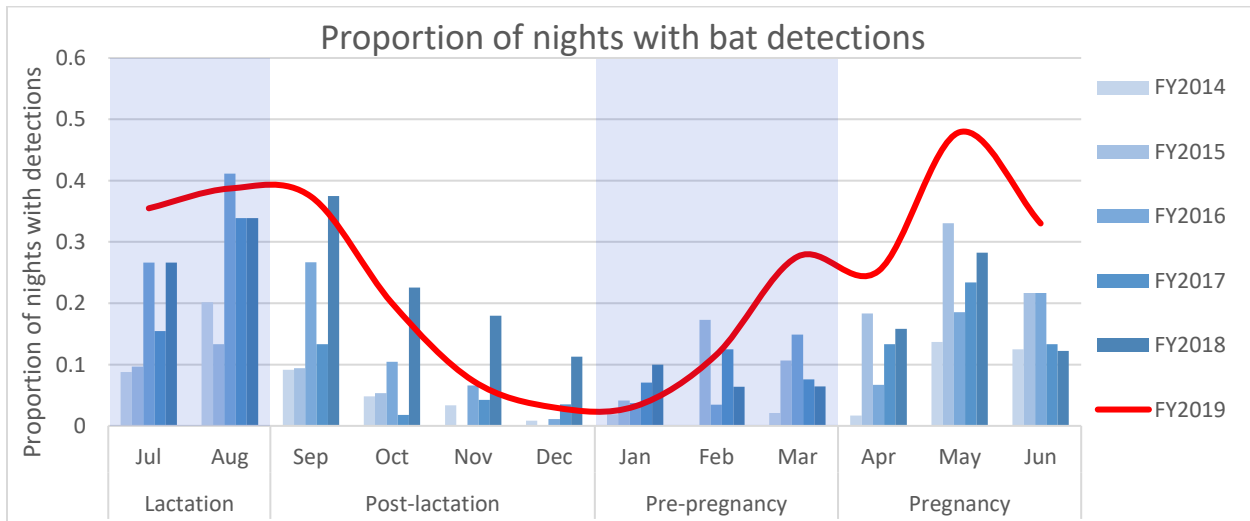


**Figure 2. Bat Acoustic Activity During Approximate Reproductive Periods for FY 2014 to FY 2019**

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[1] Breeding seasons correspond approximately with breeding seasons as defined by Starcevich et al. 2018.

Among the four sampling locations, detections rates were highest at WTG 25 in each of the four reproductive periods (Table 4). WTG 25 also had the highest detection rate of the sampling locations in FY 2017 and FY 2018.

**Table 4. Detector-Night Data for Each Location during FY 2019**

| Reproductive Period | Site Name | No. of Detector-Nights | No. of Nights with Detections | Proportion of Detector-Nights with Detections |
|---|---|---|---|---|
| Lactation | WTG 1 | 77 | 12 | 0.16 |
| | WTG 10 | 77 | 18 | 0.23 |
| | WTG 21 | 77 | 13 | 0.17 |
| | WTG 25 | 62 | 54 | 0.87 |
| Post-lactation | WTG 1 | 104 | 14 | 0.13 |
| | WTG 10 | 87 | 16 | 0.18 |
| | WTG 21 | 75 | 8 | 0.11 |
| | WTG 25 | 106 | 33 | 0.31 |
| Pre-pregnancy | WTG 1 | 105 | 3 | 0.03 |
| | WTG 10 | 79 | 4 | 0.05 |
| | WTG 21 | 71 | 15 | 0.21 |
| | WTG 25 | 88 | 22 | 0.25 |
| Pregnancy | WTG 1 | 72 | 13 | 0.18 |
| | WTG 10 | 76 | 18 | 0.24 |
| | WTG 21 | 75 | 33 | 0.44 |
| | WTG 25 | 51 | 35 | 0.69 |

In addition to the four permanent detectors, two Wildlife acoustic bat detectors were deployed at WTG 30 in September 2018 to document bat interactions with the ultrasonic acoustic bat deterrent "proof of concept" test installed on WTG 30 in July 2018 (see Section 10). One detector (referred to as WTG 30 New) was deployed on September 20, 2018 about 27 m east of the WTG 30. The second detector (referred to as WTG 30 Historical) was deployed on September 25, 2018 about 75 m east-southeast of the WTG 30 in the historical bat detector location where bat activity data is available from July 2013 to May 2015 (FY 2014 to FY 2015).

At the WTG 30 New detector, Hawaiian hoary bats were detected on 23 of 258 detector-nights (8.9 percent of detector-nights) between September 2018 and June 2019. At WTG 30 Historical detector, Hawaiian hoary bats were detected on 24 of 252 detector-nights (9.5 percent of detector-nights), which is similar to historical activity levels at this location (Kawailoa Wind 2014). Data collection will continue at these detectors throughout FY 2020.

## 9.1.2 'Uko'a Wetland (Tier 1)

Mitigation for bats and waterbirds continued at 'Uko'a Wetland during FY 2019. In FY 2016 (March 2016), USFWS and DOFAW provided written confirmation permitting adaptive management for the original bat and waterbird mitigation proposed at 'Uko'a Wetland. This included the following:

1. Reduction from 40 acres of vegetation removal to assumed open water areas, as outlined in Figure 2 of the approved 'Uko'a Wetland Hawaiian Hoary Bat Mitigation Management Plan (H.T. Harvey and SWCA 2014);

2. Omit replanting of natives with assumption of natural recruitment after invasive plant species are removed;

3. Omit mosquitofish removal component; and

4. Tie success criteria for bats to completion of all other management and monitoring components instead of increased bat activity.

In FY 2019, activities associated with Tier 1 bat mitigation included invasive vegetation removal, predator control, monitoring predator presence, fence monitoring and maintenance, bat lane maintenance, and bat acoustic monitoring. Additional details for each are provided below. Based on the approved 'Uko'a Wetland Hawaiian Hoary Bat Mitigation Management Plan (H.T. Harvey and SWCA 2014), bat acoustic monitoring will continue for 3 to 5 years post-restoration. Based on the approved 'Uko'a Wetland Management Plan for Waterbirds 2012–2032 (SWCA 2012), vegetation management, predator and ungulate control, and fence maintenance will continue for the permit term (20 years).

### 9.1.2.1 Invasive Vegetation Removal

In FY 2019, Hapa Landscaping conducted quarterly maintenance visits to remove any areas of water hyacinth (*Eichhornia crassipes*) or other non-native vegetation that regenerated in the previously cleared open water area. Scheduled visits were modified as needed to accommodate staff schedules and avoid disturbing moorhen nests and chicks in the area. Figure 3 shows a representative photograph of this ongoing maintenance.

**Figure 3. Open Water Resulting from Ongoing Removal of Water Hyacinth at ʻUkoʻa Wetland in FY 2019**

## 9.1.2.2    *Predator Control and Monitoring Predator Presence*

The Project contracted Grey Boar Wildlife Services, LLC (Grey Boar) to conduct predator and ungulate removal at ʻUkoʻa Wetland, as well as to monitor and repair the fence. Predator control first began at ʻUkoʻa Wetland in June 2014 (FY 2014). The following trap types are used throughout at ʻUkoʻa Wetland: four pig corral and two pig box traps, 100 GoodNature A24s, 12 live cages, 24 Doc-250s and 25 body grip traps (Coni-boxes). In FY 2019, a total of 192 predators were removed from ʻUkoʻa Wetland including 42 pigs, 136 mongoose, two cats, and 12 rats (Grey Boar 2018a, Grey Boar 2018b, Grey Boar 2019a, Grey Boar 2019b).

Tracking tunnels were set on five dates during FY 2019 to assess the presence of rodents, mongoose, and cats within the wetland. A total of 27 tracking tunnels were used to detect predator presence. The cards were baited with fish paste and collected one day after setting. Tracks were then counted and recorded. Percent activity (number of cards with tracks divided by total number of cards set out) is shown in Table 5.

Mongoose and cat percent activity was zero for three out of the five dates. Rat percent activity varied between zero and 11.1 percent, and mice activity ranged from zero and 14.8% percent. Overall, tracking tunnel data since 2014 shows a general reduction in predator presence, specifically mongoose, since the predator program was initiated.

**Table 5. Percent Predator Activity Based on Tracking Tunnels at ʻUkoʻa Wetland during FY 2019**

| Date | Rats | Mongoose | Mice | Cats |
|---|---|---|---|---|
| July 21, 2018 | 3.7% | 3.7% | 0.0% | 0.0% |
| September 8, 2018 | 11.1% | 0.0% | 7.4% | 0.0% |
| December 8, 2018 | 7.4% | 0.0% | 7.4% | 3.7% |
| March 23, 2019 | 7.4% | 3.7% | 7.4% | 0.0% |
| June 21, 2019 | 0.0% | 0.0% | 14.8% | 3.7% |

### 9.1.2.3    *Fence Monitoring and Maintenance*

Fence inspections were conducted by Grey Boar while checking predator control traps. The fence was visually inspected for any signs of ungulate disturbance, damage, or vandalism. During FY 2019, several sections of fence were repaired. The main cause of fence damage is by trespassers.

### 9.1.2.4    *Bat Lanes*

Oahu Tree Works, LLC finished bat lane construction in December 2017 (FY 2018). In total, there are 16 bat lanes within 10 zones throughout ʻUkoʻa Wetland (Figure 4). Bat lane maintenance occurred in Q2 and Q4 of FY 2019. Lane maintenance consists of cutting branches and trees that regrow within the 5-meter-wide bat lanes. Figure 5 shows one of the bat lanes shortly after lane maintenance.
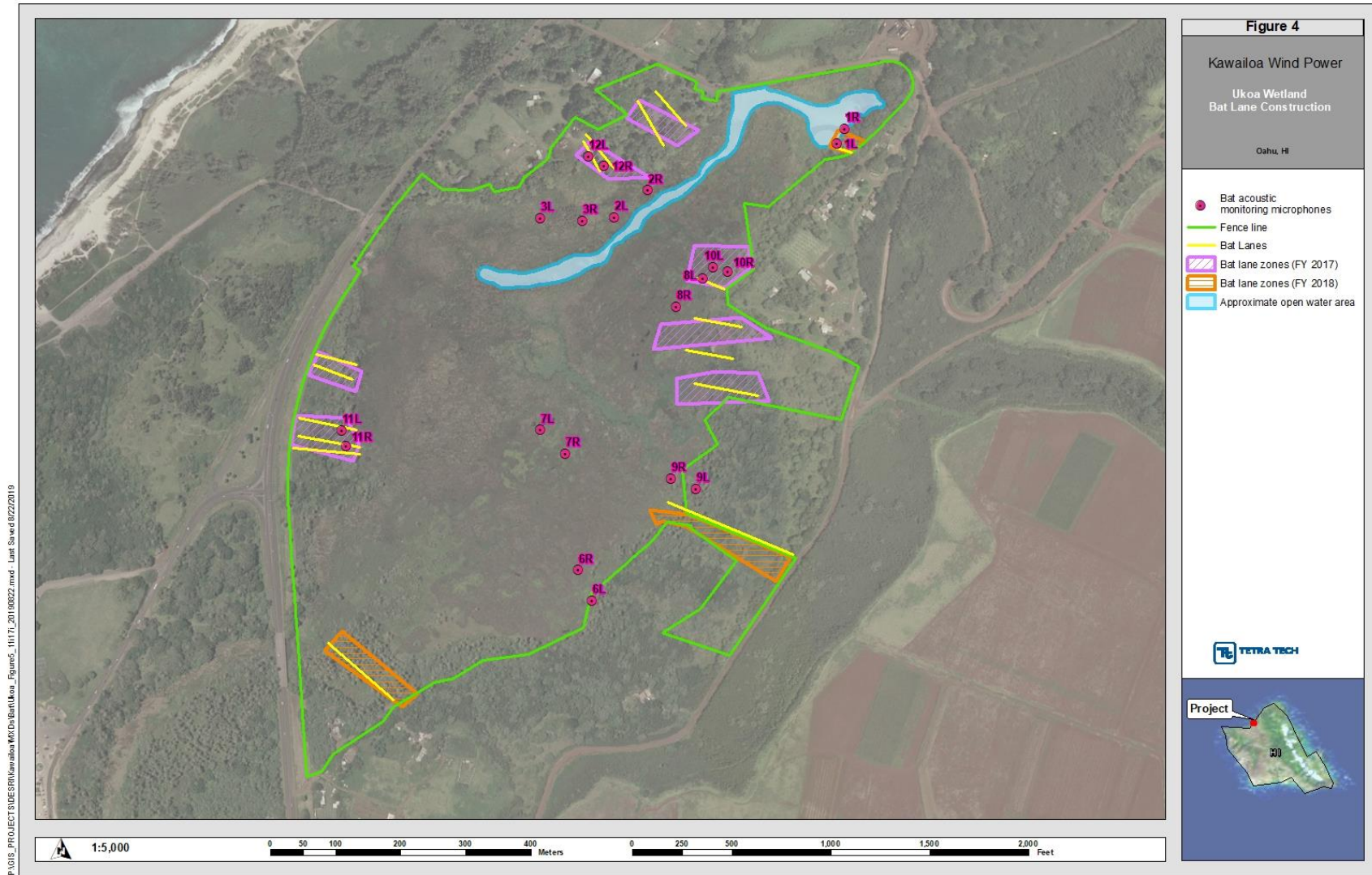
**Figure 4. Bat Lanes and Bat Acoustic Detectors at 'Uko'a Wetland**

**Figure 5. Bat Lane at 'Uko'a Wetland Adjacent to Open Water. Photo Taken in July 2019**

### 9.1.2.5        *Bat Acoustic Surveys at 'Uko'a*

In June 2017, following clearing of invasive vegetation in the open water area and while the bat lanes were being created, ten Wildlife Acoustics SM2+BAT detectors were installed in the same historical locations where detectors were deployed between 2012 and 2015 (see Figure 4). To maintain consistent methods among all years of study, two microphones were connected to each detector and bat activity levels were quantified using the proportion of detector-nights containing a bat pass (any call file containing two or more bat echolocation pulses; Gannon et al. 2003).

Hawaiian hoary bats were detected on 466 of 3,373 detector-nights (13.8 percent of detector-nights) at 'Uko'a in FY 2019 (Figure 6). This represents a slight decrease from FY 2018, which documented detections on 14.7 percent of detector-nights (Tetra Tech 2018b).
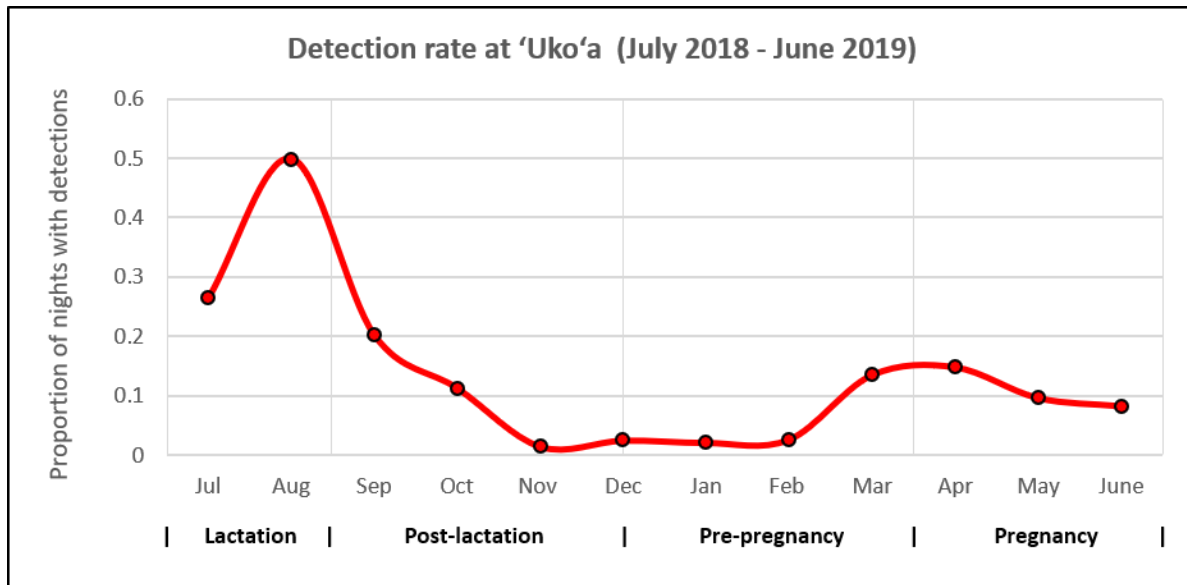
**Figure 6. Bat Acoustic Activity Rates at 'Uko'a Wetland in FY 2019**

The FY 2019 detection rate exhibited a similar temporal pattern to the detection rates recorded at 'Uko'a in previous years during the same time periods (Figure 7). Increased activity occurred during the lactation periods (Mid-June to August) followed by a decrease in activity during the post-lactation (September to Mid-December) and persisted until March of the pre-pregnancy (Mid-December to March) reproductive period when activity began to increase again. Using the proportion of nights with detections generally provides an adequate overall measure of activity for species with low detection rates (i.e., Hawaiian hoary bats); however, this metric does not always sufficiently describe the intensity of activity on specific nights.
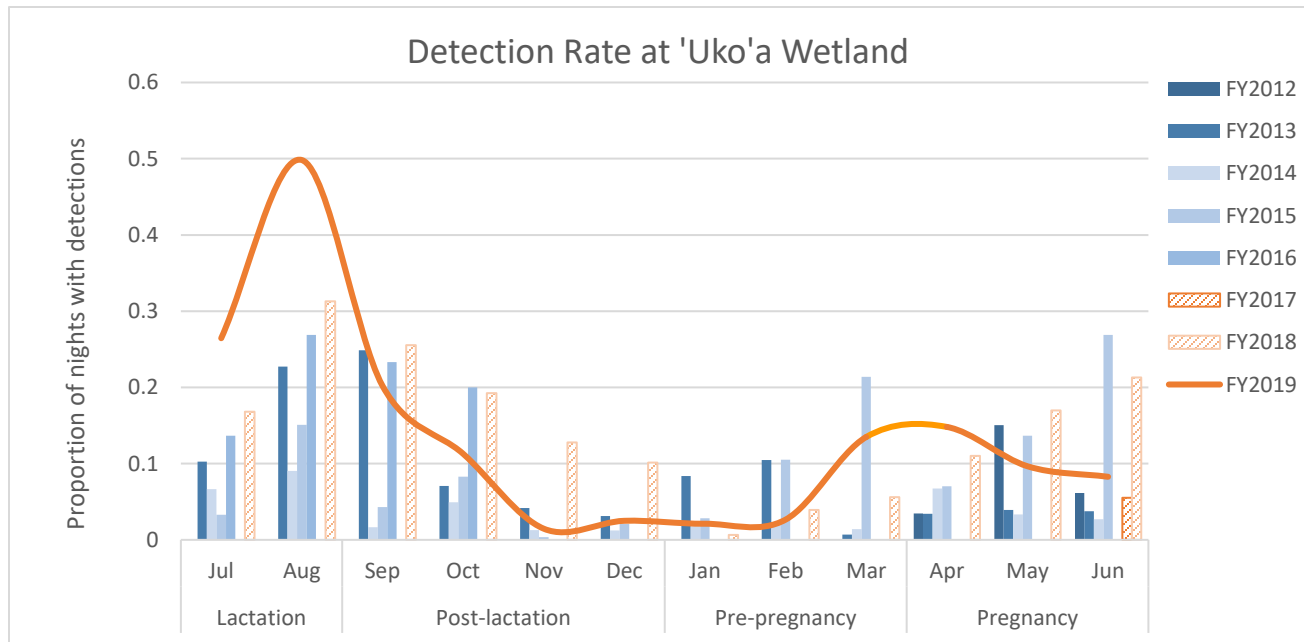


**Figure 7. Bat Acoustic Activity at 'Uko'a Wetland Before (FY 2012 – 2016) and After (FY 2017– 2019) Invasive Vegetation Removal**

Detections were relatively consistent across detectors; however, a relatively high number of bat passes were recorded at Detector #1 and Detector #12, which are the most northern detectors at 'Uko'a (see Figure 4). In March 2019, 318 passes were recorded at Detector #1 and in August 2018, 203 passes were recorded at Detector #12. These detections occurred only over a period of a few days yet resulted in 64 percent and 77 percent, respectively, of the total number of passes recorded in FY 2019 for each detector.

Table 6 and Figure 7 show the proportion of nights with bat detections between July 2012 and June 2019. Bat activity appears to have increased at 'Uko'a Wetland since sampling began in 2012, with the highest detection rates occurring in the last two years of sampling (0.143 in FY 2018 and 0.138 in FY 2019) after aquatic vegetation was removed and bat lane construction began. It is important to note that the number of nights sampled and the sampling time period is not consistent across years. Years when sampling occurred primarily during the summer months are expected to have a higher portion of detections compared to years when sampling occurred primarily during the winter months.

**Table 6. Detector-Night Data Between April 2012 and June 2019**

| Dates | Before or After Vegetation Removal | No. of Detector-Nights | Proportion of Nights with Detections |
|---|---|---|---|
| FY 2012 (April-June 2012) | Before | 256 | 0.10 |
| FY 2013 (July 2012-June 2013) | Before | 2,135 | 0.090 |
| FY 2014 (July 2013-June 2014) | Before | 2,777 | 0.036 |
| FY 2015 (July 2014-June 2015) | Before | 2,550 | 0.125 |
| FY 2016 (July -October 2015) | Before | 926 | 0.21 |
| FY 2017 (June 2017) | After | 163 | 0.06 |
| FY 2018 (July 2017-June 2018) | After | 3,544 | 0.143 |
| FY 2019 (July 2018-June 2019) | After | 3,373 | 0.138 |

In addition to overall bat activity levels, feeding buzzes were also documented and compared to previous years. A feeding buzz is classified as a burst of pulses at a very high rate with less than 11 milliseconds between pulses (Griffin et al. 1960). In the last two years since detectors have been redeployed, elevated levels of feeding buzzes were recorded at Detectors #1, #2, #6, and #12 (Figure 8). The total feeding buzzes recorded between FY 2017 and FY 2019 (6,917 total detector-nights) exceed the total feeding buzzes from FY 2012-2016 (8,635 total detector-nights) at some detector locations, which suggests more foraging activity occurred after removal of invasive vegetation from open water areas and construction of the bat lanes.
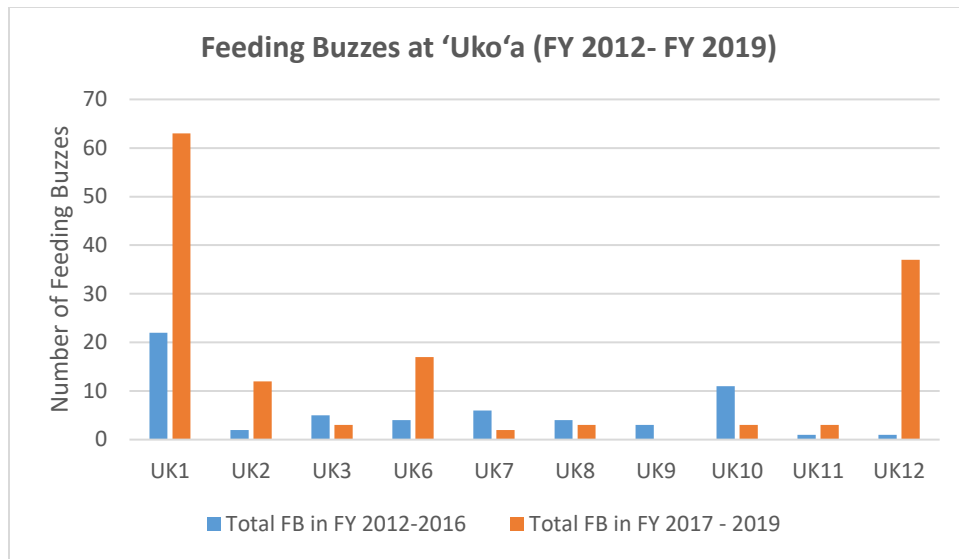
**Figure 8. Bat Feeding Buzzes (FB) Before (FY 2012-2016) and After (FY 2017-2019) Invasive Vegetation Removal**

### 9.1.3 Studies (Tier 2/3)

Kawailoa Wind finalized contracts with WEST and USGS in FY 2017 to conduct three multi-year studies as Tier 2/3 Hawaiian hoary bat mitigation. These studies were recommended to Kawailoa Wind by USFWS and DOFAW. The total funding for the three projects is over $1.6M. Kawailoa Wind continued to fund these studies in FY 2019. A summary of the work completed for these studies during FY 2019 is provided below.

USGS' *Modeling Foraging Habitat Suitability of the Hawaiian Hoary Bat* study began in February 2017. The primary objective of this study was to investigate the use of multi-state occupancy modeling to quantify foraging habitat suitability and use by Hawaiian hoary bats. This research was completed in FY 2019, and all funds were provided to USGS. The results of the research were published in an article titled "*Multi-state occupancy models of foraging habitat use by the Hawaiian hoary bat (Lasiurus cinereus semotus)*" in the Journal PLoS ONE (Gorresen et al. 2018) in October 2018 (see Appendix 3). The primary findings reported by Gorresen et al. (2018) include: 1) elevated levels of acoustic activity by Hawaiian hoary bats were found to be related primarily to beetle biomass, and 2) video-derived observations demonstrated higher and more accurate estimates of the prevalence of high bat flight activity and feeding events than acoustic sampling methods.

The objectives of USGS' *Hawaiian Hoary Bat Conservation Genetics* study are to improve the understanding of the genetic diversity of the Hawaiian hoary bat, identify bat prey items, and identify the sex of bat carcasses and any sex-specific food habits. Data on these topics will help inform conservation planning and improve host-plant selection for future habitat restoration efforts. A technical report was published for this study in November 2018 (Pinzari and Bonaccorso 2018a; see Appendix 4), and two data releases occurred in FY 2019—Version 1.0 on October 17,

2018 (Pinzari and Bonaccorso 2018b) and Version 2.0 on November 20, 2018 (Pinzari and Bonaccorso 2018c). During FY 2019, this research determined the sex of 88 Hawaiian hoary bat tissue samples using genotyping, which allows for more reliable evaluation of the ratio of males to females affected by collisions with wind turbines. The results of which indicate that 65% of observed fatalities at wind farms are male. As part of the research, DNA will continue to be extracted from any new tissue samples from bats (as acquired), and sex determination of additional bat carcasses will continue. A revised manuscript pertaining to Hawaiian hoary bat colonization time is also being drafted from this research.

The goal of WEST's multi-year *Hawaiian Hoary Bat Acoustic Surveys* study was to examine the distribution and seasonal occupancy of the Hawaiian hoary bat on Oʻahu. The Year 1 Status Report for the study (June 8, 2017 to June 29, 2018) was submitted to ESRC in October 2018 (Starcevich et al. 2018). The initial study results were presented to ESRC in March 2019. A revised report was submitted in March 2019 (Starcevich et al. 2019; see Appendix 5). Throughout FY 2019, WEST continued data downloads and processing from the detectors deployed throughout Oʻahu. As approved by ESRC in March 2019, the study also began preparation for testing drone/thermal sampling to assess ability to count bats.

### 9.1.4  Waimea Native Forest (Tier 3)

Funding the above-listed Tier 2/3 studies leaves an outstanding obligation of $353,702 for Tier 3 bat mitigation. Based on USFWS and DOFAW guidance, there are no remaining research funding gaps for joint agency sub-committee approved projects (pers. comm. Glenn Metzler, DOFAW, August 2, 2017). To fulfill the remaining uncommitted funding obligation, Kawailoa Wind will contribute the remaining funds towards the purchase of the 3,716-acre Waimea Native Forest. The land will be acquired through a partnership with The Trust for Public Land (TPL) and DOFAW, as well as other funding partners. This mitigation aligns with current USFWS and DOFAW guidance which identifies land acquisition as an appropriate mitigation approach for the Hawaiian hoary bat (DLNR 2015). On April 27, 2018, USFWS expressed via email that they were in support of the Waimea Native Forest project (pers. comm. Jiny Kim, USFWS, April 27, 2018). Kawailoa Wind is currently awaiting approval from DOFAW to provide the funds to TPL.

### 9.1.5  Helemano Wilderness Area Mitigation (Proposed Tier 4)

In response to exceedance of the authorized bat take limit under the ITP/ITL (Section 7.2), Kawailoa Wind completed additional bat mitigation in coordination with the USFWS and DOFAW. The Draft HCP Amendment identifies Tier 4 Hawaiian hoary bat mitigation as contributing $2,750,000 to TPL toward the purchase and long-term protection of the nearly 2,900-acre Helemano Wilderness Area (HWA). Kawailoa Wind proactively provided funds to TPL in October 2018, to ensure mitigation continues to occur ahead of bat take, despite uncertainty related to HCP Amendment approval. Further details of this bat mitigation are provided in the Draft HCP Amendment (Tetra Tech 2018a).

## 9.2   Waterbirds

As stated above, USFWS and DOFAW provided written confirmation permitting adaptive management for the original bat and waterbird mitigation. Some activities completed for waterbird mitigation at ʻUkoʻa Wetland overlap with bat mitigation requirements and are summarized in Section 9.1.2 above.  Tetra Tech conducts waterbird surveys as part of the waterbird mitigation, as described below.

Prior to each vegetation maintenance event, a biologist conducts waterbird surveys to identify if nests or chicks were present in the vicinity of the planned work area. These surveys are required as a Best Management Practice when contractors are working at the site to minimize impacts to endangered Hawaiian waterbirds.

Comprehensive weekly waterbird surveys began at ʻUkoʻa Wetland in January 2017, and continued throughout FY 2019 (Table 1). Generally, surveys are conducted from December to September, but in FY 2019 surveys were conducted in November as well due to observed breeding activity. A qualified biologist conducted surveys at eight point count (PC) stations set up in the vicinity of the open water and in areas with previous waterbird sightings. In addition to the PC stations, independent waterbird observations are recorded while walking between stations. The detailed protocols for these surveys were provided in the FY 2017 Annual Report (Tetra Tech 2017).

A total of 41 waterbird surveys were completed in FY 2019. The Hawaiian moorhen was the most frequently detected listed waterbird during weekly surveys. In FY 2019, moorhen were recorded at PC stations 3 - 8, but no observations were made at PC stations 1 and 2 (Figure 9). Moorhen (either adults or chicks) were observed or heard on all survey dates, except one survey date in August (Figure 10).

Moorhen breeding activity (e.g., nests or chicks) was observed at PC stations 7 and 8 between November 2018 and April 2019 (Figure 10). This consisted of four separate events. It is believed that no moorhen chicks successfully fledged from these breeding events observed at ʻUkoʻa Wetland in FY 2019. No fledglings or immature moorhen have been observed at ʻUkoʻa since February 2017.

Table 7 summarizes moorhen detectors since comprehensive waterbird surveys began in January 2017. Moorhen detections have declined each FY. The fewer detections of moorhen in FY 2019 compared to FY 2018 and 2017 could be due to reduced rainfall and water levels in FY 2019. The removal of water hyacinth in the open water area has altered habitat available to moorhen, and the birds may be using areas of the wetland that are not surveyed.

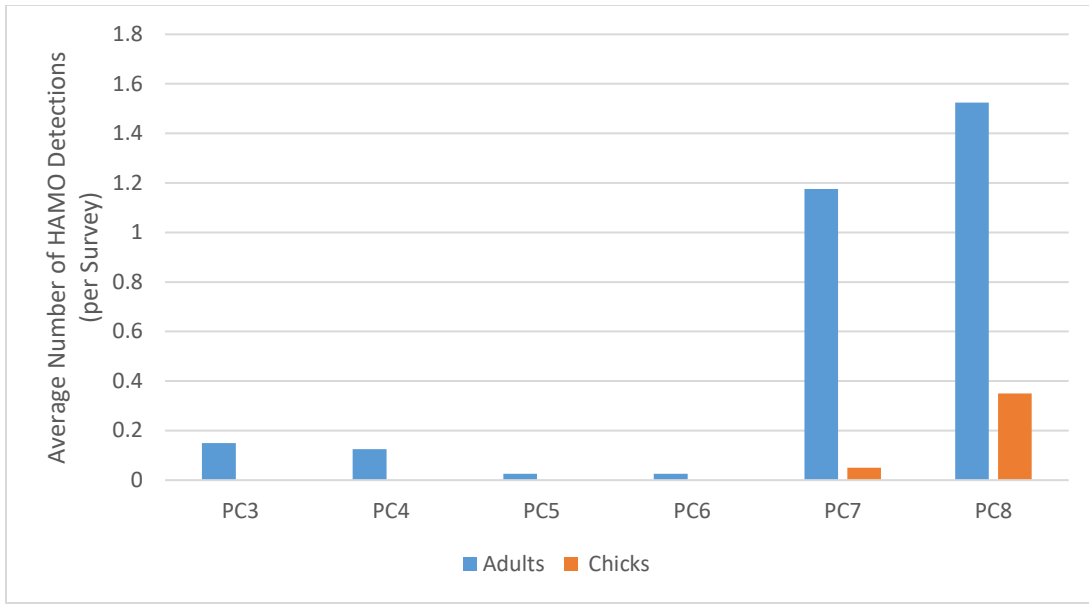**Figure 9. Average Number of Hawaiian Moorhen (HAMO) Detections Per Survey at Point Count Stations in FY 2019 (per Survey)**
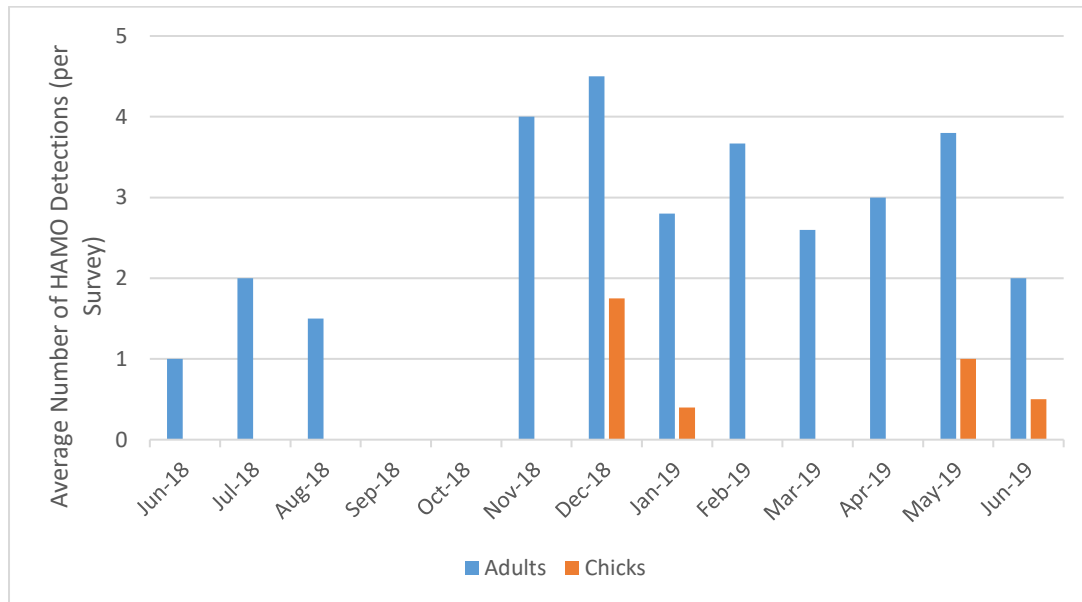


**Figure 10. Average Number of Hawaiian Moorhen (HAMO) Detections by Month (per Survey) at Point Count Stations in FY 2019**

**Table 7. Average Number of Hawaiian Moorhen Detected per Survey FY 2017 to FY 2019**

| Sampling Period | No. of Surveys | Average No. of Adults Detected per Survey | Average No. of Chicks Detected per Survey | Average No. of Fledglings Detected per Survey |
|---|---|---|---|---|
| FY 2017 (January 2017 - June 2017) | 25 | 5.7 | 0.8 | 1.0 |
| FY 2018 (July 2017 - June 2018) | 38 | 4.1 | 0.4 | 0.0 |
| FY 2019 (July 2018 - June 2019) | 41 | 3.0 | 0.4 | 0.0 |

No Hawaiian coot were seen in FY 2019. Six detections of adult Hawaiian stilt were made on two survey dates in FY 2019. Stilt were only observed flying overhead at PC 6. No Hawaiian stilt breeding activity was observed in FY 2019.

## 9.3 Seabirds

Tier 1 mitigation for Newell's shearwater was completed in FY 2015. Mitigation for the Hawaiian petrel is being proposed as part of the HCP Amendment (Tetra Tech 2018a). If approved, Kawailoa Wind will fund predator control and burrow monitoring at the Hanakāpīʻai and Hanakoa seabird colonies within the Hono O Nā Pali Natural Area Reserve (NAR) on Kauaʻi in 2020.

## 9.4 Hawaiian Short-eared Owls or Pueo

Mitigation for the pueo or Hawaiian short-eared owl is complete.

# 10.0 Adaptive Management

Kawailoa Wind is committed to the on-going implementation of operational avoidance and minimization measures described in the approved HCP and has been evaluating other options to further reduce the risk to bats since Project operations began in 2012. Kawailoa Wind has implemented multiple adaptive management steps to understand and reduce the risk to the Hawaiian hoary bat including modifying the low wind speed curtailment (LWSC) regime, implementing innovative approaches to post-construction mortality monitoring, and supporting development of the latest technologies that could reduce turbine collision risk to bats. Kawailoa Wind has proactively implemented additional measures to minimize take and has been investigating other potential minimization measures that could further reduce bat take. In FY 2018, Kawailoa Wind extended LWSC with a cut-in speed of 5.0 meters/second at all turbines to year-round from sunset to sunrise. At the end of FY 2018 (June 24, 2018), Kawailoa Wind also increased LWSC cut-in speed to 5.2 meters/second through a 0.2 meters/second hysteresis to increase the "down time" of the WTGs. Additional details are provided in the FY 2018 Annual Report (Tetra Tech 2018b) and the Draft HCP Amendment (Tetra Tech 2018a).

In FY 2019, Kawailoa Wind continued to evaluate options to minimize risk to the Hawaiian hoary bat. In the beginning of FY 2019 (July 25, 2018), Kawailoa reduced the number of stop/start events per night by extending the rolling average time from 10 to 20 minutes. Between July 2018 and October 2018, Kawailoa Wind conducted an ultrasonic acoustic bat deterrent "proof of concept" test, in collaboration with NRG Systems. NRG Systems installed an ultrasonic acoustic bat deterrent system at WTG 30, where the most bat fatalities have been detected, to evaluate effectiveness of the deterrent specific to Hawaiian hoary bats. As part of the study, Bat Conservation International, Inc., installed two thermal cameras at WTG 30 to document bat interactions in relation to the NRG deterrent system. In addition, Kawailoa Wind deployed two Wildlife acoustic bat detectors at WTG 30 to evaluate bat activity levels in the vicinity of the deterrent.  No bat fatalities have been observed at WTG 30 since the deterrent system was installed.

Subsequently, Kawailoa Wind installed acoustic deterrents at all 30 Project turbines in May and June 2019. Deterrent installation was completed on June 7, 2019. Deterrent functionality is monitored remotely to ensure the systems are functioning properly.  To date, no bat fatalities have been observed at turbines with operational bat deterrent systems.

# 11.0 Collection Permits

Annual reports for the Project's federal and state collection permits were submitted in Q3 of FY 2019. A renewal application for the State's Protected Wildlife Permit (Permit No. WL18-09) was submitted to DOFAW on February 12, 2019. The renewed Protected Wildlife Permit was issued by DOFAW on March 25, 2019. The renewal application for USFWS's special purpose utility permit was submitted to USFWS in March 2019.

# 12.0 Agency Meetings, Consultations, and Visits

Kawailoa Wind and Tetra Tech conducted or participated in 19 meetings with USFWS and DOFAW staff in FY 2019. The purpose of these meetings varied and included required semi-annual meetings, as well as discussions regarding the HCP Amendment and the Project's adaptive management strategy to reduce the risk to the Hawaiian hoary bat.

Meetings took place on:

- July 10, 2018—USFWS—Wind Energy Programmatic Environmental Impact Statement (PEIS) conference call;

- August 7, 2018—USFWS—Wind Energy PEIS conference call;

- August 8, 2018 —USFWS— conference call on draft HCP Amendment;

- September 13, 2018—USFWS—conference call on bat mitigation;

- November 28, 2018 —DOFAW—conference call on State HCP Amendment Public Hearing;

- November 28, 2018 —DOFAW—State HCP Amendment Public Hearing;

- December 4, 2018—USFWS—Wind Energy PEIS conference call;

- December 19, 2018 —USFWS—conference call about the HCP Amendment take request;

- January 9, 2019—DOFAW—Site visit to Helemano Wilderness Area mitigation site;

- February 5, 2019—USFWS—Wind Energy PEIS conference call;

- February 6, 2019—USFWS and DOFAW—HCP Amendment;

- March 5, 2019—USFWS—Wind Energy PEIS conference call;

- March 25, 2019— DOFAW—Potential mitigation for the HCP Amendment;

- April 2, 2019—USFWS—Wind Energy PEIS conference call;

- April 24, 2019—USFWS—meeting regarding PEIS;

- May 2, 2019— USFWS and DOFAW—conference call to discuss the HCP Amendment;

- May 15, 2019—USFWS—Wind Energy PEIS conference call;

- June 14, 2019— USFWS and DOFAW—conference call to discuss the HCP Amendment; and

- June 25, 2019—USFWS—Wind Energy PEIS conference call.

In addition, Kawailoa Wind and Tetra Tech presented to the Endangered Species Recovery Committee on October 25, 2018 to present the Draft HCP Amendment during the State public comment period. A site visit (for the ESRC and the public) to the Project and the HWA mitigation area occurred on February 7, 2019. Kawailoa Wind and Tetra Tech also met with the ESRC on March 6, 2019 to review the FY 2018 HCP annual report.

# 13.0 Expenditures

Total HCP-related expenditures for the Project in FY 2019 were $3,612,182 (Table 5).

**Table 8. HCP-Related Expenditures at the Project in FY 2019**

| Category | Amount |
|---|---|
| Permit Compliance | $85,000 |
| Facility Vegetation Management | $180,000 |
| Fatality Monitoring | $82,000 |
| ʻUkoʻa Wetland Mitigation Compliance | $132,000 |
| Tier 2/3 Bat Research Projects | $383,182 |
| Tier 4 Bat Mitigation | $2,750,000 |
| **Total Cost for FY 2019** | **$3,612,182** |

# 14.0 Literature Cited

Dalthorp, D., M. Huso, and D. Dail. 2017. Evidence of absence (v2.0) software user guide: U.S. Geological Survey Data Series 1055, 109 p., https://doi.org/10.3133/ds1055.

DLNR (Department of Land and Natural Resources, Division of Forestry and Wildlife). 2015. Endangered Species Recovery Committee Hawaiian hoary bat guidance document. Approved December 17, 2015.

Gorresen, P.M., K.W. Brinck, M.A. DeLisle, K. Montoya-Aiona, C.A. Pinzari, F.J. Bonaccorso. 2018. Multi-state occupancy models of foraging habitat use by the Hawaiian hoary bat (*Lasiurus cinereus semotus*). PLoS ONE 13(10): e0205150. Available at: https://doi.org/10.1371/journal.pone.0205150.

Grey Boar (Grey Boar Wildlife Services LLC). 2018a. Ukoa Predator Control Program. 3rd Quarter Report 2018. Prepared for Kawailoa Wind LLC.

Grey Boar. 2018b. Ukoa Predator Control Program. 4th Quarter Report 2018. Prepared for Kawailoa Wind LLC.

Grey Boar. 2019a. Ukoa Predator Control Program. 1st Quarter Report 2019. Prepared for Kawailoa Wind LLC.

Grey Boar. 2019b. Ukoa Predator Control Program. 2nd Quarter Report 2019. Prepared for Kawailoa Wind LLC.

Griffin, D.R., F.A. Webster, and C.R. Michael. 1960. The echolocation of flying insects by bats. Animal Behavior 8:141-154.

H. T. Harvey and SWCA (H. T. Harvey and Associates and SWCA Environmental Consultants). 2014. 'Uko'a Wetland Hawaiian Hoary Bat Mitigation Management Plan. Prepared for First Wind. Approved by USFWS and DOFAW 2014.

Kawailoa Wind, LLC. 2014. Kawailoa Habitat Conservation Plan FY 2015 Annual Report.

Kawailoa Wind, LLC. 2015. Kawailoa Habitat Conservation Plan FY 2015 Annual Report.

Pinzari, C.A. and F.J. Bonaccorso. 2018a. A Test of Sex Specific Genetic Markers in the Hawaiian Hoary Bat and Relevance to Population Studies. Hawaii Cooperative Studies Unit Technical Report HCSU-085.

Pinzari, C.A. and F.J. Bonaccorso. 2018b. Hawaiian Islands Hawaiian Hoary Bat Genetic Sexing 2009-2018 (ver. 1.0, October 2018): U.S. Geological Survey data release.

Pinzari, C.A. and F.J. Bonaccorso. 2018c. Hawaiian Islands Hawaiian Hoary Bat Genetic Sexing 2009-2018 (ver. 2.0, November 2018): U.S. Geological Survey data release. Available at: https://doi.org/10.5066/P9R7L1NS. Accessed January 23, 2019.

Pyle, R.L., and P. Pyle. 2017. The Birds of the Hawaiian Islands: Occurrence, History, Distribution, and Status. B.P. Bishop Museum, Honolulu, HI, U.S.A. Version 2 (1 January 2017) http://hbs.bishopmuseum.org/birds/rlp-monograph/.

Starcevich, L.A., J. Thompson, T. Rintz, E. Adamczyk, and D. Solick. 2018. Oahu Hawaiian Hoary Bat Occupancy and Distribution Study. Project Update and First-Year Analysis. Prepared for: Hawaii Endangered Species Research Committee.

Starcevich, L.A., J. Thompson, T. Rintz, E. Adamczyk, and D. Solick. 2019. Oahu Hawaiian Hoary Bat Occupancy and Distribution Study. Project Update and First-Year Analysis. Revised March 1, 2019 Prepared for: Hawaii Endangered Species Research Committee.

SWCA (SWCA Environmental Consultants). 2010. Kahuku Wind Power Habitat Conservation Plan. Prepared for Kahuku Wind Power LLC for DOFAW. Approved May 27, 2010.

SWCA. 2012. 'Uko'a Wetland Management Plan for Waterbirds 2012 – 2032. Prepared for First Wind. Approved by USFWS and DOFAW 2012.

Tetra Tech. 2016. Kawailoa Wind Power Habitat Conservation Plan, FY 2016 Annual Report.

Tetra Tech. 2017. Kawailoa Wind Power Habitat Conservation Plan, FY 2017 Annual Report.

Tetra Tech. 2018a. Kawailoa Wind Power Draft Habitat Conservation Plan Amendment.

Tetra Tech. 2018b. Kawailoa Wind Power Habitat Conservation Plan, FY 2018 Annual Report.

USFWS (U.S. Fish and Wildlife Service). 1998. Recovery Plan for the Hawaiian hoary bat (*Lasiurus cinereus semotus*). U.S. Fish and Wildlife Service, Portland, OR.

USFWS. 2011. Opeapea or Hawaiian Hoary Bat (*Lasiurus cinereus semotus*) 5-Year Review Summary and Evaluation. Available at: https://ecos.fws.gov/docs/five_year_review/doc5234.pdf.

USFWS. 2016. Wildlife agency guidance for calculation of Hawaiian hoary bat indirect take. USFWS Pacific Islands Field Office. Honolulu, HI. October 2016.

Young, L., E. VanderWerf, M. McKown, P. Roberts, J. Schlueter, A. Vorsino, and D. Sischo. 2019. Evidence of Newell's Shearwaters and Hawaiian Petrels on Oahu, Hawaii. The Condor. 121 (1): 1-7.

**APPENDIX 1**

**DOCUMENTED FATALITIES AT THE PROJECT DURING FY 2019**

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**Appendix 1.** Documented Fatalities at the Project during FY 2019

| Species[1] | Date Documented | WTG | Distance to WTG (meters) | Bearing from WTG (degrees) |
|---|---|---|---|---|
| *Francolinus pondicerianus* (gray francolin) | 7/3/2018 | 17 | 2 | 80 |
| *Zosterops japonicus* (Japanese white-eye) | 7/3/2018 | 22 | 40 | 100 |
| *Estrilda astrild* (common waxbill) | 7/10/2018 | 26 | 1 | 135 |
| *Acridotheres tristis* (common myna) | 7/17/2018 | 18 | 28 | 75 |
| *Lasiurus cinereus semotus* (Hawaiian hoary bat) | 7/19/2018 | 6 | 11 | 24 |
| *Estrilda astrild* (common waxbill) | 7/27/2018 | 30 | 38 | 135 |
| *Estrilda astrild* (common waxbill) | 7/27/2018 | 16 | 3 | 270 |
| *Estrilda astrild* (common waxbill) | 7/30/2018 | 08 | 2 | 180 |
| *Lasiurus cinereus semotus* (Hawaiian hoary bat) | 8/3/2018 | 17 | 24 | 298 |
| *Lasiurus cinereus semotus* (Hawaiian hoary bat) | 8/6/2018 | 11 | 26 | 228 |
| *Lasiurus cinereus semotus* (Hawaiian hoary bat) | 8/17/2018 | 24 | 15 | 268 |
| *Pterodroma sandwichensis* (Hawaiian petrel) | 8/20/2018 | 30 | 37 | 204 |
| *Bubulcus ibis* (cattle egret) | 3/22/2019 | 27 | 232 | 60 |
| *Acridotheres tristis* (common myna) | 8/27/2018 | 12 | 4 | 90 |
| *Acridotheres tristis* (common myna) | 9/3/2018 | 02 | 4 | 90 |
| *Acridotheres tristis* (common myna) | 9/6/2018 | 13 | 4 | 90 |
| *Lonchura punctulata* (scaly-breasted munia) | 10/2/2018 | 24 | 30 | 250 |
| *Geopelia striata* (zebra dove) | 10/12/2018 | 23 | 1 | 135 |
| *Lonchura punctulata* (scaly-breasted munia) | 10/23/2018 | 28 | 1 | 225 |
| *Acridotheres tristis* (common myna) | 10/25/2018 | 12 | 3 | 110 |
| *Lasiurus cinereus semotus* (Hawaiian hoary bat) | 10/26/2018 | 29 | 27 | 193 |
| *Estrilda astrild* (common waxbill) | 11/13/2018 | 16 | 1 | 120 |
| *Francolinus francolinus* (black francolin) | 11/15/2018 | 09 | 1 | 180 |
| *Geopelia striata* (zebra dove) | 11/15/2018 | 05 | 16 | 245 |
| *Spilopelia chinensis* (spotted dove) | 11/27/2018 | 16 | 5 | 225 |
| *Estrilda astrild* (common waxbill) | 11/29/2018 | 15 | 1 | 310 |
| *Geopelia striata* (zebra dove) | 12/18/2018 | 21 | 4 | 135 |
| *Spilopelia chinensis* (spotted dove) | 12/27/2018 | 02 | 15 | 270 |
| *Estrilda astrild* (common waxbill) | 1/3/2019 | 06 | 20 | 270 |
| *Lonchura punctulata* (scaly-breasted munia) | 1/8/2019 | 17 | 14 | 25 |
| *Estrilda astrild* (common waxbill) | 1/10/2019 | 06 | 1 | 90 |
| *Estrilda astrild* (common waxbill) | 2/26/2019 | 22 | 1 | 45 |
| *Lonchura punctulata* (scaly-breasted munia) | 3/21/2019 | 10 | 7 | 90 |
| *Bubulcus ibis* (cattle egret) | 3/22/2019 | 27 | 232 | 60 |

| Species[1] | Date Documented | WTG | Distance to WTG (meters) | Bearing from WTG (degrees) |
|---|---|---|---|---|
| *Acridotheres tristis* (common myna) | 4/11/2019 | 04 | 2 | 90 |
| *Spilopelia chinensis* (spotted dove) | 4/23/2019 | 18 | 1 | 210 |
| *Spilopelia chinensis* (spotted dove) | 4/30/2019 | 26 | 1 | 250 |
| *Estrilda astrild* (common waxbill) | 5/3/2019 | 24 | 15 | 110 |
| *Phaethon lepturus* (white-tailed tropicbird) | 5/20/2019 | 13 | 77 | 285 |
| *Lonchura punctulata* (scaly-breasted munia) | 5/28/2019 | 27 | 2 | 95 |
| *Acridotheres tristis* (common myna) | 5/28/2019 | 18 | 10 | 120 |
| *Spilopelia chinensis* (spotted dove) | 6/17/2019 | 14 | 2 | 92 |
| 1. Covered Species are highlighted in yellow. Species protected by the MTBA are highlighted in gray. | | | | |

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# APPENDIX 2

# DALTHORP ET AL. (2017) FATALITY ESTIMATION FOR HAWAIIAN HOARY BATS AT PROJECT THROUGH FY 2019

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**Appendix 2.** Dalthorp et al. (2017) Fatality Estimation for Hawaiian hoary bats at Project through FY 2019.[2]

Posterior Distribution of Total Fatalities over 8 years

Credibility level $(1 - \alpha) = 0.8$

Estimated baseline annual fatality rate: $\bar{\lambda} = 10.9$
95% CI = [ 7.72, 14.64]

Overall detection probability: g = 0.5297
95% CI = [ 0.5097, 0.5496]

$M^* = 79$, i.e., $P(M \leq 79) \geq 80\%$

# APPENDIX 3

# USGS' MULTI-STATE OCCUPANCY MODELS OF FORAGING HABITAT USE BY THE HAWAIIAN HOARY BAT (*LASIURUS CINEREUS SEMOTUS*) (GORRESEN ET AL. 2018)

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# Multi-state occupancy models of foraging habitat use by the Hawaiian hoary bat (*Lasiurus cinereus semotus*)

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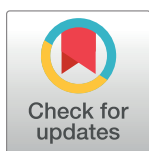**1** Hawai'i Cooperative Studies Unit, University of Hawai'i at Hilo, Hilo, Hawai'i, United States of America, **2** U.S. Geological Survey, Pacific Island Ecosystems Research Center, Hawaii National Park, Hawai'i, United States of America

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## Abstract

Multi-state occupancy modeling can often improve assessments of habitat use and site quality when animal activity or behavior data are available. We examine the use of the approach for evaluating foraging habitat suitability of the endangered Hawaiian hoary bat (*Lasiurus cinereus semotus*) from classifications of site occupancy based on flight activity levels and feeding behavior. In addition, we used data from separate visual and auditory sources, namely thermal videography and acoustic (echolocation) detectors, jointly deployed at sample sites to compare the effectiveness of each method in the context of occupancy modeling. Video-derived observations demonstrated higher and more accurate estimates of the prevalence of high bat flight activity and feeding events than acoustic sampling methods. Elevated levels of acoustic activity by Hawaiian hoary bats were found to be related primarily to beetle biomass in this study. The approach may have a variety of applications in bat research, including inference about species-resource relationships, habitat quality and the extent to which species intensively use areas for activities such as foraging.

## Introduction

Monitoring bat populations is challenging because many species occur at low densities, are difficult to detect, and have wide-ranging movement and migratory patterns that are poorly understood [1]. Foliage-roosting "tree bats" (a group of about 17 species of Lasiurus and the silver-haired bat, *Lasionycteris noctivagans*) pose a particular challenge as populations are typically "over-dispersed" [2], and may be vocally cryptic or not readily accessible to acoustic sampling or capture [3–6]. Quantifying conventional population parameters such as abundance or density for these species is not currently feasible and can hamper species status and conservation assessments [1]. For such species, occupancy analysis can be an effective method for elucidating the relationship of habitat attributes and species occurrence while also accounting for imperfect detection [7], and the approach has been used for bat studies at local [8,9], regional [10] and broader geographic extents [11,12]. However, analyses based solely on animal

presence can be inadequate because they omit information on abundance, activity levels, or behavior that may further inform assessment of habitat use and suitability. Advances in occupancy modeling have expanded its application to incorporate additional information about a range of biologically relevant states such as behavioral attributes (e.g., absent or not detected, present but possibly not reproducing, and present and reproducing; [13]) or categorical levels of activity (e.g., calling rates; [14,15]) and abundance (e.g., "none, some, many"; [16]). These "multi-state" models characterize occupancy as a hierarchical categorical variable, and accommodate uncertainty caused by imperfect detection in both the estimation of species occurrence and classification of the correct state.

Given their flexibility, multi-state occupancy models may serve to evaluate the relative use or quality of areas used by bats for foraging, roosting, or other requirements. Bat habitat suitability can be assessed in a number of ways, including for example, studies of focal use of areas by radio-tracked individuals [17], body weight [18], reproductive condition [19], and dietary richness [20]. However, these approaches typically require bat capture and handling which can be difficult or impractical in many settings. Non-invasive observations of bat detection rates and feeding behavior may provide alternate metrics that, coupled with multi-state occupancy analysis, reveal the degree to which an area is used for foraging by a resident population and the habitat attributes associated with state variables of interest (e.g., high abundance or activity), information of relevance to land managers and conservation efforts.

Optimal foraging theory posits that fitness is related to foraging efficiency, and that the rate at which energy is obtained is partly a function of time spent in a particular habitat [21,22]. Insectivorous bats expend considerable time and energy foraging (e.g., more than half the energy budget; [23]), with individuals often consuming over a quarter of their body weight in invertebrates each night, particularly during energetically demanding periods [24]. Thus, bat occurrence and activity are expected to be generally associated with insect abundance, a prediction broadly supported by various studies (e.g., [25–27]), although this relationship can be temporally variable and depend on resource availability and prey selection [28,29]. Monitoring insectivorous bat distribution and habitat use is a particularly pressing issue given observed declines in many insectivorous bird populations (e.g., [30]), a pattern that may be linked to diminished flying insect populations and ecosystem changes [31,32].

The primary objective of our study is to investigate the use of multi-state occupancy modeling to quantify foraging habitat use and suitability by Hawaiian hoary bats (*Lasiurus cinereus semotus*, Vespertilionidae), and constitutes the first application of the method to observations of bat activity and behavior. Also known as the ʻŌpeʻapeʻa, the species is the only extant native terrestrial mammal and sole bat species in Hawaiʻi state and occurs on all of the major islands [33]. It is an aerial-hawking bat that feeds primarily on Coleoptera and Lepidoptera captured and eaten while in flight [34–37]. The Hawaiian hoary bat served as the focal species in this study because it is an endangered endemic susceptible to fatality by collision with wind turbine blades [38] and the subject of management aimed at mitigating these impacts [39]. The North American subspecies, *L. c. cinereus*, also accounts for approximately 40% of all bat fatalities at turbines in continental North America [40]. We specifically examine the issue of foraging habitat use because land-cover restoration is a conservation approach applied to offset Hawaiian hoary bat fatalities and requires an understanding of current bat activity before management efforts begin and from which to compare results.

We applied the multi-state occupancy approach to analyze two distinct types of behavioral data amenable to classification as variable "states"—flight activity levels and feeding behavior. Survey methods that rely on acoustic sampling are widely used to determine species presence and relative activity, but bats may at times be vocally cryptic and remain undetected [5,6]. Therefore, we used data derived from separate visual and auditory sources, namely thermal

videography and acoustic (echolocation) detectors, jointly deployed at each sample site to secondarily compare how sampling techniques affect habitat use inferences. We speculate that prey availability may be a limiting resource regulating Hawaiian hoary bat occurrence and activity; as such, we focus exclusively on insect biomass as a predictive variable of bat occupancy and habitat use. This focus also helped ensure that models were not over-parameterized and were able to accommodate our limited sample size. We discuss the use of multi-state occupancy models to draw inference about habitat suitability and the assumptions relevant to applying such models to local populations comprised of wide-ranging individuals.

## Methods

### Study area and data collection

The field study was conducted from 10 July to 10 August 2017 in the northern Koʻolau Mountains of Oʻahu, within a 25 sq km area managed for wind energy production (centered at 21˚ 36'21"N, 158˚2'14"W). The region is comprised of a mix of active and fallow cattle pasture and forest, with the latter dominated by introduced species at lower elevations and native species at higher elevations (generally >300 m). Topography consists of incised hillsides with elevation at sample sites ranging from 145 to 360 m above sea level. Four sites, located an average of 2.5 km apart, were concurrently sampled four nights each week for a period of five weeks (20 sites total). Candidate sites based primarily on a road and trail network were obtained from a generalized random-tessellation stratified (GRTS) sampling design produced with the R package spsurvey version 3.3 [41]. Stratification was applied by dividing the study area into quadrants, and sites were selected from the candidate pool given the constraint of a 1-km minimum spacing between sites sampled concurrently. The selection of the study area for sampling and testing multi-state occupancy models was based on a previous study demonstrating bat occurrence in the region [38].

Bat activity and behavior was quantified both visually and acoustically. Visual detection of bats was achieved using surveillance cameras equipped with 19-mm lenses (Axis Q1922-E, Axis Communications, Lund, Sweden). These cameras image in the "thermal" spectrum of infrared light (approximately 9,000–14,000 nm) and require no supplemental illumination. At each sample site, a camera was set in an open area and aimed upward at a 45˚ angle so as to exclude any nearby vegetation. Previous trials showed that bats were detectable at distances of over 100 m. Video imagery was processed using custom-written code and matrix-based statistical software (Mathworks, Natick, Massachusetts, USA) to automatically detect animals flying through the video scenes. Video was recorded at 30 frames per second, and every 10th video frame was analyzed resulting in the detection of events lasting as little as 0.3 sec. All objects detected by software algorithms were visually reviewed and identified as bat, bird or insect. Bat detections occurring ≤1 minute apart were counted as a single event, and counts of these events by night and site were used to measure relative activity. Although use of a 1-minute threshold was arbitrary, about 84% of video detections of bat flight were comprised of single passes, and the time difference between all detection events averaged 21 minutes, reflecting the occasionally clustered but generally sparse distribution of the visual observations. In addition to the video-based index of activity, observations of flight trajectories that included a rapid loop or roll (accomplished in ≤1 s) were used to indicate active prey targeting. Aerial-hawking species such as *L. borealis* and *L. cinereus* that have a moderate to high wing-load (ratio of mass to wing area) and aspect ratio (wingspan$^2$/wing area) are fast and agile fliers with the ability to rapidly initiate a roll and alter their flight path while in pursuit of prey [42–44]. Nightly counts of these observations were used to quantify the number of feeding attempts at each site.

Bat echolocation was acoustically monitored with ultrasonic detectors (Song Meter 4 Bat FS, Wildlife Acoustics, Inc., Concord, Massachusetts, USA), each equipped with a directional horn-mounted SMM-U1 microphone oriented towards the air-space imaged by a video camera. Detectors began recording 30 minutes before local sunset until 30 minutes after sunrise the next morning. Acoustic events were recorded without digital compression as full-spectrum wav sound files with the following settings: sampling rate of 192 kHz; high pass filter at 16 kHz and 12 decibel gain; microphone bias off; digital high pass filter at fs/24; digital low pass filter off; trigger level 6 decibel signal-noise ratio; trigger window 3.0 sec; trigger max length 15 sec; frequency division ratio 16. Kaleidoscope Pro (version 4.1.0a; Wildlife Acoustics, Inc.) software was used to review files and filter acoustic background noise with the following settings: signal of interest between 8 and 80 kHz, 2 to 250 ms pulse duration, and a minimum of 2 pulses per event. All files classified as containing bat echolocation pulses were visually and aurally inspected as sonograms with Kaleidoscope Pro (version 3.1.0; Wildlife Acoustics) to ensure that there were no false positives. As with video detections, acoustic detections occurring ≤1 minute apart were counted as a single event, and counts of these events by night and site were used as an acoustic-based index of activity. Terminal-phase calls ("feeding buzzes" emitted just prior to an attempted insect catch [45]) were qualitatively distinguished from search and approach-phase calls by a rapid increase in the call rate. In addition to the acoustic index of activity, counts of terminal-phase call events were used to quantify feeding events per night.

To determine the abundance of potential bat prey, insects were sampled at each site with an ultraviolet fluorescent light trap (Leptraps, Georgetown, Kentucky, USA). Trapped insects were collected following each night of sampling, sorted to size and order for insects with a body length ≥5 mm, and oven-dried for 48 h at 65°C. Data used for analyses were restricted to biomass (dry weight) tallies of Coleoptera and Lepidoptera in the size class ≥5 to 20 mm (summarized in S1 Table). Moths and beetles with body lengths >20 mm made up 15% of all insect weight but comprised only 1% of counts and were generally larger than 24 mm, the maximum length of prey items consumed by Hawaiian hoary bats [36]. These captures were excluded from analyses to minimize the effect of outliers.

All sampling protocols for this study were approved by IACUC at the University of Hawaiʻi at Hilo. Access to the wind power facility was granted by D.E. Shaw Renewable Investments IV, L.L.C.

## Occupancy modeling

We used two types of multi-state models to investigate foraging habitat use by bats. The first model applied flight activity categories derived from nightly tallies of bat detections ("activity" model), and the second model used feeding attempts by bats evident from flight trajectories and terminal-phase vocalizations ("feeding" model). Both of these models were developed separately for the data obtained from the two different survey methods ("acoustic", "video"). Altogether the analyses consist of four model types: Acoustic–activity, Acoustic–feeding, Video–activity, and Video–feeding.

Each of the models produced estimates of the probability of observing the species in state 1 given its true state was 1 ($p^1$), and the probability of observing the species in state 2 given its true state was 2 ($p^2$). Put another way, for the activity model these are the probabilities of observing at least one bat at sites at which activity was truly low ($p^1$) or high ($p^2$), respectively. For the feeding model, they are the probabilities of observing at least one bat at sites where feeding was not occurring or was actually occurring, respectively. Additionally, the models estimated overall occupancy, or the probability that bats were present at a site regardless of

state ($\psi^1$), the conditional probability that state 2 (high activity or feeding) actually occurred given bat presence ($\psi^2$; the parameter of primary interest), and the probability of correctly identifying state 2 versus state 1 given the detection of bat presence ($\delta$).

A key assumption in occupancy modeling is that the occupancy state (e.g., not detected, present at low abundance, present at high abundance) remains constant for the duration of the sampling period [7]. When sample plot areas are smaller than an individual's home range and movement leads to temporary absence, occupancy estimates should instead be interpreted as the 'proportion of area used' (or for multi-state model parameterizations, 'probability of site use'), rather than as the proportion of area occupied [46, 47]. MacKenzie et al. [14] caution that the relaxation of the assumption of site closure may introduce bias. However, they do so only in the context of directional changes in the occupancy state over a survey period (e.g., seasonal trend in the breeding calls of frogs). We do not believe that Hawaiian hoary bats are likely to exhibit seasonal trends in foraging rates over the short time period (4 nights) at which it was measured at each site. MacKenzie et al. [14] also note "[w]hen such changes occur at random . . . the interpretation of the state-specific occupancy-related probabilities is the probability that that state is the highest reached during the season (i.e., the state of a unit may not always be 2, but 2 is the highest state reached at some point during the surveying)". We interpret our results along these lines such that parameter $\psi^2$ represents the mean probability that state 2 actually occurred at sample sites at some point during the survey, and represents the extent to which the survey area as a whole can support high levels of bat activity and feeding; i.e., "focal" habitat use. Furthermore, it is important to note that relaxation of the site closure assumption also applies to the interpretation of parameter $\psi^1$. That is, the parameter indicates the probability, regardless of actual state, that one or more bats were present at some point at a site during a survey period. For the totality of sites, it represents the overall prevalence of the organism in the surveyed area inclusive of "non-focal" habitat use (e.g., transit between sites).

Low and high categories of activity were distinguished based on the median detection rates from acoustic and video data obtained from a previous 1-year bat study in the same region [38]. To distinguish high from low activity, the thresholds for acoustic and video data was set at a nightly count of 1.0 and 5.0 per site, respectively. Counts greater than those values were designated as "2" in the detection history matrix used in the multi-state analyses, with "1" indicating observations of only low activity or no feeding, and "0" representing no observations of bats. The choice of the median as a threshold was arbitrary but served in our study as an example of how it may be used to identify higher-use areas; different threshold values can be obtained from pilot studies or other knowledge of a species' biology.

Multi-state model parameters can be fit with and without covariates. The basic occupancy model that included only their intercepts without covariates served as a null model against which we compared model types and covariate-fitted models. The null model specified separate parameters for the detection probabilities of state 1 and state 2, and is referred to herein as "null" with the notation $\psi^1(\cdot)\psi^2(\cdot)p^1(\cdot)p^2(\cdot)\delta(\cdot)$. To restrict the number of candidate models and likelihood of obtaining spurious results given a limited sample size, and given our study's focus on the relationship between insect prey and bat presence, we constrained our models to include only insect sample covariates that might affect overall prevalence ($\psi^1$) and the occurrence of high activity or feeding ($\psi^2$). We did not examine the use of site-specific covariate data in multi-state models because the survey area was limited to 25 sq km of relatively homogenous land-cover and physiognomic attributes that are readily traversed by resident bats (e.g., Hawaiian hoary bats tracked by radio telemetry had long-axis home range movements up to 18 km [mean = 3.4 km ± 0.8 SE]; [48]). Post-hoc trials with "single-state" occupancy models also did not demonstrate any support for the inclusion of sample-specific covariates (which can change across sampling nights) comprising mean wind speed, wind variability, total

precipitation, proportion of night with rain, and night-time sky illumination. The trials and consistent mild weather during the sampling period justified the exclusion of these variables from multi-state models (although such covariates can be readily included where sites attributes differ spatially and sampling conditions vary over a survey period). Therefore, we estimated $p^1$ and $p^2$ as constant over the 4 consecutive nights of sampling at each site. Similarly, survey night was not deemed likely to affect the probability of correctly identifying state 2 so $\delta$ was also treated as a constant over all samples.

Mean nightly Coleoptera and Lepidoptera biomass were each considered as site covariates that could independently affect bat presence (if bats preferred one food type over the other) and are referred to in models as "beetle" and "moth", respectively. We also included the combined Coleoptera and Lepidoptera biomass as an additional site covariate reflective of overall site productivity ("insect"). For models which used these covariates only for the parameter $\psi^1$, the hypothesis was that prey biomass affects overall bat prevalence but not the probability of high bat activity or feeding behavior. Conversely, where parameter $\psi^1$ was treated as a constant and parameter $\psi^2$ included covariates, the interpretation was that prey biomass determines high bat activity or feeding behavior but was not related to overall prevalence. Finally, where $\psi^1$ included the covariate "insect" and parameter $\psi^2$ included either "beetle" or "moth", the models assumed that prevalence was determined by site productivity, but the likelihood of high bat activity or feeding at a site depended on bat preference for either beetles or moths.

We used the program PRESENCE version 12.7 [49] to obtain maximum likelihood estimates for model parameters and rankings. The number of sites (n = 20) was used as the effective sample size to calculate the small-sample-corrected Akaike's Information Criterion (AIC$_c$) and relative model weights reflect evidence in favor of the respective models being the most appropriate among the members of the model set [50]. For comparability, we initially present null model (intercept-only) parameter estimates for all four model types (acoustic–activity, acoustic–feeding, video–activity, video–feeding), and subsequently examine covariate-fitted models that rank better than the null for inference of bat-prey relationships. Parameter estimates are provided with their unconditional standard errors. Bat detection histories for each of the four model types are tabulated in S2 Table, and site samples of prey biomass are presented in S1 Table and S1 Fig. Acoustic and video detections of bats are shown in S2 and S3 Figs.

## Results

Acoustic bat detections were relatively infrequent and averaged 0.8 per night/site (range = 0–5; median = 0.0), whereas video sampling resulted in an average of 5.5 detections per night/site (range = 0–35; median = 7.2). Acoustic detections were recorded at 13 of the 20 sites, yielding a naïve occupancy probability of $\tilde{\psi}^1 = 0.65$ (where "naïve" means not accounting for imperfect detection). High activity and feeding were detected acoustically at least once at 7 and 6 sites known to be occupied, respectively, yielding naïve estimates of $\tilde{\psi}^2 = 0.35$ and $0.30$ for the prevalence of these events. In comparison, bats were detected by video at all sites during the survey; that is, $\tilde{\psi}^1 = 1.0$. High activity and feeding were also detected by video at least once at 15 and 16 sites known to be occupied, respectively, yielding naïve estimates of $\tilde{\psi}^2 = 0.75$ and $0.80$ for the prevalence of these events.

The probability that a site was occupied, $\hat{\psi}^1$, was estimated as 1 by all null models (Table 1), a result substantially higher than the naïve estimates but in agreement with the scenario that Hawaiian hoary bats occur across all sites in the study area regardless of the sparsity of detections related to activity or feeding. Estimates of $\hat{p}^1$, the probability of detecting bats in state 1

**Table 1. Null model parameter estimates for each of four model types.**

| Parameter | Acoustic–activity | | Acoustic–feeding | | Video–activity | | Video–feeding | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| $\hat{\psi}^1$ | 1.00 | † | 1.00 | † | 1.00 | † | 1.00 | † |
| $\hat{\psi}^2$ | 0.48 | 0.161 | 0.48 | 0.205 | 0.78 | 0.103 | 0.89 | 0.108 |
| $\hat{p}^1$ | 0.19 | 0.076 | 0.17 | 0.078 | 0.83 | 0.101 | 0.86 | 0.156 |
| $\hat{p}^2$ | 0.52 | 0.116 | 0.70 | 0.273 | 0.98 | 0.021 | 0.96 | 0.028 |
| $\hat{\delta}$ | 0.75 | 0.131 | 0.32 | 0.118 | 0.74 | 0.101 | 0.49 | 0.073 |

† Standard error (SE) cannot be calculated for parameters estimated at boundary of parameter space.

$\psi^1$—probability that bats were present at a site regardless of state (high activity or feeding)

$\psi^2$—conditional probability that state 2 actually occurred given bat presence

$p^1$—probability of observing the species in state 1 given its true state was 1

$p^2$—probability of observing the species in state 2 given its true state was 2

$\delta$—probability of correctly identifying state 2 versus state 1 given the detection of bat presence.

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(low activity or no observed feeding), for each of the two survey methods was similar in terms of the type of bat observation (activity versus feeding) but differed between sampling methods, with the video-based method demonstrating much higher parameter values. Of greater interest, video samples yielded higher estimates and greater precision for both $\hat{p}^2$ and $\hat{\psi}^2$ compared to acoustic methods. For example, the probability of detecting high bat activity by video was almost twice that obtained by acoustic sampling: $\hat{p}^2 = 0.96$ (SE = 0.028) versus 0.52 (SE = 0.116). Likewise, the video-derived estimate of the prevalence of feeding, $\hat{\psi}^2$, was 0.89 (SE = 0.108) compared to 0.48 (SE = 0.205) for acoustic methods.

The probability of correctly classifying high activity given bat presence, $\hat{\delta}$, was one and a half to twice that of identifying feeding events, irrespective of sampling method. For example, $\hat{\delta} = 0.74$ (SE = 0.101) for video samples of bat activity, and indicated that there was a 74% chance of a nightly sample visually recording >5 separate detections. A similar result was evident from acoustic samples of bat activity. Finally, both acoustic and video-based samples of feeding activity exhibited fairly low values ($\hat{\delta} = 0.32$ and 0.49, respectively), suggesting that the probability of correctly identifying feeding from either method may be difficult and liable to underestimate true $\psi^2$. Nonetheless, estimates of $\psi^2$ for all null models were higher than the naïve estimates, a result consistent with the expectation that occupancy models adjust upward appraisals of occurrence when detection probabilities are <1.

Model selection statistics indicated that null model weights made up the plurality or majority of the support for three of the four model types (S3 Table). The exception was for the acoustic–activity set in which the three top models ranked better than the null model and together comprised 93% of total weight. The models $\psi^1(\cdot)\psi^2(\text{beetle})p^1(\cdot)p^2(\cdot)\delta(\cdot)$ and $\psi^1(\cdot)\psi^2(\text{insect})p^1(\cdot)p^2(\cdot)\delta(\cdot)$ each demonstrated that the probability of high acoustic activity occurring at a site was positively related to beetle and insect biomass, respectively (Table 2). The model $\psi^1(\text{insect})\psi^2(\text{beetle})p^1(\cdot)p^2(\cdot)\delta(\cdot)$ showed that overall bat prevalence was dependent on insect abundance, whereas the probability of high acoustic activity was related to beetle biomass. In keeping with these results, site-specific estimates of $\hat{\psi}^2$ from the top model averaged only 0.39 for the study area as a whole and revealed that high acoustic activity was not widespread, yet the estimates were fairly well correlated with Coleoptera biomass samples (r = 0.78) and suggest that high vocalization rates may be linked to available prey (S4 Fig). Non-native coprophagous dung beetles (Scarabaeidae, *Onthophagus* spp.) were the most common group of

**Table 2. Parameter estimates for top ranked models of the acoustic–activity set.** Estimates for $\psi^2$ (the probability that state 2 –high activity or feeding–actually occurred given bat presence) were obtained by averaging site-specific predicted values and their standard errors. Parameter definitions are provided in the footnote to Table 1.

| Parameter | $\psi^1(\cdot)\psi^2(\text{beetle})p^1(\cdot)p^2(\cdot)\delta(\cdot)$ | | $\psi^1(\cdot)\psi^2(\text{insect})p^1(\cdot)p^2(\cdot)\delta(\cdot)$ | | $\psi^1(\text{insect})\psi^2(\text{beetle})p^1(\cdot)p^2(\cdot)\delta(\cdot)$ | |
|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE |
| $\hat{\psi}^1$ | 1.00 | † | 1.00 | † | 1.00 | † |
| $\hat{\psi}^2$ | 0.39 | 0.123 | 0.39 | 0.126 | 0.40 | 0.130 |
| $\hat{p}^1$ | 0.20 | 0.069 | 0.20 | 0.068 | 0.22 | 0.078 |
| $\hat{p}^2$ | 0.56 | 0.111 | 0.56 | 0.109 | 0.56 | 0.113 |
| $\hat{\delta}$ | 0.79 | 0.107 | 0.79 | 0.107 | 0.79 | 0.107 |

† Standard error (SE) cannot be calculated for parameters estimated at boundary of parameter space.

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Coleoptera encountered, with high proportions of the nocturnally volant *O. sagittarius* prevalent in the light trap samples.

## Discussion

Our study is the first application of multi-state occupancy analysis of bat activity and behavior for the purpose of evaluating habitat use and quality, an assessment that can be particularly difficult to quantify for bat species. The use of this type of model in bat ecology is novel and our study may serve to inform its application to other such investigations. For example, our study demonstrated that the probability of detecting high bat activity and feeding by video was high or close to 1.0 in null models, which may argue for the use of analyses that do not require correction for imperfect detection for this type of data. Alternatively, given the interpretation of habitat use based on cumulative samples of occurrence, it also indicates that fewer video samples at sites expected to have a high frequency of detections of interest may suffice to achieve a desired level of precision in occupancy model parameters. The use of both acoustic and video sampling methods also provided a useful comparison from which to gauge in subsequent studies the relative effort necessary to balance the number of sites and repeat samples in light of the preferred sampling method [51].

Small sample sizes are common to ecological studies and conservation projects, which typically have limited resources and focus on rare species. Such studies may benefit from a larger number of samples than that available in this study to reduce the risk of over-fitting models and to accommodate covariates in addition to prey biomass. We also caution that models are expected to perform better where estimated parameters do not attain a maximum value of 1.0 and preclude estimation of standard error (i.e., "boundary effect"). This effect was evident in estimates of $\psi^1$ which indicated that the study area was "saturated" and the actual prevalence of bats was widespread (more so than anticipated during the study design). However, in our case this was not relevant given that focal habitat use as estimated by $\psi^2$ is the parameter of primary interest and detection of high activity and feeding was not ubiquitous.

It is important to note that multi-state occupancy models do not include a separate parameter to estimate availability, that is, the occurrence of individuals exposed to sampling and available for detection. Despite this, the non-identifiability of detection and availability components is not an issue where availability is relatively constant. Although the sample volume of both acoustic and video devices are considerably smaller than the total area used nightly by individual bats, the overlap of these areas and sample sites is not likely to change over short time periods. We believe this condition is met, in part, by sampling at a "fine-grained" temporal resolution over periods of short duration (in our study, nightly samples

over 4 nights). MacKenzie et al. [52] demonstrated a similar approach by sampling amphibian activity during a fixed period during which species were active and known to be consistently available for detection. Moreover, a telemetry study of Hawaiian hoary bats [48] demonstrated high foraging site fidelity in which individuals make repeated nightly use of areas within periods of at least several weeks. This, and the energetic need for bats to forage nightly (particularly during the breeding season) and general ubiquity (estimated overall prevalence was equal to 1), supports our assumption that their availability for detection is fairly invariant over short periods.

Efford and Dawson [53] distinguish asymptotic occupancy, the accumulated area used over time, from that of instantaneous occupancy, the proportion of sites occupied at a point in time. The distinction between use and occupancy is important in that it highlights the need to consider both the duration of sampling and the spatial extent of sampling if comparisons are to be made among surveys. For our study, multi-state models produced useful metrics describing the prevalence of high bat activity and feeding, results which can be used to track trends in habitat use and quality within this particular study area. Similar surveys can be applied elsewhere but the spatial and temporal grain of sampling (i.e., plot size relative to study area), survey duration and number of samples over time should be consistent if comparisons among areas or over time is the objective.

In terms of habitat use and inferences regarding habitat quality, models demonstrated that elevated levels of acoustic activity (i.e. number of calls detected nightly) by Hawaiian hoary bats were related primarily to beetle biomass at this particular place and time. Insect biomass as a covariate may also have ranked highly in acoustic–activity models because beetles comprised about three-quarters of the combined Coleoptera-Lepidotera biomass (S4 Table). Models with moth biomass as a covariate did not perform better than null models in any of the four model types, a result that might have been due to moths comprising a relatively minor amount of the insects sampled. It is possible that a relationship between Hawaiian hoary bat foraging activity and moth abundance might be more evident where and when moth availability is higher. For example, moths were the most abundant insect order and dominated the diet of Hawaiian hoary bats in open habitat, but were consumed less in cluttered habitat where they were generally smaller than the minimum prey size noted in a study on Kaua'i Island [35]. In addition, moth and beetle prevalence in the diet of hoary bats in North America appears to be seasonally variable, indicating some degree of opportunistic feeding [54–55].

Bat detection rates were higher for video than for acoustic sampling methods, but neither the video–activity nor video–feeding model sets resulted in models with moth, beetle or insect covariates that ranked better than the null. Although null model mean estimates $p^2$ and $\psi^2$ were considerably higher and precision was greater for video-based methods, measures of elevated acoustic activity appeared to be more closely associated with prey abundance. This may reflect the possibility that the higher rates of bat detections by video include commuting individuals not engaged in foraging situations. Recent observations of hoary bats not detectably vocalizing while in flight [5,6] suggest that the species may not be entirely reliant on echolocation for nocturnal navigation. Consequently, when vocalization is detected may be a more reliable indicator of prey targeting and active foraging than the number of flight passes detected visually.

Feeding activity as identified by terminal-phase calls ("feeding buzzes") were not demonstrably related to beetle or moth biomass, but this result may reflect the relative sparsity of these particular detections (only once at each of 6 sites over the 61 nights with acoustic samples) rather than the actual absence of such relationship. Terminal-phase calls are unambiguous indicators of prey targeting and likely feeding, but are typically emitted at a much lower intensity than search or approach phase calls so as to temper gain and prevent self-deafening

from echoes as a bat closes in on a target [56–58]. As such, terminal-phase calls are more difficult to detect in the field and using these calls as a measure of feeding may under-represent these events compared to tallies obtained from the acoustics of bats simply searching for prey. Notably, of the four model types, the acoustic–feeding model exhibited the highest uncertainty in correctly identifying feeding activity ($\hat{\delta}$ = 0.32; Table 1).

As with the detection of terminal-phase calls, identifying flight trajectories that included a rapid loop or roll from video recordings may undercount the actual prevalence of feeding events because Hawaiian hoary bats may be able to catch prey without such manoeuvers. Therefore, although observed detection probability was quite high ($\hat{p}^2$ = 0.96; Table 1), the state assignment was characterized by uncertainty ($\hat{\delta}$ = 0.49) and likely hindered modeling the relationships between the prevalence of this behavior and beetle and moth biomass.

The strength of bat activity and prey biomass associations can be expected to change across habitats and seasons in response to shifts in the composition and abundance of available insects. The apparent absence of bat activity and prey biomass associations for three of the four model types may be partly due to prey not actually being a limiting resource in the summer months when insect abundance is generally highest. Even reproductive bats with higher energetic demands likely can achieve a positive energy balance after only a few hours of foraging [59]. Consequently, causal factors in the spatial patterns of bat abundance, food resources, and insect predation may only be evident during seasons or periods of less favorable environmental conditions and more restricted resource availability.

Multi-state modeling has the potential to be useful in studies where investigators can obtain information at occupied sites about species status (e.g., encounter rates, behavior, etc.). Information about bat activity and the availability of foraging resources can be acquired for many species and settings, and may often be obtained non-invasively and with less effort than comparable sampling involving bat capture and handling. Assuming that sites with higher abundances, activity levels or specific behaviors (e.g. feeding or breeding) are indicative of higher habitat quality or availability, the opportunity exists to use these models to explore relationships between species status and focal resources. Multi-state occupancy modeling also can be applied to multi-season models to assess trends in specific habitat use that might not be apparent solely from short-term assessments of species presence [60]. For example, overall prevalence (as represented by $\psi^1$) of a widely ranging species may be relatively constant over time, yet the proportion of sites at which focal species activity or behavior is tracked could change in directions important to conservation management (e.g., habitat restoration, resource extraction impacts, etc.). Such models can allow inference about transition probabilities for both species occupancy state and habitat state, and the dependence of species status on habitat condition.

In summary, multi-state occupancy modeling can establish quantitative relationships in settings where sampling is difficult and animal crypsis results in imperfect detection. Although applied to coarse categories of bat activity and behavior, the resulting models are robust and explicitly incorporate uncertainty. Krebs [61] stated that "Ecology is the scientific study of the interactions that determine the distribution and abundance of organisms. We are interested in where organisms are found, how many occur there, and why". By linking animal activity, occurrence and habitat, multi-state modeling can use information of the *how many* (or how much) to effectively describe the *where* and infer the *why*.

## Supporting information

**S1 Table. Mean weight by site for samples used as covariates "beetle" (Coleoptera), "moth" (Lepidoptera) and "insect" (combined Coleoptera and Lepidoptera weights) in the multi-**

**state occupancy models.**
(DOCX)

**S2 Table. Bat detection histories by site and nightly sample for the four multi-state occupancy model types: Acoustic–activity, Acoustic–feeding, Video–activity, and Video–feeding.**
(DOCX)

**S3 Table. Model selection statistics for 9 multi-state occupancy models fit to each of four model types: Acoustic–activity, Acoustic–feeding, Video–activity, and Video–feeding.**
(DOCX)

**S4 Table.** Total weight (panel a) and count (panel b) and associated proportions (prop) of arachnid and insect samples recorded over 4 nights at each of 20 sites from 10 July to 10 August 2017 in the northern Koʻolau Mountains of Oʻahu.
(DOCX)

**S1 Fig. Coleoptera and Lepidoptera biomass (mean nightly dry weight; grams) samples by site.**
(DOCX)

**S2 Fig. Acoustic detections by site of Hawaiian hoary bats (*Lasiurus cinereus semotus*) and the subset identified as comprising feeding behavior (terminal-phase calls).**
(DOCX)

**S3 Fig. Videographic detections by site of Hawaiian hoary bats (*Lasiurus cinereus semotus*) and the subset identified as comprising feeding behavior.**
(DOCX)

**S4 Fig. Sampled Coleoptera biomass and the predicted probability of high acoustic activity by Hawaiian hoary bats (*Lasiurus cinereus semotus*) given that the site is occupied (occupancy state 2).**
(DOCX)

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# References

1. O'Shea TJ, Bogan MA. Monitoring trends in bat populations of the United States and territories: problems and prospects. Fort Collins: U.S. Geological survey, Information and Technology Report; 2003.

2. Carter TC, Menzel MA, Saugey D. Population trends of solitary foliage-roosting bats. In: O'Shea TJ, Bogan MA, editors. Monitoring trends in bat populations of the United States and territories: problems and prospects. Fort Collins: U.S. Geological survey, Information and Technology Report; 2003. pp. 41–47.

3. O'Farrell MJ, Gannon WL. A comparison of acoustic versus capture techniques for the inventory of bats. J Mamm, 1999; 80: 24–30.

4. Weller TJ, Lee DC. Mist net effort required to inventory a forest bat species assemblage. J Wildl Manage. 2007; 71: 251–257.

5. Gorresen PM, Cryan PM, Montoya-Aiona K, Bonaccorso FJ. Do you hear what I see? Vocalization relative to visual detection rates of Hawaiian hoary bats (*Lasiurus cinereus semotus*). Ecol Evol. 2017; 7: 6669–6679. https://doi.org/10.1002/ece3.3196 PMID: 28904749

6. Corcoran AJ, Weller TJ. Inconspicuous echolocation in hoary bats (*Lasiurus cinereus*). Proc R Soc B 2018; 285: 20180441. https://doi.org/10.1098/rspb.2018.0441 PMID: 29720417

7. MacKenzie DI, Nichols JD, Lachman GB, Droege S, Royle JA, Langtimm CA. Estimating site occupancy rates when detection probabilities are less than one. Ecology 2002; 83: 2248–2255.

8. Gorresen PM, Miles AC, Todd CM, Bonaccorso FJ, Weller TJ. Assessing bat detectability and occupancy with multiple automated echolocation detectors. J Mamm. 2008; 89: 11–17.

9. Weller TJ, Baldwin JA. Using echolocation monitoring to model bat occupancy and inform mitigations at wind energy facilities. J Wildl Manage. 2012; 76: 619–631.

10. Pauli BP, Zollner PA, Haulton GS. Nocturnal habitat selection of bats using occupancy models. J Wildl Manage. 2017; 81: 878–891.

11. Rodhouse TJ, Ormsbee PC, Irvine KM, Vierling LA, Szewczak JM, Vierling KT. Assessing the status and trend of bat populations across broad geographic regions with dynamic distribution models. Ecol Appl. 2012; 22: 1098–113. PMID: 22827121

12. Rodhouse TJ, Ormsbee PC, Irvine KM, Vierling LA, Szewczak JM, Vierling KT. Establishing conservation baselines with dynamic distribution models for bat populations facing imminent decline. Divers Distrib. 2015; 21: 1401–13.

13. Nichols JD, Hines JE, MacKenzie DI, Seamans ME, Gutierrez RJ. Occupancy estimation and modelling with multiple states and state uncertainty. Ecology 2007; 88: 1395–400. PMID: 17601132

14. MacKenzie DI, Nichols JD, Seamans ME, Gutierrez RJ. Modeling species occurrence dynamics with multiple states and imperfect detection. Ecology. 2009; 90: 823–835. PMID: 19341151

15. McClintock BT, Bailey LL, Pollock KH, Simons TR. Experimental investigation of observation error in anuran call surveys. J Wildl Manage. 2010; 74: 882–1893.

16. Jensen T, Vokoun JC. Using multistate occupancy estimation to model habitat use in difficult-to-sample watersheds: bridle shiner in a low-gradient swampy stream. Can J Fish Aquat Sci. 2013; 70: 1429–1437.

17. Gonsalves L, Law B, Webb C Monamy V. Foraging ranges of insectivorous bats shift relative to changes in mosquito abundance. PLOSONE. 2013; 8: e64081.

18. Wilcox A, Willis CKR. Energetic benefits of enhanced summer roosting habitat for little brown bats (*Myotis lucifugus*) recovering from white-nose syndrome. Conserv Physiol 2016; 4: 1–12. https://doi.org/10.1093/conphys/cow050

19. Nurul-Ain E, Rosli H, Kingston T. Resource availability and roosting ecology shape reproductive phenology of rain forest insectivorous bats. Biotropica. 2017; 49: 382–394.

20. Clare EL, Symondson WO, Broders H, Fabianek F, Fraser EE, MacKenzie A, et al. The diet of *Myotis lucifugus* across Canada: assessing foraging quality and diet variability. Mol Ecol. 2014; 23: 3618–3632. https://doi.org/10.1111/mec.12542 PMID: 24274182

21. Charnov EL. Optimal foraging, the marginal value theorem. Theor Popul Biol. 1976; 9: 129–136. PMID: 1273796

22. Pyke GH. Optimal foraging theory: a critical review. Annu Rev Ecol Evol Syst. 1984; 15: 523–575.

23. Kurta A, Bell GP, Nagy KA, Kunz TH. Energetics of pregnancy and lactation in freeranging little brown bats (*Myotis lucifugus*). Phys Zool. 1989; 62: 804–818.

24. Kunz TH, de Torrez EB, Bauer D, Lobova T, Fleming TH. Ecosystem services provided by bats. Ann N Y Acad Sci. 2011; 1223: 1–38. https://doi.org/10.1111/j.1749-6632.2011.06004.x PMID: 21449963

25. Black HL. A north temperate bat community: structure and prey populations. J Mamm. 1974; 55: 138–157.

26. Fukui DAI, Murakami M, Nakano S, Aoi T. Effect of emergent aquatic insects on bat foraging in a riparian forest. J Anim Ecol. 2006; 75: 1252–1258. https://doi.org/10.1111/j.1365-2656.2006.01146.x PMID: 17032357

27. de Oliveira LQ, Marciente R, Magnusson WE, Bobrowiec PED. Activity of the insectivorous bat *Pteronotus parnellii* relative to insect resources and vegetation structure. J Mamm. 2015; 96: 1036–1044.

28. Rydell J. Variation in foraging activity of an aerial insectivorous bat during reproduction. J Mamm, 1993; 74: 503–509.

29. Clare EL, Symondson WO, Fenton MB. An inordinate fondness for beetles? Variation in seasonal dietary preferences of night-roosting big brown bats (*Eptesicus fuscus*). Mol Ecol. 2014; 23: 3633–3647. PMID: 25187921

30. Nebel S, Mills A, McCracken JD, Taylor PD. Declines of aerial insectivores in North America follow a geographic gradient. Avian Conserv Ecol. 2010; 5: 1.

31. Collen B, Böhm M, Kemp R, Baillie JE. Spineless: status and trends of the world's invertebrates. Zoological Society of London; 2012.

32. Hallmann CA, Sorg M, Jongejans E, Siepel H, Hofland N, Schwan H, et al. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLOSONE 2017; 12: e0185809.

33. Tomich PQ. Mammals in Hawai'i. Bishop Museum Special Publication 76. Honolulu: Bishop Museum Press; 1986.

34. Whitaker JO Jr, Tomich PQ. Food habits of the hoary bat, *Lasiurus cinereus*, from Hawaii. J Mamm. 1983: 64: 150–151.

35. Belwood JJ, Fullard JH. Echolocation and foraging behavior in the Hawaiian hoary bat, *Lasiurus cinereus semotus*. Can J Zool. 1984; 62: 2113–2120.

36. Jacobs DS. The diet of the insectivorous Hawaiian hoary bat (*Lasiurus cinereus semotus*) in an open and a cluttered habitat. Can J Zool. 1999; 77: 1603–1608.

37. Todd CM. Effects of prey abundance on seasonal movements of the Hawaiian hoary bat (Lasiurus cinereus semotus). M.Sc. Thesis, University of Hawai'i at Hilo. 2012.

38. Gorresen PM, Cryan PM, Huso MM, Hein CD, Schirmacher MR, Johnson JA, et al. Behavior of the Hawaiian hoary bat (*Lasiurus cinereus semotus*) at wind turbines and its distribution across the North Ko'olau Mountains, O'ahu. University of Hawai'i at Hilo, Hawai'i Cooperative Studies Unit. 2015 Technical Report 064.

39. Mykleseth K. Wind farms killing more bats than expected. Honolulu Star Advertiser. 14 Jan 2017. Available from: http://www.wind-watch.org/news/2017/01/15/wind-farms-killing-more-bats-than-expected/

40. Arnett EB, Baerwald EF. Impacts of wind energy development on bats: implications for conservation. In: Adams RA, Peterson SC, editors. Bat evolution, ecology, and conservation. New York: Springer Science Press; 2013. pp. 435–456.

41. Kincaid TM, Olsen AR. Spsurvey: Spatial Survey Design and Analysis. R package version 3.3. 2016. Available from: https://cran.r-project.org/web/packages/spsurvey/index.html. Accessed 2017 March 12.

**42.** Norberg UM. Some advanced flight manoeuvres of bats. J Exp Biol. 1976; 64: 489–495.

**43.** Norberg UM, Rayner JM. Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. Philos Trans R Soc Lond B Biol Sci. 1987; 316: 335–427.

**44.** Altringham JD. Flight. In: Bats: From evolution to conservation. New York: Oxford University Press; 2011. pp. 37–60.

**45.** Griffin DR. Listening in the dark: The acoustic orientation of bats and men. New Haven: Yale University Press; 1958.

**46.** MacKenzie DI, Royle JA. Designing occupancy studies: general advice and allocating survey effort. J Appl Ecol. 2005; 42: 1105–1114.

**47.** Latif QS, Ellis MM, Amundson CL. A broader definition of occupancy: Comment on Hayes and Monfils. J Wildl Manage. 2016; 80: 192–194.

**48.** Bonaccorso FJ, Todd CM, Miles AC, Gorresen PM. Foraging range movements of the endangered Hawaiian hoary bat, *Lasiurus cinereus semotus* (Chiroptera: Vespertilionidae). J Mamm. 2015; 96: 64–71.

**49.** Hines JE. PRESENCE—Software to estimate patch occupancy and related parameters. USGS-PWRC. Available from: http://www.mbr-pwrc.usgs.gov/software/presence.html. Accessed 2018 September 1.

**50.** Burnham KP, Anderson DR. Model selection and multimodel inference: a practical information-theoretic approach. 2nd ed. New York: Springer-Verlag; 2002.

**51.** Guillera-Arroita G, Ridout MS, Morgan BJ. Design of occupancy studies with imperfect detection. Methods Ecol Evol. 2010; 1: 131–139.

**52.** MacKenzie DI, Nichols JD, Lachman GB, Droege S, Andrew Royle J, Langtimm CA. Estimating site occupancy rates when detection probabilities are less than one. Ecology. 2002; 83: 2248–2255.

**53.** Efford MG, Dawson DK. Occupancy in continuous habitat. Ecosphere. 2012; 3: 1–15.

**54.** Valdez EW, Cryan PM. Food habits of the hoary bat (*Lasiurus cinereus*) during spring migration through New Mexico. Southwest Nat. 2009; 54: 195–200.

**55.** Valdez EW, Cryan PM. Insect prey eaten by hoary bats (*Lasiurus cinereus*) prior to fatal collisions with wind turbines. West N Am Nat. 2013; 73: 516–524.

**56.** Kick SA, Simmons JA. Automatic gain control in the bat's sonar receiver and the neuroethology of echolocation. J. Neurosci. 1984; 4: 2725–2737. PMID: 6502201

**57.** Hartley DJ. Stabilization of perceived echo amplitudes in echolocating bats. II. The acoustic behavior of the big brown bat, *Eptesicus fuscus*, when tracking moving prey. J Acoust Soc Am. 1992; 91: 1133. PMID: 1556313

**58.** Corcoran AJ, Conner WE. Predator counteradaptations: stealth echolocation overcomes insect sonar-jamming and evasive-manoeuvring defences. Anim Behav. 2017; 132: 291–301.

**59.** Salcedo HDLC, Fenton M, Hickey M, Blake R. Energetic consequences of flight speeds of foraging red and hoary bats (*Lasiurus borealis* and *Lasiurus cinereus*; Chiroptera: Vespertilionidae). J Exp Biol. 1995; 198: 2245–2251. PMID: 9320162

**60.** MacKenzie DI, Bailey LL, Hines JE, Nichols JD. An integrated model of habitat and species occurrence dynamics. Methods Ecol Evol. 2011; 2: 612–622.

**61.** Krebs CJ. Ecology: The experimental analysis of distribution and abundance. New York: Harper & Row; 1972.

# APPENDIX 4

# A TEST OF SEX SPECIFIC GENETIC MARKERS IN THE HAWAIIAN HOARY BAT AND RELEVANCE TO POPULATION STUDIES (PINZARI AND BONACCORSO 2018)

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Technical Report HCSU-085

# A TEST OF SEX SPECIFIC GENETIC MARKERS IN THE HAWAIIAN HOARY BAT AND RELEVANCE TO POPULATION STUDIES

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## ABSTRACT

We tested the utility of a protocol using genetic markers that previously proved successful to identify the sex of Vespertilionid bats on tissues collected from live bats and carcasses of varying age from the Hawaiian hoary bat (*Lasiurus cinereus semotus*). This molecular method is based on genes unique to X and Y chromosomes in mammals and previously was used successfully on North American hoary bats (*L*. c. cinereus). We amplified two markers within intron regions of the zinc-finger-X (Z*fx*) and zinc-finger-Y (Z*fy*) genes using a multiplexed polymerase chain reaction technique and obtained product bands that were easily visualized using gel electrophoresis. Genotyping determined the sex of 36 individual Hawaiian hoary bat carcasses previously assigned sex only by external genitalia and identified sex for 29 "unknown" bat carcasses that could not be classified by external genitalia. Employing this method for sexing Hawaiian hoary bats will permit more reliable evaluation of the ratio of males to females in subpopulations affected by fatalities from emerging threats. This is critical to the conservation and management of this endangered bat.

## INTRODUCTION

The Hawaiian hoary bat was listed as a federally endangered species in 1970 under the Endangered Species Conservation Act of 1969 that protects native fish and wildlife found to be threatened with extinction under 16 USC 668aa(c). A Recovery Plan for the Hawaiian hoary bat was published in 1998 and lists research as the overall recovery strategy because of insufficient information on abundance, distribution, critical habitat needs, and population status of the Hawaiian hoary bat (USFWS 1998). In Hawai'i's modern environment, potential threats to population recovery for this species include timber harvest practices (especially during the pupping season), entanglement on barbed-wire fencing, exposure to pesticides, and fatal collisions with wind turbines (USFWS 2011).

In the last decade, turbines have been deployed to harvest wind power in many areas throughout the world, including Hawai'i. Hawaiian hoary bats are experiencing collisions with wind turbines on the islands of Hawai'i, Maui, and O'ahu. Information on sex ratios of downed bats may provide more accurate fatality estimates and help understand the potential impact of wind energy-associated fatalities to Hawaiian hoary bats (Arnett *et al.* 2013, Cryan *et al.* 2012, Frick *et al.* 2017, Hein & Schirmacher 2016).

The goal of our study was to apply a method of sex determination based on molecular genetics previously proven effective on Vespertilionid bats to test the probability of correct sex identification from Hawaiian hoary bat carcasses and live individuals. Carcasses in our study were estimated to range from less than one to seven plus days post mortem. We also discuss some benefits this research may provide toward improving the accuracy of modeling population take and impacts of wind energy on Hawaiian hoary bats.

## METHODS

### Sample Collection
The U.S. Geological Survey holds collections of biological samples from Hawaiian hoary bats dating from 2007. This tissue collection includes wing membrane and muscle tissue samples representing approximately 200 male, female, and unknown sex individuals from four Hawaiian

Islands (Hawai'i, Kaua'i, O'ahu and Maui). These tissue samples were obtained from live capture and release or from carcasses provided by wind energy facilities, Hawai'i State wildlife offices, and the U.S. Geological Survey's National Wildlife Health Center Honolulu Field Station. While new samples continue to be acquired periodically; our present study is limited to an analysis of tissues from 73 Hawaiian hoary bats obtained between 2009 and 2016 from the islands of Hawai'i, Maui and O'ahu (Figure 1 & Table 1).
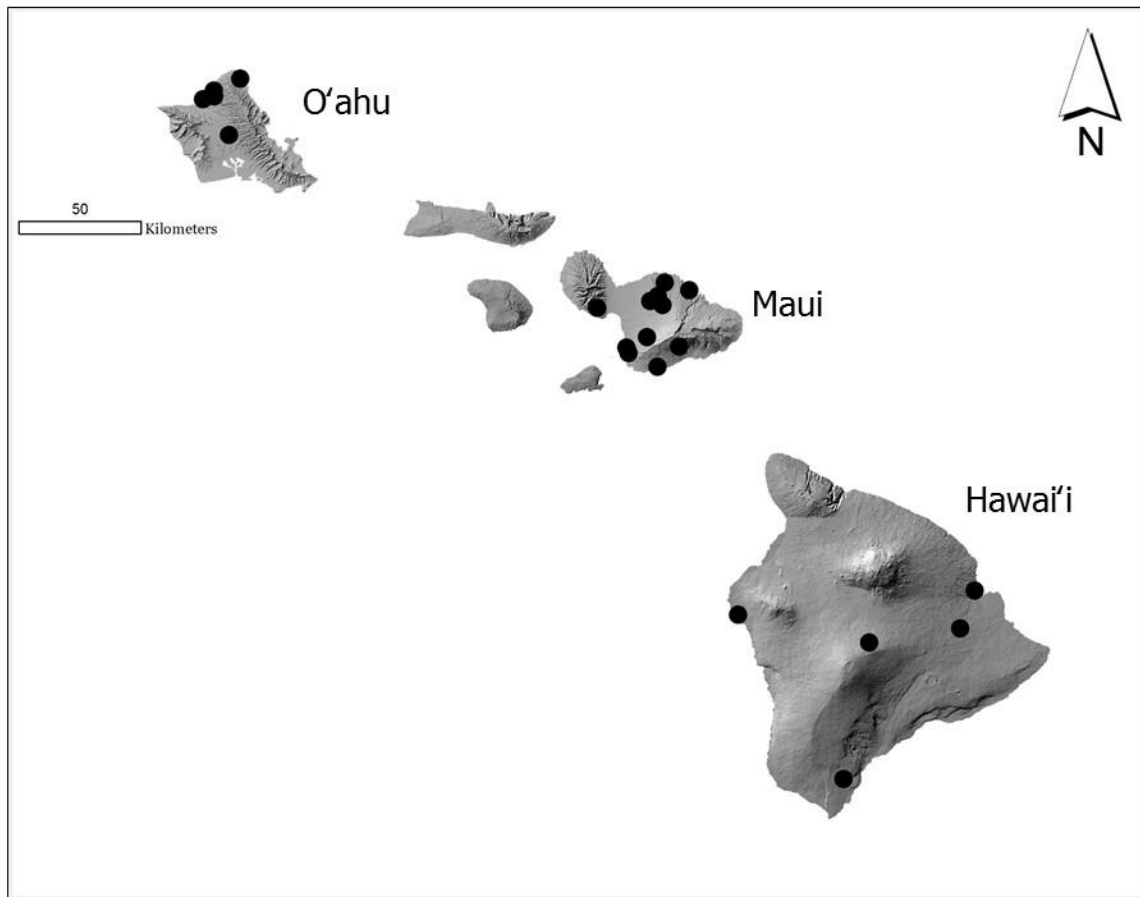


Figure 1. Map of the sampling locations for bats used in genetic sex testing.

Both samples from carcasses (n=67) and samples from captures (n=6) were used to test the genotyping method. For bat carcasses with associated downed wildlife reports and necropsies, we used the sex and the estimated time since death written on the report from the field observer. Field observers included both biologists and wind facility staff. In cases of wind fatality reports, estimated time of death was based on the frequency at which observers performed carcass searches and the condition of the carcass at the time of discovery. We used a $\chi^2$ test of independence to assess the influence of carcass age (estimated time since death) on the number of bats with sex assigned as unknown. Only 55 case reports associated with carcasses provided time estimated since death, and we limited our $\chi^2$ analysis of sex

determination on reports by external genitalia alone to this dataset. The sex of captured bats (n=6) was recorded during mist netting by wildlife biologists experienced in working with bats, and these samples were used to confirm the accuracy of the genotyping technique. We calculated sex ratios based both on identifications from external genitalia and genetic DNA testing. Sex ratio calculations were restricted to 65 bat carcasses previously categorized from examination of external genitalia as male, female, and unknown that had DNA successfully amplified during PCR. We used Fischer's exact test to assess if sex ratio was influenced by method of identification (observation of external genitalia or DNA genotyping) used to determine sex of bat carcasses.

Table 1. Numbers and types of bat specimens used for sex genotyping of Hawaiian hoary bats.

| Island | Specimen Type | n | Recorded Sex[1] | n |
|--------|--------------|---|------------|---|
| Hawaiʻi | Capture | 2 | Male | 1 |
|  |  |  | Female | 1 |
|  | Carcass | 3 | Male | 2 |
|  |  |  | Unknown | 1 |
| Maui | Capture | 2 | Male | 2 |
|  | Carcass | 28 | Male | 10 |
|  |  |  | Female | 5 |
|  |  |  | Unknown | 13 |
| Oʻahu | Capture | 2 | Male | 1 |
|  |  |  | Female | 1 |
|  | Carcass | 36 | Male | 14 |
|  |  |  | Female | 5 |
|  |  |  | Unknown | 17 |
| Total |  |  |  | 73 |

[1]Sex that was recorded during collection of the bat and observed from external genitalia.

Live bats were captured using four-shelf, nylon mist nets (Kunz & Parsons 2009). Nets were opened at sunset across roads, trails, gulches, and ponds where bats had been observed flying. On some occasions, we used playback of Hawaiian hoary bat social calls to lure bats into mist nets. Nets were checked every 15 minutes and bats were placed individually in cloth holding bags until processing. Biological data collection included: sex and age class, reproductive condition, forearm length, and body mass (Kunz & Parsons 2009). We used a sterile 3 mm biopsy punch to obtain tissue samples from each wing. Bats were released at the site of capture within 40 minutes of netting.

Our methods follow the guidelines for capture, handling, and care of mammals recommended by the Institutional Animal Care and Use Committee (IACUC #04-039-12) of the University of Hawaiʻi at Hilo and the American Society of Mammologists (Sikes and Gannon 2011). We collected biological samples from bats as specified by US Fish and Wildlife Service permit TE003483-31 and Hawaiʻi Department of Land and Natural Resources permit WL 16-04.

Carcasses were refrigerated or frozen upon discovery, and tissue samples from necropsies were stored at -20 °C. Carcasses were assessed for external sexual morphology. Tissue samples were taken from soft wing membrane (Figure 2) with a sterile 3 mm circular biopsy tool. In some

cases, muscle tissue was cut away from the breast area of a fresh carcass with a sterile scalpel. All tissue samples were stored in 1.5 ml tubes containing NaCl-saturated 20% DMSO or on silica gel desiccant beads at ambient temperature in the field and later frozen at -20 °C until DNA extraction.



Figure 2. Examples of Hawaiian hoary bats used to test genetic sex determination, a live individual captured by mist net (left), and a desiccated carcass of unknown sex (right).

**Genotyping Technique**
DNA was isolated from bat tissues using a Qiagen DNeasy Blood and Tissue kit following the manufacturer's protocol for purification of total DNA from animal tissues (Qiagen, Valencia, CA, USA). After extraction, DNA quantity was estimated, and quality assessed with two different methods. First, 2 µL of the DNA sample was electrophoresed through a 1.5% agarose gel and visualized under UV illumination against a 1 kb bp ladder after staining with SYBR Safe (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Second, UV absorbance was measured using spectrometry in 3µL of each DNA sample on a NanoDrop 1000 machine. Isolated DNA was used in polymerase chain reactions (PCR) to amplify two gene sections on the X and Y sex chromosomes of Hawaiian hoary bats.

Sex determination PCR primers were multiplexed and consisted of two groups of fragment sizes (Korstian *et al.* 2013): the first primer set for the region within the *Zfx* intron yielded fragments of ~245 base pairs in length (F-ZFXBat: AGTCAAGGGRTGTCCATCR, R-ZFXBat: GTTTGYASACCAGGTTCCTC) and the second primer set for the region within the *Zfy* intron yielded fragments of ~80 base pairs in length (F-ZFYBat: GGTRAGDGCACAYRAGTTCCACA, R-ZFYBat: TGCYATTACAAAACCTTTRTAGATAC). DNA fragments were amplified using Qiagen's Multiplex Reaction Kit master mix following standard protocol. PCRs were 10 µL volume reactions each containing 20 ng template DNA, 0.5 µM of each X-primer, 0.35 µM of each Y-primer, 1X Multiplex Master Mix with HotStarTaq, Multiplex PCR Buffer containing 3mM MgCl2

pH 8.7, and dNTPs Mix. Cycling parameters were 1 cycle at 95 °C for 15 minutes, then 30 cycles of 30 seconds at 94 °C, 15 seconds at 57 °C, 30 seconds at 72 °C, and were carried out on an Eppendorf Pro S Thermal Cycler.

PCR products were checked for desired fragment size using gel electrophoresis and UV light visualization; the entire 10 µL volume reaction for each product was loaded into separate wells on a 1% agarose gel at 110 volts for 30 minutes and stained using SYBRSafe gel dye, with a 100 bp ladder for reference. The sex of each bat was determined based on the number of bands present for the individual in the gel lane (Figure 3). Males produced two bands, one resulting from the X chromosome intron, and another from the Y chromosome intron. Females produced only one large, very bright band, from the X-chromosome intron. The X-chromosome bands appeared at ~245 bp product size, and Y-chromosome bands at ~80 bp.

For a subset of 14 samples; 4 known males, 3 known females, 4 unknown males and 3 unknown females, we repeated both the PCR and gel electrophoresis two additional times to confirm repeatable banding patterns and correct genotype identification.
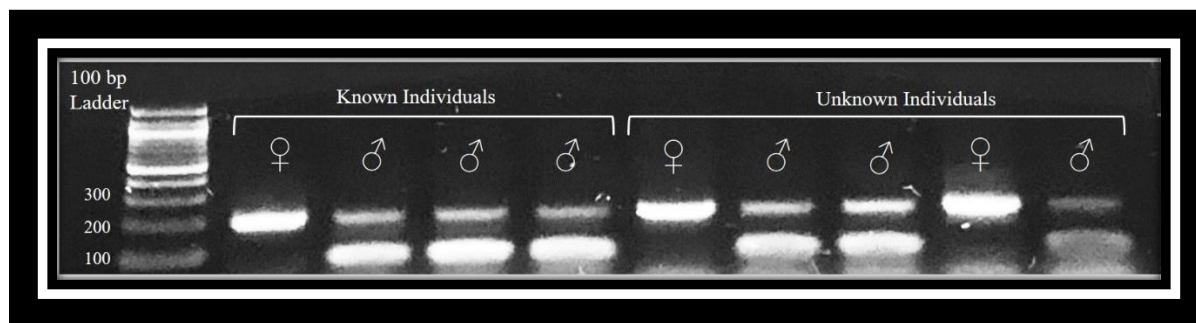


Figure 3. Bat genotype banding patterns on an agarose gel after successful amplification of zinc-finger X and Y specific PCR products and electrophoresis.

## RESULTS

We determined sex by genotyping the X and Y chromosome introns (*Zfx* and *Zfy*) for 71 of 73 (97%) Hawaiian hoary bats sampled, including both capture and carcass samples. Extremely low DNA quantity and quality attributed to advanced decomposition of carcasses >7 days post-mortem likely were responsible for two samples that did not amplify during PCR. Samples from males (n=41) produced two bands, one resulting from the X chromosome intron, and another from the Y chromosome intron. Females (n=30) produced a single large, very bright band from the X-chromosome intron. X-chromosome bands appeared at ~245 bp product size, and Y-chromosome bands at ~80 bp (Figure 3). All 14 individuals of both sexes whose genotyping was replicated three times had a 100% confirmation among the three resulting outcomes.

PCR product bands from electrophoresis provided an 88.8% (32 of 36) match with the sex observed from external genitalia of bat carcasses. For all carcasses (Figure 4) that had sex recorded by a field observer (n=36), we calculated an 11.2% error for female bat carcasses

originally recorded as males; there were no male bat carcasses that had been recorded as females using external genitalia alone. Additionally, this genetic method provided identification of the sex of 29 individuals whose carcasses were categorized as unknown by field observation.
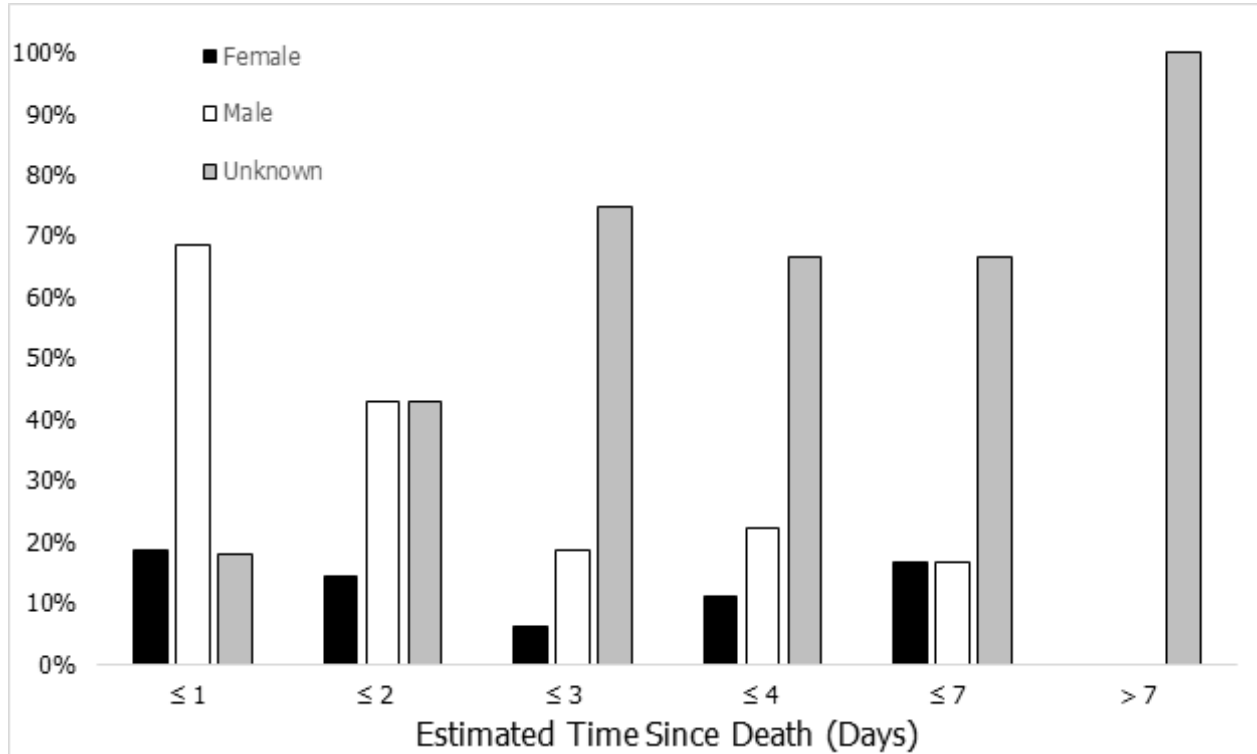


Figure 4. Sex identifications of carcasses (n=55) based on external genitalia recorded in wildlife reports as a function of the estimated time since death in days.

The accuracy of sex information generated by observers from carcasses in the field changed with time after death. Observers noted similar if not higher percentages of males to females from carcasses ≤4 days old. Carcasses of unknown sex dominated each category, except for carcasses ≤1 day, and often accounted for more than 50% of all carcasses. Due to extremely rapid rates of decomposition in the tropical climate of Hawaiʻi, the ability to assign sex to bat carcasses using external genitalia decreased significantly with estimated time since death (chi square $\chi^2$=15.53, P=0.04). The sex ratio of Hawaiian hoary bat carcasses collected was significantly different using the two methodologies (Fischer's exact test, P=0.04; Table 2).

Table 2. Sex ratios based on external genitalia versus DNA genotyping for Hawaiian hoary bat carcasses.

| Method | No. ♀ | No. ♂ | No. Unknown | Total | Sex Ratio/100 (♀:♂) |
|---|---|---|---|---|---|
| External Genitalia | 10 | 26 | 29 | 65 | 28:72 |
| DNA Genotyping | 28 | 37 | 0 | 65 | 43:57 |

## DISCUSSION

Sex genotyping was informative for 100% of the individuals analyzed in this study for which quality DNA was available, while only 88.8% of individual carcasses were correctly assigned sex using external morphology alone. For a subset of bats of known sex (3 ♀, 4 ♂) previously identified by external genitalia during capture by bat biologists, sex genotyping was 100% accurate, thus validating the utility of this method for Hawaiian hoary bats. We demonstrated that two published primer sets developed and validated for North American hoary bats, amplified regions on the *Zfx* and *Zfy* introns correctly and produced sex-specific variants in Hawaiian hoary bats. This method has shown itself to be useful in the identification of sex from bat carcasses found at wind facilities and submitted by citizens to wildlife agencies. Unless a bat carcass was quite fresh (≤2 days old), the sex of decayed or partially scavenged carcasses were difficult or nearly impossible to identify from external observation, leading to a male biased dataset in which females often were identified as unknown (Korstian *et al.* 2013, Nelson *et al.* 2018, this study). Observers noted males more often than females, but overwhelmingly they noted unknown sex. Sex determination of bat carcasses based on external genitalia often is unreliable unless limited to fresh carcasses and identifications are performed by trained personnel (Nelson *et al.* 2018). When valid sex identification is important, it can be confirmed by genetic analysis. The genotyping method that we describe also provides the opportunity to gain sex information even if a body fragment is all that was available. For example, we were able to genotype the sex of a bat from a single wing fragment.

Sex ratios of Hawaiian hoary bats may differ based on location, cause of mortality, and annual variation. In our study we grouped all carcasses together to provide a statewide overview, 43♀:57♂. Two previous studies reported sex ratios for the North American hoary bat (*Lasiurus cinereus*) using this sex genotyping technique and ratios varied by geographic location. Korstian *et al.* (2013) report hoary bats collected from northern Texas wind facilities at a ratio of 48♀:52♂, while Nelson *et al.* (2018) report hoary bats collected from wind facilities in Indiana were 61♀:39♂ (n=117). Although sex ratios from carcasses identified by the two different methods were statistically different in our study, our sample size of 65 carcasses was relatively small, and the ratio may change as more samples are analyzed. For example, Korstian *et al.* (2013) sampled 500 carcasses before a significant difference in the sex ratio of red bats (*Lasiurus borealis*) was detected.

Identification of sex from carcasses can be of great value in evaluating the impact of wind energy on local bat populations because the sex and age of bat carcasses often cannot be determined morphometrically. Hoary bats are sexually dimorphic, females are slightly larger than males, thus sex and age classification of a Hawaiian hoary bat cannot be identified reliably based on size alone (Jacobs 1996). For example, adult male forearms overlap with the range of

measurements for juvenile females. Genetic analysis allows accurate determination of sex from both adult and juvenile hoary bat (*Lasiurus cinereus*) carcasses (Nelson *et al.* 2018).

For an endangered bat species with limited live field collection opportunities, the importance of carcasses should be noted. A fresh carcass can contribute much more than hereditary genetic information, it also can provide details on bat foraging activity and prey types (Valdez & Cryan 2009). For example, Foo *et al*. (2017) was able to gain dietary information from genetic analysis of stomach content from hoary bat fatalities through the use of carcasses. It may soon be possible to genetically determine age from freshly collected bat wing tissues, researchers in Europe have developed and tested a molecular DNA methylation assay to establish age structure in the endangered Bechstein's bat (*Myotis bechsteinii,* Wright *et al.* 2018).

Sex information from genotyping is being employed in the study of genetic diversity, population structure, and historic population size in North American hoary bats (Korstian *et al.* 2015). Future studies of the evolutionary and phylogenetic relationships could compare Hawaiian hoary bat zinc-finger gene DNA sequences easily obtained from PCR products with this assay to that of the North American hoary bat. We suggest that this methodology has many potential applications for research across mammalian population genetics. Many mammal specimens throughout museum collections, especially those from 19th and early 20th Century collections lack records of specimen sex. Although this method of sex identification has not been tested yet on tissues from preserved museum specimens, we believe it could provide local collections with increased demographic information that was not captured by the original collectors.

## LITERATURE CITED

Arnett, E.B., and E.F. Baerwald. 2013. Impacts of wind energy development on bats: implications for conservation, In: Adams R.S., Pedersen S.C., eds. *Bat evolution, ecology, and conservation*. New York: Springer Science and Business Media, pp. 435–456.

Cryan, P.M., J.W. Jameson, E.F. Baerwald, C.K. Willis, R.M. Barclay, E.A. Snider, E.G. Crichton. 2012. Evidence of late-summer mating readiness and early sexual maturation in migratory tree-roosting bats found dead at wind turbines. PLOS One 7: e47586.

Frick, W.F., E.F. Baerwald, J.F. Pollock, R.M.R. Barclay, J.A. Szymanski, T.J. Weller, A.I. Russell, S.C. Loeb, R.A. Medellin, and L.P. McGuire. 2017. Fatalities and wind turbines may threaten population viability of a migratory bat. Biological Conservation 209: 172–177.

Foo, C.F., V.J. Bennett, A. M. Hale, J.M. Korstian, A.J. Schildt, and D. A. Williams. 2017. Increasing evidence that bats actively forage at wind turbines. PeerJ 5: e3985.

Hein, C.D., and M.R. Schirmacher. 2016. Impact of wind energy on bats: a summary of our current knowledge. Human-Wildlife Interactions. 10(1): 19-27, Spring 2016. https://digitalcommons.usu.edu/hwi/vol10/iss1/4.

Jacobs, D.S. 1996. Morphological divergence in an insular bat, *Lasiurus cinereus semotus*. Functional Ecology 10: 622–630.

Korstian, J.M., A.M. Hale, V.J. Bennett, and D.A. Williams. 2013. Advances in sex determination in bats and its utility in wind-wildlife studies. Molecular Ecology Resources, 13: 776–780.

Korstian, J.M., A.M. Hale, and D.A. Williams. 2015. Genetic diversity, historic population size, and population structure in 2 North American tree bats. Journal of Mammalogy 96: 972–980.

Kunz, T.H. & S. Parsons (eds.). 2009. Ecological and behavioral methods for the study of bats. 2nd edition. John Hopkins University Press, Baltimore, Maryland, USA, 901 pp.

Nelson, D.M., Nagel, J., Trott, R., Campbell, C.J., Pruitt, L., Good, R.E., Iskali, G. and P.F. Gugger. 2018. Carcass age and searcher identity affect morphological assessment of sex

of bats. The Journal of Wildlife Management.
https://onlinelibrary.wiley.com/do/epdf/10.1002/jwmg.2155.

Sikes, R.S., and W.L. Gannon. 2011. Guidelines of the American Society of Mammalogists for
the use of wild mammals in research. Journal of Mammalogy, 92: 235–253.

Valdez, E.W., and P.M. Cryan. 2009. Insect prey eaten by hoary bats (*Lasiurus cinereus*) prior
to fatal collisions with wind turbines. Western North American Naturalist 73: 516–524.

U.S. Fish and Wildlife Service. 1998. Recovery plan for the Hawaiian hoary bat. Portland,
Oregon: U.S. Fish and Wildlife Service, 50 pp.
https://ecos.fws.gov/docs/recovery_plan/980511b.pdf

U.S. Fish and Wildlife Service. 2011. Ōpeʻapeʻa or Hawaiian Hoary Bat (*Lasiurus cinereus
semotus*), 5-Year Review Summary and Evaluation. Honolulu, HI: Pacific Islands Fish
and Wildlife Service, 13 pp. https://ecos.fws.gov/docs/five_year_review/doc3865.pdf

Wright, P.G.R., Matthews, F., Schofield, H., Morris, C., Burrage, J., Smith, A., Dempster, E.L.,
and P.B. Hamilton. 2018. Application of a novel molecular method to age free-living wild
Bechstein's bats. Molecular Ecology Resources. https://doi.org/10.1111/1755-
0998.12925

# APPENDIX 5

# OAHU HAWAIIAN HOARY BAT OCCUPANCY AND DISTRIBUTION STUDY PROJECT UPDATE AND FIRST-YEAR ANALYSIS, REVISED MARCH 2019 (STARCEVICH ET AL. 2019)

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# OAHU HAWAIIAN HOARY BAT OCCUPANCY AND DISTRIBUTION STUDY

Project Update and First-Year Analysis



**Prepared for:**

**Hawaii Endangered Species Research Committee**

**Prepared by:**

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**Revised March 1, 2019**
**(Replaces October 16, 2018 version)**

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## INTRODUCTION

In response to a request for proposals (RFP) issued in 2016 by Hawaii's Endangered Species Recovery Committee (ESRC), Western EcoSystems Technology, Inc. (WEST) developed a 5-year study proposal to investigate the distribution and seasonal occupancy of Hawaiian hoary bat (HAHOBA; *Lasiurus cinereus semotus*) on the island of Oahu. The initial study proposal, submitted in fall 2016, was modified as a result of two meetings and discussions with the ESRC Bat subcommittee which occurred in January and February, 2017. Based on the discussions with the ESRC and Bat subcommittee, it was decided that the initial goals of the study were to examine the distribution and seasonal occupancy of HAHOBA, with study plans for subsequent years to be based on the results of the initial year of data.

A final study plan developed in early 2017 that focused on HAHOBA distribution and occupancy was considered to be consistent with the recommendations and priorities of the ESRC bat subcommittee. The objectives of the initial year of study were to 1) provide information on bat occupancy, distribution, and detection probabilities for the island of Oahu, 2) examine seasonal changes in distribution by estimating seasonal changes in occupancy, and 3) collect data that could be used later to assess HAHOBA habitat use relationships.

While field studies are ongoing, the first year of data collection for the HAHOBA Occupancy and Distribution Study (Occupancy Study) has been completed. This preliminary report has been developed to update the ESRC and other cooperating entities on the status of the Occupancy Study and initial analysis results.

In this report we describe the sampling design and methods used to collect and analyze the data. We then summarize the results of the first year of field studies and occupancy analysis, and conclude with an evaluation of sample sizes needed for precise occupancy estimation. This report is a revision of the first-year analysis report dated October 16, 2018 and information presented herein supersedes that reported in the October 2018 report. Revisions include edits to text, tables, and figures in response to comments received on the October 2018 version, and in response to refinements in data management processes and anomalies discovered during on-going data review and analysis. We provide this revised report to ensure data resulting from and presented for the initial year-1 analysis period are as accurate and informative as possible. While changes in site-specific results are presented, these changes do not alter the overall patterns observed in terms of spatial or temporal bat activity patterns nor the interpretation of the initial year-1 results. Readers should also recognize that this is an interim report addressing the first year of data from a multi-year project and that revised analyses and results, which may affect interpretation of results, will be forthcoming as additional data are collected.

## METHODS

In this section, we describe the sampling design, field data collection methods, and occupancy modeling techniques used to address HAHOBA distribution on Oahu.

Sampling Design

The sampling design was developed to form the basis for island-wide inference. A sampling frame of 787 grid cells was obtained by overlaying a grid of 2.3 km$^2$ cells across the island of Oahu. To allow for island-wide inference, no areas on Oahu were omitted from the sampling frame except for small nearshore islands. From the grid of 787 cells, an equiprobable generalized random tessellation stratified sample (GRTS; Stevens and Olsen 2003, 2004) of 100 grid cells was selected for placement of acoustic bat detectors (Figure 1). An oversample of 150 grid cells was also selected to provide an extra set of spatially-balanced sites to use if the main sample of 100 grid cells could not be completely surveyed. Reasons that a grid cell might not be surveyed every year include inaccessibility due to safety issues, landowner denial of access, and possible relocation of detectors to new grid cells if the sampling design is amended.

Field Data Collection

Wildlife Acoustics Song Meter SM4Bat full spectrum bat detectors fitted with model SMM-U1 ultrasonic microphones (Wildlife Acoustics, Inc., Concord, Massachusetts) were used for all data collection conducted by WEST; however, data from cooperating entities sometimes was collected using older Wildlife Acoustics full spectrum bat detectors (e.g., SM3bat) outfitted with newer microphones (i.e. SMM-U1). The SM4Bat detectors are small, measuring roughly 8 inches (in) tall x 5 in wide x 3 in deep and are fully self-contained (Figure 2). Some of the detectors located in easily accessible areas with relatively high risk of theft or vandalism were operated on internal batteries to minimize their detectability by people, while most detectors utilized a small external battery and accompanying solar panel as a power source (Figure 2). Those units operates on internal batteries were checked roughly every 10-14 days to minimize potential data loss, while those operated on external batteries were checked less frequently, with the interval dependent on ease of access and extending up to in excess of two months as the most remote locations where the use of helicopters was required.

Detectors were attached to existing structures (e.g., fence posts, light poles) or to newly installed t-posts, via attachment of a 10 foot (ft) length of 0.75 in diameter metal conduit used to extend the microphone to approximately 10 ft AGL (Figure 2). In some cases, the 10 ft pole was supported by small guy wires. The detector, and external battery and solar panels when used, were mounted low on the pole and the microphone at the top (Figure 2). In some developed areas, units were contained in a small (~15") toolbox and placed on top of an appropriately sized outbuilding (approximately 10 ft AGL).
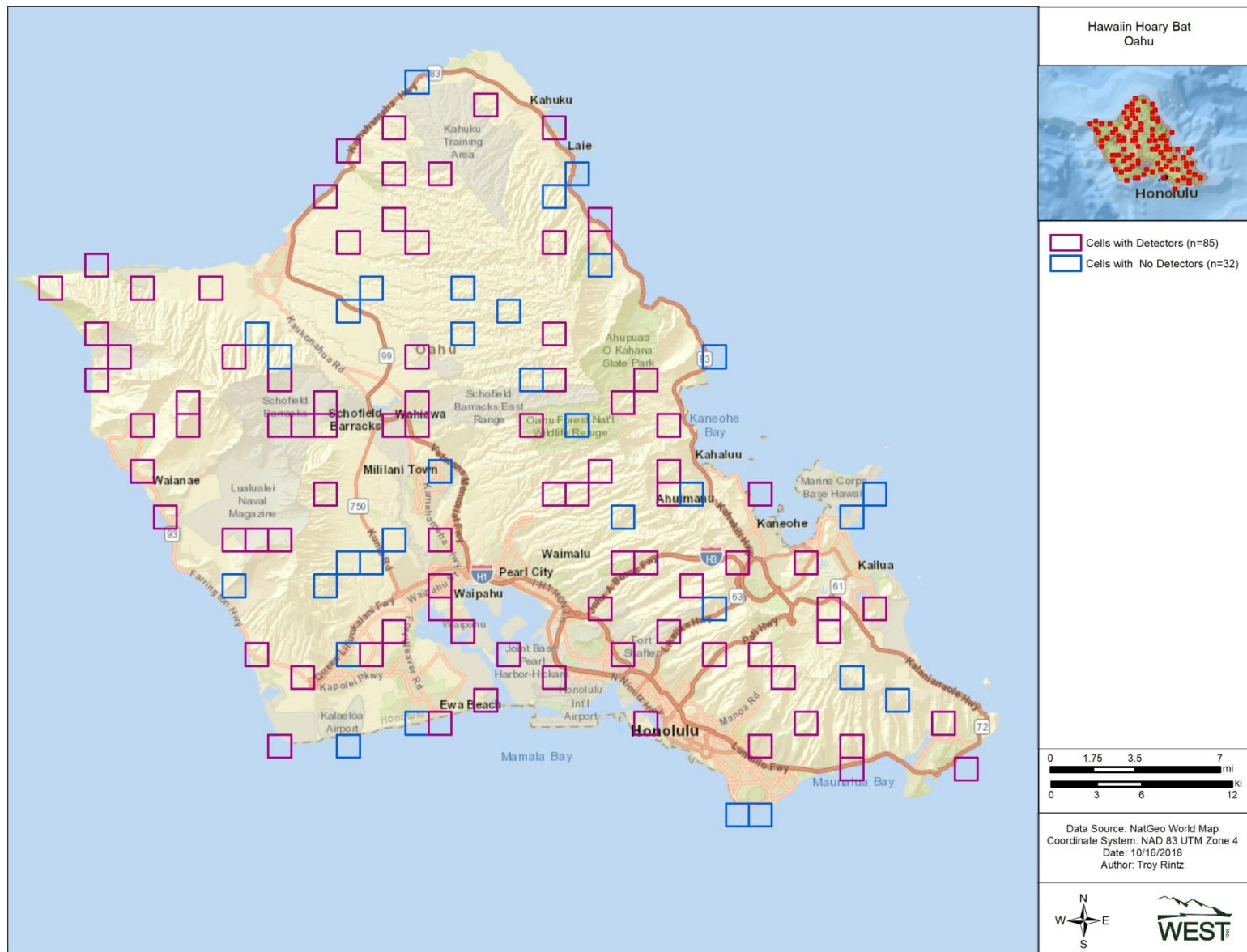
**Figure 1: Equiprobable GRTS sample of 100 grid cells.**
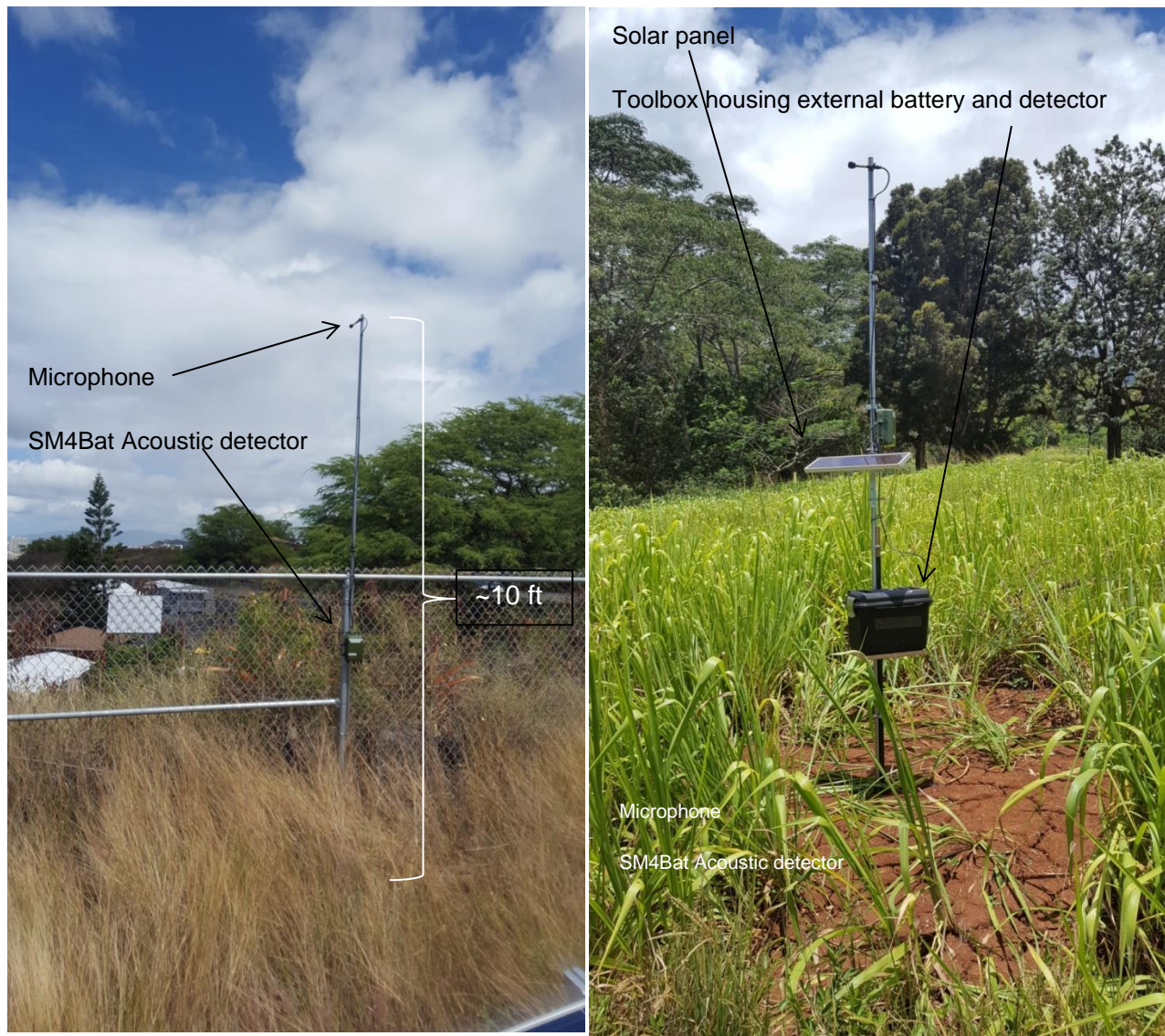
**Figure 2. Examples of commonly used set-ups of the Wildlife Acoustics SM4Bat detectors used in the Hawaiian hoary bat Occupancy and Distribution study on Oahu.**

Detectors remained in the field year round at the location of original placement. Detectors were programmed to operate nightly, from approximately one hour prior to sunset to approximately one hour after sunrise. Within the SM4 Detector Configurator the following settings were selected: detector sample rate of 192 kilohertz (kHz); gain of 12 decibels (dB); minimum signal duration of 1.5 milliseconds; maximum signal duration off; minimum trigger frequency of 10 kHz; trigger level of 12 dB; and trigger window of 3 seconds. Detectors were visited regularly to swap data cards and ensure detectors were functioning properly. Following initial set-up, detectors were checked once at 7-14 day intervals to ensure proper function and data collection. After these initial checks, sites were checked less frequently. At sites with external power sources, detectors were checked every 1-2 months, with sites that mandated helicopter access sometimes extending to more than two months between checks. At sites where the detectors were powered by internal batteries, units would be visited every 10-14 days on average.

To expedite call analysis, call recordings were processed with the Kaleidoscope Pro 5 software package (Wildlife Acoustics 2018) to convert the full-spectrum call files to zero-cross files and removed noise (i.e., non-bat) files. For all files classified as containing a bat echolocation call, the zero-cross call files were manually reviewed by a biologist using program Analook (Titley Scientific) to ensure that detections contained a minimum of two distinct pulses and confirm the recording was consistent with that of a HAHOBA. Manual review by a bat biologist of all recorded bat calls helped to ensure no false positives were included in the final dataset. Social calls and feeding buzzes were also recorded during the manual review process for later assessment of behavioral activity as sites. Initially, some call files were recorded as having multiple individuals in a single file; however, upon further review and discussion with other acoustic call experts, it was decided that these call files be reassigned to a single individual, which resulted in a slight reduction in total calls from that reported in the October 2018 version of the Year-1 report. A subset of noise files was also examined to ensure that detectors were functioning properly when several consecutive nights with no recordings occurred.

Occupancy modeling

Nightly detector data were used to model occupancy rates and detection probabilities of HAHOBA on Oahu (MacKenzie et al. 2006), with the appropriate model type depending on model assumptions. If we can assume that grid-cell occupancy of HAHOBA is static over the course of the year, then a single-season occupancy model is appropriate. If HAHOBA occupy different sites during the year, then a multi-season occupancy model that accounts for site-level extinction (the probability that an occupied site will be unoccupied the next season) and local colonization (the probability that an unoccupied site will be occupied the next season) is needed to meet the closure assumption within a season. We used the HAHOBA reproductive season definitions of Gorresen et al. (2013; as adapted from Menard 2001) as the basis for our seasonal models. Gorresen et al. (2013) classified the lactation season as mid-June to August, the post-lactation season as September to mid-December, the pre-pregnancy season as mid-December to March, and the pregnancy season as April to mid-June. Differences in detections among seasons could be due to a seasonal detection effect, differences in occupancy by season, or both. We examined two main models: (1) the single-season occupancy model with seasonal differences explained by seasonal differences in detection and (2) the multi-season occupancy model where site-level occupancy is expected to change for at least one reproductive season. Occupancy analysis was conducted with the *unmarked* package (Fiske and Chandler 2011) in R (2016). Single-season models are specified by the covariates used to model occupancy ($\Psi$) and detection ($p$), while multi-season models also include the extinction ($\varepsilon$) and local colonization ($\gamma$) parameters.

Occupancy modeling requires assumptions of equal probability of occupancy across sites, equal probability of detection across sites, population closure during the survey period, independence of detections across sites, and independence among detections at a site (MacKenzie et al. 2006). The first two assumptions can be relaxed if covariates related to the occupancy and detection processes are included in the models. If HAHOBA occupy different areas of the island during different reproductive seasons within each year, then the assumption of population closure among seasons may need to be accommodated with a multi-season model. We assume

independence among sites based on the probabilistic and spatially balanced sampling design. Independence among detections at a site may be violated if detections observed over time are temporally correlated. When temporal correlation is present, detection occasions may be separated in time to avoid temporal correlation (Wright et al. 2016). We evaluated the independence of nightly detection data with goodness of fit tests and partial autocorrelation plots of site-level data. The join count chi-square test (Wright et al. 2016) compares the number of temporal "joins" (number of detections in consecutive time periods) to an expected number based on the assumption of independence. We applied the join count test to data sets obtained from systematic random samples of detector nights taken by site at various intervals to determine the appropriate temporal spacing of detector nights to meet the assumption of independent sampling occasions for a given site. We also used the join count test to examine the assumption of a first-order Markov detection process (Hines et al, 2010, 2014) to account for correlated detections. For this model, the probability of detection is a function of local presence for the current and previous sampling occasion and detection given local presence.

## RESULTS

Data collection began in June of 2017 and has been ongoing since. Gaining access to sample sites progressed steadily, but more slowly than anticipated in the original proposal. Ultimately, while our initial goal was to deploy 100 detectors across the island, we were unable to do achieve the goal of 100 detectors in Year 1 using the probabilistic sampling design. Land access was the most common reason for not getting units deployed within sequentially selected cells, with lack of suitable sample sites also causing some cells to be skipped. Land access issues were most often associated with cells that were mostly owned and or managed by larger private landowners (e.g., agricultural entities and developers) that would not grant permission or from whom we could not a get response to our request for access. Lack of suitable sites within cells resulted from a lack of safely accessible sites or simply the lack of a suitable location to mount or locate a detector. As a result, we extended our sample effort to include 19 of the oversample cells; however these same issues also affected some of the oversample cells.

In total, 86 detectors have been placed in the field by WEST during the study period, with 84 of the 86 located in the randomly-selected grid cells and two located at sites not within randomly selected cells (Figure 3). One additional randomly-selected grid cell is located in the Kuhuku Wind Project, where Mitchell Craig of Terraform provided data from two detectors already being monitored; from which one was randomly selected to incorporate into the analysis. This brings the total number of detectors being monitored to 87. Of the two detectors not placed in randomly selected cells, one was initially used as a test site and is located in Waialua at the home of WEST's field biologist, while the other was placed at Hamakua Ponds at the request of Department of Forestry and Wildlife (DOFAW) staff. WEST has collected, processed, and incorporated data from 83 of the 85 detectors placed in randomly selected grid cells into the current analysis. Data from two detectors were not yet analyzed and available for inclusion, but will be incorporated into future analyses.

Detectors were placed in the field as access permissions were obtained, therefore the temporal distribution of data varied among detectors. As such, seasonal data sets differ in the number of detectors that contributed data. We now discuss the survey results, including tabular and graphical summaries of the detector data and an initial analysis of occupancy and detection rates.

Detector Data

A total of 4,808 HAHOBA detections were recorded across the 83 detectors from June, 2017 to June 2018. Site-level detections ranged from 0 to 1,512 (Figure 4) and the number of detector nights by site ranged from 18 to 356 (Table 1). At least one detection was recorded at 51 of the 83 (61%) sites. The mean number of site-level detections per detector night ranged from 0 to 4.34 (Table 1, Figure 5) across all seasons and 0 to 21.54 for within-season summaries (Figure 6). The proportion of nights with detections by site ranged from 0 to 0.33 (Table 1, Figure 7) across all seasons (for all sites with more than one season of data) and from 0 to 0.84 for within-season proportions (Figure 8). Feeding buzzes (297 detections) and social calls (31 detections) were identified from call files recorded at 22 detectors. The presence of feeding buzzes and social calls is reported here for informational purposes, but may be incorporated into future analyses in a more formal way.

**Figure 3: Distribution of deployed detectors.**

**Table 1. Total detections, total detector nights, mean detections per night, and proportion of nights with detections by site from June 2017 – June 2018.**

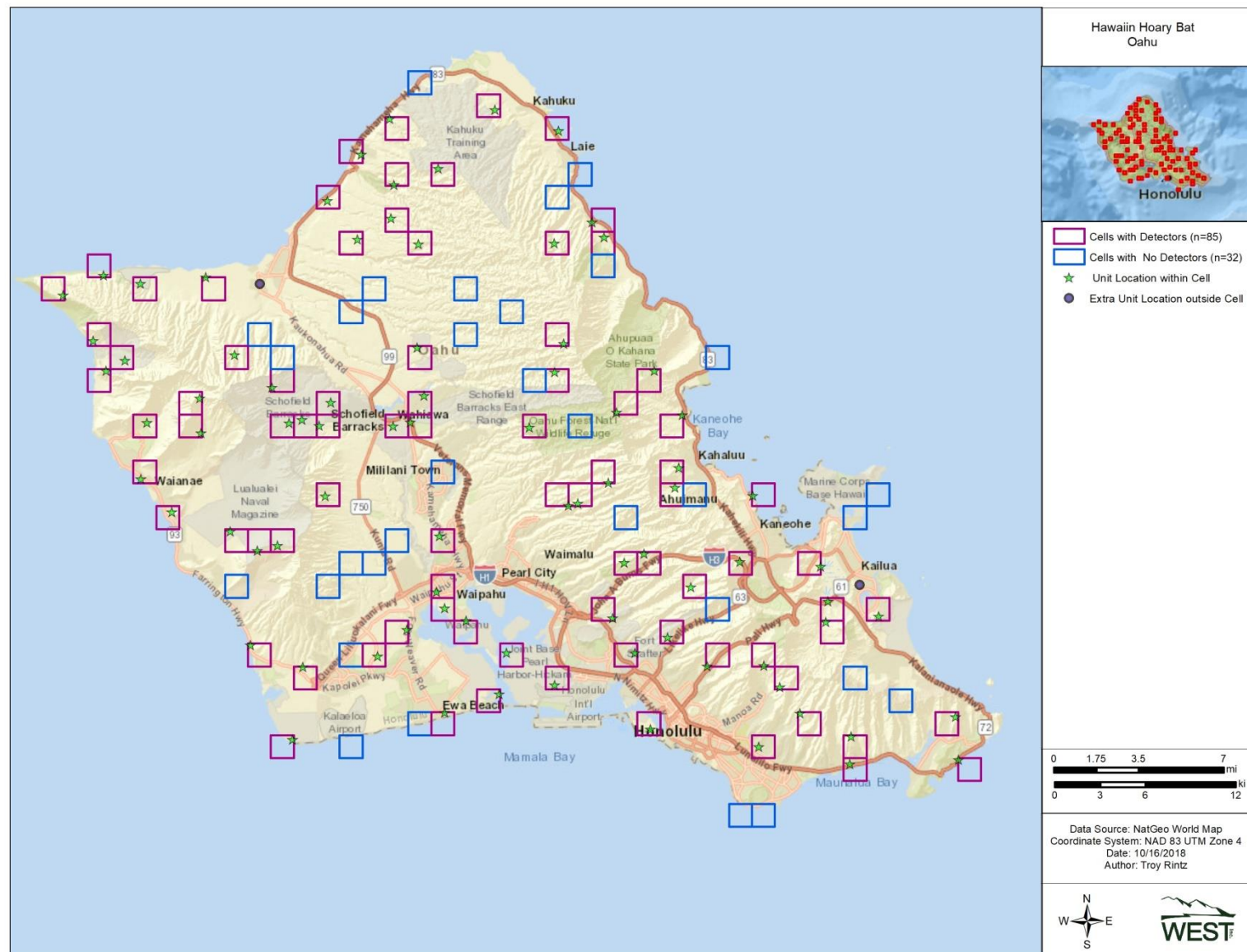| Site ID | Site Name | Detections | Nights with Detections | Detector Nights‡ | Mean Detections Per Detector Night | Proportion of Detector Nights with Detections |
|---|---|---|---|---|---|---|
| Site-000 | Goodale Tribe | 19 | 17 | 338 | 0.0562 | 0.0503 |
| Site-002 | TTHTT | 8 | 8 | 300 | 0.0267 | 0.0267 |
| Site-004 | Army Nat Res | 6 | 6 | 301 | 0.0199 | 0.0199 |
| Site-006 | Waihee Res | 0 | 0 | 130 | 0.0000 | 0.0000 |
| Site-008 | Ewa Beach Park | 0 | 0 | 80 | 0.0000 | 0.0000 |
| Site-009 | Waianae HS | 0 | 0 | 211 | 0.0000 | 0.0000 |
| Site-011 | Burn Camp | 10 | 9 | 253 | 0.0395 | 0.0356 |
| Site-013 | KAW Gate | 188 | 79 | 352 | 0.5341 | 0.2244 |
| Site-016 | Radar Hill Rd | 3 | 2 | 30 | 0.1000 | 0.0667 |
| Site-018 | Dillingham Air | 9 | 8 | 278 | 0.0324 | 0.0288 |
| Site-020 | Wahiawa botanical | 7 | 7 | 315 | 0.0222 | 0.0222 |
| Site-021 | Lualualei 1 | 24 | 16 | 220 | 0.1091 | 0.0727 |
| Site-022 | Kahana Wedding | 0 | 0 | 213 | 0.0000 | 0.0000 |
| Site-023 | Waimea Valley | 168 | 69 | 322 | 0.5217 | 0.2143 |
| Site-024 | Ft Shafter | 3 | 3 | 286 | 0.0105 | 0.0105 |
| Site-025 | Schofield | 82 | 56 | 332 | 0.2470 | 0.1687 |
| Site-026 | Kawainiui | 0 | 0 | 293 | 0.0000 | 0.0000 |
| Site-029 | KAW Rd | 28 | 25 | 253 | 0.1107 | 0.0988 |
| Site-030 | Sacred Falls | 0 | 0 | 213 | 0.0000 | 0.0000 |
| Site-031 | Plantation Village | 3 | 3 | 334 | 0.0090 | 0.0090 |
| Site-032 | Nuuanu Watershed | 0 | 0 | 155 | 0.0000 | 0.0000 |
| Site-033 | Camp Erdman | 19 | 16 | 311 | 0.0611 | 0.0514 |
| Site-034 | Barbers Point | 0 | 0 | 147 | 0.0000 | 0.0000 |
| Site-035 | Helemano | 21 | 20 | 332 | 0.0633 | 0.0602 |
| Site-036 | Kroc Center | 1 | 1 | 224 | 0.0045 | 0.0045 |
| Site-038 | Moanalua Trail | 1 | 1 | 245 | 0.0041 | 0.0041 |
| Site-039 | Pupukea | 1,512 | 102 | 348 | 4.3448 | 0.2931 |
| Site-040 | Hickham AFB | 0 | 0 | 144 | 0.0000 | 0.0000 |
| Site-041 | Schofield 3 | 105 | 29 | 332 | 0.3163 | 0.0873 |
| Site-043 | Manana Trail 1 | 0 | 0 | 351 | 0.0000 | 0.0000 |

**Table 1. Total detections, total detector nights, mean detections per night, and proportion of nights with detections by site from June 2017 – June 2018.**

| Site ID | Site Name | Detections | Nights with Detections | Detector Nights‡ | Mean Detections Per Detector Night | Proportion of Detector Nights with Detections |
|---------|-----------|-----------|------------------------|------------------|-----------------------------------|-----------------------------------------------|
| Site-044 | Royal Hawaiian Golf | 1 | 1 | 256 | 0.0039 | 0.0039 |
| Site-046 | Poamoho | 2 | 2 | 297 | 0.0067 | 0.0067 |
| Site-048 | Chaminade Univ. | 3 | 3 | 356 | 0.0084 | 0.0084 |
| Site-049 | Lualualei NAVY | 0 | 0 | 143 | 0.0000 | 0.0000 |
| Site-050 | HECO Kahe Point | 1 | 1 | 318 | 0.0031 | 0.0031 |
| Site-053 | Kumaipo LZ | 906 | 80 | 242 | 3.7438 | 0.3306 |
| Site-054 | Anchor Church | 0 | 0 | 154 | 0.0000 | 0.0000 |
| Site-055 | Schofield Waikane | 6 | 6 | 328 | 0.0183 | 0.0183 |
| Site-057 | McCarthy Field | 58 | 37 | 332 | 0.1747 | 0.1114 |
| Site-058 | Kailua Heights | 0 | 0 | 154 | 0.0000 | 0.0000 |
| Site-059 | Moanalua Red Hill | 0 | 0 | 155 | 0.0000 | 0.0000 |
| Site-060 | Hawaii Loa Booster | 0 | 0 | 155 | 0.0000 | 0.0000 |
| Site-061 | Mt Kaala | 150 | 91 | 307 | 0.4886 | 0.2964 |
| Site-064 | Kamehameha Res | 1 | 1 | 115 | 0.0087 | 0.0087 |
| Site-065 | Makua Valley | 3 | 2 | 185 | 0.0162 | 0.0108 |
| Site-066 | Wheeler | 12 | 11 | 279 | 0.0430 | 0.0394 |
| Site-067 | Honouliuli FR | 3 | 3 | 218 | 0.0138 | 0.0138 |
| Site-068 | Waikane Valley | 0 | 0 | 153 | 0.0000 | 0.0000 |
| Site-069 | MitchDetector | 2 | 1 | 312 | 0.0064 | 0.0032 |
| Site-071 | Makaha Res | 0 | 0 | 148 | 0.0000 | 0.0000 |
| Site-072 | Waihee Wells | 0 | 0 | 143 | 0.0000 | 0.0000 |
| Site-073 | Kipapa NF | 0 | 0 | 179 | 0.0000 | 0.0000 |
| Site-074 | Hawaii Loa | 0 | 0 | 155 | 0.0000 | 0.0000 |
| Site-075 | Peerson | 395 | 112 | 343 | 1.1516 | 0.3265 |
| Site-076 | Kaipapau FR | 5 | 1 | 296 | 0.0169 | 0.0034 |
| Site-077 | Manana Trail 2 | 1 | 1 | 351 | 0.0028 | 0.0028 |
| Site-078 | Sand Island | 0 | 0 | 288 | 0.0000 | 0.0000 |
| Site-079 | Makua Ridge | 58 | 34 | 286 | 0.2028 | 0.1189 |
| Site-081 | KAW 2 | 21 | 20 | 352 | 0.0597 | 0.0568 |
| Site-083 | Lualualei 2 | 56 | 24 | 220 | 0.2545 | 0.1091 |

**Table 1. Total detections, total detector nights, mean detections per night, and proportion of nights with detections by site from June 2017 – June 2018.**

| Site ID | Site Name | Detections | Nights with Detections | Detector Nights‡ | Mean Detections Per Detector Night | Proportion of Detector Nights with Detections |
|---------|-----------|------------|------------------------|------------------|------------------------------------|-----------------------------------------------|
| Site-084 | Aiea Loop Ridge | 0 | 0 | 214 | 0.0000 | 0.0000 |
| Site-085 | Kaw 1 | 6 | 5 | 352 | 0.0170 | 0.0142 |
| Site-087 | Schofield 1 | 35 | 28 | 332 | 0.1054 | 0.0843 |
| Site-088 | Kawainui Marsh 1 | 0 | 0 | 293 | 0.0000 | 0.0000 |
| Site-089 | Waiawa Snot | 0 | 0 | 330 | 0.0000 | 0.0000 |
| Site-090 | Kau Crater Trail | 0 | 0 | 250 | 0.0000 | 0.0000 |
| Site-093 | Pouhala Marsh | 4 | 3 | 286 | 0.0140 | 0.0105 |
| Site-094 | Manoa Falls | 2 | 2 | 349 | 0.0057 | 0.0057 |
| Site-095 | Kuaokala Game Area | 6 | 4 | 255 | 0.0235 | 0.0157 |
| Site-097 | Malaekahana SP | 151 | 8 | 248 | 0.6089 | 0.0323 |
| Site-098 | West Loch Golf | 1 | 1 | 217 | 0.0046 | 0.0046 |
| Site-100 | Heeia State Park | 4 | 3 | 300 | 0.0133 | 0.0100 |
| Site-101 | Pupukea Paumalu | 45 | 11 | 18 | 2.5000 | 0.6111[a] |
| Site-102 | Pearl Harbor | 0 | 0 | 144 | 0.0000 | 0.0000 |
| Site-103 | Schofield Forest | 418 | 84 | 328 | 1.2744 | 0.2561 |
| Site-105 | Aiea Loop Trail 1 | 12 | 11 | 295 | 0.0407 | 0.0373 |
| Site-106 | Puu Pia Trail | 1 | 1 | 251 | 0.0040 | 0.0040 |
| Site-109 | Central Oahu Park | 0 | 0 | 149 | 0.0000 | 0.0000 |
| Site-110 | Halone Blowhole | 0 | 0 | 150 | 0.0000 | 0.0000 |
| Site-111 | YMCA Waianae | 0 | 0 | 118 | 0.0000 | 0.0000 |
| Site-113 | Hauula Dist. Park | 0 | 0 | 143 | 0.0000 | 0.0000 |
| Site-114 | Waipio Soccer | 0 | 0 | 145 | 0.0000 | 0.0000 |
| Site-115 | Waianae Valley | 215 | 32 | 258 | 0.8333 | 0.1240 |
| Site-119 | Makua Cave | 0 | 0 | 185 | 0.0000 | 0.0000 |
| Site-999 | Hamakua Pond | 7 | 6 | 289 | 0.0242 | 0.0208 |

\* Denotes subjectively-selected grid cells

†Denotes grid cells where feeding buzzes or social calls were detected

‡ Denotes nights that the detector was functional

[a] data from a single season only

**Figure 4: Total detections by site between June 2017 and June 2018.**
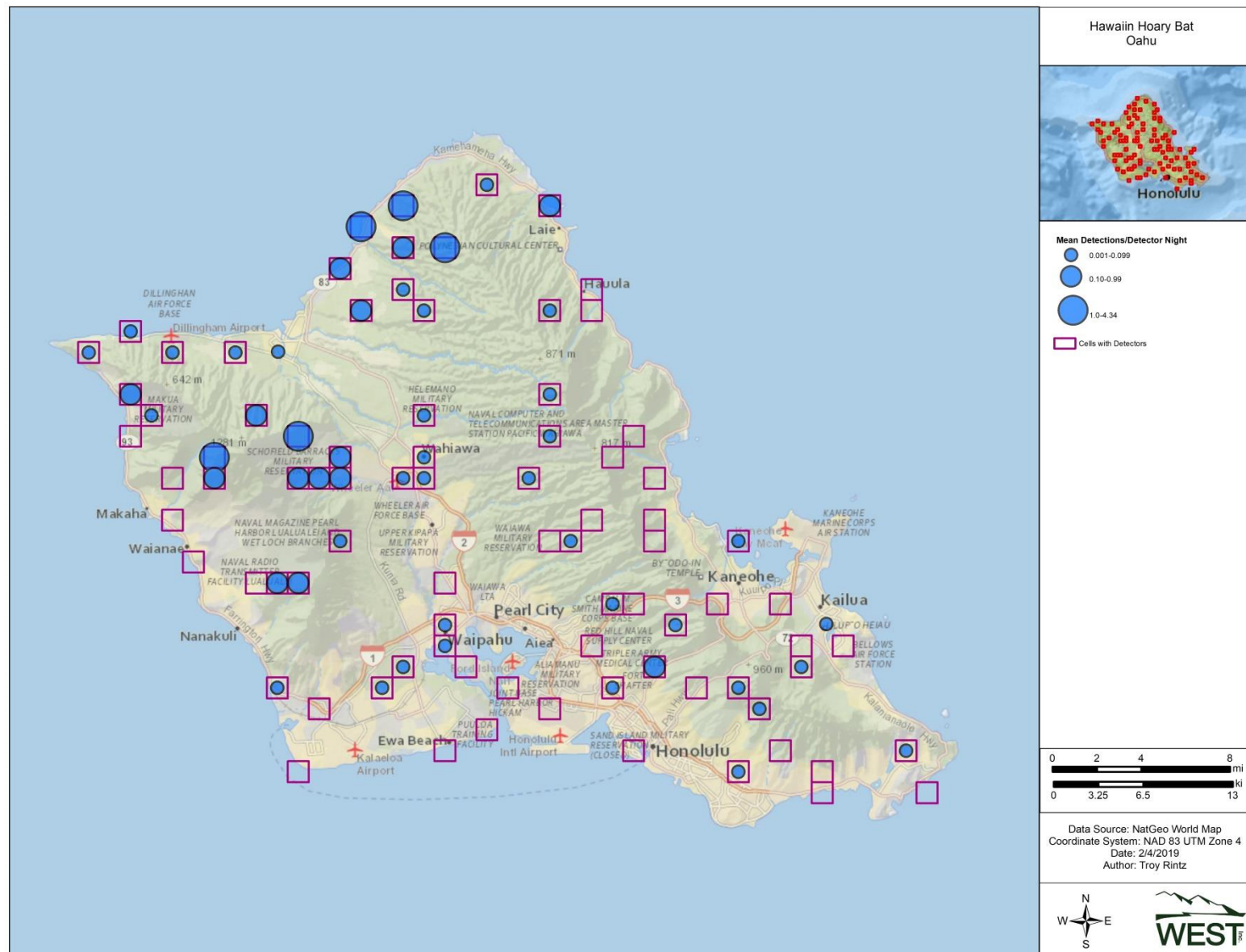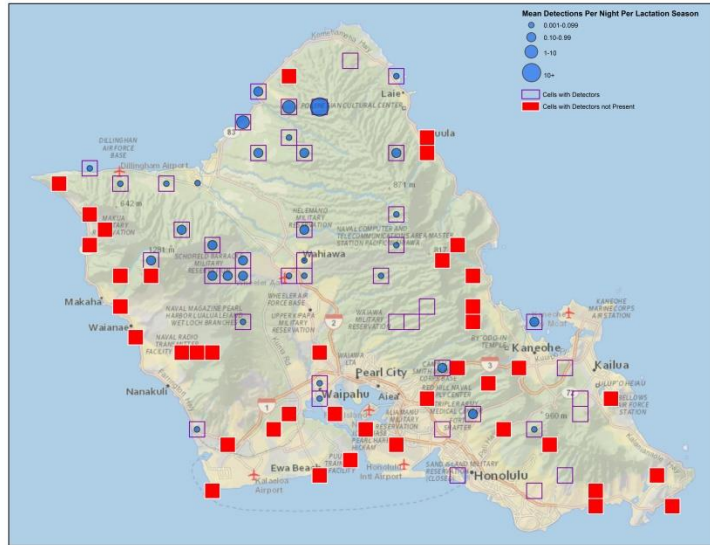
**Figure 5: Mean detections per night by site between June 2017 and June 2018.**

Lactation season (mid-June through August)

Post-Lactation season (September through mid-December)

Pre-Pregnancy season (mid-December through March)

Pregnancy season (April through mid-June)

**Figure 6: Mean detections per night by site and season. June 2017 – June 2018.**

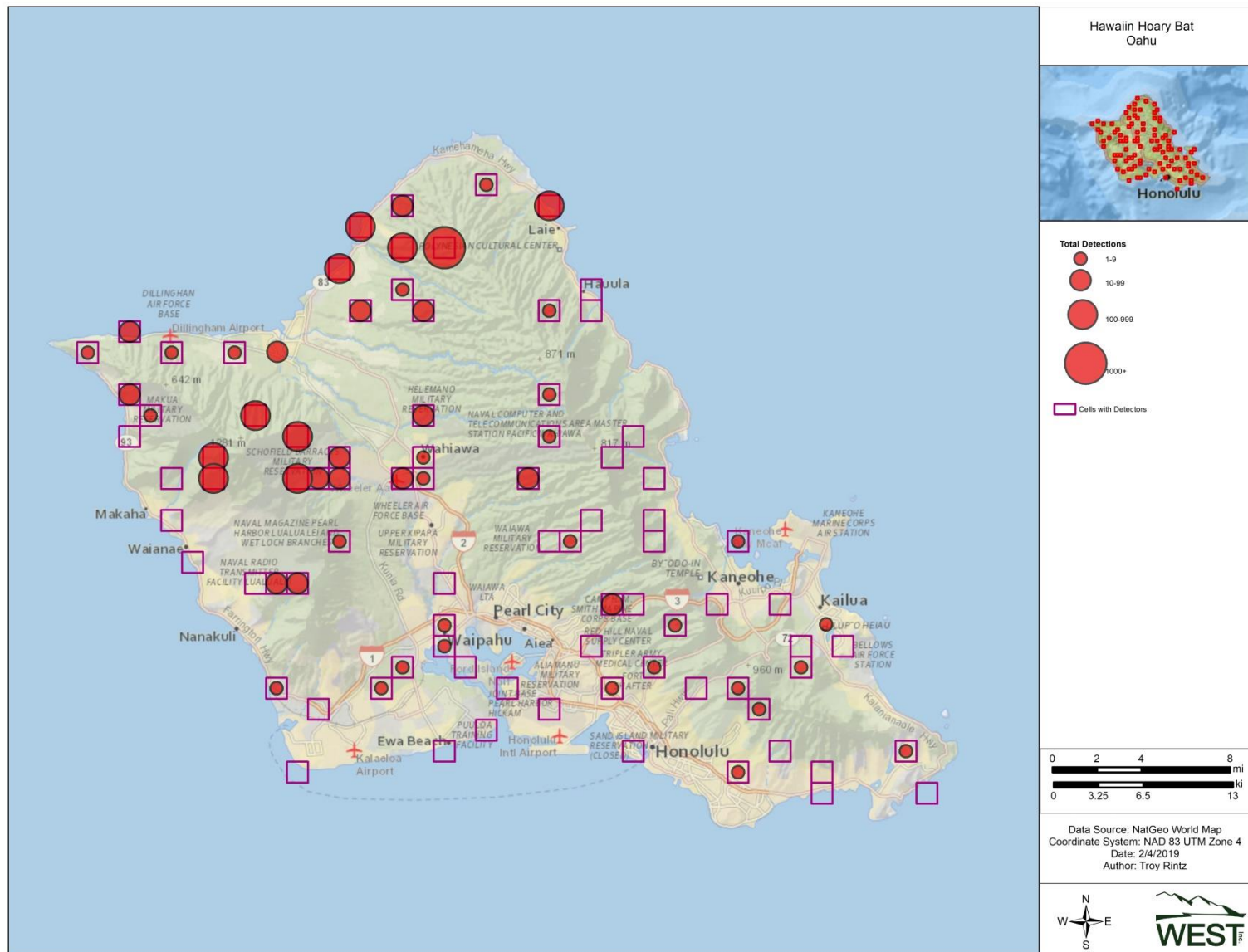**Figure 7: Proportion of nights with detections by site between June 2017 and June 2018.**

Lactation season (mid-June through August)

Post-Lactation season (September through mid-December)

Pre-Pregnancy season (mid-December through March)

Pregnancy season (April through mid-June)

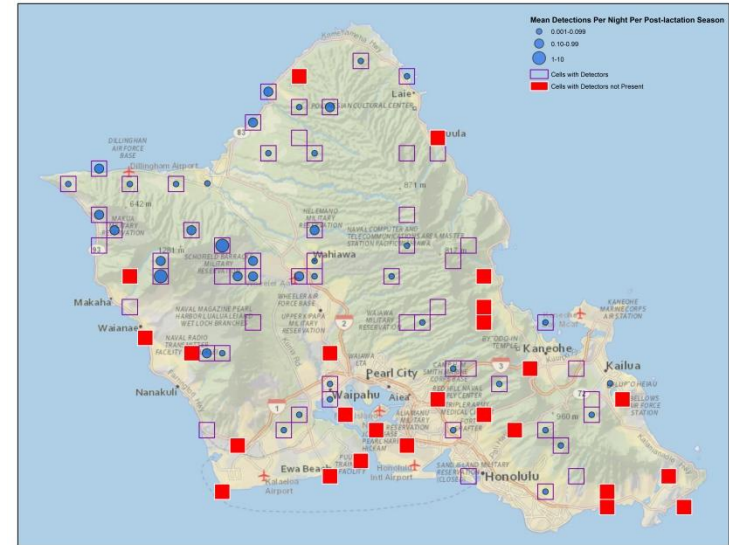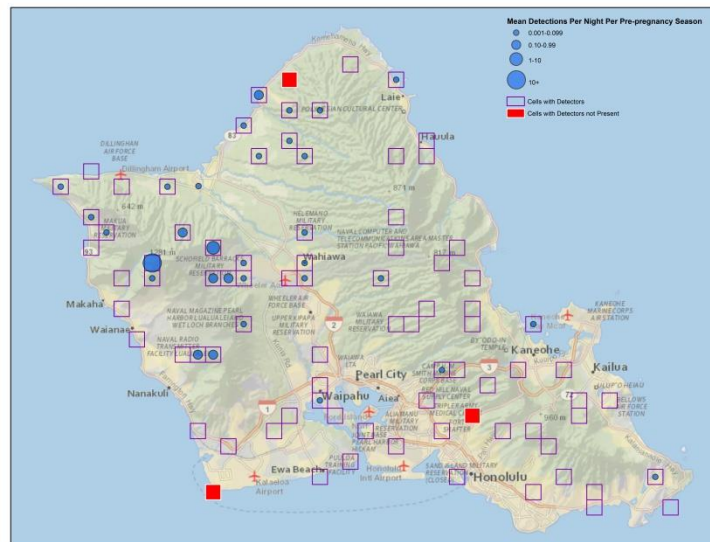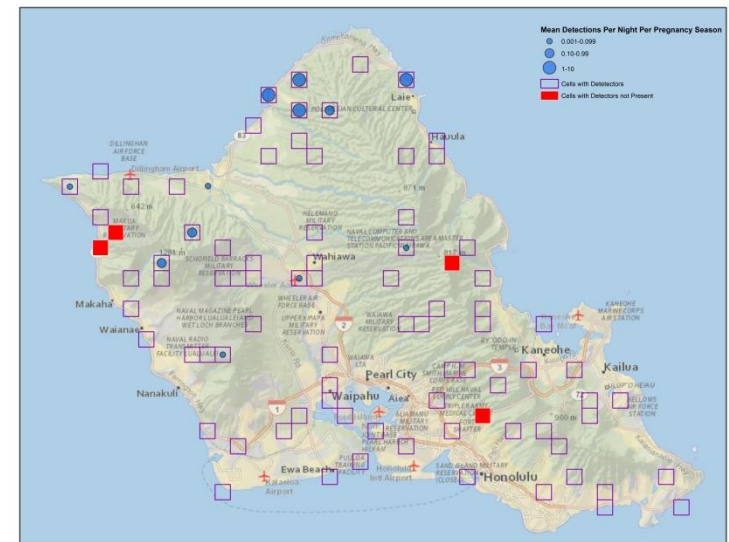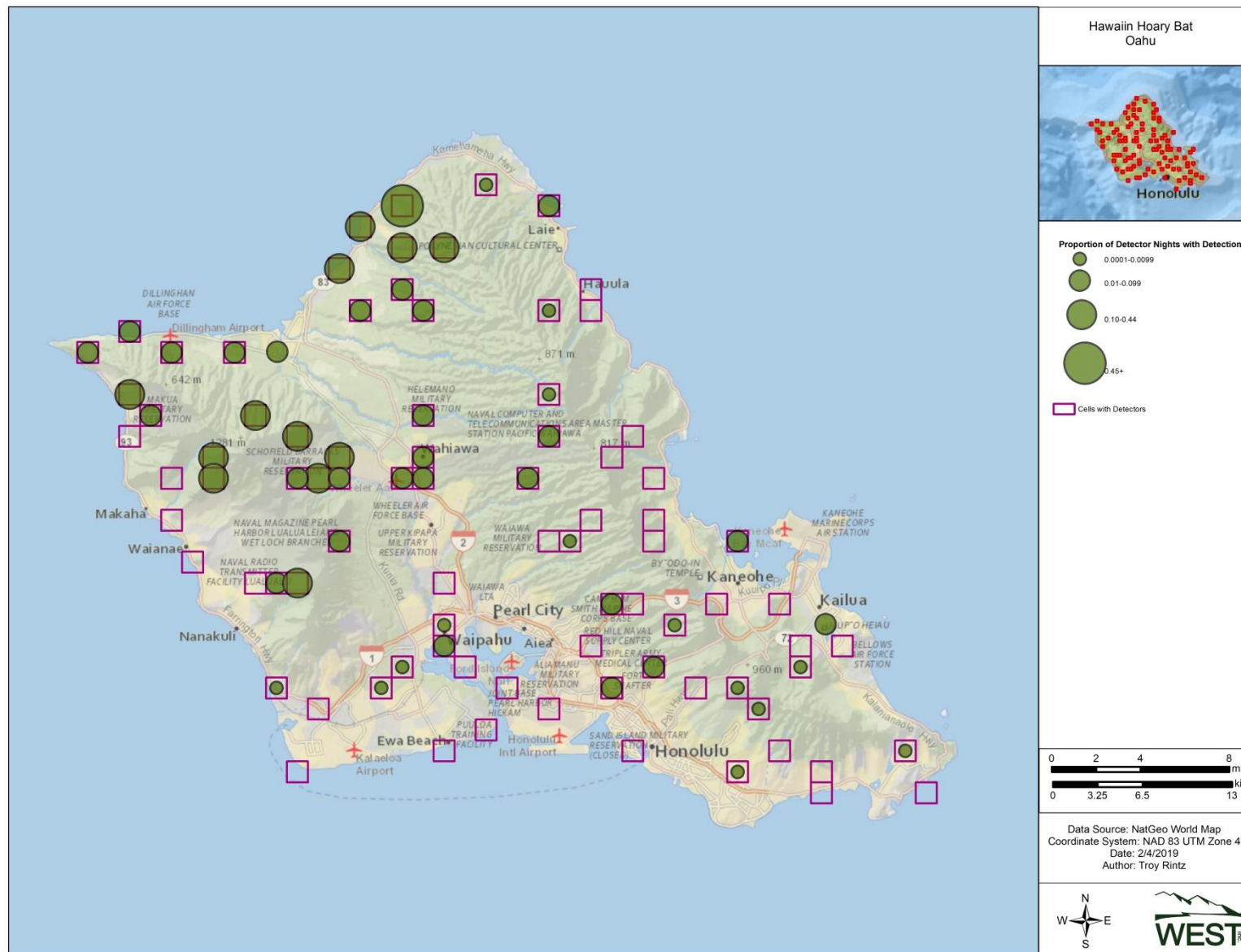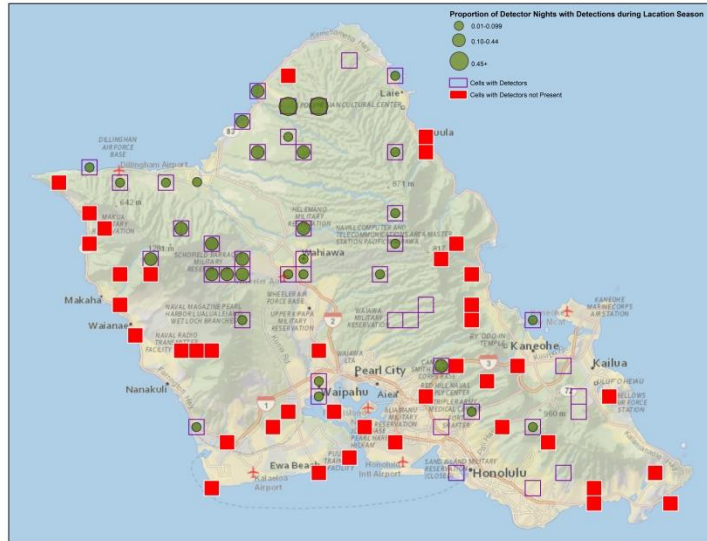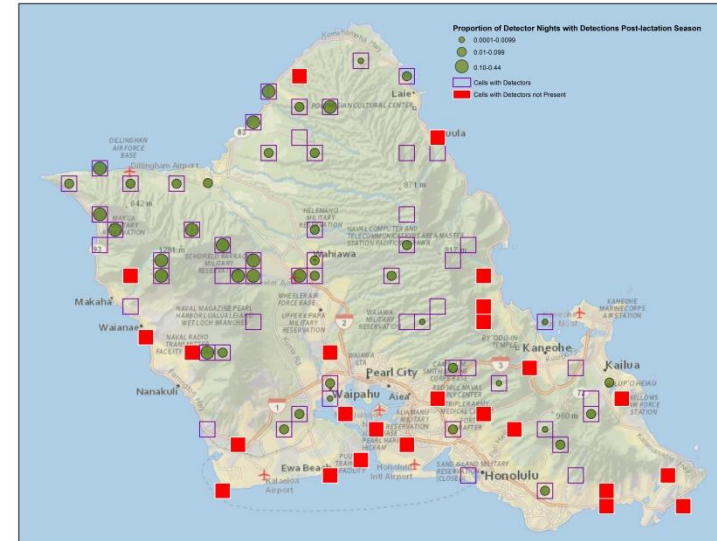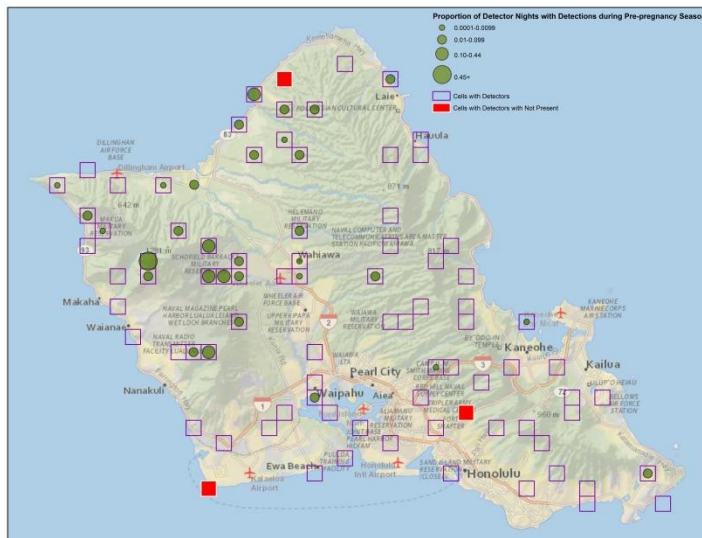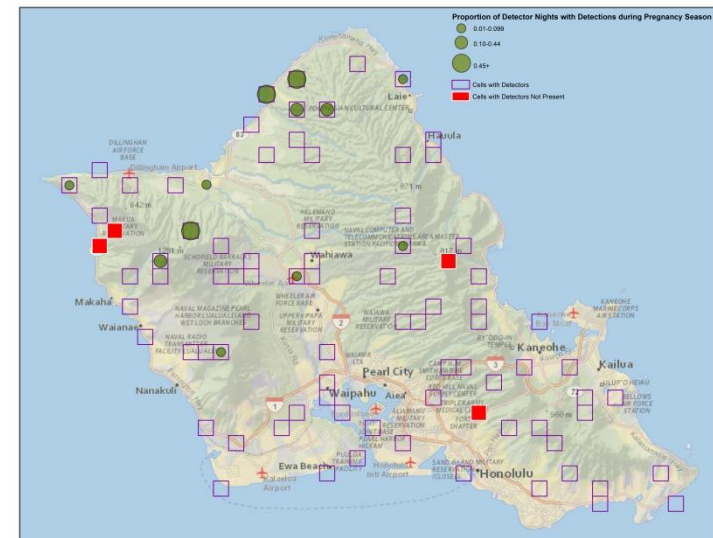**Figure 8: Proportion of detector nights with detections by site and season between June 2017 and June 2018.**

Occupancy modeling

The sample data available as of June 2018 were examined in an occupancy analysis to obtain initial estimates of occupancy and detection rates. The results presented herein are preliminary and are for informational purposes only. Formal reviews have not been completed and the results should not be cited outside of project planning discussions. We begin with an assessment of the assumptions for occupancy modeling, report the results of the occupancy modeling exercise, and determine if the current sample size of detectors is sufficient to meet the goal of estimating the HAHOBA occupancy rate on Oahu.

The independence of nightly detection data was evaluated by applying join count chi square tests to data sets obtained by systematically sampling the nightly detector data every 4th, every 7th, every 10th, and every 14th night (Table 2). We attempted to apply the test to the nightly data, but the dimension of the resulting data set was too large to process. Small p-values from the join count test indicate that the number of temporal joins exceeded the expectation under the independence assumption. To accommodate a multi-season or single-season occupancy model, we examined the independence assumption by season. The assumption of independence resulted in a poor fit for at least one season in all but the 14-day subsample data set. The partial autocorrelation plots for the sites with at least 60 total detections (Appendix A) indicate that significant temporal correlation persists in the data sets taken at intervals of 4, 7, and 10 days apart (as evidenced by vertical lines that exceed the blue broken 95%-confidence interval line in Appendix A) and for at least one site in the 14-day data set. Based on the join count test and exploratory autocorrelation plots, we assume that temporal correlation is not substantial when examining detections from every 14 days and use this data set for occupancy modeling.

**Table 2: Temporal correlation test results for join count $\chi^2$ tests.**

| Interval | Markov Model | Lactation Join count $\chi^2$ | p-value | Post-Lactation Join count $\chi^2$ | p-value | Pre-Pregnancy Join count $\chi^2$ | p-value | Pregnancy Join count $\chi^2$ | p-value |
|---|---|---|---|---|---|---|---|---|---|
| 4 days | No | 14856.64 | <0.0001 | -- | -- | -- | -- | 9.96 | **0.2340** |
| 7 days | No | 4.80 | **0.5260** | 4.81 | **0.3160** | 6.01 | **0.1760** | 13.93 | 0.0240 |
| 10 days | No | 11.04 | 0.0140 | 1.59 | **0.6240** | 1.72 | **0.6940** | 2.58 | **0.8260** |
| 14 days | No | 1.68 | **0.8420** | 1.11 | **0.6480** | 1.16 | **0.2180** | 0.95 | **0.8520** |
| 4 days | Yes | 450.75 | <0.0001 | -- | -- | -- | -- | 10.43 | **0.6360** |
| 7 days | Yes | 4.39 | **0.6720** | 4.60 | **0.7740** | 3.93 | **0.5020** | 7.70 | **0.1020** |

We also assessed the assumption of a first-order Markov model of correlated detections (Hines et al. 2010, 2014) by season. We found that when the Markov model was applied to account for correlated detections during the Lactation season (the period of highest apparent occupancy) the correlation among detections was explained by the first-order Markov model when a 7-day was used, but not when a 4-day interval was used (Table 2). Therefore, we examined dynamic

occupancy models assuming independence for a subsample taken every 14 detector nights and assuming a first-order Markov detection process for a subsample taken every 7 detector nights.

The multi-season occupancy model with detection probabilities modeled by Season generated the lowest AIC value of the models we explored and was selected as the best fitting occupancy model (Appendix B) for the revised 14-day data set. The multi-season intercept only model had been reported as having the lowest AIC in the October 2018 report. Derived estimates by season (Weir et al. 2009) and bootstrapped 95% confidence intervals of occupancy and detection probability by season are provided in Table 3. The derived initial occupancy rate was estimated as 0.47 (SE = 0.12; Table 3). The detection rate (i.e. the probability of detecting a HAHOBA on a given night at a given detector conditional on occupancy) varied from 0.13 to 0.43 depending on season (Table 3), and the local extinction and colonization rates were estimated as 0.53 (SE = 0.10) and 0.03 (SE = 0.02), respectively (Table 4).

**Table 3: Derived occupancy estimates by Season and bootstrapped 95%-confidence intervals**

| Season | Est. Occupancy Rate | SE | 95%-CI | Est. Detection Probability | SE | 95%-CI |
|---|---|---|---|---|---|---|
| Lactation | 0.47* | 0.12 | (0.23, 0.70) | 0.34 | 0.08 | (0.20, 0.51) |
| Post-Lactation | 0.25 | 0.06 | (0.14, 0.37) | 0.19 | 0.04 | (0.12, 0.28) |
| Pre-Pregnancy | 0.11 | 0.04 | (0.03, 0.18) | 0.13 | 0.05 | (0.06, 0.25) |
| Pregnancy | 0.08 | 0.03 | (0.02, 0.13) | 0.43 | 0.13 | (0.21, 0.68) |

\* Note that the derived estimate of initial occupancy during the Lactation season differs slightly from that reported in Table 4 because the derived estimates are calculated conditionally on the data. See Weir et al. (2009) for more information.

The results of the occupancy analysis model selection exercise (Table 4) are organized by the model covariates used to model occupancy ($\Psi$) and detection (p) for both single- and multi-season occupancy models and the extinction ($\varepsilon$) and local colonization ($\gamma$) parameters for multi-season occupancy models. Models that include only an intercept term are denoted as "(.)". Detection models included only an intercept term, a linear Month effect, an intercept for each Month, and an intercept for each Season. Single-season occupancy models included only an intercept term for the occupancy rate. Seasonal effects were considered for multi-season occupancy models in the extinction and colonization parameters, but colonization models containing Season effects did not converge. Model selection was based on AIC.

Note that the data set used for this modeling exercise is based on a subsample of detector nights taken at a 14-day interval within each site. Therefore, a larger population of subsamples exists that could form the basis for occupancy modeling of independent detection probabilities. To explore the distribution of occupancy estimates, we drew 1,000 14-day-interval subsamples and calculated the initial occupancy estimate for the Lactation period and provide that distribution (Figure 9) to illustrate the range of estimates that could be derived from the year-1 dataset. The range of the occupancy estimates is 0.25 - 0.71, which is nearly identical to the bootstrap confidence interval of 0.23 - 0.70 for the Lactation Period.

**Table 4: Occupancy analysis results for two occupancy models for detector data collected every 14 days.**

| Occupancy model | Model covariates | Initial occupancy rate estimate (SE) | Detection probability (p) estimate (SE) | | Extinction rate (SE) | Colonization rate (SE) | AIC |
|---|---|---|---|---|---|---|---|
| Single-season | Ψ(.)p(.) | 0.30 (0.06) | 0.14 (0.02) | | - | - | 481.47 |
| Single-season | Ψ(.)p(Month) | 0.32 (0.06) | **Month**<br>1<br>2<br>3<br>4<br>5<br>6<br>7<br>8<br>9<br>10<br>11<br>12 | **p**<br>0.07 (0.02)<br>0.08 (0.02)<br>0.09 (0.02)<br>0.10 (0.02)<br>0.11 (0.02)<br>0.12 (0.02)<br>0.13 (0.02)<br>0.15 (0.02)<br>0.16 (0.02)<br>0.18 (0.02)<br>0.20 (0.03)<br>0.21 (0.04) | - | - | 473.98 |
| Single-season | Ψ(.) p(MonthFactor) | 0.35 (0.07) | **Month**<br>1<br>2<br>3<br>4<br>5<br>6<br>7<br>8<br>9<br>10<br>11<br>12 | **p**<br>0.05 (0.03)<br>0.18 (0.06)<br>0.13 (0.05)<br>0.10 (0.04)<br>0.02 (0.02)<br>0.06 (0.03)<br>0.08 (0.04)<br>0.12 (0.04)<br>0.04 (0.04)<br>0.34 (0.09)<br>0.27 (0.07)<br>0.24 (0.07) | - | - | 468.86 |
| Single-season | Ψ(.) p(Season) | 0.38 (0.07) | **Season**<br>Lact.<br>Post-Lact.<br>Pre-Preg.<br>Preg. | **p**<br>0.29 (0.05)<br>0.15 (0.03)<br>0.06 (0.02)<br>0.09 (0.03) | - | - | 464.33 |

**Table 4: Occupancy analysis results for two occupancy models for detector data collected every 14 days.**

| Occupancy model | Model covariates | Initial occupancy rate estimate (SE) | Detection probability (p) estimate (SE) | Extinction rate (SE) | Colonization rate (SE) | AIC |
|---|---|---|---|---|---|---|
| Multi-season | $\Psi(.)\varepsilon(.)\gamma(.)p(.)$ | 0.47 (0.11) | 0.23 (0.04) | 0.57 (0.10) | 0.03 (0.02) | 400.51 |
| Multi-season | $\Psi(.)\varepsilon(.)\gamma(.)p(Season)$ | 0.43 (0.11) | Season p SE<br>Lact. 0.34 0.08<br>Post-Lact. 0.19 0.04<br>Pre-Preg. 0.13 0.05<br>Preg. 0.43 0.13 | 0.53 (0.10) | 0.03 (0.02) | **395.13** |
| Multi-season | $\Psi(.)\varepsilon(Season)\gamma(Season)p(.)$ | 0.43 (0.10) | 0.24 (0.04) | Season ε SE<br>Lact. 0.26 0.17<br>Post-Lact. 0.84 0.09<br>Pre-Preg. 0.69 0.28 | Season γ SE<br>Lact. 0.00 0.02<br>Post-Lact. 0.04 0.03<br>Pre-Preg. 0.05 0.04 | 402.15 |
| Multi-season | $\Psi(.)\varepsilon(Season)\gamma(.)p(.)$ | 0.41 (0.10) | 0.24 (0.03) | Season ε SE<br>Lact. 0.28 0.18<br>Post-Lact. 0.84 0.10<br>Pre-Preg. 0.68 0.28 | 0.04 (0.02) | 398.51 |
| Multi-season | $\Psi(.)\varepsilon(.)\gamma(.)$ p(Month) | 0.47 (0.11) | Month p SE<br>1 0.22 (0.08)<br>2 0.22 (0.07)<br>3 0.22 (0.06)<br>4 0.22 (0.06)<br>5 0.22 (0.05)<br>6 0.23 (0.04)<br>7 0.23 (0.04)<br>8 0.23 (0.04)<br>9 0.23 (0.04)<br>10 0.24 (0.04)<br>11 0.24 (0.04)<br>12 0.24 (0.05) | 0.56 (0.10) | 0.03 (0.02) | 402.47 |

**Table 4: Occupancy analysis results for two occupancy models for detector data collected every 14 days.**

| Occupancy model | Model covariates | Initial occupancy rate estimate (SE) | Detection probability (p) estimate (SE) | | | Extinction rate (SE) | Colonization rate (SE) | AIC |
|---|---|---|---|---|---|---|---|---|
| Multi-season | $\Psi(.)\varepsilon(.)\gamma(.)$ p(Month Factor) | 0.56 (0.11) | **Month** 1 2 3 4 5 6 7 8 9 10 11 12 | **p** 0.12 0.24 0.16 0.14 0.05 0.10 0.30 0.47 0.14 0.42 0.37 0.26 | **SE** (0.07) (0.08) (0.06) (0.06) (0.05) (0.07) (0.15) (0.20) (0.13) (0.12) (0.11) (0.12) | 0.50 (0.10) | 0.03 (0.02) | 404.48 |

**Figure 9: Distribution of occupancy estimates from 1,000 systematic random 14-day subsamples by site.**

Using the join count test, we determined that a first-order Markov detection model adequately explained the temporal correlation in the 7-day interval data set (Table 2). This data set was examined with the multi-season dynamic occupancy model for correlated detections with program PRESENCE (Hines 2006). The intercept-only dynamic occupancy model for correlated detections (AIC = 714.11) was preferred over the intercept-only dynamic occupancy model for independent detections (AIC = 716.17) and the dynamic occupancy model for correlated detections calculated by Season (AIC = 716.37). The occupancy estimates from the top model (Table 5, Appendix B) were similar to those from the model assuming independent detections for the 14-day interval data (Table 3), with the occupancy estimates for the Lactation and Pre-Pregnancy seasons slightly higher for the correlated detection model than during the other seasons.

**Table 5: Occupancy estimates by season from the dynamic occupancy model for correlated detections**

| Season | Est. Occupancy Rate | SE | 95%-CI | Est. Detection Probability | SE | 95%-CI |
|---|---|---|---|---|---|---|
| Lactation | 0.54 | 0.10 | (0.34, 0.72) | | | |
| Post-Lactation | 0.26 | 0.06 | (0.15, 0.37) | 0.45 | 0.09 | (0.28, 0.63) |
| Pre-Pregnancy | 0.14 | 0.04 | (0.06, 0.21) | | | |
| Pregnancy | 0.08 | 0.03 | (0.02, 0.14) | | | |

Sample Size Assessment

We conducted a sample size approximation to determine the number of sites (i.e., sample size) needed to achieve relative precision levels of 0.1, 0.2, and 0.3 for Type I error rates of 0.05 and 0.10. For example, given an occupancy estimate of 0.50, a confidence interval of ±10%, or 0.40 - 0.60, that includes the true occupancy parameter in 95% of confidence intervals obtained from repeated surveys would attain 20% relative precision for an alpha level of 0.05. The sample size approximation uses the estimates of Lactation season occupancy and detection from final models for independent detections and for correlated detections (Markov model) and the approximate estimate of the variance of the maximum likelihood estimate of occupancy (MacKenzie et al. 2006, p. 96). For the estimated occupancy and detection rates from the Lactation season, assuming the first-order Markov detection model for the 7-day interval data, the current sample size of 85 installed detectors appears suitable to obtain occupancy estimates with precision adequate to obtain 90%- and 95%-confidence intervals with half-widths within 20% of the occupancy estimate (Table 6). Based on the 14-day interval data, our sample of 85 detectors should achieve 90% confidence interval coverage with 20% precision (Table 6).

**Table 6: Sample size approximation for three levels of precision based on estimates of detection and occupancy rates for the Lactation season, three levels of desired relative precision, and 7- or 14- day sample intervals.**

| Interval | Est. Occupancy rate ($\Psi$) | Est. Detection rate ($p$) | Relative Precision | Sample size ($\alpha = 0.05$) | Sample size ($\alpha = 0.10$) |
|---|---|---|---|---|---|
| 14-day | 0.47 | 0.34 | 0.1 | 433 | 305 |
| 14-day | 0.47 | 0.34 | 0.2 | 108 | 76 |
| 14-day | 0.47 | 0.34 | 0.3 | 48 | 34 |
| 7-day | 0.54 | 0.45 | 0.1 | 327 | 231 |
| 7-day | 0.54 | 0.45 | 0.2 | 82 | 58 |
| 7-day | 0.54 | 0.45 | 0.3 | 36 | 26 |

## DISCUSSION

Temporal correlation among detections violates assumptions of independent detections required for standard occupancy modeling. One approach to accommodate this requirement is to reduce the detector nights to a subset of data for which independent detections may be assumed (Wright et al. 2016). Removing detection occasions reduces the sample size of observations which may make modeling with covariates more difficult. However, the standard errors of occupancy and detection rates obtained from the reduced data set will result in more reliable confidence interval coverage. Furthermore, data reduction may eliminate incidental detections from HAHOBA that may simply be transiting through a grid cell and not truly occupying the cell for biological reasons, therefore helping to better address occupancy for grid cells with consistent use. Subsampling to examine the behavior of occupancy estimates for data subsets may provide a reasonable range of possible occupancy values for consideration. Detection models that account for a first-order Markov process (Hines et al. 2014) provide a promising approach for retaining more of the detector data for occupancy analysis.

MacKenzie's (2006) recommendation on survey designs for assessing habitat use when detection probabilities are less than one is to select a single sample and revisit the same sites over time. This helps ensure that the detection probability is accurately estimated, and that habitat and detection variables are not confounded. Because the detection probability in our study is estimated to be well below one, we recommend leaving the detectors at their current locations in order to provide the most appropriate data set for incorporating habitat variables into the occupancy analysis.

The final occupancy model for our data set to date assumed closure within each reproductive season and modeled the detection, local colonization, and extinction rates as equal across all seasons. For the 14-day data set, the colonization parameter was estimated as 0.03 (SE = 0.02) and the extinction parameter was estimated to be 0.53 (SE = 0.10). The colonization rate near zero and the high extinction rate may indicate that HAHOBA distribution is more widespread during the lactation season, when our surveys began, and then becomes more restricted during the subsequent reproductive seasons. The distribution of HAHOBA across the island appears to be most widespread during the lactation and post-lactation seasons with detections reduced to only 12 grid cells in the pregnancy season (Figures 4 through 8). Additional data will provide more information to assess how occupancy might change by season. In the current analysis presented herein, lower AIC values were obtained from models of occupancy that assumed open populations between seasons rather than single-season models that assumed population closure among seasons. While our first year of acoustic data suggests that HAHOBA are more widespread during the lactation and post-lactation periods, it is also possible that changes in detection probability due to behavioral changes in HAHOBA activity or call rates may be responsible for the changes in occupancy across seasons. Future analyses will consider the possibility that detection probabilities, rather than distribution, are varying by season by including detection heterogeneity by season in the models.

An assessment of sample size indicates that the current sample size is sufficient to provide 90%- and possibly 95%-confidence intervals that are within 20% of the occupancy estimate. We

are currently collecting other potential variables (e.g., elevation and vegetation type) to examine in the occupancy models to describe habitat selection. A general rule of thumb for occupancy analysis is to survey 10 sites per model parameter (Donovan and Hines 2007). There are four parameters in the intercept-only dynamic occupancy model; therefore, for a sample of 85 grid cells, we can expect to include eight or nine covariate levels. The current sample size should accommodate four or five additional parameters. Convergence should improve for the current sample size as site-level replication over time increases. If the current sample size is adequate for the primary project goal of assessing distribution, then habitat modeling may be limited to initial univariate models and broad habitat inference.

Analysis is ongoing, and current tasks include incorporating habitat variables such as elevation, human population density, and percent forest as predictors in the occupancy analysis to assess HAHOBA habitat selection. Examining occupancy for subsets of data such as urban vs. non-urban areas or other types of subpopulations may also be of interest. If model convergence becomes an issue, then Bayesian occupancy analysis methods may be explored.

# REFERENCES

Donovan, T. M. and J. Hines. 2007. Exercises in occupancy modeling and estimation. Available online: http://www.uvm.edu/rsenr/vtcfwru/spreadsheets/?Page=occupancy/occupancy.htm

Hines, J. E., J. D. Nichols, J. A. Royle, D. I. MacKenzie, A. M. Gopalaswamy, N. Samba Kumar, et al. 2010. Tigers on trails: occupancy modeling for cluster sampling. Ecol. Appl. 20:1456–1466.

Hines, J.E., Nichols, J.D. and Collazo, J.A., 2014. Multiseason occupancy models for correlated replicate surveys. *Methods in Ecology and Evolution*, *5*(6), pp.583-591.

Fiske, I. and R. Chandler. 2011. unmarked: An R Package for Fitting Hierarchical Models of Wildlife Occurrence and Abundance. Journal of Statistical Software, 43(10):1-23. URL http://www.jstatsoft.org/v43/i10/.

Gorressen, P.M., Bonaccorso, F., Pinzari, C., Todd, C., Montoya-Aiona, K. and Brinck, K. 2013. A five-year study of Hawaiian hoary bat (*Lasiurus cinereus semotus*) occupancy on the Island of Hawaii. Hawai'i Cooperative Studies Unit. Technical Report HCSU-041.

Hines, J. E. (2006). PRESENCE- Software to estimate patch occupancy and related parameters. USGS-PWRC. http://www.mbr-pwrc.usgs.gov/software/presence.html.

Menard, T. 2001. Activity patterns of the Hawaiian hoary bat (*Lasiurus cinereus semotus*) in relation to reproductive time periods. M.S. thesis. University of Hawai`i, Honolulu, HI.

MacKenzie, D.I. 2006. Modeling the probability of resource use: the effect of, and dealing with, detecting a species imperfectly. The Journal of Wildlife Management 70(2):367-374.

MacKenzie, D. I., J. D. Nichols, J. A. Royle, K. H. Pollock, L. L. Bailey, and J. E. Hines. 2006. Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence. Academic Press, New York, NY. 344 pp.

R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Stevens, D. L. & Olsen, A. R. 2003. Variance estimation for spatially balanced samples of environmental resources. Environmetrics 14: 594-610.

Stevens, D. L. & Olsen, A. R. 2004. Spatially balanced sampling of natural resources. *Journal of the American Statistical Association* 99(465):262-278.

Weir, L., Fiske, I.J. and Royle, J.A., 2009. Trends in anuran occupancy from northeastern states of the North American Amphibian Monitoring Program. Herpetological Conservation and Biology, 4(3), pp.389-402.

Wildlife Acoustics. 2018. Kaleidoscope Pro 5 Analysis Software.

Wright, W.J., Irvine, K.M. and Rodhouse, T.J., 2016. A goodness-of-fit test for occupancy models with correlated within-season revisits. Ecology and Evolution 6(15):5404-5415.

**APPENDIX A: Partial autocorrelation plots for sites with at least 60 total detections**

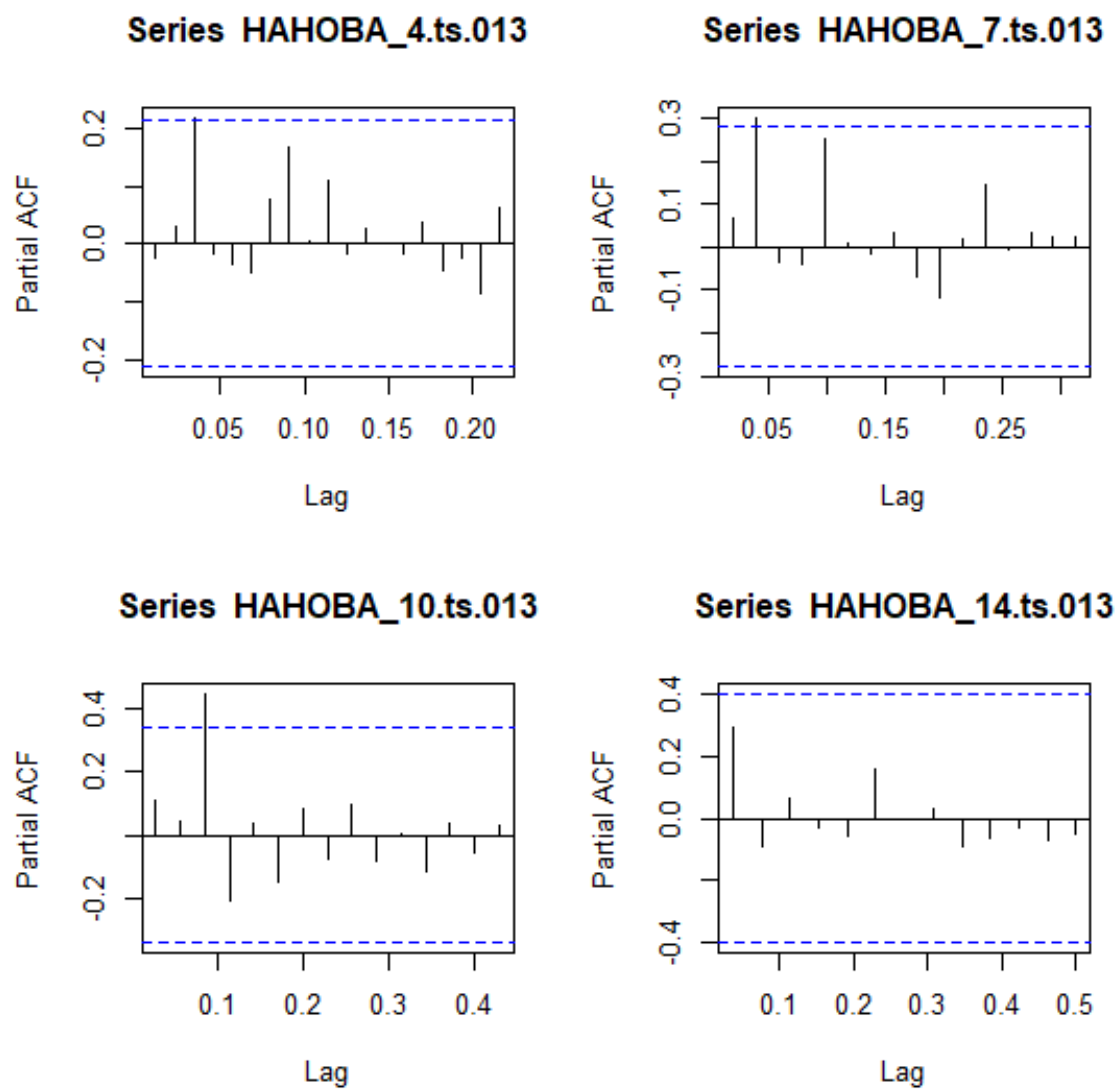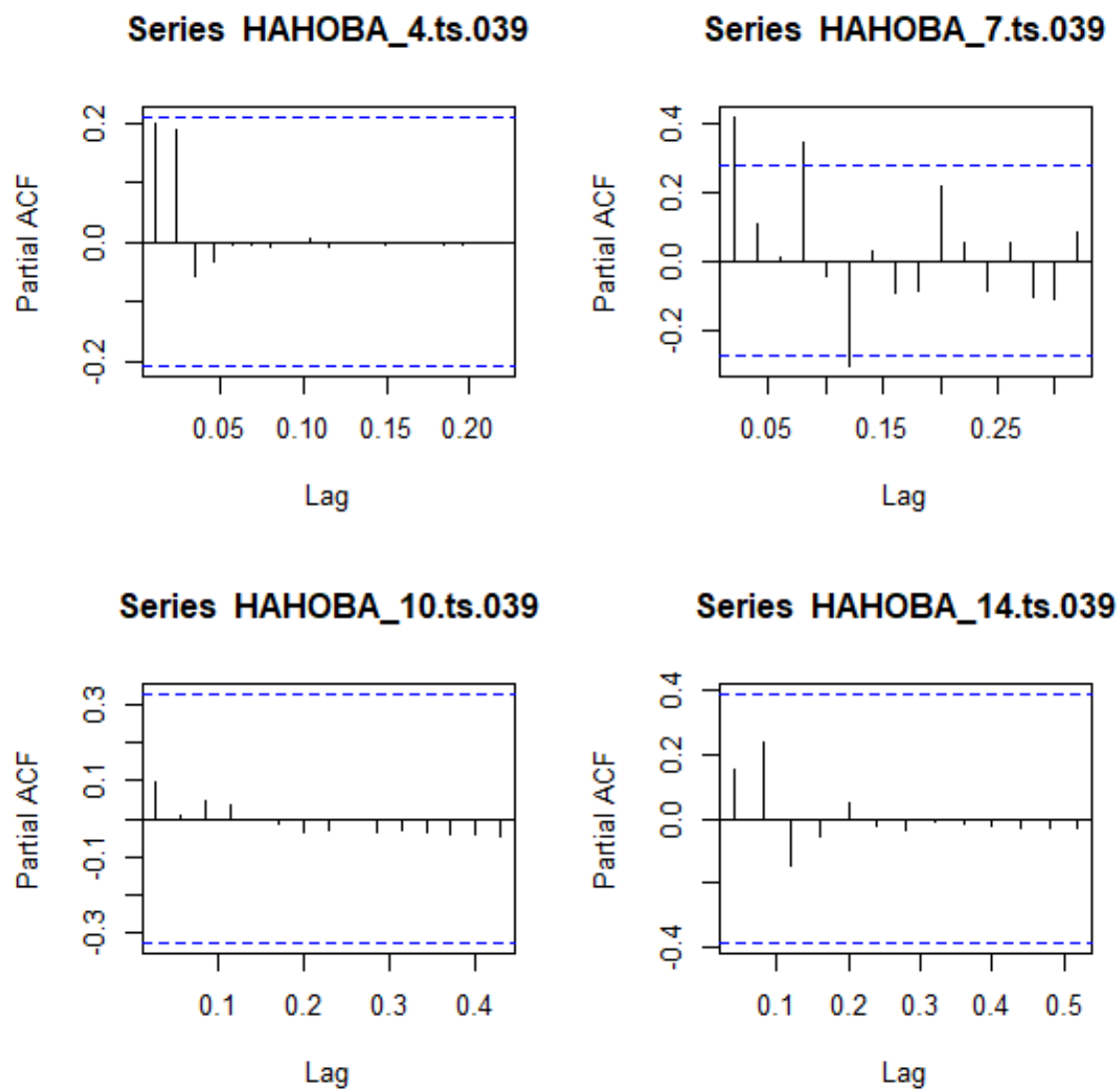Figure A1: Partial autocorrelation plots for site 13 for 4 time-intervals.

Figure A2: Partial autocorrelation plots for site 39 for 4 time-intervals.
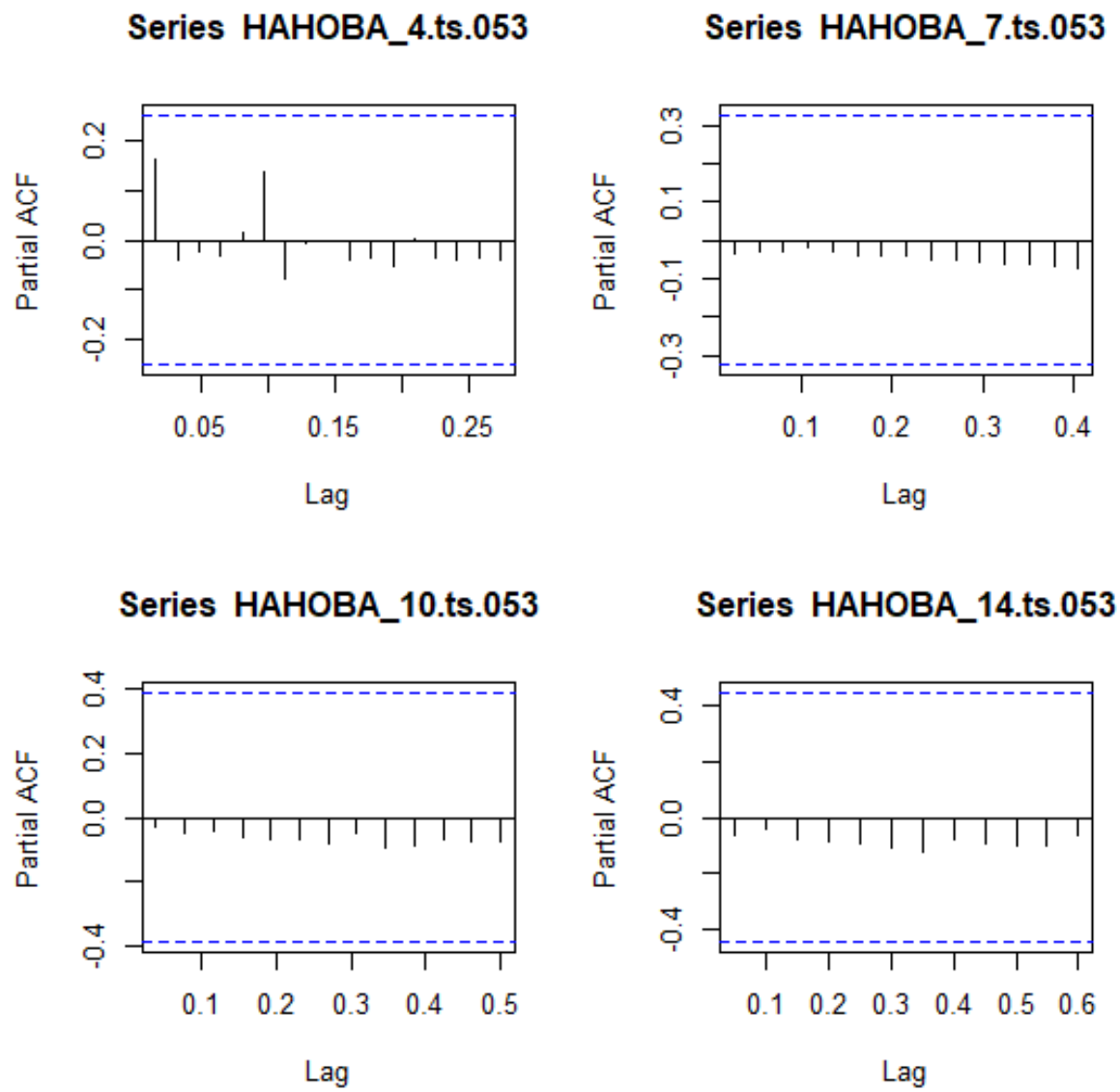
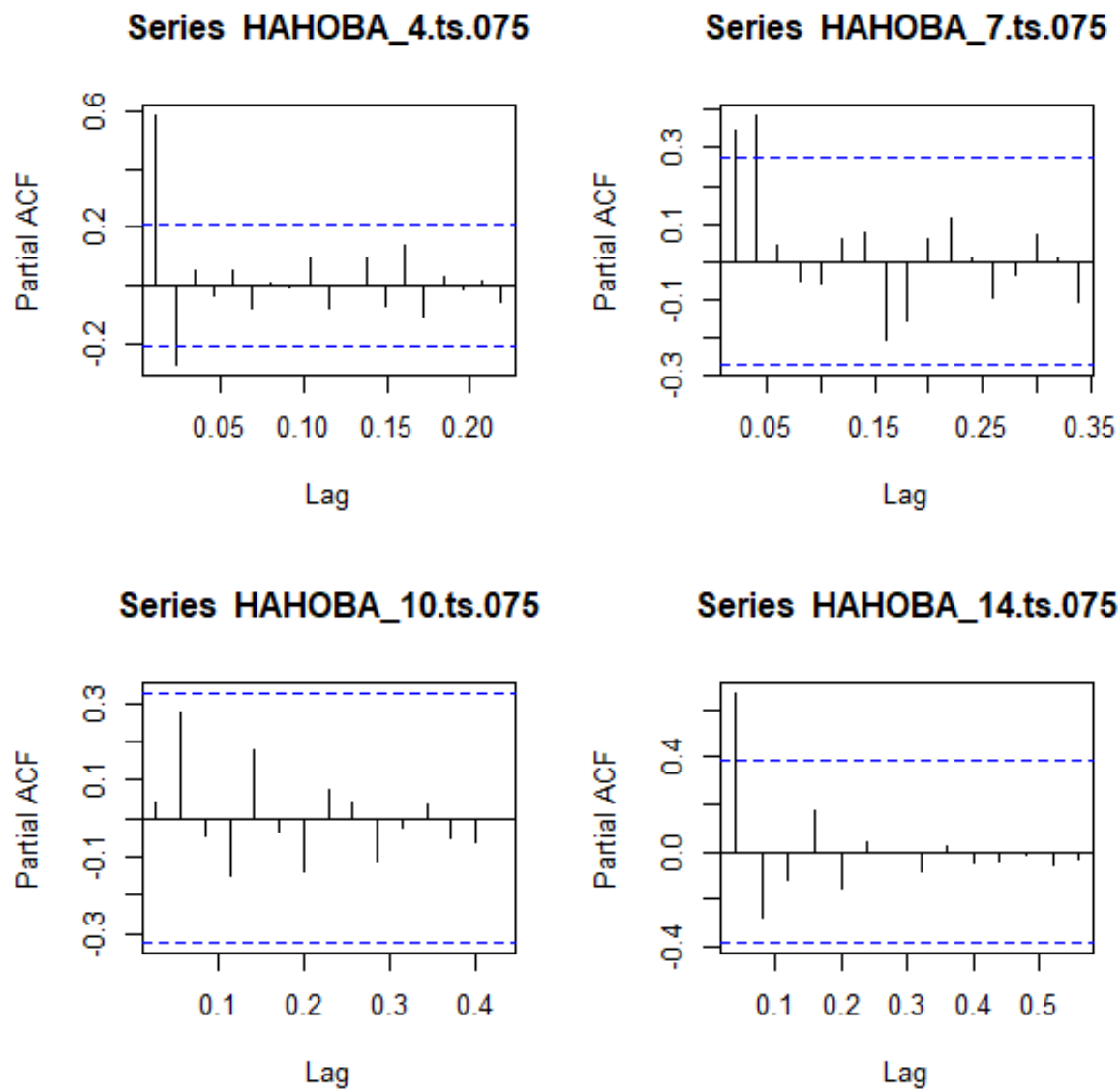Figure A3: Partial autocorrelation plots for site 53 for 4 time-intervals.

Figure A4: Partial autocorrelation plots for site 75 for 4 time-intervals.
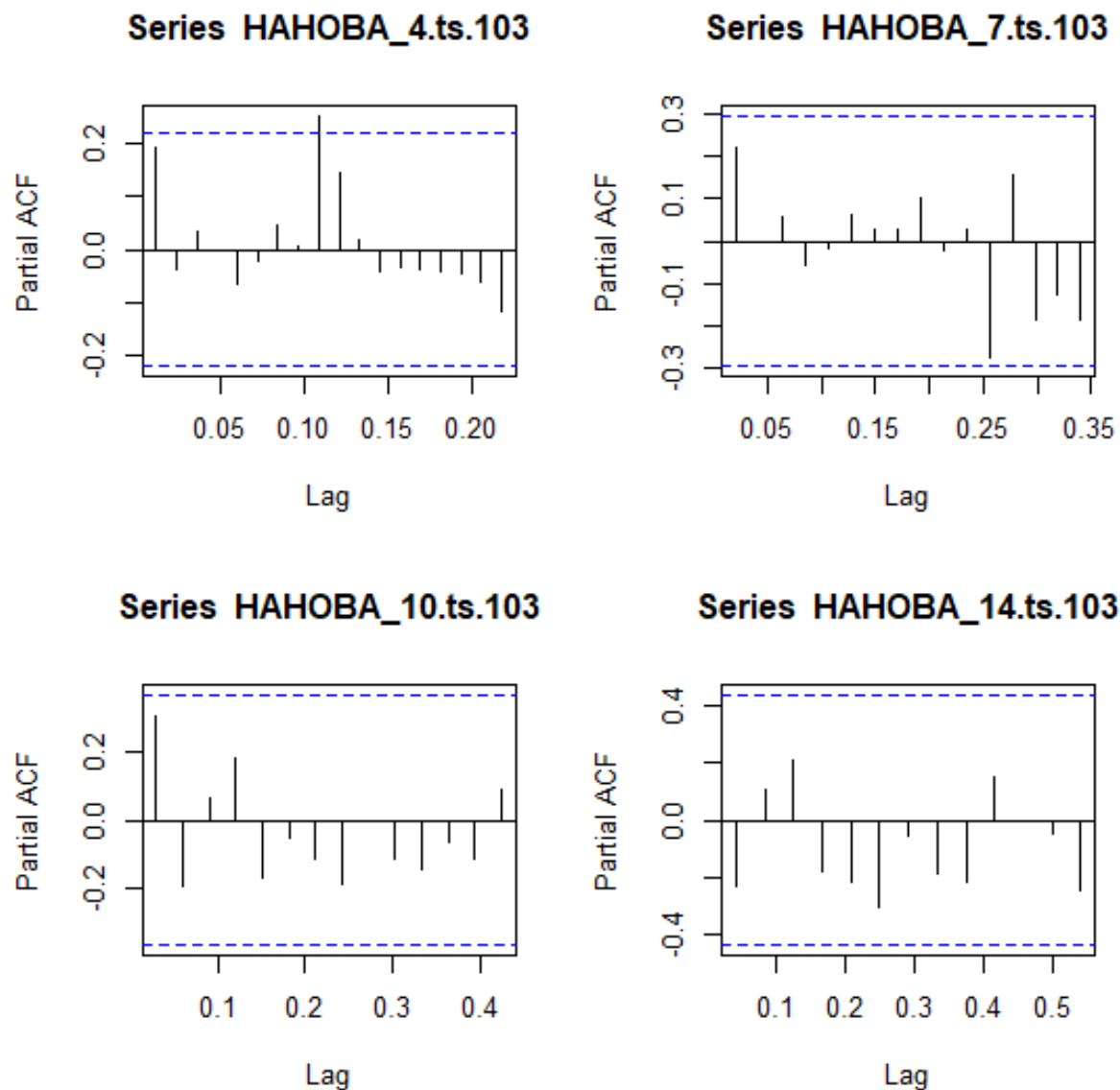
Figure A5: Partial autocorrelation plots for site 103 for 4 time-intervals.

**APPENDIX B: Output of Final Occupancy Models**

14-day interval data, dynamic occupancy model assuming independent detections (unmarked package in R)

```
fit.multiOcc_14.1 <- colext(psiformula= ~ 1, gammaformula = ~ 1, epsilonformula = ~ 1,
pformula = ~ SeasonObs, data = Occ_HAHOBA_14_MultiFrame, method="BFGS")
summary(fit.multiOcc_14.1)

Call:
colext(psiformula = ~1, gammaformula = ~1, epsilonformula = ~1,
    pformula = ~SeasonObs, data = Occ_HAHOBA_14_MultiFrame, method = "BFGS")

Initial (logit-scale):
 Estimate    SE      z P(>|z|)
    -0.29 0.429 -0.676   0.499

Colonization (logit-scale):
 Estimate    SE      z P(>|z|)
    -3.58 0.849 -4.22 2.47e-05

Extinction (logit-scale):
 Estimate    SE     z P(>|z|)
    0.107 0.392 0.273   0.785

Detection (logit-scale):
                         Estimate    SE     z P(>|z|)
(Intercept)                -0.663 0.359 -1.85  0.0649
SeasonObsPost-Lactation    -0.810 0.419 -1.94  0.0530
SeasonObsPre-Pregnancy     -1.228 0.520 -2.36  0.0182
SeasonObsPregnancy          0.376 0.649  0.58  0.5620

AIC: 395.1346
Number of sites: 83

# occupancy
backTransform(fit.multiOcc_14.1, type='psi')
Estimate    SE LinComb (Intercept)
   0.428 0.105   -0.29           1
expit(confint(fit.multiOcc_14.1, type='psi'))
          0.025     0.975
psi(Int)0.2439642 0.6342892

# detection
# detProb_14_1 = predict(fit.multiOcc_14.1, type = 'det', newdata =
# data.frame(SeasonObs=names(table(HAHOBA$Season))), appendData=TRUE)
# detProb_14_1[,1:4] = round(detProb_14_1[,1:4],2)
# detProb_14_1 = detProb_14_1[,c(5,1:4)]
# detProb_14_1
      SeasonObs Predicted   SE lower upper
1      Lactation      0.34 0.08  0.20  0.51
2 Post-Lactation      0.19 0.04  0.12  0.28
3  Pre-Pregnancy      0.13 0.05  0.06  0.25
4      Pregnancy      0.43 0.13  0.21  0.68

expit(confint(fit.multiOcc_14.0, type='det'))
#                                0.025      0.975
# p(Int)                    0.20324140 0.5102603
# p(SeasonObsPost-Lactation) 0.16374814 0.5025840
# p(SeasonObsPre-Pregnancy)  0.09553813 0.4479753
# p(SeasonObsPregnancy)      0.28989604 0.8387166
```

```
# extinction
backTransform(fit.multiOcc_14.0, type='ext')
# Estimate      SE LinComb (Intercept)
#    0.527 0.0978   0.107             1
expit(confint(fit.multiOcc_14.0, type='ext'))
#               0.025     0.975
# ext(Int) 0.340333 0.7060253


# colonization
backTransform(fit.multiOcc_14.0, type='col')
#Estimate      SE LinComb (Intercept)
#   0.0272 0.0224   -3.58             1
expit(confint(fit.multiOcc_14.0, type='col'))
#               0.025     0.975
#col(Int) 0.005262454 0.1283372
```

7-day interval data, dynamic occupancy model assuming correlated detections (Presence)

**DYNAMIC OCCUPANCY WITH CORRELATED DETECTIONS**
Untransformed Estimates of coefficients for covariates (Beta's)
```
=======================================================================
                                        estimate    std.error
A1   psi.a1                        :    0.149805    0.408589
A2   th0[4-13].a2                  :   -1.652755    0.415361
A3   th1[4-13].a3                  :    1.363999    0.630989
B1   gam(3).b1                     :   -4.073508    1.110801
C1   eps(3).c1                     :    0.103425    0.331947
D1   P[4-13].d1                    :   -0.200054    0.368891
E1   th0pi(4).e1                   :   -0.436870    0.694560

    Individual Site estimates of <psi>
                 Site                 estimate   Std.err    95% conf. interval
psi            1 "1"              :    0.5374    0.1016     0.3428 -  0.7212
    Individual Site estimates of <th0[1-1]>
                 Site                 estimate   Std.err    95% conf. interval
th0[1-1]       1 "1"              :    0.1607    0.0560     0.0782 -  0.3018
    Individual Site estimates of <th1[14-1]>
                 Site                 estimate   Std.err    95% conf. interval
th1[1-1]       1 "1"              :    0.7964    0.1023     0.5318 -  0.9309
    Individual Site estimates of <gam(1)>
                 Site                 estimate   Std.err    95% conf. interval
gam(1)         1 "1"              :    0.0167    0.0183     0.0019 -  0.1305
    Individual Site estimates of <eps(1)>
                 Site                 estimate   Std.err    95% conf. interval
eps(1)         1 "1"              :    0.5258    0.0828     0.3665 -  0.6801
    Individual Site estimates of <P[1-1]>
                 Site                 estimate   Std.err    95% conf. interval
P[1-1]         1 "1"              :    0.4502    0.0913     0.2843 -  0.6278
    Individual Site estimates of <th0pi(1)>
                 Site                 estimate   Std.err    95% conf. interval
th0pi(1)       1 "1"              :    0.3925    0.1656     0.1421 -  0.7160
DERIVED parameters
     th0(1) = th0pi*th0 + (1-th0pi)*th1 = Pr(1st segment is used)

       Site                      th0(1)   Std.err     95% conf. interval
th0(1)        1 "1"              :    0.4102    0.1137     0.1875 - 0.6330


 DERIVED parameters - psi2,psi3,psi4,...

       Site                      psi(t)   Std.err      95% conf. interval
psi( 2)     1     "1"     :        0.2625   0.0573      0.1502 - 0.3749
psi( 3)     1     "1"     :        0.1368   0.0394      0.0595 - 0.2141
psi( 4)     1     "1"     :        0.0793   0.0285      0.0235 - 0.1351

DERIVED parameters - lam2,lam3,lam4,...

       Site                      lam(t)   Std.err      95% conf. interval
lam( 2)     1     "1"     :        0.4886   0.0785      0.3348 - 0.6424
lam( 3)     1     "1"     :        0.5212   0.0714      0.3812 - 0.6611
lam( 4)     1     "1"     :        0.5797   0.0973      0.3890 - 0.7704
```