Technical Report HCSU-090

HAWAIIAN HOARY BAT (*LASIURUS CINEREUS SEMOTUS*) ACTIVITY, DIET AND PREY AVAILABILITY AT THE WAIHOU MITIGATION AREA, MAUI

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ABSTRACT

Habitat use, diet, prey availability, and foraging ecology of the endangered Hawaiian hoary bat (*Lasiurus cinereus semotus*, Vespertilionidae), was examined in the east Maui region inclusive of the Waihou Mitigation Area, Pu'u Makua Restoration Area and the wind energy facility operated by Auwahi Wind Energy, LLC. The study was conducted to inform the mitigation and management requirements of Auwahi Wind Energy. Acoustic monitoring over the three-year period demonstrated that bats are present and actively forage year-round at the Waihou Mitigation Area. Over an 8-month span, 11 bats were uniquely color-banded and released, three of which were pregnant or lactating females, and highlights the importance of the area to breeding residents. Our study included the first genetic analysis of Hawaiian hoary bat diet, and confirms the inclusion of Coleoptera, Lepidoptera, Diptera, Hemiptera, and Blattodea among the prey items of this bat identified in previous microscopy-based studies. Hawaiian hoary bats consumed both native and non-native insect species, including several invasive species damaging to crop agriculture. Moths were the primary dietary component, both in prevalence among individual bats and the proportion of gene sequence counts. Through genetic analysis, we identified 18 Lepidoptera families (dominated by Noctuidae, Geometridae, Crambidae, Oecophoridae and Tortricidae) including 24 genus- or species-level taxa. Lepidoptera collected as caterpillars directly from vegetation did not appear in the diet of the 8 bat guano samples at the genus or species level. However, the occurrence of moth larva on native plants suggests that reforestation that includes host plants for these insect families may provide food for locally foraging bats.

INTRODUCTION

Wind energy has emerged as a potential threat to the Hawaiian hoary bat (*Lasiurus cinereus semotus*, Vespertilionidae), a federally and state listed endangered subspecies (USFWS 1998) and the only land mammal endemic to the Hawaiian Islands. Also known as the ʻŌpeʻapeʻa, the species occurs on all of the high islands (Tomich 1986). The Hawaiian hoary bat is closely related to the North American subspecies (*L. c. cinereus*), the latter of which makes up about 40% of all bat fatalities at wind turbines in the United States and Canada (range: 650,000–1,306,000 fatalities for all species in 2010–2011; Arnett and Baerwald 2013). Although in absolute numbers, turbine fatalities of hoary bats in Hawaiʻi are few compared to continental North America, population-level susceptibility of Hawaiian hoary bats to turbines remains unknown. Presently, Hawaiʻi has 206 megawatts of installed wind turbine capacity on the islands of Hawaiʻi, Maui, and Oʻahu (AWEA 2019), and Hawaiian hoary bat fatalities have been recorded at every wind energy facility on these islands. Bat fatalities may influence decisions concerning future wind energy development in Hawaiʻi.

To fulfill requirements for mitigating bat fatalities under its approved incidental take permit, Auwahi Wind Energy, LLC (Auwahi Wind) provided funding for a research project focused on the ecology of Hawaiian hoary bats on the Waihou Mitigation area. The objectives of the study presented herein were to determine within and in the vicinity of the Waihou Mitigation Area on east Maui: (1) bat occurrence and seasonal activity patterns; (2) the availability and diversity of nocturnal aerial insect prey; and (3) diet composition of captured bats. More specifically, the study objectives included quantitatively demonstrating current foraging activity for the purpose of evaluating the area's baseline importance to bats as recently planted native vegetation

matures over the course of long-term restoration efforts within the Waihou Mitigation Area. Additionally, this report summarizes observations of the Hawaiian hoary bat and insect prey in the study area over a 37-month period from March 2015 to March 2018.

METHODS

Study Area

The study area was located on ʻUlupalakua Ranch on east Maui, and was comprised of the Waihou Mitigation Area and a separate property in proximity to the wind energy facility operated by Auwahi Wind (Figure 1, Table 1). The mitigation area is situated adjacent to the Kula Forest Reserve and the Kanaio Natural Area Reserve, and is dominated by pasture, with a small tract of land containing native koa (*Acacia koa*) and ʻōhiʻa (*Metrosideros polymorpha*) forest, and non-native conifers (Figures 2 and 3). Land-cover upslope from the area is comprised of non-native coniferous forest plantations. An ungulate-proof exclosure comprising the 53 ha (130 acre) Puʻu Makua Restoration Area within the Waihou Mitigation Area is protected by a conservation easement and the focus of native trees and shrub out-planting and restoration efforts by Auwahi Wind. In addition to the Puʻu Makua Restoration Area, the study area also included the mid-section of the Waihou Mitigation Area; referred herein as "upper Waihou". Elevation in the parcel ranges from 1,611 m (5,285 ft) above sea level (asl) at the top of a steep, south-facing slope to 1,298 m (4,259 ft) asl in the gently sloping pasture below. The area adjacent to the Auwahi Wind facility spans a low elevation gradient (10–363 m [33–1,191 ft] asl) dominated by dryland vegetation composed of open grassland, wiliwili (*Erythrina sandwicensis*) groves, kīawe (*Prosopis juliflora)*, and an ephemeral anchialine pond on ʻaʻā lava substrate close to the coast (Figures 4 and 5).

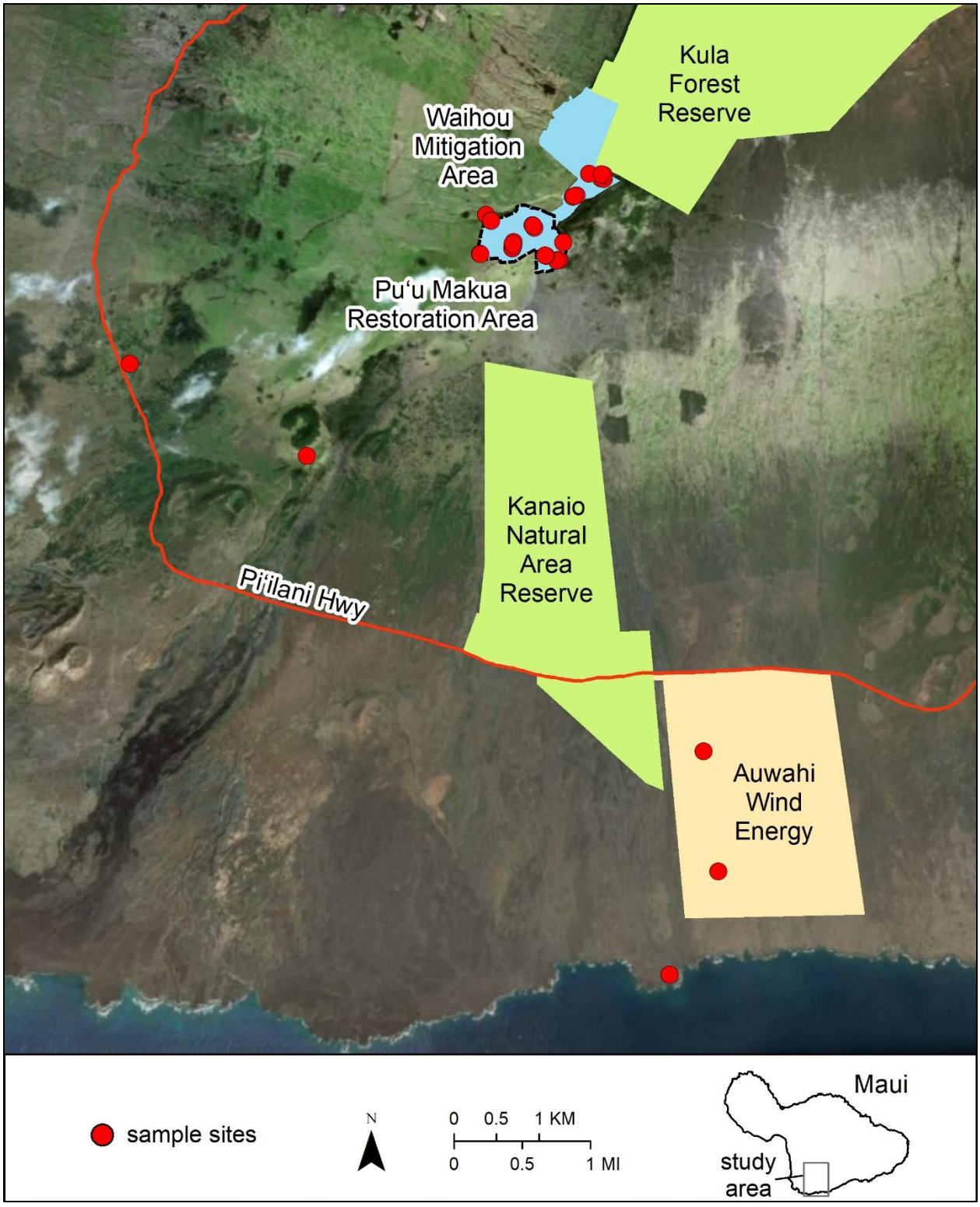

Figure 1. The east Maui study area, including the Pu'u Makua Restoration Area (dash outline) within the Waihou Mitigation Area (blue polygon) and the Auwahi Wind Energy facility (orange polygon). Red circles represent sample sites used for acoustic monitoring, bat netting, and/or insect collection. Site names are shown in subsequent study area figures.

Table 1. Site names, coordinates (Easting and Northing, Universal Transverse Mercator Zone 4 WGS 1984), elevation (m), general location, sample type (bat acoustic, "A"; bat netting, "N"; insect collection, "I-light" or "I-malaise"). Sites are listed by sample type and general location. Coordinates and elevations collected using Garmin eTrax 10 GPS unit.

Site	Easting, Northing	Elevation	Location	Sample type
AUW1	775756, 2286710	1,611	Pu'u Makua	A
AUW2	776015, 2286954	1,606	Pu'u Makua	A
AUW3	776353, 2286781	1,607	Pu'u Makua	A
AUW4	776315, 2286576	1,515	Pu'u Makua	A
AUW5	775447, 2287108	1,396	Pu'u Makua	A
AUW6	776465, 2287322	1,644	upper Waihou	A
AUW7	776801, 2287532	1,647	upper Waihou	A
AUW8	778005, 2280800	363	wind energy facility	A, I-malaise
AUW9	777602, 2278171	10	below facility	A
AUW10	778179, 2279384	150	wind energy facility	A
CABIN	776512, 2287336	1,633	upper Waihou	N
POND1	776831, 2287528	1,659	upper Waihou	N
POND2	776660, 2287589	1,606	upper Waihou	N
RANCH	771254, 2285353	595	below Pu'u Makua	N, I-light
TANK	773335, 2284271	883	below Pu'u Makua	N
RIDGE	775764, 2286741	1,593	Pu'u Makua	N
REST1	775502, 2287044	1,370	Pu'u Makua	I-light
REST1	775507, 2287036	1,370	Pu'u Makua	I-malaise
REST2	775376, 2286650	1,413	Pu'u Makua	I-light
REST2	775380, 2286647	1,413	Pu'u Makua	I-malaise
MAM1	776298, 2286581	1,507	Pu'u Makua	I-light
MAM1	776276, 2286570	1,507	Pu'u Makua	I-malaise
MAM2	776149, 2286627	1,517	Pu'u Makua	I-light
MAM2	776139, 2286632	1,517	Pu'u Makua	I-malaise
PUU1	775992, 2286984	1,587	Pu'u Makua	I-light
PUU1	775997, 2286978	1,587	Pu'u Makua	I-malaise
PUU2	775771, 2286786	1,604	Pu'u Makua	I-light
PUU2	775761, 2286767	1,604	Pu'u Makua	I-malaise
PINE1	776479, 2287325	1,649	upper Waihou	I-light
PINE1	776479, 2287325	1,649	upper Waihou	I-malaise
PINE2	776833, 2287576	1,657	upper Waihou	I-light
PINE2	776805, 2287583	1,657	upper Waihou	I-malaise

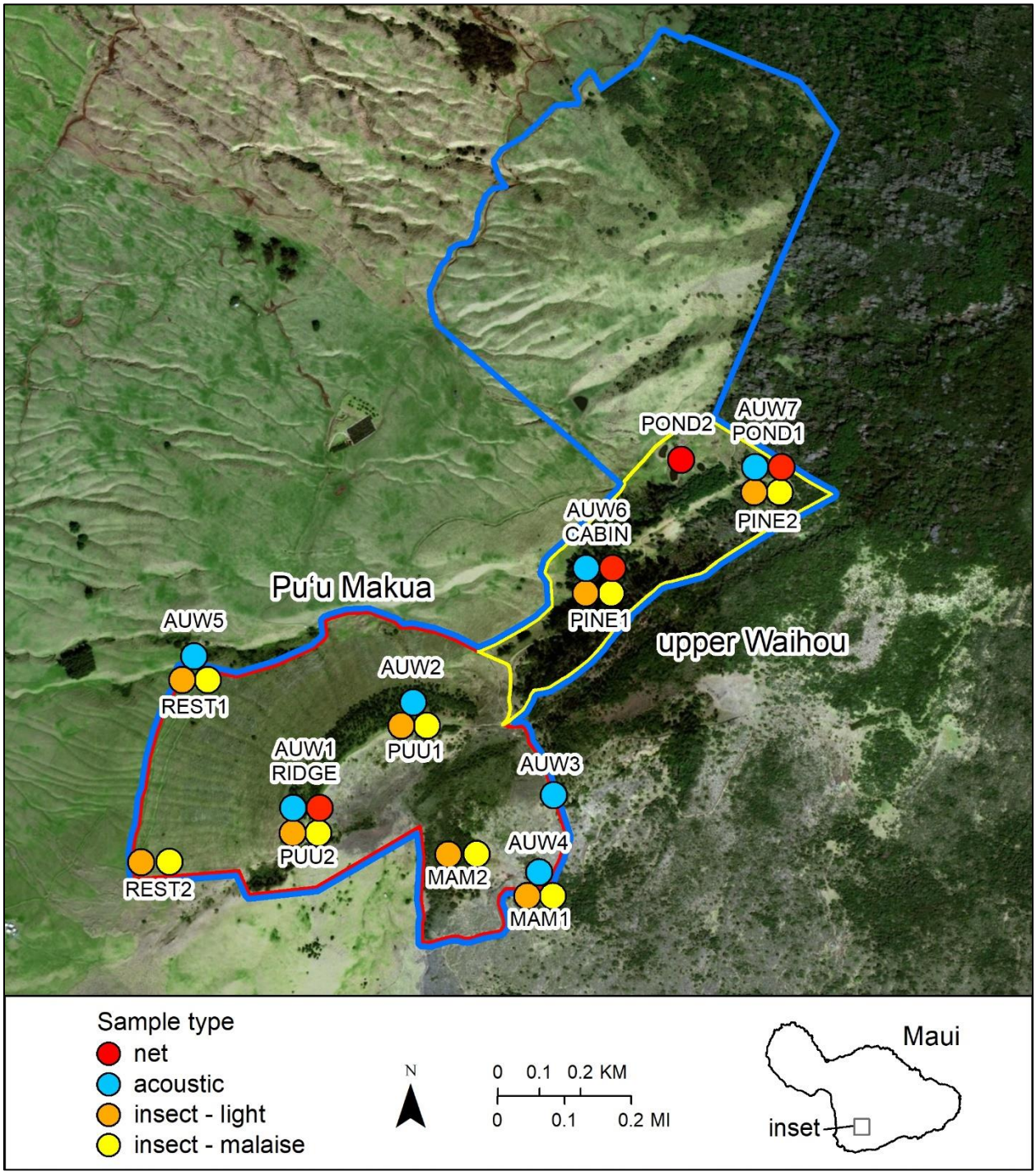

Figure 2. Sample sites within the Waihou Mitigation Area (blue polygon) include the mid-section (referred herein as "upper Waihou"; yellow polygon) and the Pu'u Makua Restoration Area parcel (red polygon) on east Maui, Hawai'i. Sample types include seven acoustic recording sites (blue circles; AUW1 through AUW7), eight paired light and malaise traps (orange and yellow circles) and Hawaiian hoary bat mist net locations (red circles).

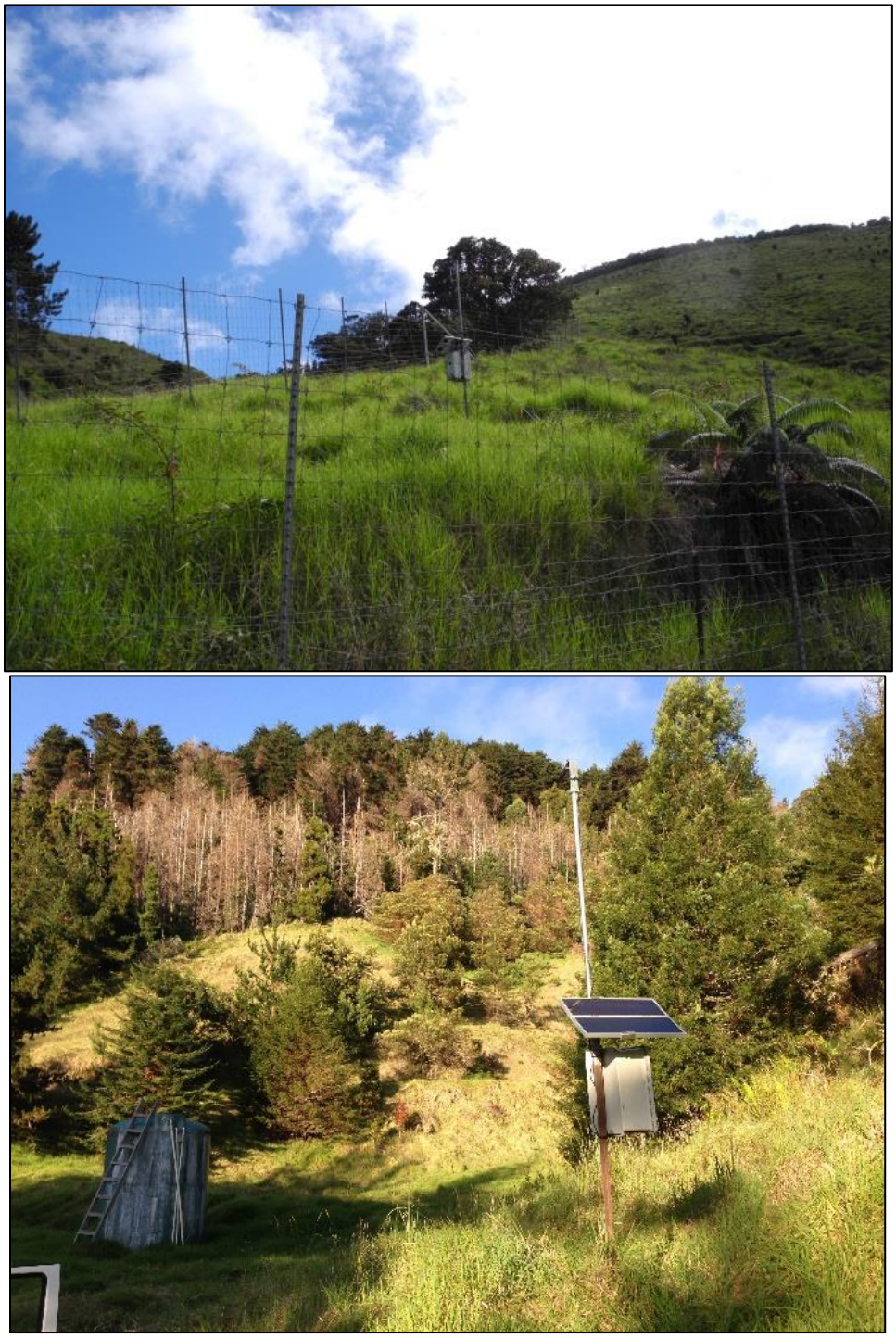

Figure 3. Bat acoustic recording site AUW5 in the Pu'u Makua Restoration Area (top). Bat monitoring site AUW7 next to Pond1 (bottom).

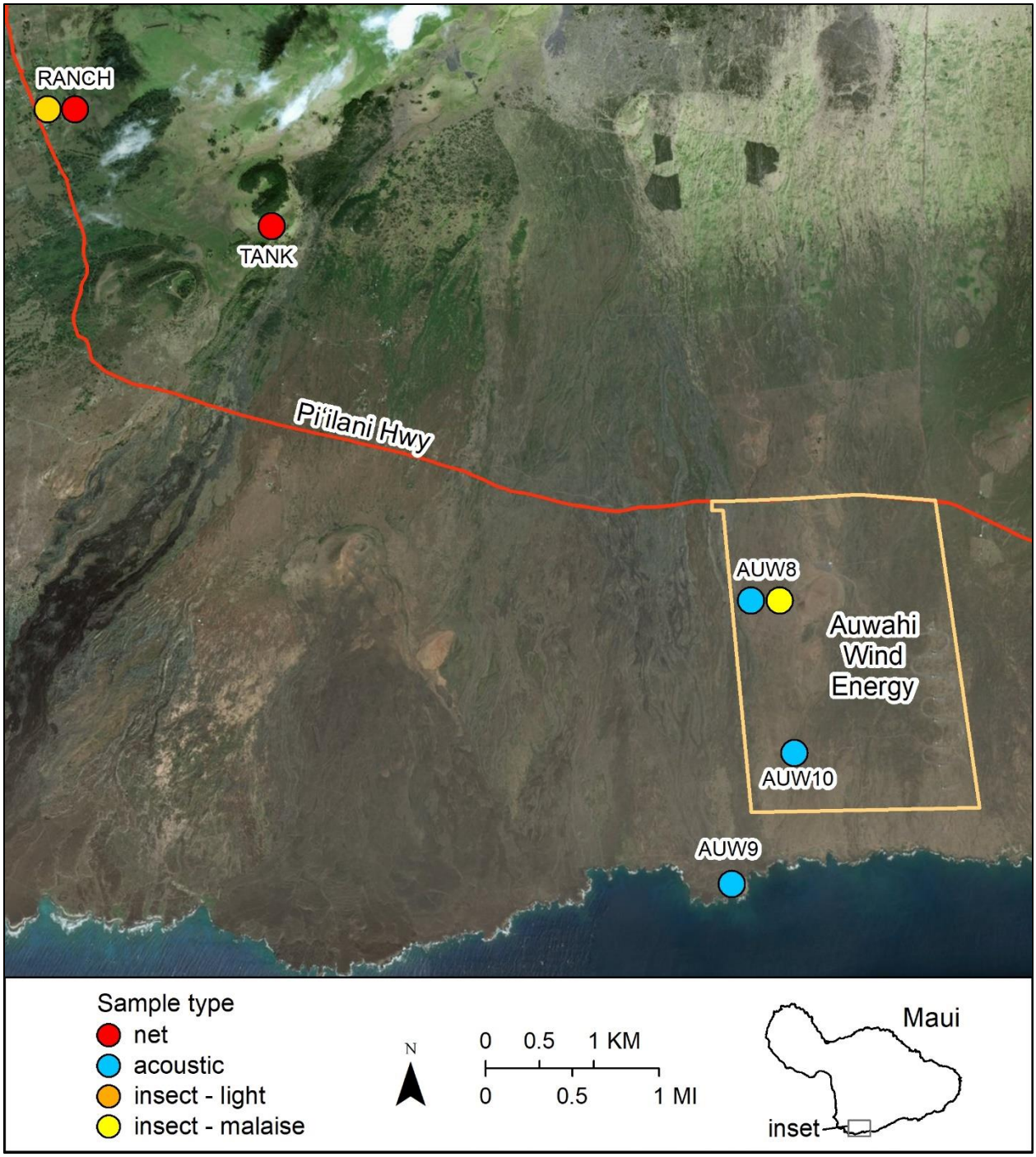

Figure 4. Sample sites within and adjacent to the Auwahi Wind Energy facility on east Maui, Hawai'i. Sample types include three acoustic recording sites (blue circles; AUW8 through AUW10), insect light (yellow circle) and malaise traps (orange circle), and Hawaiian hoary bat mist net locations (red circles).

Figure 5. Bat acoustic recording sites AUW8 (top), AUW9 (center), and AUW10 (bottom) at the Auwahi Wind Energy facility and surrounding habitats.

Acoustic Monitoring

Acoustic sampling of bat occurrence and activity was conducted between March 2015 and March 2018 (Figures 2 and 4; Table 1). On 17 March 2015, five sites were established within the Pu'u Makua Restoration Area (sites AUW1–AUW5) and one outside the parcel at a site deemed potentially good for mist-net capture of bats (AUW6). One additional site (AUW7) was established on 18 April 2017 at POND1, one of four artificial ponds constructed for wetland bird habitat in the area. These seven sites were located at an average elevation of 1,575 m asl. Three additional acoustic monitoring sites (AUW8, AUW9, AUW10) were activated between 13–20 June 2017 adjacent to the Auwahi Wind Energy facility at elevations ranging from 10 to 363 m asl. Additional photographs of bat acoustic recording sites showing equipment and surrounding habitat are presented in Appendix I.

Each site consisted of an SM2BAT+ detector equipped with an SMX-US microphone that records ultrasound between 10 and 100 kHz (Wildlife Acoustics Inc., Concord, MA), and powered by a 6V external battery connected to a 6W solar panel. Between October 13 and October 15 2016, the SMX-US microphones were replaced with upgraded model SMX-U1 microphones. A 6-week session of acoustic recording to compare microphone model performance was conducted at two sites following the replacement, and the results of microphone recording differences are presented in Appendix II. Each detector had the microphone affixed to the top of metal conduit 2 to 3 m above the ground and connected by cable to the microphone port. Both the SMX-US and the SMX-U1 are omnidirectional and capable of detecting bat calls at distances up to 30 m (Adams *et al.* 2012) under ideal conditions (i.e. no wind or rain, low humidity). To ensure quality recordings, detectors were equipped with new microphones every three to four months.

The ultrasonic, full spectrum detectors were triggered by acoustic signals and operated every night for up to three months, from one hour prior to sunset until one hour after the following sunrise. Acoustic events were recorded without digital compression as full-spectrum Waveform Audio File format (.wav) sound files onto Secure Digital (SD) cards with a sampling rate of 192 kHz; analog high pass filter at 1 kHz and 36 decibel gain (SMX-US) or 12 decibel gain (SMX-U1); microphone bias off; digital high pass filter at fs/24; digital low pass filter off; trigger level 18 SNR signal-noise ratio; trigger window 2.0 sec; trigger max length 8 sec; frequency division ratio 16. Detectors were checked at 2 to 3-month intervals to exchange SD cards and to test battery levels and microphone function.

Kaleidoscope Pro (version 4.1.0a; Wildlife Acoustics, Inc.) software was used to filter acoustic background noise with the following settings: 10–70 kHz, 1–7 ms pulse duration, 250 ms maximum inter-syllable gap, and a minimum of 2 pulses per event. Subsequently, all files containing bat echolocation pulses were visually and aurally inspected as sonograms with Kaleidoscope Pro to ensure that there were no false positives. Ultrasonic vocalizations by Hawaiian hoary bats were categorized by type, and terminal-phase calls ("feeding buzzes" emitted just prior to an attempted insect catch; Griffin *et al.* 1960) were qualitatively distinguished from search and approach-phase calls by a rapid increase in the call rate (Figure 6). Call files were visually assessed for evidence that there were two or more bats concurrently vocalizing at a site.

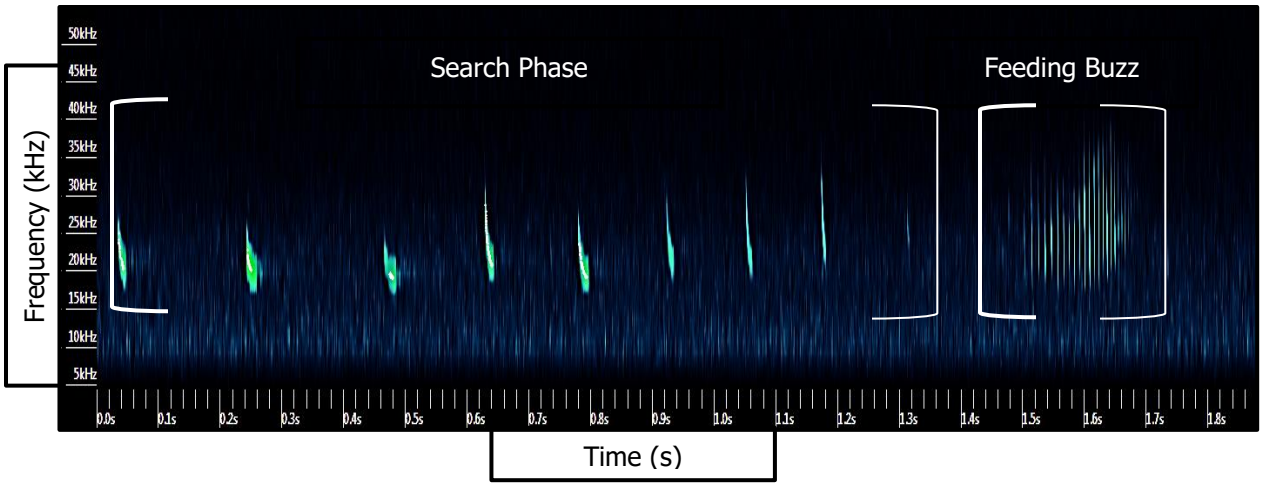

Figure 6. Spectrogram of a Hawaiian hoary bat echolocation call-event with search-phase pulses (left hand bracket set) followed by a terminal-phase or feeding buzz call (right hand bracket set).

The frequency of echolocation as determined by the number of bat acoustic files and the incidence of feeding buzzes were each summed by recording site and night. Acoustic data are available at https://doi.org/10.5066/P9U0KRMY (Pinzari *et al.* 2019). The resulting detection history was used to calculate the proportion of total nights with observed bat activity throughout the year. For the subset of sites with at least one detection, the frequency of detection per month was calculated for each survey location as the total number of nights with detections divided by the total number of nights sampled (effectively weighting the values by sampling effort).

Hawaiian hoary bat reproductive or "breeding" season (as adapted from Menard 2001) includes periods of pregnancy (May to June) and lactation (late June to August). The remainder of the year includes a fledging/post-lactation period (September to October) and a pre-pregnancy period or "post-partum" (November to April), during which there is no reproductive or parental care shown by adult females.

Because the change of microphone model in October 2016 resulted in a noticeably higher rate of calls, comparisons of bat occurrence and activity were necessarily limited to the late autumn and winter periods of 2016 and 2017, corresponding to the end of the fledging/post-lactation and pre-pregnancy periods. For the high elevation sites (AUW1–AUW6) we used comparable monthly data collected after the improved microphones were added in October 2016. This resulted in two six-month periods with which to compare bat occurrence and activity over two years: winter 2016 (October 2016 to March 2017) and winter 2017 (October 2017 to March 2018).

To assess how the frequency of acoustic detections changed between winter periods we used log-linear regression to model the number of call files as a function of year, month, and the interaction of year and month. To account for zero values, 0.5 was added to all nightly tallies of detections. Prevalence of bat occurrence as determined by acoustic detections, and more specifically, feeding activity as indicated recordings of feeding buzzes was compared for the two

winter periods with repeated measures logistic regression. The effect of year (as a fixed effect coded by the year in which the six-month span began), month (as a random effect), and year + month as an interactive variable were modeled and compared to a null model of random variation among sites. We used the Akaike Information Criterion corrected for small sample size (AICc) to compare models. Prevalence of bat occurrence per month or site is presented herein as percentages and calculated as monthly means weighted by the number of sample nights in each month.

Bat Capture

Mist-netting to capture bats was conducted between October 14 and December 7, 2016 (13 nights), and again between June 8 and July 5, 2017 (20 nights). Netting locations included a clearing around site AUW6 (CABIN site) and over water at the POND1 and POND2 sites (Figure 7). A combination of single high and triple high nets of various lengths (6-, 9-, 12-, and 18-m) were used within the first five hours after sunset. A UltraSoundGate Player BL Light acoustic lure (Avisoft Bioacoustics, Glienicke, DE) broadcasting locally recorded hoary bat social calls was deployed for 2 nights in 2016 and 12 nights in 2017. Capture rates for each of the two netting periods were calculated as the total net-hours (length of mist nets times hours deployed each night) divided by number of bats captured.

We recorded age class, sex, reproductive condition, weight, forearm length, and noted the collection of wing tissue biopsies, guano samples, and hair clippings. Each captured bat received uniquely colored plastic bands on the right forearm. The protocol for handling bats was approved by the Institutional Animal Care and Use Committee (IACUC #04-039-12) of the University of Hawai'i at Hilo and followed guidelines of the American Society of Mammologists. Biological samples were collected under permits USFWS TE003483-31 and Hawai'i DLNR-DOFAW WL16-04.

Insect Sampling

Insect sampling focused on Lepidoptera (moths) and Coleoptera (beetles), the primary prey of Hawaiian hoary bats (Whitaker and Tomich 1983, Jacobs 1999, Todd 2012). The insect prey base available to Hawaiian hoary bats in the Waihou Mitigation Area was examined over late autumn and early summer periods using malaise traps, ultraviolet (UV) light traps, and by shaking insects from vegetation using cloth beating sheets (Figure 8). Paired malaise and light traps were placed at eight sites, two of each which comprised paired samples for each of four sites: REST, PUU, MAM, PINE (Figure 2; Table 1). Malaise traps operated continuously from October 25 to December 7, 2016, and June 7 to July 3, 2017. Light traps were run for the first three hours of the night during October 26 to December 1, 2016, and June 20 to 22, 2017. An opportunistic site (AUW8) was established in proximity to the Auwahi Wind Energy facility.

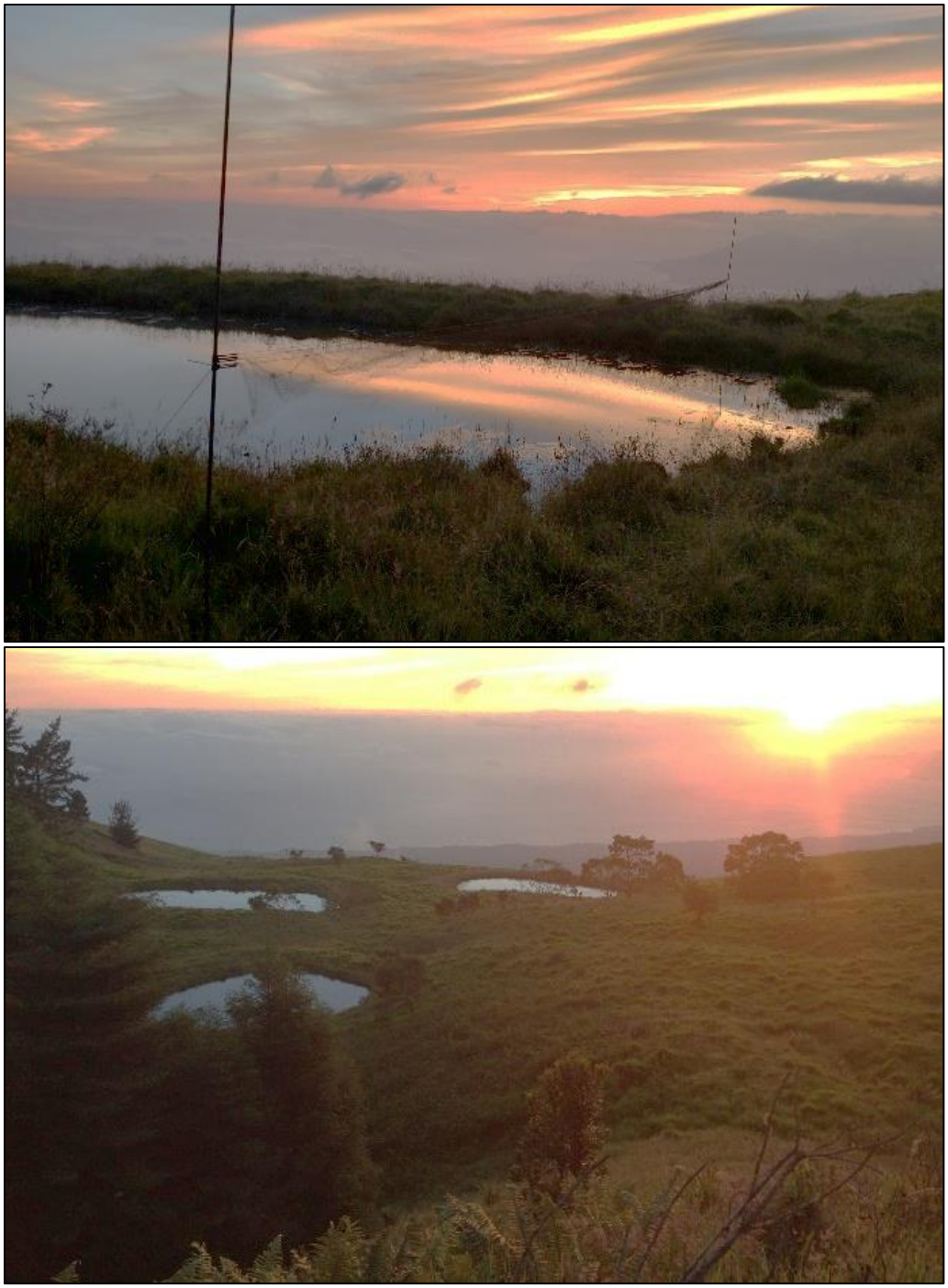

Figure 7. POND2 site (top) in the upper portion of the Waihou Mitigation Area (see Fig. 2). Bats were captured in mist nets at these artificial ponds constructed for wetland bird habitat (bottom).

Each light trap was set at ground level and positioned a minimum of 200 meters from acoustic detectors. Light traps consisted of a 22W UV light bulb situated above a funnel and bucket trap (Model #2851M; Bioquip Products Inc., Rancho Dominguez, CA). Light traps were powered by

12-V marine deep cycle batteries. A timer allowed the light to begin operating 30 min after sunset. Samples were collected the following morning. The malaise traps (Model 2875DG; Bioquip Products Inc., Rancho Dominguez, CA) were deployed slightly above ground level, and were emptied of contents at approximately weekly intervals. Trap samples were preserved by freezing for later sorting. During sorting, insects were identified to family level using keys from the Insects of Hawaii Series (Zimmerman 1958a, 1958b, 1978).

While malaise and light traps were aimed at sampling the general prey base at each site, sampling directly from vegetation was applied to identify larval Lepidoptera (caterpillars) and Coleoptera associated with particular plant species. Insects associated with restoration plantings and pasture grasses were sampled from the dominant plant species within each area by gently shaking foliage October 26–28 and November 28–30, 2016, and on June 20, 2017. The plant species searched include ʻaʻaliʻi (*Dodonaea viscosa*), an unidentified grass, koa (*Acacia koa*), māmaki (*Pipturus albidus*), māmane (*Sophora chrysophylla*), naio (*Myoporum sandwicense*), ʻōhiʻa (*Metrosideros polymorpha*), pūkiawe (*Leptecophylla tameiameiae*), black wattle (*Acacia mearnsii*), redwood (*Sequoia sempervirens*) and Monterey pine (*Pinus radiata*). Caterpillars collected on these plants were conveyed to the lab and reared to the adult stage to facilitate identification.

Insect counts at each site were adjusted by sampling effort to produce separate indices of capture rates (number per trap night) for malaise and light trap samples, and collections directly from vegetation (Appendix III). The capture rate indices for malaise and light trap samples were subsequently combined to provide a measure of overall abundance for each site. The body lengths of all moths were measured and samples were assigned to size classes: small (<10 mm), medium (10–15 mm) and large (>15 mm). Representative insect samples are held as a voucher collection at Kīlauea Field Station. Insect count data are available at https://doi.org/10.5066/P9U0KRMY (Pinzari *et al.* 2019).

Bat Diet Sampling and Genetic Analyses

Insect reference library

Genomic sequence data on public databases are not well represented for Hawaiian arthropods (i.e., records with accurate species identifications do not exist). Therefore, a reference library of potential bat prey items collected in the vicinity of the Waihou Mitigation Area was prepared for comparison to items in the bat diet subsequently identified by genomic analysis. Seventy insects collected using the three trapping techniques (light, malaise, vegetation beating) were preserved for barcoding to create the reference library. Insects represented taxa from four orders and included 62 Lepidoptera (10 families), five Coleoptera (three families), one Diptera, and two Hemiptera. The Diptera and Coleoptera families were collected from cow dung in the pasture below the Waihou Mitigation Area. The insects selected for barcoding, while a subset of the entire dataset, included many of the most common species available as potential bat prey. One to three legs were removed from moths, while for some smaller specimens, such as small moths, flies and beetles, the whole body was used for DNA extraction. Insect DNA was extracted using a DNAeasy Blood and Tissue Kit (Qiagen, USA) A mortar and pestle were used to grind insects following modifications in the Qiagen Supplementary Protocol for Purification of Total DNA from Insects.

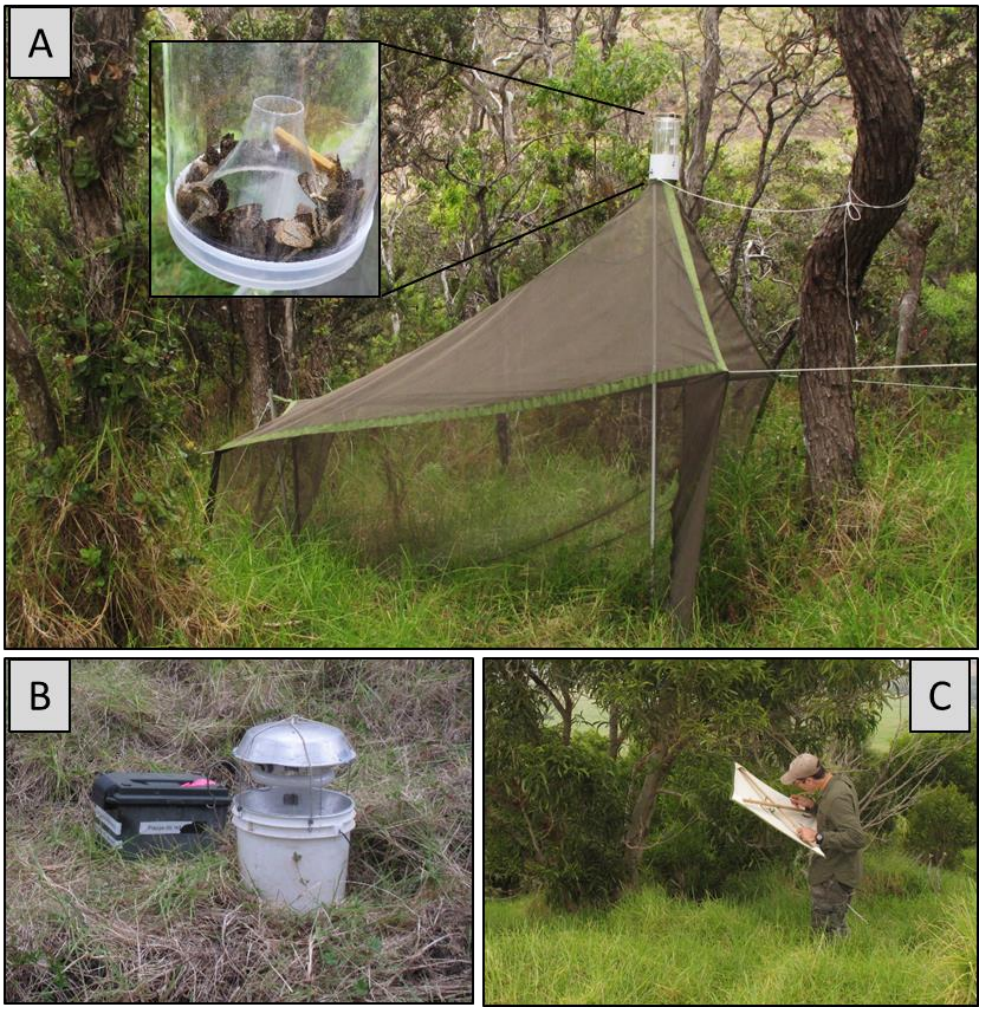

Figure 8. Methods used to collect Lepidoptera and Coleoptera across the study area include malaise traps ((A); inset shows numerous geometrid moths within the collection chamber), battery operated light traps (B), and shaking vegetation to dislodge insects (C).

Polymerase chain reaction (PCR) sequencing of mitochondrial DNA was used to develop the insect reference library and identify arthropod species in bat guano samples. Barcoding of the reference library samples was conducted as follows: Folmer primers (Folmer *et al.* 1994) were used to amplify an approximately 657 base pair [bp] region of the COI gene sequence for reference library specimens (Table 2). Extracted DNA was amplified via PCR using Illustra Hot Start mix PCR beads (GE Healthcare, USA) in 25 µL reaction volumes, each containing 20.5 µL sterile water, 0.5 µL of each primer (10 µM concentration), and 2.5 µL of genomic DNA template. PCR cycling conditions consisted of 1 cycle at 94 °C for 1 minute; 5 cycles of 1 minute at 94 °C, 1 minute 30 seconds at 45 °C, 1 minute 30 seconds at 72 °C; 35 cycles of 1 minute at 94 °C, 1 minute 30 seconds at 50 °C, 1 minute at 72 °C, and a final extension period of 5 minutes at 72 °C (Folmer *et al.* 1994) and was carried out on an Eppendorf Pro S Thermal Cycler (Eppendorf, USA). PCR products were checked for desired fragment size using 1.5% gel electrophoresis and a 100-base pair (bp) ladder. PCR products were cleaned of excess

nucleotides and primers using Exo-Sap (Affymetrix, Thermo Fischer, USA) according to manufacturer's protocol and quality checked using UV spectrometry. Sanger sequencing of both the forward and reverse primer PCR products was performed on an ABI Prism 3500 Genetic Analyzer (Applied Biosystems, USA) at the University of Hawai'i at Hilo Core Genomics Facility. Sequence chromatograms were manually trimmed, edited, and consensus DNA sequences were formed using Sequencher v5.2.4 (Gene Codes 2014).

Resulting sequences were compared to publicly available COI sequence data using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool Nucleotide "BLASTn" (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed February 2019) as well as using the Identification Engine on Barcode of Life Data System "BOLD" (Bold Systems v3) (http://www.boldsystems.org/index.php/IDS_OpenIdEngine, accessed February 2019). Order and family matches to arthropod sequences were considered informative for matches ≥87%, and followed in agreement with visual identifications. Genus and species level matches were considered for matches ≥93%, and which also agreed with visual identification given to insect before DNA extraction. The geographic validity of arthropod taxon matches and native/non-native status were confirmed by referencing the Hawaiian Terrestrial Arthropod Checklist, Fourth Edition (Nishida 2002), the online database at the Insect Museum at the University of Hawai'i (https://www.ctahr.hawaii.edu/insectmuseum/insectholdings.htm, accessed February 2019), consulting local state and university entomologists, and literature searches. Reference library insect barcode data are available at https://doi.org/10.5066/P9U0KRMY (Pinzari *et al.* 2019).

Guano collection and DNA extraction

Guano was collected from nine adult bats (3 females, 6 males) (Table 3). Eight live captures in the Waihou Mitigation Area vicinity during November 2016 and June/July 2017 provided guano. One guano pellet was obtained from a fresh female bat carcass collected at Auwahi Wind Energy Facility on August 15, 2016. One to four guano pellets were used for DNA extraction per individual (average 2.2 pellets per individual), and no more than 20 micrograms of guano were used per extraction. Pellets were combined and homogenized during DNA extraction. DNA was extracted using a Qiagen DNA PowerSoil Kit (Qiagen, USA) following the manufacturer's protocol (2018 version), with modifications as described in Alberdi *et al.* 2018. We included one negative extraction control, and extractions were performed in a dedicated pre-PCR laboratory space.

Table 2. Details of the two primer sets used to explore diet of the Hawaiian hoary bat on Maui, and the primer set used to create barcodes for the insect reference library. Length refers to amplicon size excluding primers. Adapted from Alberdi *et al.* 2018.

General name	Region	Primer names	Forward primer 5′-3′	Reverse primer 5′-3′	Length (bp)	Target Taxa	Reference
Epp	16s	F: Coleop_16sc R:Coleop_16Sd	TGCAAAGGTAGCATAATMATTAG	TCCATAGGGTCTTCTCGTC	106 [102–107]	coleoptera	Epp *et al.* (2012)
Zeale	COI	F: ZBJ-ArtF1c R: ZBJ-ArtR2c	AGATATTGGAACWTTATATTTTATTTTTGG	WACTAATCAATTWCCAAATCCTCC	157 [157–159]	arthropoda	Zeale *et al.* (2011)
Folmer	COI	F: HCO2198 R: LCOI490	TAAACTTCAGGGTGACCAAAAAATCA	GGTCAACAAATCATAAAGATATTG	657	arthropoda	Folmer *et al.* (1994)

Table 3. Bats contributing guano samples for diet analysis. See Table 1 for details on sample locations.

Bat ID	Sex	Date	Time	Location	Number of pellets	Primers
M41	female	8/15/2016	unknown	Turbine 2	1	Epp, Zeale
M47	male	11/3/2016	18:50	POND1	4	Epp, Zeale
M48	male	11/15/2016	19:36	POND1	2	Epp, Zeale
M49	male	11/28/2016	21:00	POND1	2	Epp, Zeale
M50	male	6/20/2017	21:00	CABIN	4	Epp, Zeale
M51	male	6/20/2017	21:05	POND1	4	Epp, Zeale
M54	female	6/26/2017	22:05	POND1	2	Epp, Zeale
M55	male	6/28/2017	22:08	POND2	1	Epp only
M57	female	7/4/2017	22:13	POND2	1	failed

Metabarcode sequencing of bat guano

Bat guano samples are generally comprised of degraded and fragmented DNA, as such, sequencing required a "mini-barcode" approach (Alberdi et al 2012). For diet analyses, we used Zeale and Epp primers to target short COI gene sequences (Table 2). We used Zeale and Epp primers jointly to characterize taxonomic diversity because they cover different regions of the mitochondrial gene and Epp primers specifically target Coleoptera (Alberdi *et al.* 2018).

The following metabarcode library preparation and sequencing was performed at the Genomics Core Facility of the University of Tennessee. Each sample of amplified arthropod DNA obtained from bat guano was duplicated for each of the Zeale and Epp primer sets and included one reaction blank per primer set. Target gene sequences were amplified for each primer set by modifying the primers with adapters on the 5' and 3' end for the Illumnia MiSeq platform (Illumnia, USA). PCR was used for each primer-adapter set to amplify prey DNA in guano samples plus replicates, as well as two reaction blanks of water which were carried through the entire sequencing process. Amplification success was confirmed with gel electrophoresis. Initial PCR products with adapters were cleaned with Agencourt AMPure XP beads (Beckman Coulter, USA), and a second round of PCR and a Nextera XT library kit (Illumnia, USA) was used to attach unique combinations of MID tags (Illumnia, USA), allowing us to reference prey sequences to individual bats. Indexed PCR products received another round of purification with Agencourt AMPure XP beads, then were quantified using a fluorometer, combined into approximately equimolar pools and quantified on a Bioanalyzer (Agilent Technologies, USA) to verify MID tag additions and calculate final loading concentrations for sequencing. Samples and blanks were duplicated, diluted to 4 pM, combined with PhiX control DNA at a ratio of 10 % PhiX, then loaded onto a MiSeq Reagent Kit v2 250-cycle flow cell set for a paired-end read of 175 bases each (2 X 175). After sequencing, Illumina reads were automatically demultiplexed and MID tags and adapters were removed.

Bioinformatic analyses

The data analysis pipeline and taxonomic assignment methodology follow the procedure described in the R Notebook tutorial (Divoll *et al.* 2018; http://github.com/tdivoll/Bat-Diet-Metabarcoding/ accessed February 2019). Several tools were used to take raw sequence reads

produced in the above section and create a table of filtered prey taxa for each individual bat. FastQC (Andrews 2010) was used to assess Illumina sequencing performance and determine quality-filtering thresholds. Analyses were performed using the Quantitative Insights Into Microbial Ecology software (QIIME version 1.9.1; Caporaso *et al.* 2010) run inside a Linux virtual machine (Oracle Virtual Box version 5.0.8 r103449).

Tools within QIIME and FASTX Toolkit 0.0.12 (Gordon and Hannon 2010) were used to join the forward and reverse paired end Illumina reads and determine average quality at expected read length (211 bp for Zeale, and 148 bp for Epp), then filter out base calls under Q25 Phred quality score and remove sequences less than 200 bp for Zeale and 137 bp for Epp. Sequences were clustered into operational taxonomic units (OTUs) with the SWARM method (Mahe *et al.* 2014), allowing for a 2 bp difference (98.5%; Hope *et al.* 2014). A high threshold allowed for greater representation of rare OTUs. Potential over inflation of OTUs and removal of chimeric sequences was accounted for by filtering to remove OTUs that did not occur ≥10 times in a sample. We used the custom Python script (provided in the tutorial) and employed the 'pandas' package (McKinney 2010) to filter by the threshold and extract the most abundant sequences for each OTU. After OTUs were generated from QIIME, taxonomic assignment and further filtering were done using R (version 3.5.2, R Core Team 2018).

Taxonomic matches were retrieved from the Barcode of Life Database (BOLD v4, February 2019; Ratnasingham and Hebert 2007) with the 'bold' package (Chamberlin 2017), using the 'dyplr' package (Wickham and Francois 2016) to filter out matches with <95% similarity. For each OTU, only the top 40 specimen matches were kept, then manually assigned to a taxon at ≥98.5% similarity from output tables (see *Manual Vetting of Results* section in the R Notebook tutorial from Divoll *et al.* 2018). OTUs assigned to the same taxonomy were collapsed into one OTU-based prey taxon. This approach assumes that potential chimeric sequences do not match any specimens in the BOLD reference database; however, substitution errors or single nucleotide polymorphisms may still persist, even when a match is ≥98.5%. False negatives may be increased by discarding prey that do not have records in BOLD and by collapsing higher taxonomic assignments into single prey taxa (e.g., two OTUs of different species in the same genus and the same percent similarity collapsed into one genus-based prey taxa). Taxonomy was mostly assigned using the final filtered dataset (consensus among top 40 matches), but representative OTU sequences were also manually input into BOLD to resolve discrepancies and in many cases, we left assignments at order or family if not resolved at the genus level. Species level assignments were rare, and all genus- and species-level matches were manually checked, with assignment restricted to those with known occurrence in Hawai'i. If multiple assignments shared the highest matching scores, taxonomy was assigned to Hawaii-present species or downgraded to the highest taxonomic level (usually family). Ultimately, ordinal-level taxonomy was assigned at >95% similarity, familiar-level taxonomy at >96.5%, and species-level ≥ 98% following Alberdi *et al.* 2018. This data analysis pipeline is appropriate for comparisons of identified prey (OTUs with assigned taxonomy). We used this pipeline to produce a set of OTUs for each desired gene, COI and 16s, and recorded which OTUs and taxonomic assignments appeared within one or both members of a duplicated guano sample. We also noted those that were unique to Zeale or Epp primer sets.

The dietary composition identified for individual bats and taxa identified to genus and species level are presented using only Zeale primer results. The Zeale primer produced a more robust dataset than the Epp primer, which previous bat diet studies have shown to be unreliable for species identifications (Alberdi *et al.* 2018, Kaunisto *et al.* 2017). Moreover, for maximum

confidence, we report the order and family of prey items consumed by individual bats if OTUs were recovered from both members of a duplicated sample sequenced with Zeale primers. Current review of genetic dietary analysis recommends sequencing bat guano samples in duplicate or triplicate fashion (referred to as technical replication) and reporting prey items that are recovered from multiple samples of a set to counter stochasticity arising from DNA sequencing (Alberdi *et al.* 2018, Mata *et al.* 2019). However, to allow for a more inclusive assessment of dietary diversity (but with a concomitant decrease in the confidence of taxa identifications), we also report results for Epp primers. Given a low rate of OTU recovery for sequences obtained with Epp primers, we include taxa that were identified even when derived from only a single member of a duplicated sample. Relative representation of order and lepidopteran families were graphed as the prevalence among individual bats (i.e., proportion of bats in which the taxon was detected) and sequences recovered (i.e., proportion of sequences within an assigned taxon). The number of OTUs (i.e., read counts) is interpreted as an approximation of the relative abundance or biomass of insect taxa consumed by bats (Deagle *et al.* 2019). Insect OTU data used in analyses are available at https://doi.org/10.5066/P9U0KRMY (Pinzari et al. 2019).

RESULTS

Acoustic Monitoring

Bat occurrence and activity was observed at all ten sites from the onset of acoustic sampling on March 17, 2015, through March 21, 2018 (Appendix IV). Activity as indicated by the number of acoustic files per night demonstrated higher mean bat encounter rates during May to October when females are pregnant and lactating, and during the fledging/post-lactation period relative to the months when bats are reproductively quiescent from November to April (Figure 9). This seasonal pattern of higher encounter rates was observed at the higher elevation Waihou sites (AUW1–AUW6) in 2015 and 2016. Although bat activity was much lower at the Auwahi Wind Energy stations (AUW8–AUW10), a seasonal pattern of higher encounter rates persisted from July through October. However, the three-year time series collected in Waihou also demonstrated a marked difference in encounter rates at the resulting from the change in detector microphones in October 2016. Consequently, year-to-year comparisons are limited to periods with the same microphone type (i.e., "winter" periods spanning the months of October to March in both 2016 and 2017).

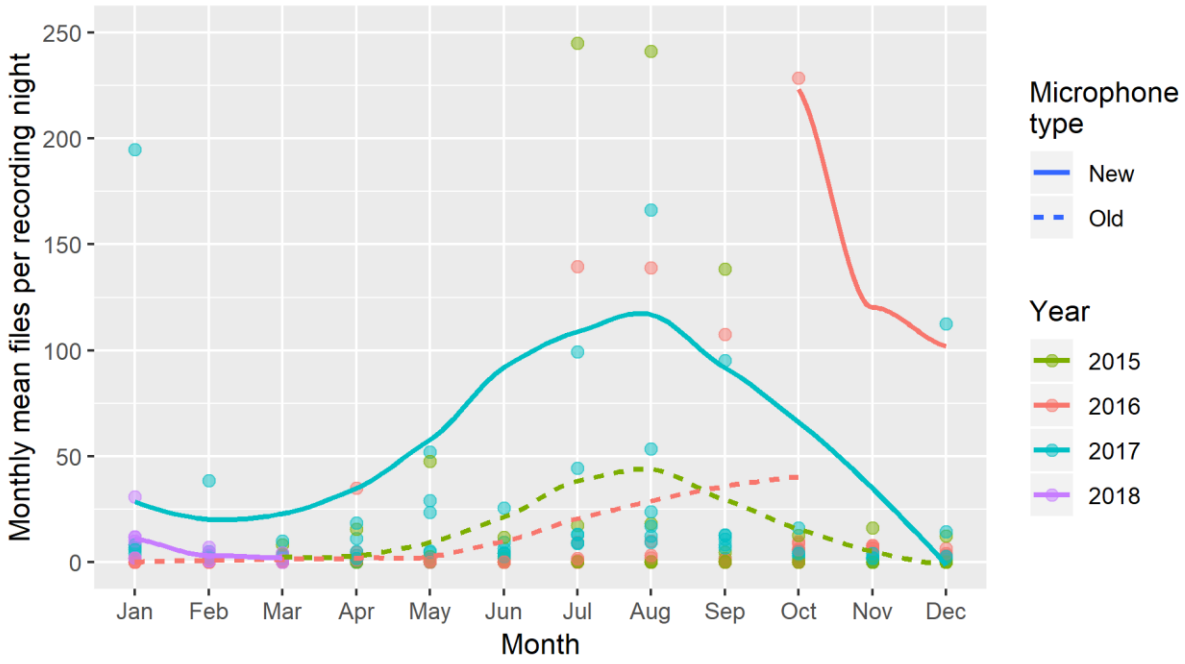

Figure 9. Frequency of bat detection by month from March 2015 to March 2018 in the Waihou Mitigation Area. Frequency is derived from the monthly mean number of nightly acoustic files with bat detections recorded at sites AUW1–AUW7 (points). Overall trend is shown with a LOESS smooth curve weighted by number of sample nights in a month. The change of detector microphones in October 2016 is distinguished by the dashed and solid lines. Periods were determined as the span of months for which detectors were both equipped with new microphones. See Appendix IV for acoustic detection details.

The frequency of bat detections, as measured by the monthly mean number of files per recording night, demonstrated lower within-night rates during winter 2017 compared to winter 2016 in both the monthly and site assessments (Figures 10 and 11; Tables 4 and 5). Log-linear regression (log of monthly mean files per night + 0.5) identified "month + year" as the top model, with 96% of the relative weight assigned by AICc (Table 6). The overall mean number of files were 9.0 per night in winter 2016 and 4.7 per night in winter 2017.

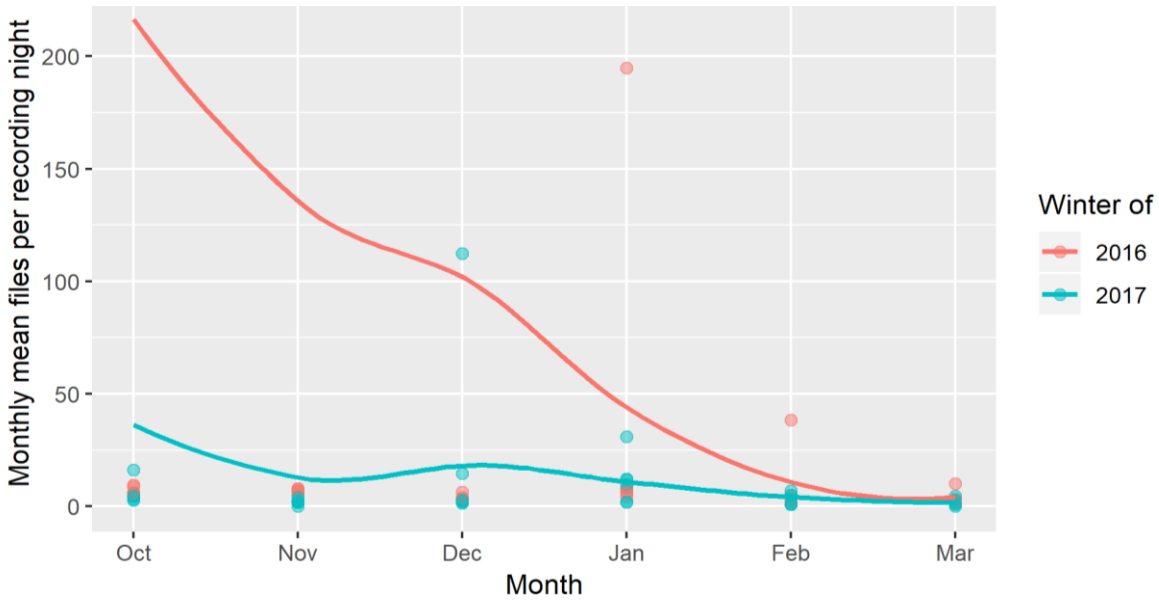

Figure 10. Mean number of nightly bat detections by month for the winters of 2016 and 2017 at sites AUW1–AUW7. Overall trend is shown with a loess smooth curve weighted by number of sample nights/month.

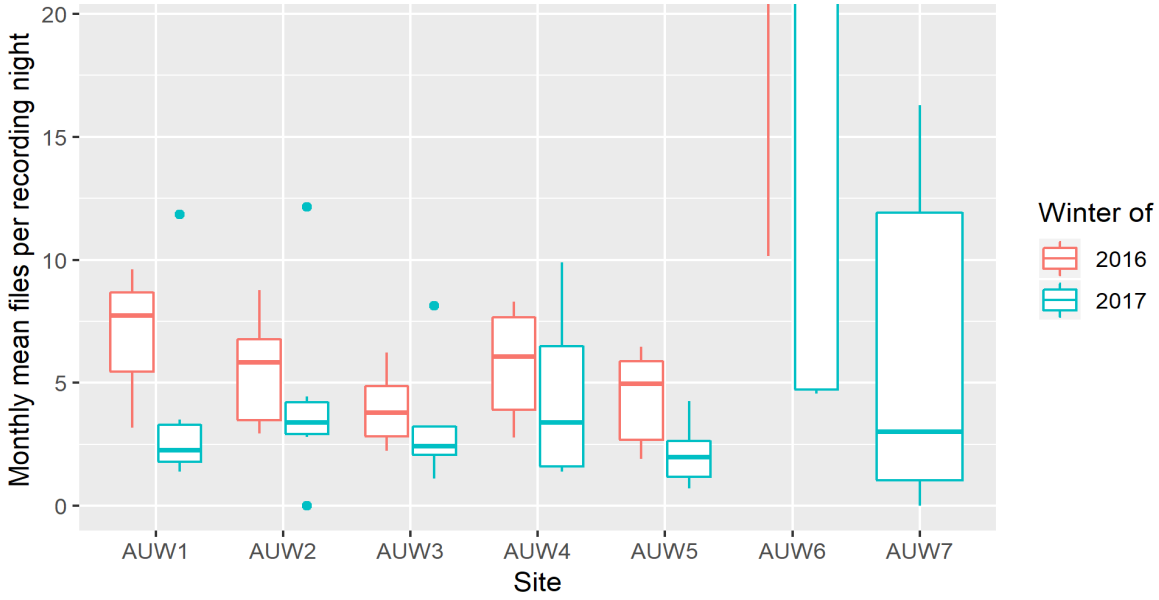

Figure 11. Mean number of nightly bat detections by site for the winters of 2016 and 2017. Boxplot whiskers denote values within 1.5 times the interquartile range above the 75th percentile and below the 25th percentile. To enhance the difference in monthly frequencies the y-axis is truncated at a maximum value of 20 (see Table 5 for mean values).

Table 4. Frequency of nightly bat detections by month for winter 2016 (AUW1–AUW6) and 2017 (AUW1–AUW7) monitoring periods, as measured by the mean number of detections weighted by number of sample nights in a month.

Winter	Oct	Nov	Dec	Jan	Feb	Mar
2016	223.1	120.3	101.9	44.0	10.4	4.1
2017	39.1	2.0	18.0	11.0	3.1	1.9

Table 5. Frequency of nightly bat detection by site for winter 2016 (AUW1–-AUW6) and 2017 (AUW1–AUW7) monitoring periods, as measured by the mean number of files weighted by number of sample nights in a month.

Winter	AUW1	AUW2	AUW3	AUW4	AUW5	AUW6	AUW7
2016	6.8	5.3	3.8	5.8	4.3	421.8	NA
2017	4.0	4.5	3.6	4.6	2.2	95.7	6.8

Table 6. Results of log-linear regression models of monthly mean number of files per recording night (AUW1–AUW6) for winter 2016 and 2017 monitoring periods. The model "month + year" tests for a year effect while controlling for month and site.

Model	Df	AICc	Δ AICc	AICcWt
month + year	5	217.3	0	98%
year	4	226.0	8.7	1%
month	4	227.1	9.8	1%
null	3	231.6	14.3	0%

Bat occurrence, as measured by the percent of nights within a month with at least one bat detection, demonstrated lower nightly prevalence of bats in winter 2017 compared to winter 2016 in both monthly and site assessments (Figures 12 and 13; Tables 7 and 8). Regression analysis identified the model "month + year" as having the best fit, with 100% of the relative model weight assigned to it by AICc (Table 9). The model estimated a significant declining effect for year from 2016 to 2017, such that the chance of detecting bats was 43% for a given night in 2017 relative to 2016.

Foraging activity, as measured by the percent of nights with at least one feeding buzz detection, demonstrated lower nightly foraging activity of bats in winter 2017 compared to winter 2016 in both monthly and site assessments (Figures 14 and 15; Tables 10 and 11). Regression analysis of the frequency of feeding buzz detections during the winters of 2016 and 2017 identified the model "month + year" as having the best fit, with 100% of the relative model weight assigned to it by AICc (Table 12). The model estimated a significant declining detection effect from 2016 to 2017, such that on average the chance of detecting a feeding buzz in 2017 was less than 10% that for a given night in 2016.

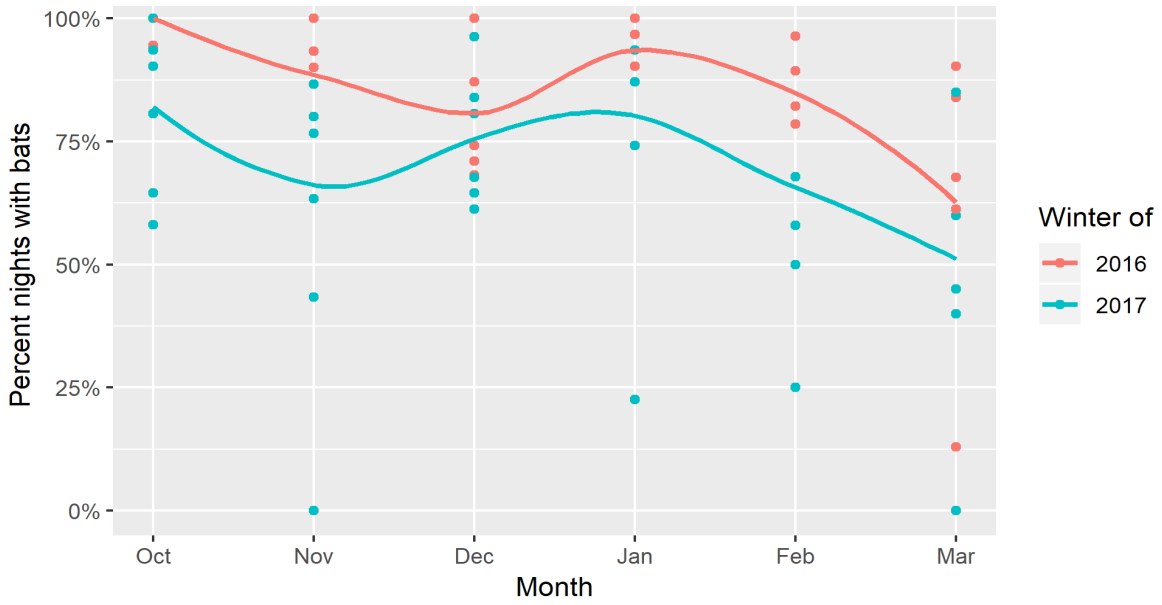

Figure 12. Percent of nights with bat occurrence by month for the winters of 2016 and 2017 at sites AUW1–AUW6. Overall trend is shown with a loess smooth curve weighted by number of sample nights/month.

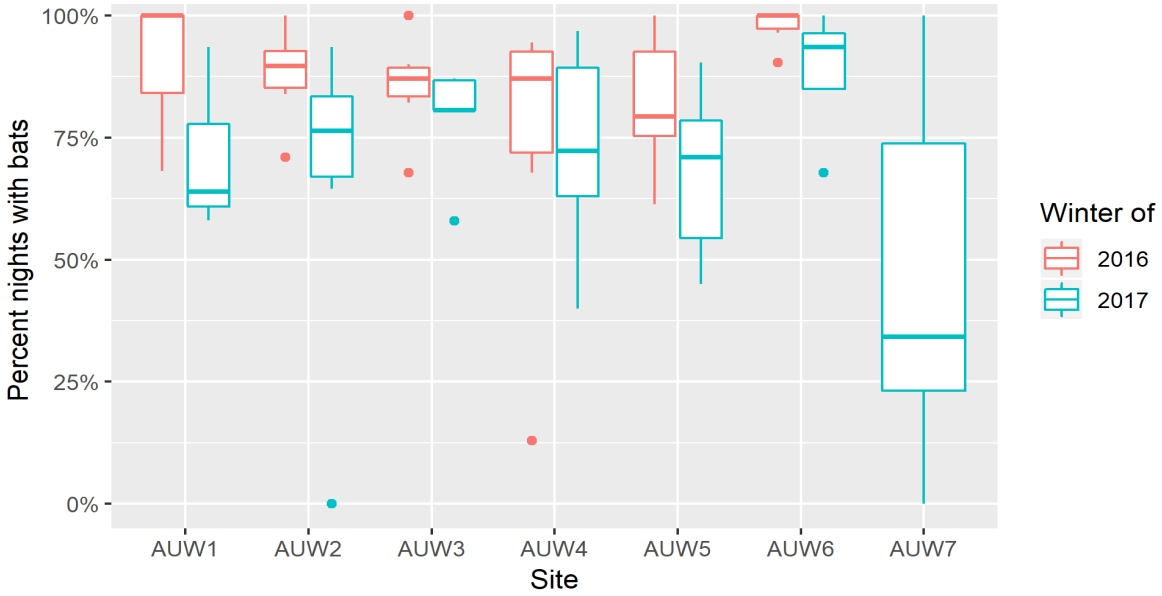

Figure 13. Percent of nights with bat occurrence by site for the winters of 2016 and winter 2017. Boxplot whiskers denote values within 1.5 times the interquartile range above the 75th percentile and below the 25th percentile.

Table 7. Mean percent of nights with bat detections by month for the winter monitoring of 2016 (AUW1–-AUW6) and 2017 (AUW1–AUW7).

Winter	Oct	Nov	Dec	Jan	Feb	Mar
2016	99%	92%	81%	94%	83%	63%
2017	82%	58%	75%	80%	61%	53%

Table 8. Mean percent of nights with bat detections by site for the winter monitoring of 2016 and 2017.

Winter	AUW1	AUW2	AUW3	AUW4	AUW5	AUW6	AUW7
2016	90%	87%	85%	72%	80%	98%	NA
2017	71%	65%	80%	75%	70%	86%	49%

Table 9. Logistic regression models of bat occurrence (determined by one or more acoustic detections) per night and by site for AUW1–AUW6 during the winter monitoring periods of 2016 and 2017. The model "month + year" tests for a year effect while controlling for month and site.

Model	Df	AICc	Δ AICc	AICc Wt
month + year	4	571.5	0	100%
year	3	615.5	44	0%
month	3	673.6	102.1	0%
null	2	702.8	131.3	0%

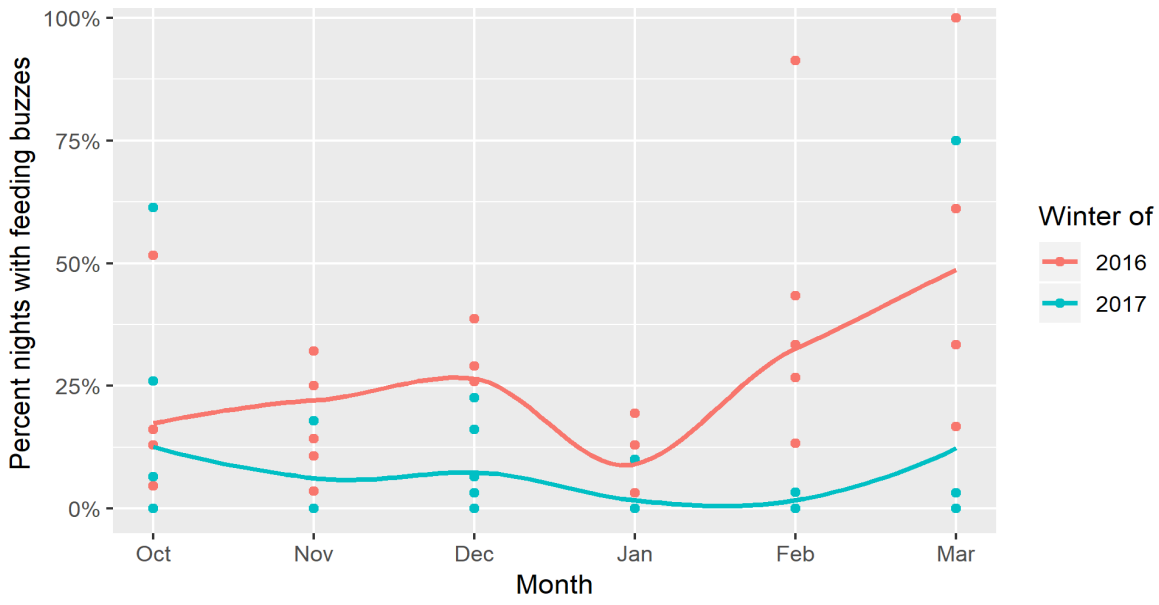

Figure 14. Percent of nights with feeding buzz detections by month for the winters of 2016 and 2017 at sites AUW1–AUW6. Overall trend is shown with a loess smooth curve weighted by number of sample nights/month.

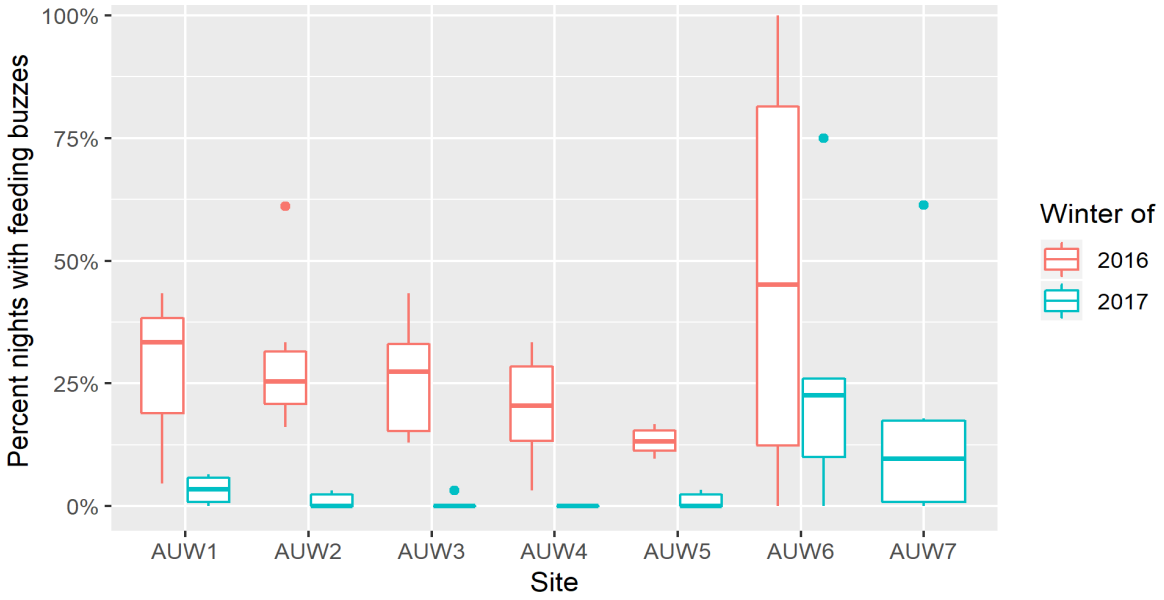

Figure 15. Percent of nights with feeding buzz detections by site for the winters of 2016 and 2017. Boxplot whiskers denote values within 1.5 times the interquartile range above the 75th percentile and below the 25th percentile.

Table 10. Mean percent of nights with feeding buzzes detected by month comparing the winter monitoring periods of 2016 (AUW1–AUW6) and 2017 (AUW1–AUW7).

Winter	Oct	Nov	Dec	Jan	Feb	Mar
2016	46%	40%	19%	26%	17%	9%
2017	4%	1%	13%	7%	3%	2%

Table 11. Mean percent of nights with feeding buzzes detected by site comparing the winter monitoring periods of 2016 and 2017.

Winter	AUW1	AUW2	AUW3	AUW4	AUW5	AUW6	AUW7
2016	29%	28%	25%	19%	13%	42%	NA
2017	4%	1%	1%	0%	1%	17%	18%

Table 12. Logistic regression models comparing bat feeding activity (determined by one or more detections of feeding buzz calls) per night and site at AUW1–AUW6 during the winters of 2016 and 2017. The model "month + year" tests for a year effect while controlling for month and site.

Model	Df	AICc	Δ AICc	AICcWt
month + year	4	412.3	0	100%
year	3	457	44.7	0%
month	3	611.7	199.5	0%
null	2	648.7	236.4	0%

The occurrence of multiple bat detections, as measured by the percent of nights within a month with at least one such event, demonstrated low occurrence that averaged 10% for both the winters of 2016 and 2017 (Figures 16 and 17; Tables 13 and 14). Note, however, that percent values of multiple bat detections for some months were occasionally large; these indicate months for which relatively few nights of sampling were available. Regression estimated a modest but not significant declining effect from 2016 to 2017 such that on average the chance of detecting multiple bats in 2017 was about 80% of that for a given night in 2016 (Table 15).

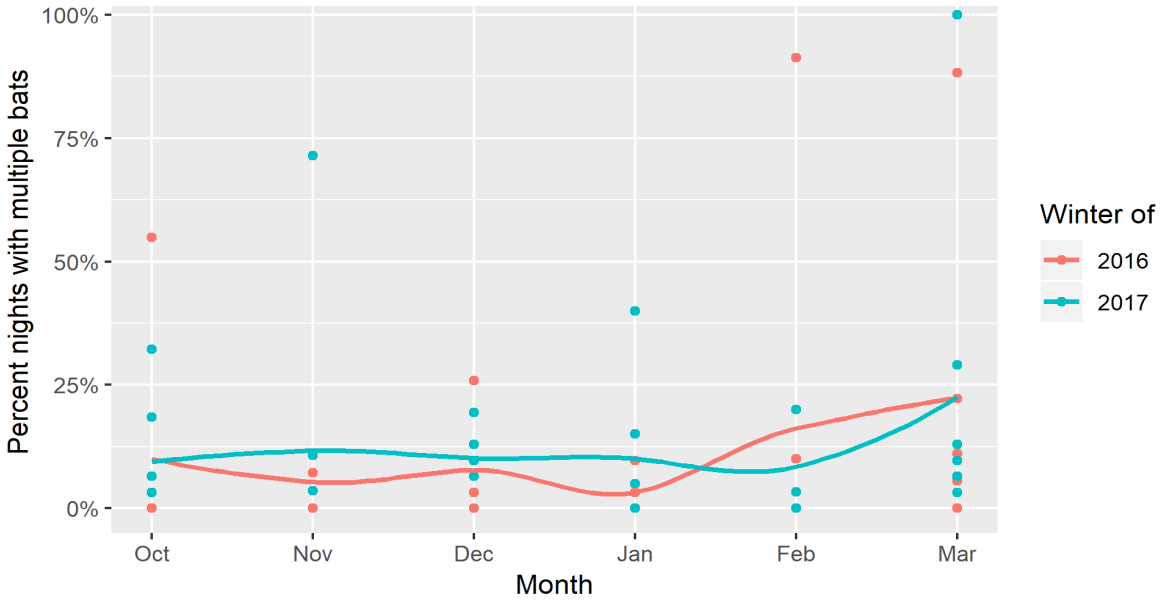

Figure 16. Percent of winter nights with multiple bats detections for the winters of 2016 and 2017 at sites AUW1–AUW6. Monthly trends are shown with LOESS smooth curves weighted by number of sample nights/month. Large values generally indicate months for which relatively few nights of sampling were available.

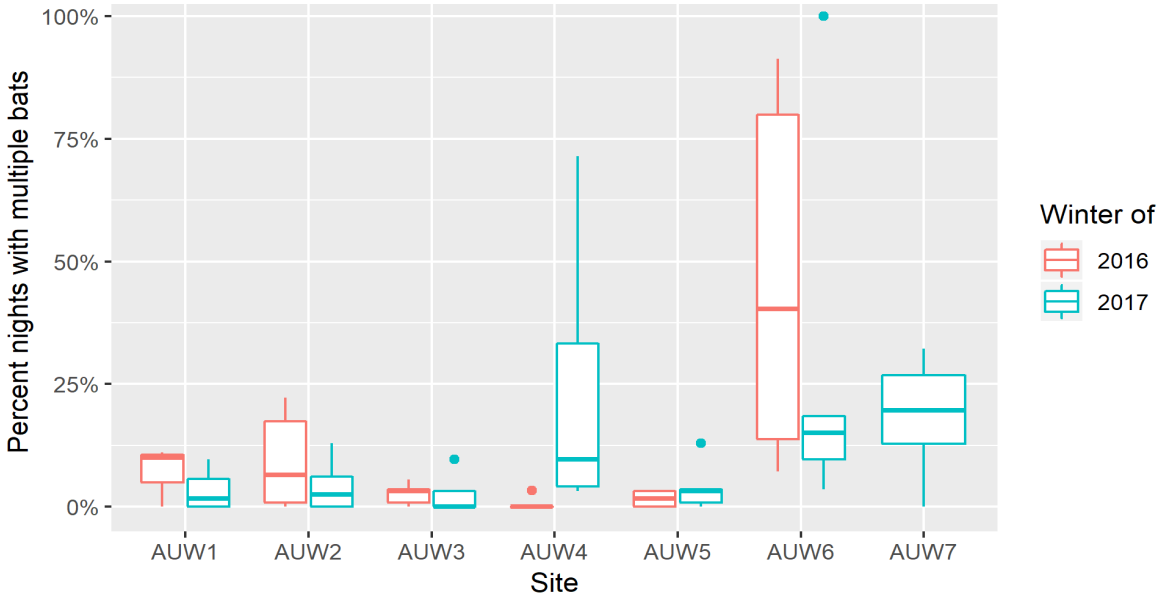

Figure 17. Percent of winter nights with multiple bats detections by site for the winters of 2016 and 2017. Boxplot whiskers denote values within 1.5 times the interquartile range above the 75th percentile and below the 25th percentile.

Table 13. Mean percent of winter nights from 2016 (AUW1-AUW6) and 2017 (AUW1-AUW7) with multiple bats detected by month.

Winter	Oct	Nov	Dec	Jan	Feb	Mar
2016	11%	2%	8%	3%	18%	21%
2017	9%	13%	10%	10%	4%	13%

Table 14. Mean percent of winter nights from 2016 and 2017 with multiple bats detected by site.

Winter	AUW1	AUW2	AUW3	AUW4	AUW5	AUW6	AUW7
2016	7%	8%	2%	1%	2%	41%	NA
2017	4%	4%	3%	21%	4%	15%	20%

Table 15. Logistic regression model results of winter nights from 2016 and 2017 with multiple bats detections per night and site for AUW1-AUW6. The model "month" controls for both a year and site effect, and "month + year" tests for a year effect while controlling for month and site.

Model	Df	AICc	Δ AICc	AICcWt
month	3	458.4	0.0	74%
month + year	4	460.5	2.1	26%
null	2	474.8	16.4	0%
year	3	476.8	18.4	0%

Bat Capture

Bats were captured by mist-net in the Waihou Mitigation Area during fall and summer months (Table 16). In 2016, nets were deployed for 13 nights for a total of 1,630 net-hours (averaging 125 net-hours and 30 meters of net/night) and produced a bat capture rate of 0.0025 bats/net-hour. Three adult male bats were captured adjacent to the pond at the net site CABIN (Figure 18). The male captured on November 3 was recaptured on November 15, over the pond where it was previously caught. None of the males exhibited externally visible signs of enlarged testes that is indicative of spermatogenesis. Fecal pellets were collected from two of these males for dietary analysis.

In 2017, nets were deployed for 20 nights for a total of 2,075 net-hours (averaging 104 net-hours and 24 meters of net/night) and resulted in a capture rate of 0.0039 bats/net-hour. Eight adult bats were captured in mist nets: five males and three females (Figure 19). There were no recaptures of marked bats during this effort. Two female bats were pregnant, confirming presence of reproductive females in the vicinity of the Waihou Mitigation Area. None of the males exhibited visible signs of enlarged testes. Fecal pellets were collected from five of these individuals (Table 16).

Insect Abundance

Insect captures at the Waihou Mitigation Area consisted almost entirely of Lepidoptera. The small number of Coleoptera collected included Coccinelidae (ladybugs), Curculionidae (weevils) and Elateridae (click beetles), and comprised <0.1% of the malaise and light trap samples in late autumn, with none collected in early summer by either method. In late autumn 3,697 and 709 individual lepidopterans were collected in the malaise and light traps, respectively (Appendix III). In early summer, malaise and light traps captured 1,356 and 687 Lepidoptera. Lepidoptera abundance and composition as measured by capture rates differed among the four sites and among seasons (Figure 20; Appendix III). Undetermined lepidopteran taxa (mostly <10 mm in body length) comprised the majority of the samples, ranging from 45% in early summer to 48% in late autumn at the PINE site. Samples identified to family or superfamily that made up >5% of overall samples included Crambidae, Erebidae, Gelechioidea, Geometridae, Noctuidae, Tineidae and Tortricidae. Noctuidae (owlet and miller moths) and Tortricidae (tortrix or leaf roller moths) together represented 40% of the late autumn captures and were most abundant at the REST site. In early summer the composition was dominated by Geometridae (geometer moths) and Noctuidae (52% of total). Gelechioidea were relatively abundant at all

Table 16. Bats captured in the Waihou Mitigation Area, November 2016 and June–July 2017.

Bat ID	Date	Time	Location	Sex	Weight (g)	Forearm (mm)	Band	Fecal	Reproductive condition
M47	11/3/2016	18:50	POND1	male	14.0	47.8	green/white	yes	testes not enlarged
M48	11/15/2016	19:36	POND1	male	16.5	49.0	orange	yes	testes not enlarged
M49	11/28/2016	21:00	POND1	male	15.8	48.5	blue	yes	testes not enlarged
M50	6/20/2017	21:00	CABIN	male	17.5	47.5	purple	yes	testes not enlarged
M51	6/20/2017	21:05	POND1	male	15.5	47.4	blue/red	yes	testes not enlarged
M52	6/22/2017	21:35	POND2	female	24.3	50.0	red/white	no	pregnant
M53	6/26/2017	20:05	POND1	male	19.3	49.0	yellow/orange	no	testes not enlarged
M54	6/26/2017	22:05	POND1	female	21.8	50.2	yellow/green	yes	lactating
M55	6/28/2017	22:08	POND2	male	18.0	46.5	white	yes	testes not enlarged
M56	7/3/2017	22:10	POND2	male	19.0	49.2	red/green	no	testes not enlarged
M57	7/4/2017	22:13	POND2	female	23.5	50.3	green	yes	pregnant

Figure 18. Adult Hawaiian hoary bats captured in 2016; male, bat M47 (top); male, bat M48 (bottom).

Figure 19. Adult Hawaiian hoary bats captured in 2017; male, bat M55 (top); female, bat M54, showing prominent nipples (bottom).

sites during this period, with Geometridae primarily caught at the PINE and PUU sites, and Noctuidae most numerous at the REST site. Although absent in the late autumn sample, large-bodied Erebidae were relatively abundant in early summer at the REST site (22% of the sample in this period).

During October and November 2016, and June 2017, 69 caterpillars were collected from seven host plants (ʻaʻaliʻi, koa, māmane, naio, redwood, black wattle and Monterey pine). Fifty-seven of these caterpillars emerged as adults or were identifiable to genus as caterpillars (Table 17). An unidentified species of the endemic *Scotorythra* genus (Geometridae) was the most common moth and occurred on the widest range of host plants sampled, including invasive black wattle, that is widespread in part of the study area. The non-native *Amorbia emigratella* (Tortricidae) was the second most common species and was reared from ʻaʻaliʻi, koa and redwood. The endemic butterfly *Udara blackburni* (Lycaenidae) and indigenous moth *Uresiphita polygonalis* (Crambidae) were collected from ʻaʻaliʻi, koa and black wattle.

Insect Reference Library

Of the initial 70 insect samples, 57 samples produced quality sequences for reference library inclusion (Table 18). Of these 57 samples, 49 were obtained from Lepidoptera, four from Coleoptera, one from Diptera, and two from Hemiptera.

Bat Diet Composition

Eight of the nine guano samples amplified successfully during PCR and were suitable for metabarcoding library preparation. One sample (pregnant female bat) failed to amplify PCR products and could not be sequenced possibly due to its small size (1 µg). We used two primer sets (Zeale, Epp) to evaluate diet composition, and each sample was successfully duplicated (Table 2). We sequenced both Epp and Zeale products in seven samples, however only Epp products were sampled in M55, due to space on the sequencing plate and the need to include blanks (Table GUANO). Blanks are necessary to check for artificially introduced contamination (such as in reagents) during the sequencing process, our blanks returned no concerns for contamination.

Using the Zeale primer, 145 arthropod OTUs were identified, while 14 OTUs were identified with the Epp primer. Thus, we identified 42 unique prey taxa from seven arthropod orders and 32 families (excluding three additional unknown families; Table 19, Figures 21 and 22). The taxa identified with Zeale data when considering single and duplicate sample OTU recoveries comprised all seven orders and 32 families, including 15 Lepidoptera families. OTU identifications with BOLD for Zeale data provided species confirmation for 13 species. Epp data identified three orders and 11 families, including eight Lepidoptera families. Gracillariidae, Momphidae [Batrachedridae], and Pyralidae were detected solely with Epp primers. Families that were confirmed by both primer sets included Nitidulidae and Scarabaeidae among Coleoptera, Cydnidae (Hemiptera), and among Lepidoptera, Crambidae, Hesperiidae, Noctuidae, Oecophoridae, Tortricidae. We also detected in the guano samples of Hawaiian hoary bats, fleas (Order Siphonaptera, Ceratophyllidae, *Orchopeas caedens*), freshwater ostracods (Order Podocopida, Cyprididae, Heterocypris sp.), and parasitic nematodes (order Rhabditida, Rhabditidae) that are not likely prey taxa in the bat's diet. These were removed from prey analysis but may warrant further study.

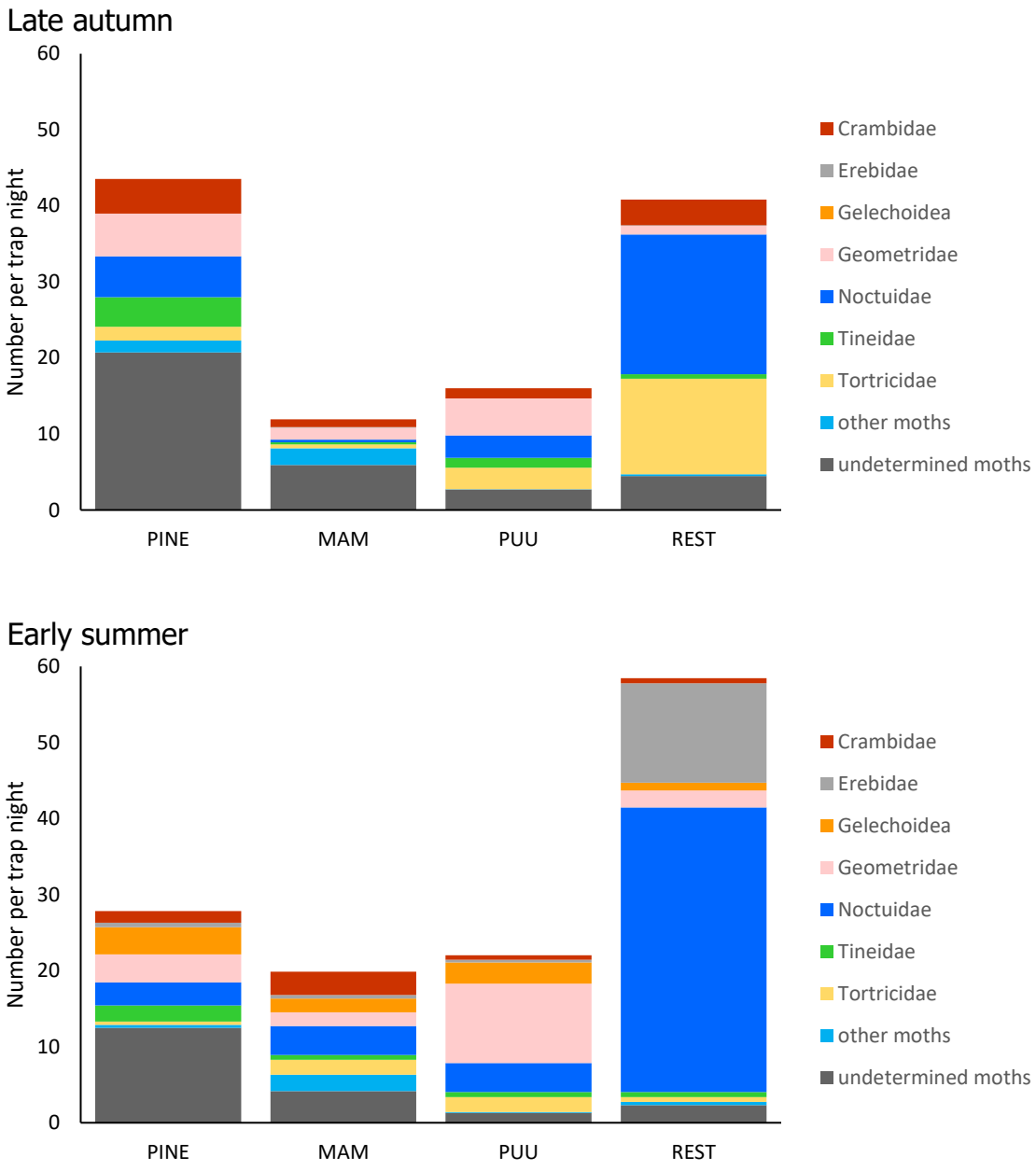

Figure 20. Site capture rates for Lepidoptera in late autumn (October 25–December 7, 2016) and early summer (June 7–July 3, 2017). Counts are adjusted for sampling effort from combined malaise and light traps for each pair of site samples. For graphical clarity, Lepidoptera families and superfamilies that comprised <5% of the overall seasonal sample were included as "other moths". Counts, sampling effort and capture rate details are presented in Appendix III.

Table 17. Lepidoptera reared from caterpillars collected on host plants in 2016 and 2017.

	Host plant					
Lepidoptera species	ʻaʻaliʻi	koa	māmane	naio	redwood	black wattle
Amorbia emigratella	11	1	0	0	5	0
Scotorythra sp.	15	7	0	5	0	4
Udara blackburni	3	1	0	0	0	2
Uresiphita polygonalis	0	0	3	0	0	0
Total	29	9	3	5	5	6

After filtering, Zeale raw reads (2,289,427 total) were reduced to 21,321 reads, and Epp raw reads (1,232 total) reduced to 262 reads. After removing chimeric sequences, the number of unique OTUs was reduced to 158 (0.74% of unfiltered reads) for Zeale and 14 (5.34% of unfiltered reads) for Epp. The mean number of OTUs detected per individual bat was 26.8 ± 19.0 using Zeale primers, and 4 ± 3.1 using Epp primers. Dietary composition by order and family as determined by Zeale data from duplicate samples was relatively similar among the seven bats captured at the Waihou Mitigation Area (Figure 23). At the order level, OTUs belonging to Lepidoptera were found in a preponderance of the diets of individual bats and accounted for the highest percentages of sequence counts (Figure 21). All other orders occurred in fewer than half of the bats. Coleopterans were identified in only three of the individuals, accounting for less than 20% of the Zeale sequence counts. The lepidopteran families Crambidae and Geometridae were found in all individual bats; whereas Noctuidae, Oecophoridae, Tortricidae, Xyloryctidae were identified in about three-quarters of these individuals (Figure 22). Other families comprised smaller proportions of Zeale-derived sequence counts (i.e., Crambidae and Oecophoridae ~40%, Noctuidae ~20% of counts). Although geometrids occurred in all the sampled bats, it accounted for ≤ 10% of the total sequence counts. The diet of the female captured in June included the highest number of lepidopteran families identified, and also included the dipteran families Culicidae (mosquitos) and Muscidae (flies) (Figure 23). In November, the diet of the three males captured in the Waihou Mitigation Area, was composed almost exclusively of Lepidoptera. The female bat found in August under a turbine at the Auwahi Wind Energy facility, had the most diverse diet of the eight bats examined, including all six orders identified by PCR. About a third of OTUs in this sample were from the dung beetle *Digitonthophagus gazella* (Coleoptera, Scarabaeidae).

Although ground-based insect traps and airborne bats may only mutually sample a fraction of the available prey, the captured insects used to develop the reference library were well represented at the order- and family-levels in the bat diet analysis. Four of the six orders and 11 of the 16 families captured were also present in guano samples (Tables 18 and 19). Furthermore, eight out of 29 genus-or species-level assignments matched that found in the bat diet.

Table 18. List of order, family, subfamily or genus, and species (where available) for insects barcoded for inclusion in the reference library. Taxa that may include species endemic to Hawai'i are denoted with an asterisk (*). Taxa included in the insect reference library samples and also found in bat diet samples (see Table 19) are indicated with a dagger (†).

Order	Family	Subfamily or Genus and Species	Individuals barcoded
Coleoptera	Hydrophilidae	*Sphaeridium scarabaeoides*	1
	Scarabaeidae †	*Aphodiinae* sp.	1
		Digitonthophagus gazella †	1
	Staphylinidae	*Philonthus* sp.	1
Diptera	Sepsidae	*Sepsis thoracica*	1
Hemiptera	Cydnidae†	*Pangaeus bilineatus*	1
	Lygaeidae	*Lygaeidae* sp.	1
Lepidoptera	Carposinidae †	*Carposina* sp.*	1
	Cosmopterigidae †	*Hyposmocoma* spp.*	5
	Crambidae †	*Omiodes* spp.*	4
		Udea sp.	2
		Uresiphita polygonalis *	1
		unknown sp.	2
	Erebidae †	*Melipotis indomita*	1
		Schrankia sp.	3
	Geometridae †	*Eupithecia* sp.*	2
		Scotorythra spp.*	7
		unknown sp.	1
	Noctuidae †	*Athetis thoracica*	3
		Chrysodeixis eriosoma	1
		Feltia subterranea †	1
		Ophiusa disjungens	1
		Peridroma saucia †	3
		Pseudaletia unipuncta †	1
		Spodoptera exempta	1
		unknown sp.	1
	Tineidae	*Opogona sacchari*	1
	Tortricidae †	*Acleris* spp.	2
		Amorbia emigratella	2
		Crocidosema sp. †	1
		unknown sp.	1
	Sphingidae †	*Hyles lineata*	1
	Xyloryctidae †	*Thyrocopa* sp.*	1

Table 19. List of prey items and associated information identified in Hawaiian hoary bat guano from the Waihou Mitigation Area and Auwahi Wind Energy facility. Order and family identifications are based Zeale and/or Epp primers sequencing, although and genus and species level identifications are based only on Zeale primer data. Total counts of operational taxonomic units (OTUs) by primer are noted in columns "s" if an item occurred in only one of a duplicate sample set, or under "d" if it's occurred in both samples. OTU counts apply only to the family level. Family and genus-level taxa that include species endemic to Hawai'i are denoted with an asterisk (*). Taxa found in bat diet samples included in the insect reference library samples are indicated with a dagger (†).

Order	Family	Zeale s	Zeale d	Epp s	Epp d	Genus	Species	Element or Vegetation
Blattodea	Kalotermitidae	1	3	0	0	*Neotermes*		wood
Coleoptera	Corylophidae *	1	0	0	0	*Sericoderus* *		fungal spores
	Nitidulidae	0	1	1	0	*Phenolia*		decaying fruit
	Scarabaeidae †	0	5	0	3	*Digitonthophagus* †	*gazella* †	mammal dung
Diptera	Cecidomyiidae *	1	0	0	0			various plants
	Culicidae	0	3	0	0			nectar, blood
	Muscidae *	3	1	0	0	*Coenosia*		insect predators
	Tachinidae	0	1	0	0	*Eucelatoria*	*armiger*	insect parasitoids
	Sarcophagidae	1	0	0	0	*Blaesoxipha*	*plinthopyga*	carrion
	unknown	0	2	0	0			
Ephemeroptera	unknown	1	0	0	0			associated with water
Hemiptera	Cicadellidae *	1	1	0	0			various plants
	Pentatomidae †	1	2	1	0	*Nezara*	*viridula*	legumes, macadamia
						Piezodorus		legumes
	unknown	2	0	0	0			
Lepidoptera	Blastobasidae	1	0	0	0	*Blastobasis*		legumes
	Carposinidae *†	0	1	0	0	*Carposina* *†		unknown
	Coleophoridae	0	3	0	0	*Coleophora*		thistle
	Cosmopterigidae *†	0	7	0	0	*Pyroderces*		decaying vegetation
	Crambidae *†	1	32	3	6	*Nomophila*	*noctuella*	grasses

Order	Family	Zeales	Zealed	Epps	Epped	Genus	Species	Element or Vegetation
						Nomophila sp.		various plants
						Herpetogramma	*licarsisalis*	grasses
	Erebidae †	2	2	0	0	*Hypena*		grasses
	Gelechiidae *	6	3	0	0	*Dichomeris*		sourbush (*Pluchea*)
	Geometridae *†	5	15	0	0	*Eupithecia *†*		predatory, native plants
	Gracillariidae	0	0	2	2			various plants
	Hesperiidae	0	1	2	0			various plants
	Lycaenidae *	0	3	0	0			various plants
Lepidoptera (continued)	Momphidae (Batrachedridae)	0	0	0	1			various plants
	Noctuidae *†	11	46	2	0	*Athetis †*		grasses
						Feltia †	*subterranea* †	various plants
						*Peridroma *†*	*saucia* †	various plants
						*Pseudaletia *†*	*unipuncta* †	various plants
	Oecophoridae *	18	31	3	2			decaying vegetation
	Pyralidae *	0	0	2	0			various plants
	Sphingidae †	1	0	0	0			various plants
	Tortricidae *†	2	11	3	0	*Cryptophlebia*		fruit, seeds
						Crocidosema †	*lantana*	*Lantana camara*
	Xyloryctidae *†	6	12	0	0			decaying vegetation
	Gryllidae *	1	0	0	0	*Gryllus*	*bimaculatus*	various plants
Orthoptera	Tettigoniidae *	0	1	0	0	*Conocephalus*		various plants
	Trigonidiidae *	1	0	0	0	*Trigonidomorpha*	*sjostedti*	various plants

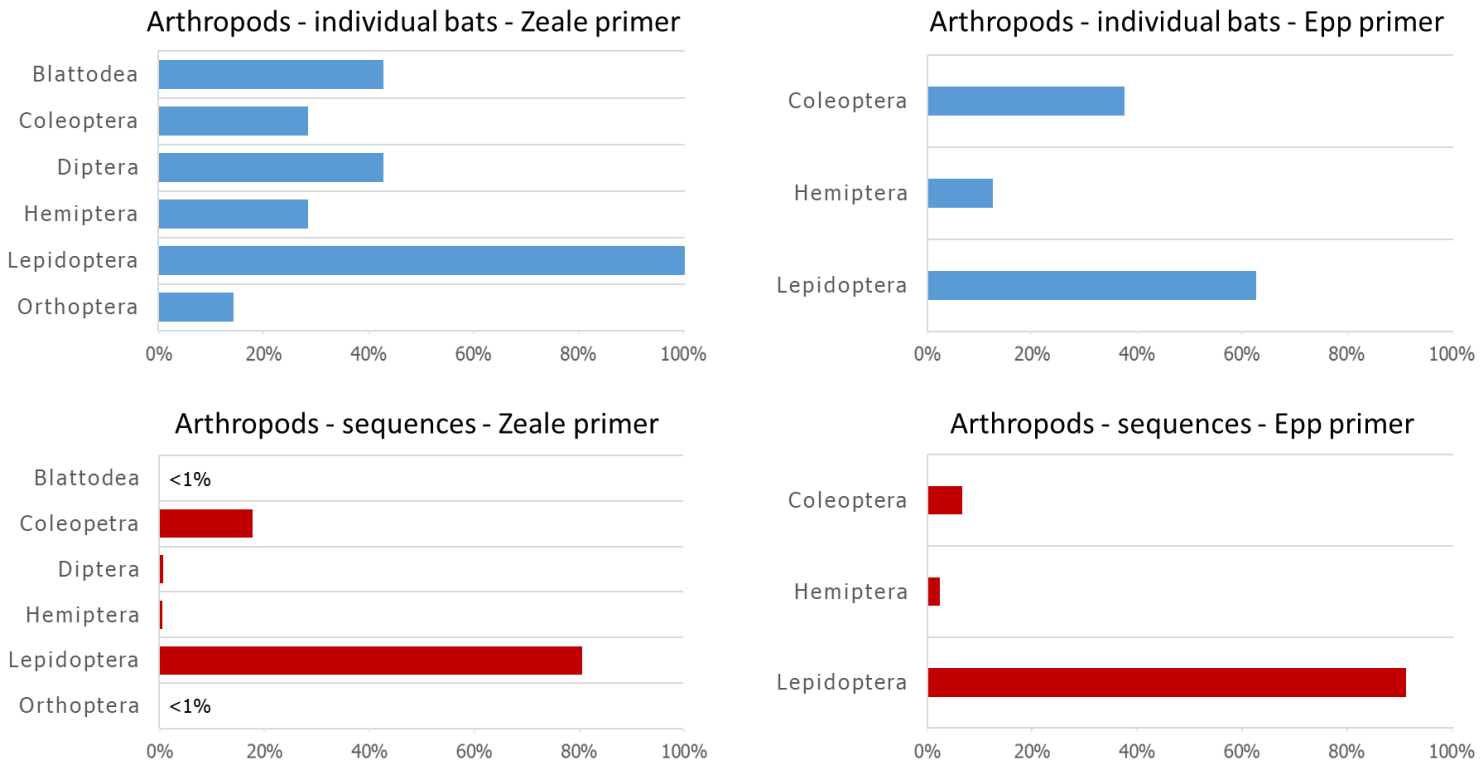

Figure 21. Proportion of arthropod orders identified in the Hawaiian hoary bat guano samples by prevalence (number of individual bats in which orders were detected; upper panels) and sequence occurrence (number OTUs; lower panels) using Zeale and Epp primers (left versus right panels). Values noted as "<1%" indicate non-zero counts.

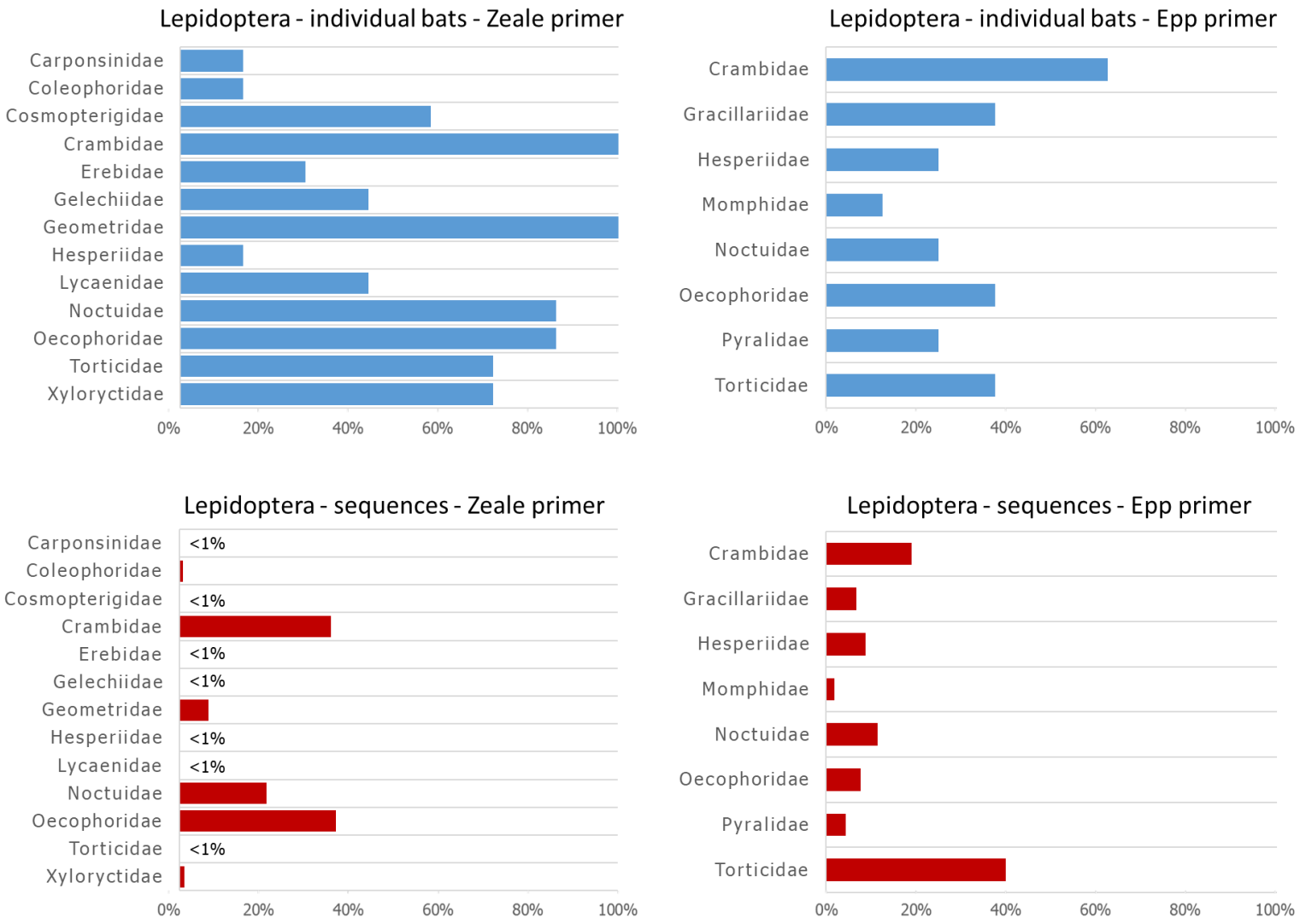

Figure 22. Proportion of Lepidoptera families identified in the Hawaiian hoary bat guano samples by prevalence (number of individual bats in which families were detected; upper panels) and sequence occurrence (number OTUs; lower panels) using Zeale and Epp primers (left versus right panels). Values noted as "<1%" indicate non-zero counts.

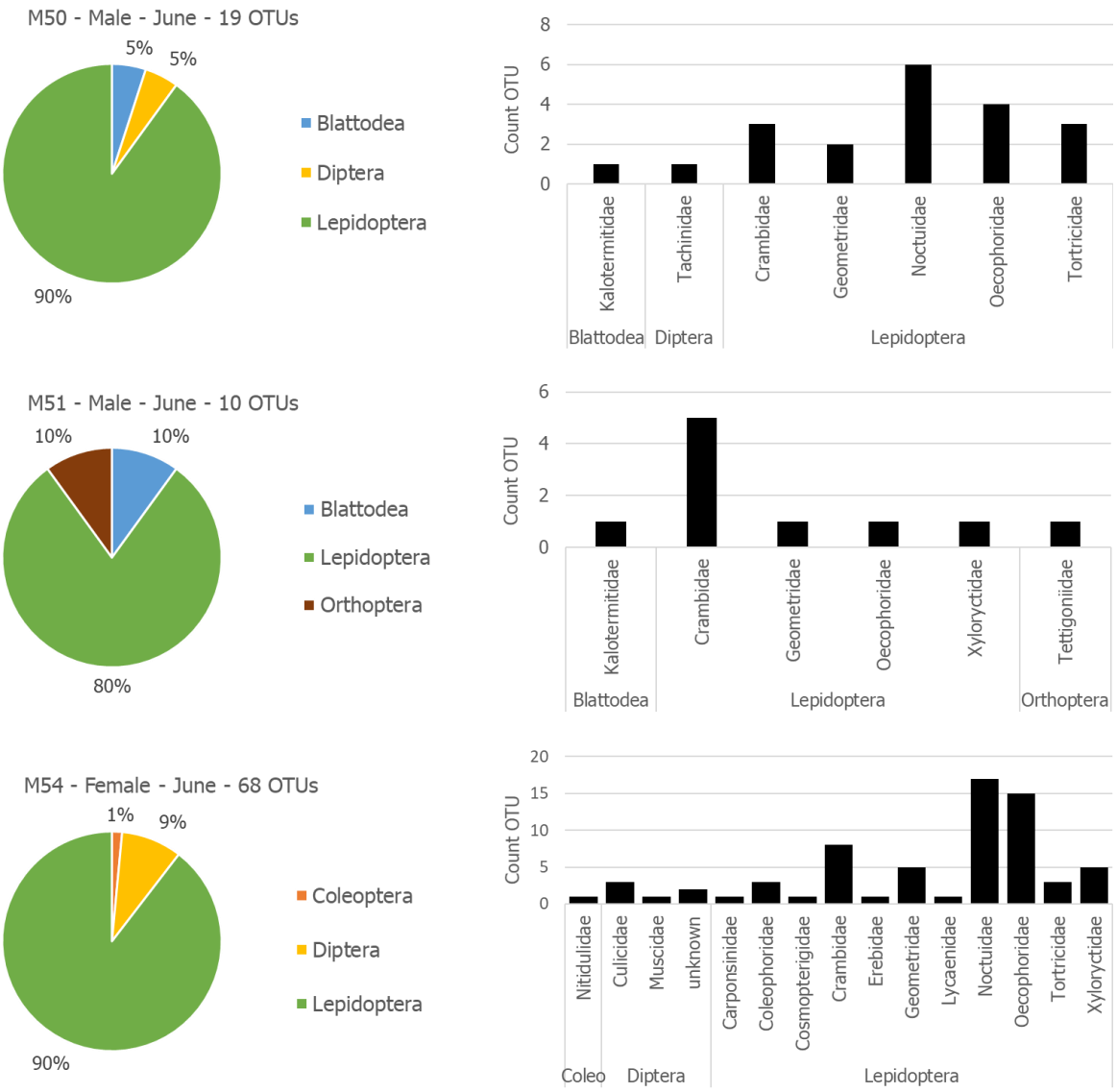

Figure 23. Diet composition and proportion of OTUs by order and family recovered from both members of Zeale primer samples for individual bats.

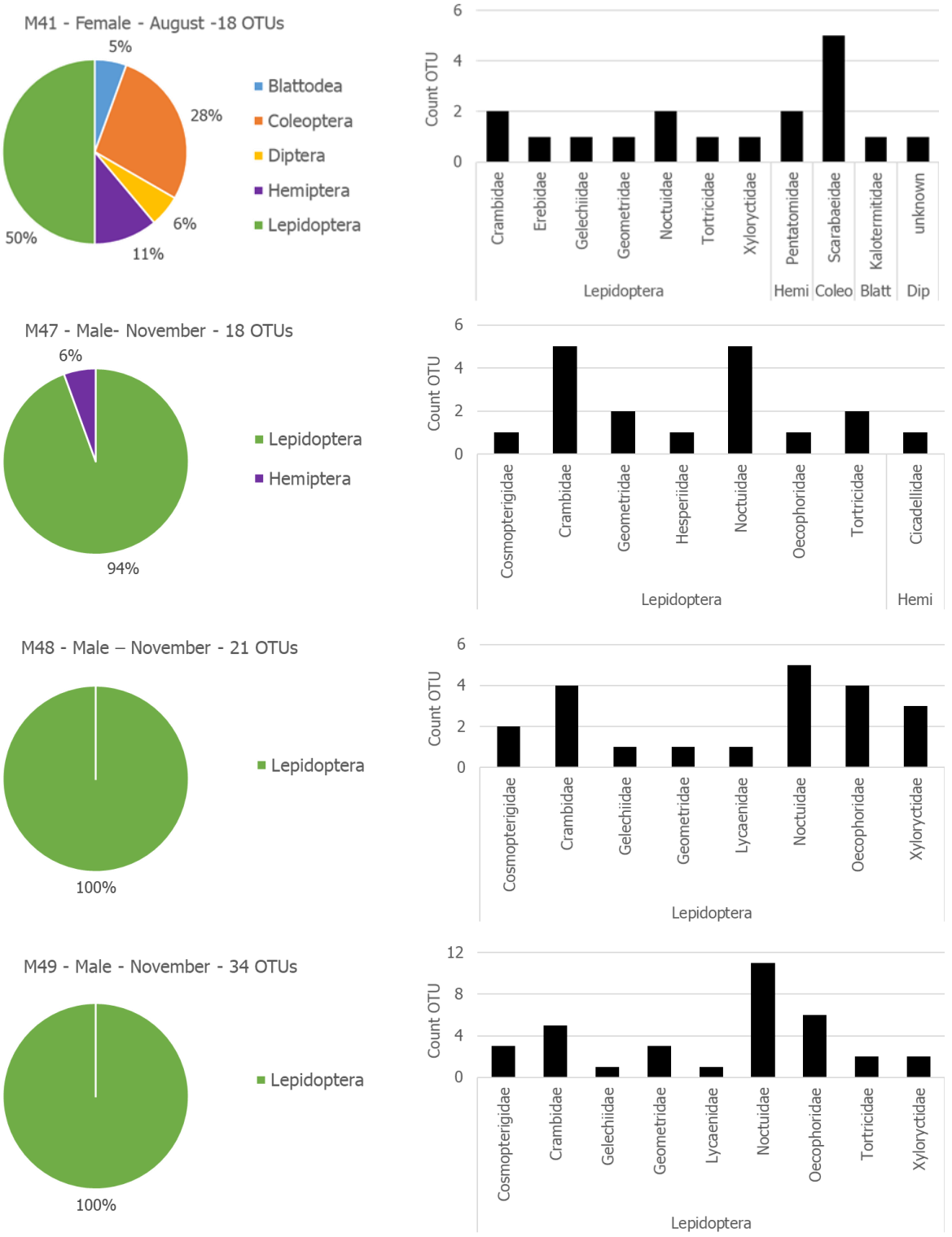

Figure 23 (continued).

DISCUSSION

This study included a three-year acoustic survey of bat occurrence and seasonal activity obtained from sampling echolocation calls and provides information on the sex, age, and reproductive status of individual bats present in the Waihou Mitigation Area (inclusive of the Pu'u Makua Restoration Area). Additionally, this is the first study to report with genetic analysis, the diet of the Hawaiian hoary bat in relation to potential prey availability.

Acoustic surveys confirm the year-round occurrence of Hawaiian hoary bats in the Waihou Mitigation Area throughout the three years of sampling. Hawaiian hoary bats exhibited a seasonal pattern of presence similar to that previously observed on the islands of Hawai'i (Gorresen *et al.* 2013) and O'ahu (Starcevich *et al.* 2019). These patterns showed peak acoustic activity between May and November, broadly encompassing the portion of the annual cycle that includes pregnancy, lactation, and fledging/post-lactation periods. During this time adult females can be expected to have their highest energy demands and efforts needed to forage to support reproductive activities. Thus, higher acoustic detection rates during those periods also may be attributable to increased foraging associated with greater insect availability, a trend indicated by the larger number of Lepidoptera trapped during summer and autumn. Acoustic detections during the autumn likely reflect the contribution of newly volant juveniles that accompany mothers in tandem flight during the first months of foraging (Hickey and Fenton 1990, Pfalzer and Kusch 2003).

Between-year comparisons demonstrated lower acoustic detection in 2017 relative to 2016 for all site and monthly metrics (i.e., mean call files recorded each night, percent of nights with at least one detection, percent of nights with at least one feeding buzz detected, and percent of nights with recordings of multiple bats). However, considerable inter-annual variability is typical of acoustic surveys (e.g., Gorresen *et al.* 2013, Rodhouse *et al.* 2012), and inference about trends are generally not possible from short-term datasets (i.e., <4 years). In addition, acoustic sampling is prone to imperfect detection for both methodological and biological reasons. For example, detection may be affected by factors associated with the propagation and detection of sound, cryptic foraging strategies, and conspecific presence (Gorresen *et al.* 2017). Furthermore, bat detectability may be affected by stochastic seasonal or weather related factors such as precipitation and wind velocity. Additional periodic or long-term acoustic monitoring of the Waihou Mitigation Area could more effectively assess resident bat trends and responses to management aimed at forest restoration and habitat enhancement.

Bat captures over an 8-month span confirmed 11 individuals, two of which were pregnant and one that was lactating, thus highlighting the importance of the area to resident and breeding individuals. The relatively high acoustic activity in the upper Waihou area at sites AUW6 and AUW7 also included a high proportion of nights with multiple bat detections. Moreover, the capture of eight adult males over two sampling periods spanning eight months and within the limited area suitable for netting bats, indicated a degree of co-occurrence as-yet not observed elsewhere. It is particularly notable given the typically agonistic behavior and structured use of foraging space by adult males (Bonaccorso *et al.* 2015), and the fact that agonistic interactions may occur among insectivorous bats when prey is scarce (Barlow and Jones 1997). The presence of multiple adult males suggests either that prey was not limiting during the period of survey, and/or the area supports a limited open water resource requiring a degree of mutual tolerance among bats, perhaps facilitated by a high rate of temporal-spatial turnover of individual bats (i.e., bats using the area for short periods of time).

The high acoustic activity recorded in the upper Waihou area is difficult to attribute to any single factor, as the area includes ponds, tall trees and is located close to larger tracts of contiguous forest at higher elevations. Although these features were largely absent in the immediate surroundings at the other acoustic sampling sites, the proximity of the sample sites and the broad extent of bat home ranges precludes any inference about selective use of relatively small areas such as the Pu'u Makua Restoration Area. However, the detection of feeding buzzes at all sampling sites does indicate that the entire area is used by foraging individuals.

The genetic analysis of Hawaiian hoary bat diet from samples collected in the study area confirms the major arthropod orders (Coleoptera, Lepidoptera, Diptera, Hemiptera, and Blattodea) found in previous studies that used dissection and microscopy of guano samples to identify prey items (Whitaker and Tomich 1983, Todd 2012, Bernard and Mautz 2016). However, genetic analysis of guano samples may afford greater taxonomic resolution and better prey identification of soft-bodied insects such as moths and flies than does morphological methods. For example, the hard carapaces of beetles may differentially survive digestion resulting in its over-representation in morphological analysis, while the taxa with soft parts are under-represented (Clare *et al.* 2009, 2014).

Genetic analysis showed Lepidoptera as the primary component in the diet of bats captured at the Waihou Mitigation Area, both in terms of its ubiquity among individuals (100%) and the mean proportion of Zeale sequence counts recovered (92%), the latter of which is potentially indicative of the amount of biomass consumed (Deagle *et al.* 2019). This finding was also supported by the results for Epp primer data. Two guano samples were entirely composed of moths, and the remaining four bats from this same area included only one to two other orders with relatively low OTU counts. A notable exception was the sample from bat M41, the fatality from the Auwahi Wind facility 7 km to the south and located in low elevation dryland habitat. The guano of M41 contained 50% moths but also included Coleoptera, Diptera, Hemiptera, and Blattodea. Although difficult to speculate about habitat-dependent effects on insect availability, it is possible that the feeding of bat M41 reflected either a more diverse local assemblage of insects, or its foraging over a large multi-habitat area on the night sampled. As the two fecal samples from females contain Coleoptera and Diptera, it may also indicate dietary preference for prey that are easier targets than fast flying moths. Previous studies have suggested that pregnant or lactating females (and juveniles) preferentially select less-maneuverable prey such as beetles (Anthony and Kunz 1977, Bellwood and Fenton 1976, Bellwood and Fullard 1984, Brack and LaVal 1985, Valdez and Cryan 2009). Moreover, some noctuid and geometrid moth species have evolved the capacity to hear bat echolocation calls and engage in evasive flight maneuvers to avoid bats (Fullard 2001). Moths in general may therefore be more difficult to catch compared to beetles, true bugs, and some and other insects. The preponderance of Lepidoptera in the diet of bats at the Waihou Mitigation Area may also reflect overall insect availability as our trapping in the area consisted almost entirely of moths. Other orders made up <0.1% of the insect sampled in late autumn and none were collected in early summer. This pattern of captures differs with the findings of Whitaker and Tomich (1983), which demonstrated a greater proportion of Coleoptera and Hemiptera in the diets of bats collected along the northeast coast of Hawai'i Island at elevations between sea level and 800 m. The pattern of insect composition also differs from that of Gorresen *et al.* (2018) that showed the abundance of Coleoptera (primarily dung beetles) to be significantly associated with areas in which Hawaiian hoary bats concentrated foraging activity at elevations <400 m on O'ahu.

However, the high proportion of moths in our study is consistent with insects collected at high elevations (≥1,200 m) on windward Hawai'i Island (Todd 2012).

The dietary items of Hawaiian hoary bats sampled in this study share some similarities to that of hoary bats in North America (*L. cinereus cinereus*). Guano samples obtained from migrating hoary bats in New Mexico (Valdez and Cryan 2009) and those identified from stomach and intestinal contents of bats collected at wind energy facilities in Texas and New York (Valdez and Cryan 2013, Foo *et al.* 2017) also contain Lepidoptera as the predominant diet item. Other major items common to the diet of both subspecies include Coleoptera, Hemiptera, and Diptera. Orthoptera (specifically *Gryllus spp*.) were found in the stomachs of North American hoary bats (Foo *et al.* 2017), and *Gryllus bimaculatus* (two-spotted cricket) was detected in the Hawaiian hoary bat diet. Notable differences include Blattodea (likely the forest tree termite, *Neotermes connexus*) present in three of the seven Hawaiian hoary bat samples (albeit with a small amount of recovered OTUs), but was not noted in any of the North American studies. Conversely, Hymenoptera and Neuroptera were noted in the diet of *L. cinereus cinereus*, and appear in small proportions in other studies in Hawai'i (Jacobs 1999, Todd 2012), but were not apparent in our samples. The detection of Ephemeroptera (mayflies) and Ostracoda in the guano samples is interesting as these taxa are associated with fresh water, and most of the bats in our study were captured over ponds. The mayflies were likely captured by bats as these insects emerged from ponds, however the presence of ostracods could occur from drinking pond water.

Five dipteran families were present in three guano samples, and although OTU counts indicate that the volume consumed was relatively small, and Dipterans have been reported in the diet of Hawaiian bats, our samples include taxa not previously reported by either Jacobs (1999) or Todd (2012). Culicidae (mosquitos) and Cecidomyiidae (gall midges) found in our study are noteworthy since most species are usually <4 mm in length, a size that may approach the limit at which hoary bats can detect prey with echolocation (Barclay *et al.* 1999). The small size of these insects may be the reason they have been overlooked in previous studies. Although chironomid midges made up a negligible amount of the dipterans consumed by older juvenile or adult hoary bats in North America, it was a major component in the diet of conspecific juveniles during the 1st week of flight, apparently made more readily available to young juveniles due to their lower wing loading and greater maneuverability (Rolseth *et al.* 1994). *Lasiurus cinereus semotus* weighs almost half as much and has a considerably lower wing loading as does *L. cinereus cinereus* (Jacobs 1996). As such, Hawaiian hoary bats may be more maneuverable with the ability to capture and feed on smaller prey than their North American counterparts.

Our results confirm that the Hawaiian hoary bat is a feeding generalist. It feeds on a diverse range of insect taxa and on a large range in prey size. It is also a generalist in exploiting a range of habitats, being capable of foraging in both open grasslands and over ponds (e.g., Pu'u Makua parcel) and in vegetation-cluttered airspace (e.g., amongst trees in the upper Waihou Mitigation Area), and where it is active in the latter type of habitat, it tends to consume smaller prey (<15 mm; Jacobs 1999). The ubiquity of "micro-moth" families Crambidae, Oecophoridae, and Xyloryctidae in our Hawaiian hoary bat guano samples demonstrates that they often consume relatively small moths (although these families also include some larger species). However, the presence of families such as Noctuidae, Geometridae, and occurrence of Sphingidae (at low OTU counts), in the guano samples indicates these bats also consume large prey (>15 mm).

The overall diversity of Lepidoptera taxa (18 families) in the diet from bats sampled in the Waihou/Auwahi area indicates a wide breath of prey, averaging ~7 families (range 4 to 11) per bat based on Zeale primer sequencing. Twenty-four moth taxa identified compare similarly to the 20 moth species identified from stomach contents of hoary bats sampled in Texas (Foo *et al.* 2017). The prevalence of Noctuidae and Geometridae in the diet of *L. cinereus semotus* confirms a similar observation by Todd (2012), and its frequency in the diet of *L. cinereus cinereus* has been noted by Valdez and Cryan (2009, 2013). The families Crambidae, Oecophoridae and Tortricidae, showed recovered OTUs potentially indicative of a high volume of consumption, and were common to most of the bats sampled, and to our knowledge constitutes prey taxa for which no previous records exist for the Hawaiian hoary bat. These taxa were trapped seasonally at relatively high rates at various locations in the study area and likely constitute a prey base readily available to foraging bats.

The four species of Lepidoptera collected as caterpillars (*Scotorythra* sp., *Amorbia emigratella*, *Udara blackburni*, *Uresiphita polygonalis*) directly from vegetation did not appear in the diet of the bats sampled for guano in our study at the genus and species level, but OTUs associated with their families (Geometridae, Tortricidae, Lycaenidae, and Crambidae) were found in bat diet. Bat consumption of *Scotorythra* during moth outbreaks has been assumed on Hawai'i Island (Banko *et al.* 2014), and its occurrence in the diet of bats on Maui is also likely given the large numbers of Geometridae OTUs detected. Its prevalence on 'a'ali'i, koa and naio indicates that reforestation that include these plant species may provide food for locally foraging bats. In addition, the indigenous moth *Uresiphita polygonalis* has been recorded in Hawai'i feeding on koa and māmane (Leen 1997), and its potential as bat prey may be enhanced by reforestation efforts.

Direct insect-plant associations are difficult to make at this time, as we were not able to identify many of the recovered OTUs to a genus- or species-level, largely because Hawaiian arthropods are not well represented in public barcode libraries. Zeale primers, although widely used in insectivorous predator studies, have been shown to poorly resolve family-level taxa within Lepidoptera (Brandon-Mong *et al.* 2015). To better understand if the Hawaiian hoary bat is moth specialist in some habitats, future studies should consider different primers in addition to Zeale, and development of reference libraries specific to Hawai'i and with a focus on Lepidoptera taxa.

Our results also indicate that Hawaiian hoary bats consume both native and non-native insect species. Identified in the bat's diet are agricultural pests in Hawai'i (Funasaki *et al.* 1988) and elsewhere that include the noctuid moths *Feltia subterranean (granulate cutworm) (*Prestes 2014*)*, *Peridroma saucia (variegated cutworm)* and *Pseudaletia unipuncta* (army worm). Another agricultural pest fed upon by Hawaiian hoary bats is *Nezara viridula* (southern green stink bug; Follett *et al.* 2009).

Items detected in the diet also include species deliberately introduced to Hawai'i as biological control agents, such as *Crocidosema lantana* (lantana tortricid moth) used to manage the highly invasive plant *Lantana camara* (Funasaki *et al.* 1988), and *Digitonthophagus gazella* (gazelle scarab) which was brought as an aid to agriculture and ranching because of the beetle's ability to recycle dung and reduce horn fly infestations (Markin and Yoshioka 1998). Given the high proportion of adventive insects introduced to Maui (e.g., 80%; Howarth *et al.* 2012) and elsewhere in the state, genetically evaluating the diet of bats in agricultural habitats may assist in the detection of new pest species (Maslo *et al.* 2017), as well as improve understanding of

the role of bats in the biological suppression of pests (Boyles *et al.* 2011, McCraken *et al.* 2012, Maine and Boyles *et al.* 2015).

In conclusion, acoustic surveys confirm the year-round use of habitat by Hawaiian hoary bats in the Waihou Mitigation Area. Moreover, genetic analysis of the species' diet indicates that Hawaiian hoary bats feed on a diverse variety of insect prey items and range of habitats. Prey items include native and non-native insects, including agricultural pests, and indicate that the Hawaiian hoary bat is largely a food and habitat generalist. Genetic identification of guano samples has greatly expanded our understanding of the diet of this endangered species. Additional use of this technique will further enhance understanding of bat diet and contribute to planning habitat restoration across varied habitats in Hawai'i.

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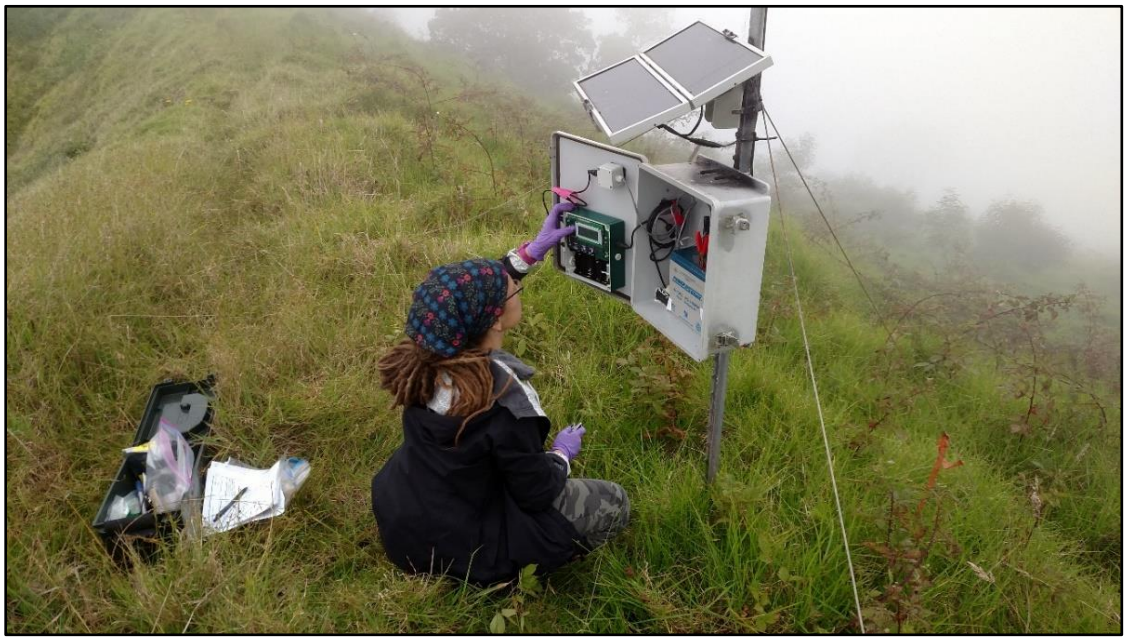
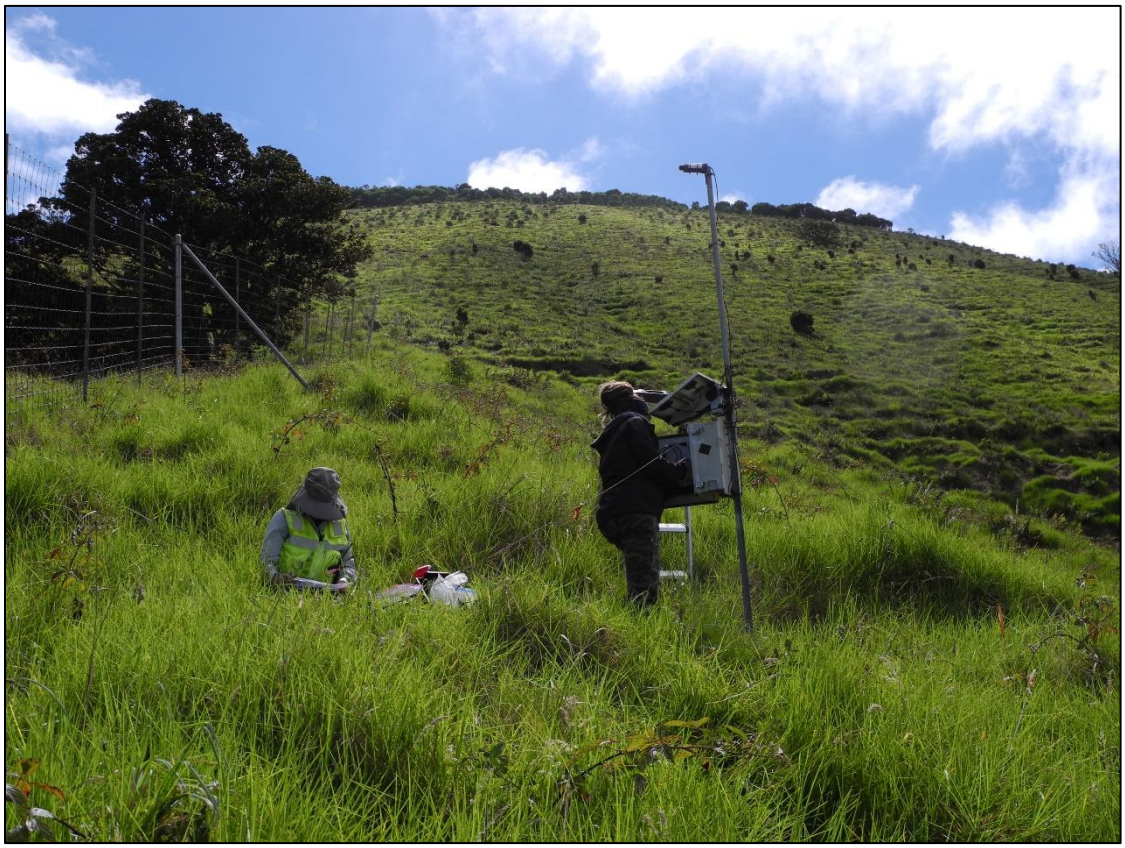
APPENDIX I. BAT ACOUSTIC RECORDING SITES



Appendix I Figure 1. Pu'u Makua bat acoustic recording site AUW1.

Appendix I Figure 2. Pu'u Makua bat acoustic recording site AUW2.

Appendix I Figure 3. Pu'u Makua bat acoustic recording site AUW4. The two microphone models (SMX-US and SMX-U1) are shown attached to the top of the pole, demonstrating comparison testing set up.



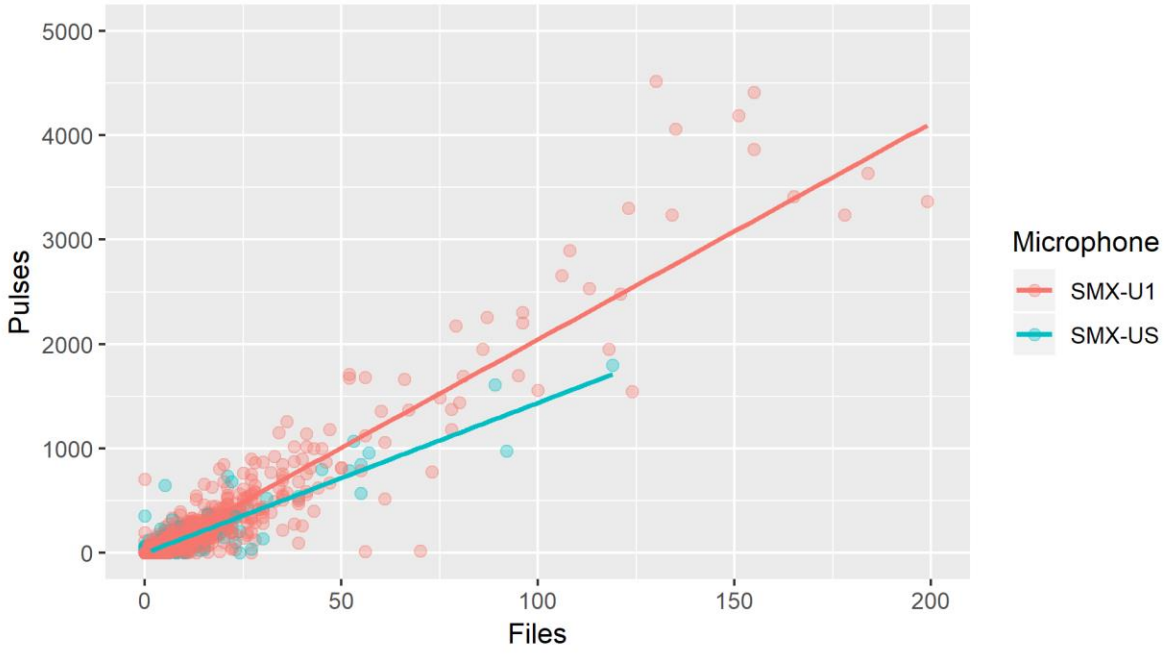
Appendix I Figure 4. Pu'u Makua bat acoustic recording site AUW5.

Appendix I Figure 5. Pu`u Makua bat acoustic recording site AUW6.

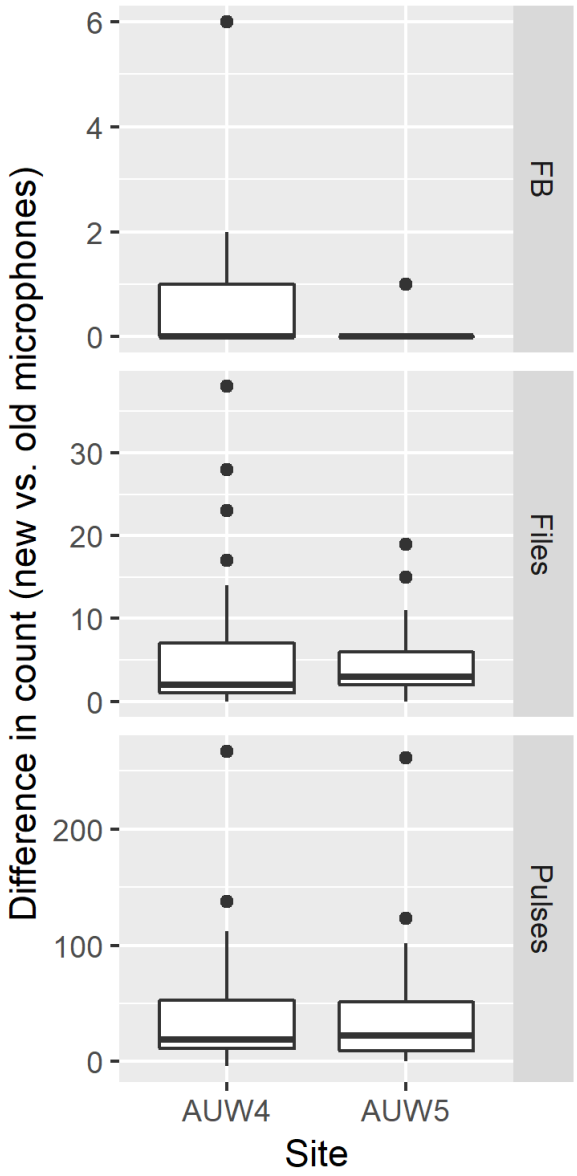
APPENDIX II. ACOUSTIC MICROPHONE COMPARISON

A comparison of simultaneous recordings between the SM2 acoustic detectors equipped with SMX-US and newer SMX-U1 ultrasonic microphones was performed to assess whether the detection data from the older SMX-US could be corrected and made comparable to that obtained from SMX-U1. The test was conducted at two stations (AUW4 and AUW5) from October 14 to November 27, 2016. The SM2 detector at each of the two stations were equipped with an SMX-US and an SMX-U1 positioned at the same height above ground and aimed into the same airspace (Appendix I Figure 3). Acoustic microphone comparison data are available at https://doi.org/10.5066/P9U0KRMY (Pinzari *et al.* 2019).

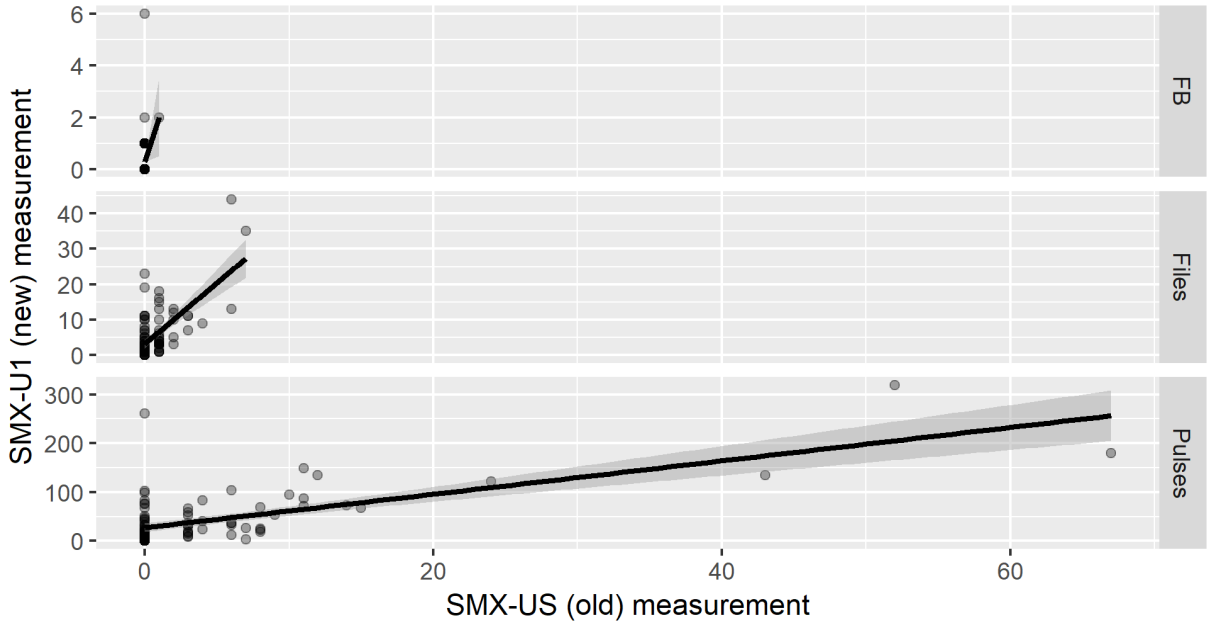
The SMX-U1 microphones samples detected more pulses, feeding buzzes, and generated more files than the SMX-US samples (Figures 1 & 2). In addition, the comparison of detections between microphone types demonstrated the following correlations for number of feeding buzzes ($r = 0.233$, $p = 0.027$), pulses ($r = 0.665$, $p < 0.0001$), and total files ($r = 0.652$, $p < 0.0001$) (Figure 3). A regression estimator of SMX-US to SMX-U1 file counts had a mean slope of 3.44 (95% confidence interval [CI]= 2.59-4.29) and an intercept of 3.13 (95% CI=1.84-4.43) (Figure 4). In general, a detector equipped with a SMX-US microphone detects about 1/3 the number of files of a detector with an SMX-U1 microphone. Although these data were obtained from only 2 acoustic stations over a 6-week sample period, the results indicate that the correlations between the microphone models were low and the confidence intervals around regression estimator were large. Consequently, applying the regression estimator with the objective of extrapolating and making comparable detections from SMX-US and SMX-U1-equipped detectors is not advisable.



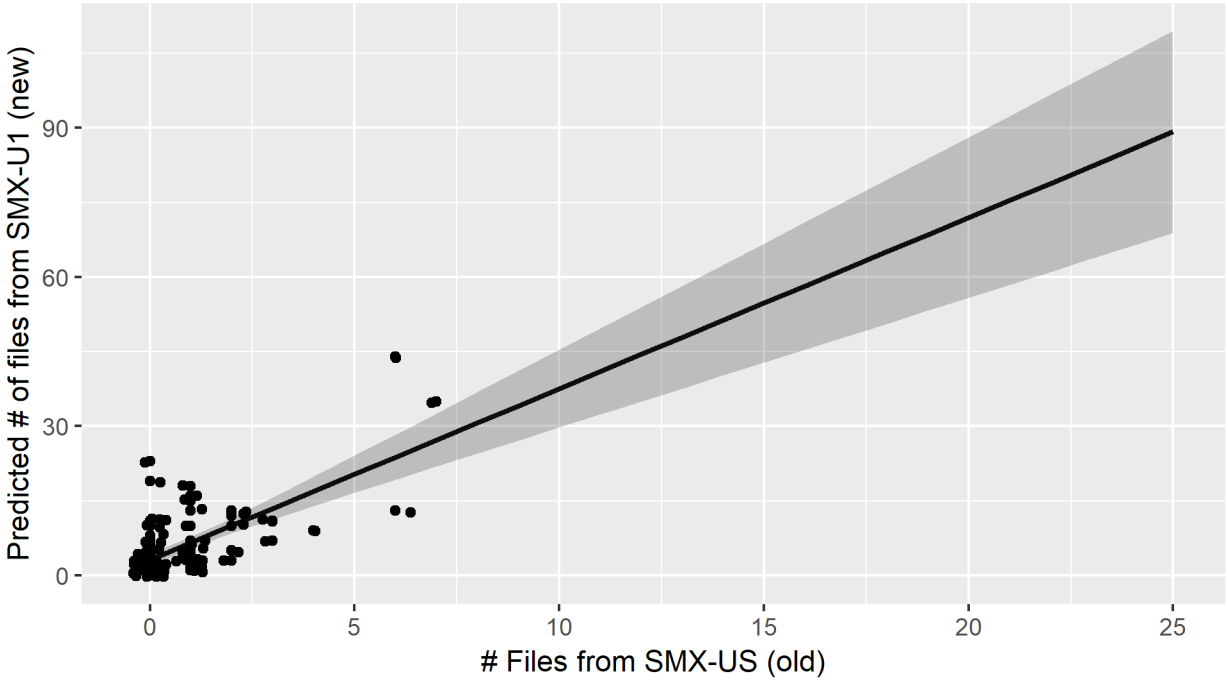
Appendix II Figure 1. Number of bat echolocation pulses relative to the number of files recorded from acoustic detectors equipped with SMX-US ("old") and SMX-U1 ("new") microphones at stations AUW4 and AUW5 from October 14 to November 27, 2016.



Appendix II Figure 2. Box plots demonstrating differences in count between SMX-U1 ("new") and SMX-US ("old") microphones from stations AUW4 and AUW5 for feeding buzzes (FB), number of files, and number of pulses.



Appendix II Figure 3. Correlation plots of simultaneous measurements between SMX-US ("old"; black dots) and SMX-U1 ("new"; grey dots) for feeding buzzes (FB), number of files and pulses from microphones at stations AUW4 and AUW5 from October 14 to November 27, 2016.



Appendix II Figure 4. Regression of detections obtained from SMX-US ("old") microphones relative to the number of files from detectors equipped with SMX-U1 ("new") microphones. It is important to note the lack of files generated from the SMX-US microphones, no more than 7 files were recorded on any given night during this test, whereas the number of files recorded from the SMX-U1 on a given night may be higher than 15 files.

APPENDIX III. INSECT CAPTURE DATA

Appendix III Table A. Insect sample counts by season/year, trap type and site.

Light trap counts	PINE	MAM	PUU	REST
Late autumn (2016) totals	287	54	92	276
Crambidae	45	9	11	17
Erebidae	0	1	0	1
Geometridae	40	8	13	9
Noctuidae	58	3	32	201
Pterophoridae	13	0	0	0
Sphingidae	1	0	0	2
Tineidae	5	0	7	1
Tortricidae	9	2	9	17
Xyloryctidae	1	0	0	0
undetermined	115	31	20	28
Early summer (2017) totals	62	83	188	354
Carposinidae	0	3	0	0
Crambidae	6	13	2	0
Erebidae	2	2	1	78
Gelechioidea	6	2	7	0
Geometridae	10	8	39	13
Noctuidae	18	19	22	224
Sphingidae	0	0	0	1
Tineidae	4	2	3	1
Tortricidae	1	8	7	0
Xyloryctidae	1	0	0	1
undetermined	14	26	107	36
Malaise trap counts	PINE	MAM	PUU	REST
Late autumn (2016) totals	1,461	369	656	1,211
Crambidae	41	9	28	140
Geometridae	166	64	318	24
Lycaenidae	3	0	0	0
Noctuidae	8	5	4	7
Pterophoridae	2	4	2	2
Tineidae	287	23	57	40
Tortricidae	85	26	172	849
Xyloryctidae	7	0	0	0
undetermined	862	238	75	149
Early summer (2017) totals	450	303	412	191
Carposinidae	3	83	1	0
Cosmopterigidae	6	0	3	0
Crambidae	25	24	12	37
Erebidae	13	4	10	3
Gelechioidea	120	75	88	57
Geometridae	95	11	213	2
Gracillariidae	0	0	1	0
Lycaenidae	0	1	0	2
Noctuidae	2	2	8	6
Pterophoridae	0	0	0	2
Tineidae	67	11	10	27
Tortricidae	13	20	43	34
Xyloryctidae	1	1	0	0
undetermined	105	71	23	21

Appendix III Table B. Number of samples days per trap, season/year and site.

Number of light trap-days	PINE	MAM	PUU	REST
Late autumn (2016)	11	10	11	11
Early summer (2017)	6	5	6	6
Number of malaise trap-	PINE	MAM	PUU	REST
Late autumn (2016)	84	84	86	77
Early summer (2017)	47	54	54	54

Appendix III Table C. Capture rates (number per trap night) per season/year and site.

Late autumn (2016)	PINE	MAM	PUU	REST	Total	Proportion
Carposinidae	0.06	2.14	0.02	0.00	0.55	1.9%
Cosmopterigidae	0.00	0.00	0.00	0.00	0.00	0.0%
Crambidae	4.58	1.01	1.33	3.36	2.57	9.1%
Erebidae	0.00	0.10	0.00	0.09	0.05	0.2%
Gelechioidea	0.00	0.00	0.00	0.00	0.00	0.0%
Geometridae	5.61	1.56	4.88	1.13	3.36	11.9%
Gracillariidae	0.00	0.00	0.00	0.00	0.00	0.0%
Lycaenidae	0.04	0.00	0.00	0.00	0.01	<0.1%
Noctuidae	5.37	0.36	2.96	18.36	6.91	24.5%
Pterophoridae	1.21	0.05	0.02	0.03	0.33	1.2%
Sphingidae	0.09	0.00	0.00	0.18	0.07	0.2%
Tineidae	3.87	0.27	1.30	0.61	1.53	5.4%
Tortricidae	1.83	0.51	2.82	12.57	4.28	15.2%
Xyloryctidae	0.17	0.00	0.00	0.00	0.04	0.2%
undetermined	20.72	5.93	2.69	4.48	8.51	30.2%
Early summer	PINE	MAM	PUU	REST	Total	Proportion
Carposinidae	0.06	2.14	0.02	0.00	0.55	1.7%
Cosmopterigidae	0.13	0.00	0.06	0.00	0.04	0.1%
Crambidae	1.53	3.04	0.56	0.69	1.38	4.3%
Erebidae	0.61	0.47	0.35	13.06	3.75	11.6%
Gelechioidea	3.55	1.79	2.80	1.06	2.28	7.0%
Geometridae	3.69	1.80	10.44	2.20	4.58	14.1%
Gracillariidae	0.00	0.00	0.02	0.00	0.00	<0.1%
Lycaenidae	0.00	0.02	0.00	0.04	0.01	<0.1%
Noctuidae	3.04	3.84	3.81	37.44	12.39	38.2%
Pterophoridae	0.00	0.00	0.00	0.04	0.01	<0.1%
Sphingidae	0.00	0.00	0.00	0.17	0.04	0.1%
Tineidae	2.09	0.60	0.69	0.67	0.99	3.0%
Tortricidae	0.44	1.97	1.96	0.63	1.22	3.8%
Xyloryctidae	0.19	0.02	0.00	0.17	0.10	0.3%
undetermined	12.50	4.15	1.30	2.32	5.05	15.6%

APPENDIX IV. BAT ACOUSTIC DATA

Appendix IV Table A. Summary of bat presence and acoustic information recorded at detector sites in the Waihou Mitigation Area between March 17, 2015 and October 13, 2016. Recordings were made with model SMX-US microphones.

Site	Elevation (m)	Microphone model	Recording nights	Nights bats present	Percent nights with bats present	Number of files with bat activity	Feeding buzzes	Files with multiple bats
AUW1	1,611	SMX-US	576	131	23	1,452	88	59
AUW2	1,606	SMX-US	483	62	13	153	17	8
AUW3	1,607	SMX-US	183	152	83	734	146	4
AUW4	1,515	SMX-US	577	131	23	536	65	10
AUW5	1,396	SMX-US	520	79	15	236	15	0
AUW6	1,644	SMX-US	576	389	68	38,902	168	328

Appendix IV Table B. Summary of bat presence and acoustic information recorded at detector sites in the Waihou Mitigation Area and surrounding sites between October 14, 2016 and March 21, 2018. Recordings were made with model SMX-U1 microphones, which are more sensitive than the previously deployed microphones (SMX-US) to echolocation activity and feeding buzzes. Asterisk (*) indicates detector site with a 2017 start date, less than 12 months of calendar year sampling effort.

Site	Elevation (m)	Microphone model	Recording nights	Nights bats present	Percent nights with bats present	Number of files with bat activity	Feeding buzzes	Files with multiple bats
AUW1	1,611	SMX-U1	407	323	79	7,404	138	326
AUW2	1,606	SMX-U1	523	424	81	4,164	178	132
AUW3	1,607	SMX-U1	494	416	84	2,278	75	26
AUW4	1,515	SMX-U1	517	366	71	4,288	53	423
AUW5	1,396	SMX-U1	523	410	78	2,265	28	36
AUW6	1,644	SMX-U1	391	362	93	155,094	1,111	1,878
AUW7*	1,647	SMX-U1	337	246	73	12,110	1,334	178
AUW8*	363	SMX-U1	280	39	14	56	0	0
AUW9*	10	SMX-U1	219	61	28	136	0	1
AUW10*	150	SMX-U1	266	61	23	111	1	0