



Proposal for



Basic research of the home ranges, seasonal movements, habitat utilization, diet, and prey availability of the Hawaiian hoary bat on the island of Maui

In Response to the RFP: Hawaiian Hoary Bat Research



Prepared for:

Kate Cullison State of Hawaii Endangered Species Recovery Committee 1151 Punchbowl Street, Room 325 Honolulu, HI 9822





Prepared by:

H. T. Harvey & Associates

July 15, 2016

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1. Cover Letter

July 15, 2016

Endangered Species Recovery Committee Hawaiian Hoary Bat Request for Proposals C/O Kate Cullison 1151 Punchbowl Street, Room 325 Honolulu, HI 96822

Subject: Basic Research of the Home Ranges, Seasonal Movements, Habitat Utilization, Diet, and

Prey Availability of the Hawaiian Hoary Bat on the Island of Maui

In response to Hawaiian Hoary Bat Research RFP, June 15, 2016

Dear Ms. Cullison:

We propose to conduct basic research of the home ranges, seasonal movements, habitat utilization, diet, and prey availability of the Hawaiian hoary bat on Maui. Our research is proposed over a period of 3 years, from 2016 through 2019. Our proposal supports two of the goals presented in the request for proposals: (1) basic research and (2) identify limiting factors. In support of basic research, we plan to measure the foraging range, core-use area, and long axis of home ranges in our primary study area, presumed to be breeding habitat on Maui, and compare those data with the measurements of home ranges from previous studies. To study some of the potential limiting factors that influence populations of the Hawaiian hoary bat, we will investigate what is suitable habitat and what is the food availability for the species.

We believe this type of research will prove to be tremendously valuable in guiding and assisting conservation effort, and understanding the factors that limit the survival and reproductive success of individuals will inform decisions regarding how to increase bat population sizes and create net recovery benefits.

We look forward to your thoughtful consideration of this proposal.

Sincerely,

Ron Duke President

H. T. Harvey & Associates



2. Project Narrative

a. Summary

Little is known about the ecology of the Hawaiian hoary bat, and the lack of information is hampering efforts to develop and evaluate effective mitigation and recovery plans. We plan to investigate the home range, seasonal movements, foraging and roosting habitat use, diet, and prey availability for the Hawaiian hoary bat at two study sites—one that supports breeding habitat and one that supports wintering habitat—on the island of Maui. To define suitable habitat, we plan to deploy nine bat detectors—six in the breeding habitat study area and three in the wintering habitat study area—that will rotate 25 times per year (once every 2 weeks) for 2 years.

We anticipate capturing 16 – 20 bats (8 – 10 each year) starting in May or June of 2017 or sooner if we believe we've collected enough acoustic data to determine hot spots of activity where bats can be caught. Captured bats will be fitted with transmitters for radiotelemetry studies so that the bats' core use areas (CUAs) can be mapped onto habitat maps, thus providing a fine-grain scale of habitat use. Fecal pellets will be collected from captured bats, and the diets of the bats will be determined by analyzing DNA fragments in faeces using high throughput metabarcoding on an illumina next seq and comparisons of this to the reference library of DNA barcodes generated from potential prey types and existing reference collections (Genbank and BOLD).

As we delineate a home range, we will operate light traps and flight interceptor traps (FIT) to sample the availability of prey at habitats within the home range starting with the capture site. Using the Hawaiian hoary bat's known preferences for size (>10 millimeters [mm] long) and taxa (beetles and moths), we will develop a reference database of DNA sequences from the 5' end of the cytochrome c oxidase subunit 1 region (a DNA barcode) for potential prey species from the study areas' insect sampling.

In addition to using mobile handheld antennae to determine the positions of radio-tagged bats, we will set up a series of semipermanent receiver towers in and adjacent to the two study areas to triangulate positions of bats and to document larger movements, such as seasonal movements outside the study areas. Bat activity patterns for habitats, based on the results of acoustic monitoring, will be compared to the activity patterns for habitats defined from the radio-tracking data. Our home range, foraging, and roost data will be compared to data from other main islands (e.g., Bonaccorso et al. [2015] for the island of Hawaii and H. T. Harvey & Associates [2014] for Oahu). Additionally, we will determine the relationships of home ranges to food availability based on the abundance of known prey types within each home range. Capture efficiency will be increased with the use of high definition acoustic lures and strategically placed macronets up to 30 feet high and 100 feet long.

One of the strengths of this study is that it involves several layers of inquiry that augment each other, allowing the same data to be used for several inquiries. Some objectives (e.g., defining suitable habitat and determining the relationships of distribution to suitable habitat) can be approached from two different methods, such as by acoustic means and by radiotelemetry, so that data sets can be compared. Another strength is that the study areas have known records suggesting that a lot of time will not be required to identify areas where bats are foraging and roosting. Our budget for 2016 is \$71,051; for 2017, \$295,837; for 2018, \$339,181, and for 2019, \$45,345.



b. Goals

Our proposal supports two of the goals presented in the request for proposals: (1) basic research and (2) identify limiting factors. In support of basic research, we plan to measure the foraging range, core-use area, and long axis of home ranges in our primary study area (Figure 1), presumed to be breeding habitat on Maui, and compare those data with the measurements of home ranges determined by U.S. Geological Survey (USGS) studies on the island of Hawaii (Bonaccorso et al. 2015), studies at the Waihou mitigation site on Maui (in progress), and H. T. Harvey & Associates studies on Oahu (H. T. Harvey & Associates 2014).

Bats' home range sizes may be influenced by the distribution of prey (Womack et al. 2013). The authors studied home ranges in rural Missouri, where bats' home ranges encompassed fragmented habitats, such as is found throughout much of the Hawaiian Islands, including in our study area. Therefore, we expect similar results—that bats' CUAs will be influenced by the fragmented nature of the suitable habitats.

We also plan to study some of the potential limiting factors that influence populations of the Hawaiian hoary bat, namely what is suitable habitat and what is the food availability for the species. The Hawaiian hoary bat recovery plan (USFWS 1998) suggests that limiting factors possibly include the alteration of prey availability attributable to the introduction of insects. To date, no studies have examined diet at the level of detail needed to answer this question because it is difficult to determine actual prey at the species level, and few studies provide adequate sampling of the availability of prey. Our multifaceted study provides the means to identify an estimated 200 species of likely potential prey types, determine which insect species the bats consume, and determine whether populations of the Hawaiian hoary bat are indeed limited by food resources in at least parts of their range. We expect that in our study sites, the availability of prey is not uniformly distributed over space and time and that we will find areas that have limited potential food resources for the Hawaiian hoary bat.

c. Objectives

In support of our first goal of conducting basic research that will guide and assist conservation efforts, we plan to document home range and nightly and seasonal movements. As mentioned above, after documenting foraging and roosting behavior, we will compare those data with the measurements of home ranges determined by others on different islands. Additionally, we will document the seasonal movements of bats in our summer study site as they presumably move higher in elevation for the winter months. We also will document the foraging and roosting behavior of bats during the winter months and compare those home range (foraging range, core-use area, and long axis of home ranges) and roosting data with any other available data associated with this winter period.

In support of our second goal of identifying limiting factors, we plan to define suitable habitat with our acoustic sampling and our radiotelemetry activities. Acoustic sampling can be useful for addressing questions regarding habitat selection, particularly at the stand level, as they relate to foraging and commuting (Kalcounis et al. 1999, Miller et al. 2003), but radiotelemetry can be useful for studying roosting habitat and general habitat use at multiple scales, which is not possible with acoustic sampling. Thus, the two approaches complement each other, and using both will allow us to compare habitat use data obtained by the two methods. In defining suitable habitat, we also will include many parameters of roosting habitat, including tree characteristics and microhabitats for both breeding and nonbreeding seasons.



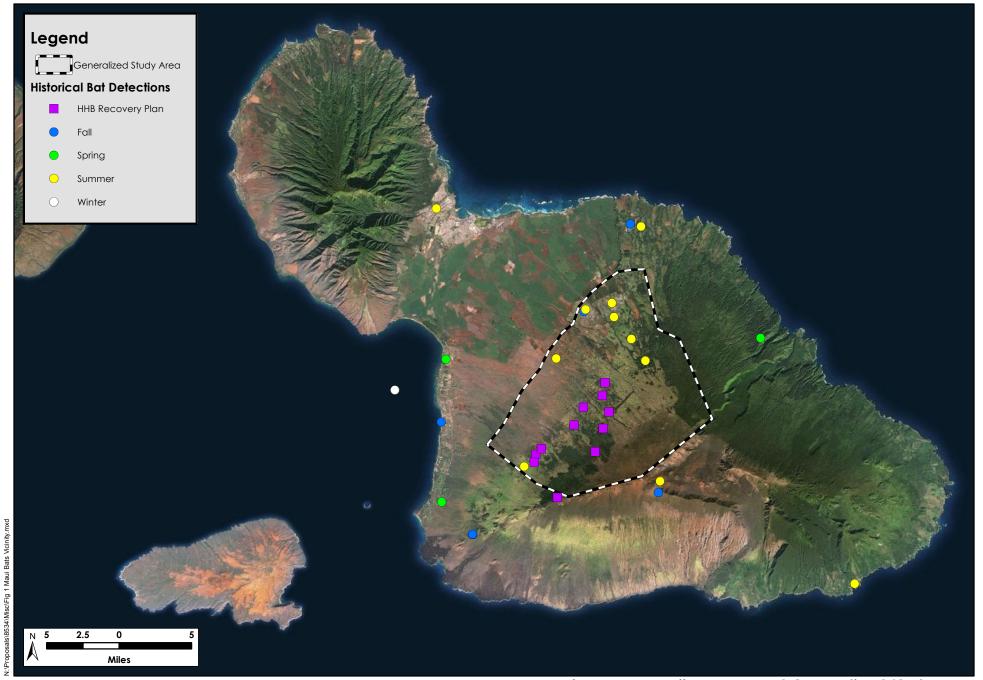




Figure 1. Hawaiian Hoary Bat Generalized Study Area
Proposal 8534A
July 2016

Also in support of our goal of identifying limiting factors, we will identify the diets of Hawaiian hoary bats through the DNA barcoding of the available prey and the combination of this technology with highthrough put sequencing to determine the species represented by trace (eDNA) in the bats' guano. After determining what bats eat in specified areas, with known home ranges and activity levels identified through acoustic monitoring, we can examine the relationship of bats foraging and home range to food availability.

We hypothesize that limiting factors are complex and that it is a combination of factors such as vegetation characteristics, roost characteristics, other habitat characteristics (e.g., altitude, landscape structure), and the availability of prey that determines suitable habitat. No single factor, such as the availability of prey, can be used to predict whether a habitat is occupied. Hagen and Sabo (2011) found that in riverine habitat, bats were not distributed according to prey resources found along the longitudinal axis of the river; rather, they were distributed according to the lateral (cross section) structure of the habitat. Our study is designed to provide new information on ecological (biological and physical) parameters in a habitat that supports or does not support occupancy by the Hawaiian hoary bat.

d. Tasks and Activities

To define suitable habitat, we will use acoustic monitoring and radiotelemetry, each method providing similar information but by different techniques. For the acoustic monitoring, we will begin in fall 2016 by using the Generalized Random Tesselation Stratified (GRTS) (Stevens and Olsen 2004) survey design to select sampling sites in our study areas. The GRTS sampling design is a probability survey design that produces spatially balanced random sample points, provides a representative sample of the sampling domain, and reduces the potential for spatial auto correlation. Simple random sampling, which is a form of probability design, is widely used for sample site selection but often produces clustering of sample sites and unrepresentative samples of the population. Originally created for surveys of linear aquatic systems, the GRTS sampling design has been applied to acoustic bat research in linear systems but also can be applied to bat research projects with list-based polygon sample units (e.g., vegetation or habitat units) (Rodhouse et al. 2011).

To provide a sufficiently robust sample set of monitoring sites, we plan to survey 15 sets of the habitat types determined by the GRTS sampling design, with each of nine habitat types represented (six for the breeding habitat and three for the nonbreeding habitat) by an acoustic station. Staff will deploy acoustic equipment on specified days, and the equipment will record bat calls for the ensuing three nights.

We will apply for the renewal of State of Hawaii and United States Fish and Wildlife Service (USFWS) permits to capture and study the Hawaiian hoary bat.

To help identify limiting factors and to determine food availability and identify diet, we will begin with sampling insects at each habitat type within the home range of a given bat for three days during radiotracking activities. Additionally, we will sample insects in habitat types not otherwise included in a home range at the rate of three nights per month, during a midmonth period of the acoustic monitoring. Collecting additional insect data in habitats occurring outside known home ranges, but near acoustic stations that record feeding buzzes, will also help determine if bat occupancy is prey dependent. One FIT and one light trap will be deployed for each of the habitats found within home ranges. Insects greater than 4 mm long will be identified to species through experts and comparisons to museum collections and then sent to Dr. Elizabeth Clare's lab where a DNA barcode library will be generated. This library can be used to identify unknown sequences recovered from fecal



pellets from captured bats. Starting with the mist-netting and capturing of bats for the radiotelemetry studies, guano will be collected and sent to Dr. Clare's lab for dietary analysis to the species level for beetles and moths, the recognized prey types for the Hawaiian hoary bat (Todd 2015).

In 2017, we will begin our radiotelemetry work to measure home ranges in support of conducting basic research and to help define suitable habitat. We will capture bats by using high definition acoustic lures with small mist nets and by using very large mistnets strategically placed in flyways. Mist-netting, sites will be selected based on the bat activity as measured by the acoustic monitoring, prior experience catching hoary bats, observations of foraging hoary bats, and habitat features conducive to mist-netting. Typically, this work will be conducted by two staff and two volunteers. Use of an acoustic lure has been shown to increase capture rates in difficult capture locations for mainland hoary bats, as well as in Hawaii. Dave Johnston and Gabe Reyes of H. T. Harvey & Associates introduced the use of acoustic lures to bat biologists in Hawaii in 2012.

Additionally, we will erect three fixed-antennae stations to be used as an automated receiving system. This system will continuously collect triangulation data to document bat movements. Recording continuous automated data greatly improves the resolution of the CUAs. Where there is a concern that antennae towers could create a collision risk for birds, the short antennae pieces can be attached to tree branches instead of a tower with guy wires. Otherwise, antennae will be placed on a 20-foot-tall single-pole-style tower with wide stripes that birds can easily see and avoid. Antennae masts will be relocated as needed to track bat movements, particularly during fall because bats are presumed to move up in elevation for their winter habitats.

e. Outputs

In Year 2016: Field work starts end of 2016. No deliverables anticipated.

In Year 2017: 1) Dataset of acoustic detections per habitat over time with feeding buzzes and spatial hot spots of activity separated out (habitat occupancy) and 2) Dataset of habitat characteristics per habitat

In Year 2018: 1) Dataset of insects collected at an estimated nine habitats separated by order, size, biomass, and month. 2) Dataset of species identified for approximately 100 moths and 100 beetles collected in traps (likely prey species for the Hawaiian hoary bat), 3) Dataset of DNA barcodes for approximately 200 insects representing species that potentially are eaten by Hawaiian hoary bats.

In Year 2019: 1) Radiotelemetry data for locations of bats in time and space and an analysis of data to determine CUAs for home ranges of individual bats with maps and descriptive statistics 2) Dataset of species identified for approximately 100 moths and 100 beetles collected in traps (likely prey species for the Hawaiian hoary bat) 3) Dataset of DNA barcodes for approximately 200 insects representing species that potentially are eaten by Hawaiian hoary bats, 4) Analysis of habitat occupancy based on a comparison of occupancy determined using acoustic data and occupancy based on CUAs for home ranges identified through radiotelemetry 5) Dataset of roost locations and characteristics, 6) Map of suitable habitat based on habitat characteristics, roost characteristics, and available prey, 7) Map of suitable habitat compared to map of CUAs for bats, 8) Correlation analysis of distribution of available prey vs. distribution of bat activity based on CUAs and acoustic data.



f. Outcomes

Knowing the characteristics and locations of roosts, the CUA for home ranges, and nightly and seasonal movements based on radiotelemetry studies will contribute to the ecological knowledge of the Hawaiian hoary bat (defined in the RFP as basic research), which should generally help inform management decisions for the species. In addition, the acoustic monitoring data for habitats and the data for CUAs for home ranges will be used to define habitat preferences for the Hawaiian hoary bat on Maui for the breeding and nonbreeding periods. The analyses of these data, coupled with an analysis of occupancy with derived habitat characteristics based on our study of the area, should help determine whether suitable habitat, in some cases, remains unoccupied. If the suitable habitat remains unoccupied, habitat management and restoration would not necessarily contribute to the recovery of the species. If it appears that adequate habitat is occupied, then knowing these habitat characteristics will directly help agencies and landowners to better manage lands to support and increase the populations of the Hawaiian hoary bat.

By knowing the diets of bats on Maui, we should be able to develop a list of plants that are host to the prey species. Restoration ecologists can then use this information to grow plants on restoration sites that will support an increase in prey abundance for the Hawaiian hoary bat. To help understand whether the Hawaiian hoary bat populations on Maui are limited by food resources, we will first compare the dietary data of the bats caught with the insect samples collected throughout the study sites to determine the availability of prey in the landscape. Then we will compare the distribution of available prey against the CUAs of home ranges, and with the distribution of acoustic detections, to determine if the available prey is correlated with the distribution of bat activity. Knowing whether bats are limited by food resources is critical when deciding whether restoration sites should be planted with host plant species that would support preferred prey types.

g. Materials and Methods

1. Acoustic monitoring. For the acoustic monitoring, we will use nine SM4 bat detectors (Wildlife Acoustics Inc., Maynard, Massachusetts, USA) for habitat characteristics and four of the same type of bat detectors to help establish specific areas of high densities of bat activity for radio telemetry work.

To determine the spatial placement of the bat detectors, we will use the Generalized Random Tesselation Stratified (GRTS, Stevens and Olsen 2004) survey design to select our acoustic sampling sites in our study area. The GRTS sampling design is a probability survey design that produces spatially balanced random sample points, provides a representative sample of the sampling domain, and reduces the potential for spatial auto correlation. Simple random sampling, which is a form of probability design, is widely used for sample site selection, but often produces clustering of sample sites and unrepresentative samples of the population. Originally created for surveys of linear aquatic systems, the GRTS sampling design has been applied to acoustic bat research in linear systems, but can also be applied to bat research projects with list-based polygon sample units (e.g., vegetation or habitat units) (Rodhouse et al. 2011).



We will incorporate existing digital vegetation maps from the USGS National Gap Analysis Program (GAP) Land Cover Data Portal as a list of grid cells (sample units) to be randomly selected using the GRTS survey design. Depending on the number of different vegetation types present in the survey area, we will employ the GRTS survey design to randomly select vegetation types from which to sample. Based on our initial assessment of the GAP maps, the lower elevation area supports 1) agricultural vegetation, 2) developed and other human use, 3) forest and woodland, 4) introduced and semi natural vegetation, 5) non-vascular & sparse vascular rock vegetation, and 6) shrubland & grassland, and the upper elevation area supports 1) shrubland and grassland, 2) non-vascular & sparse vascular rock vegetation, and 3) introduced and semi natural vegetation. We will not assess the open water classification, which is present in the lower elevation portion of the study area, because this vegetation type is underrepresented and cannot be replicated sufficiently to make statistical inferences about habitat selection. We will employ the GRTS survey design to select a spatially balanced random sample of 15 replicates of each vegetation type from which to sample in the lower elevation (n=90 sites) and upper elevation (n=45 sites) elevation areas. Additionally, we will employ the GRTS survey design to randomize the order that each site will be sampled. Sample selection and survey order using the GRTS sample design algorithm will be conducted in the R statistical computing environment (v. 3.2.0; R Development Core Team 2015).

At the lower elevation gradient we will deploy six detectors, one in each of six habitat types found there, and in the upper elevation gradient we will deploy 3 detectors, one in each of the three habitat types found at these higher elevations. At each site we will record bat activity for 15 three-night periods spaced out across the year to capture bat activity during the reproductive (April through October) and non-reproductive (November through March) periods for Hawaiian hoary bat (Todd 2013). Sets of units (nine total) will be moved five times during the months of monitoring (i.e., every other month). For example in November, six bat detectors in the lower elevation gradient and 3 bat detectors in the upper elevation gradient (one for each habitat type) will be deployed five times, each time for 3 nights totaling 45 different sites. No acoustic monitoring will occur in December. This pattern is then repeated for the remainder of the two years.

We will analyze calls using callviewer v.18 (Skowronski and Fenton 2008) and Sonobat v.3.2 (Szewczak 2015). To characterize foraging activity in relation to each habitat type, we will label files with feeding buzzes and files with search phase calls. We will report the total number of minutes of activity detected per night, the number of feeding buzzes, and search phase calls detected per night. We will analyze activity in R (v. 3.2.0; R Development Core Team 2015). Detections will be analyzed as binary daily data (i.e., presence / absence of at least one bat recording per night).

To analyze but activity in relation habitat types we will conduct a logistic regression of presence of but over 10-minute intervals in R (R core team 2013). Additionally, weather variables will be collected with Hobo data loggers (Onset Computer Corp., MA). We will analyze presence of the but over 10-minute intervals in relation to temperature, humidity, precipitation, and moon illumination.



2. Food availability. We will be using Universal black light traps (Bioquip Products, Inc. Rancho Dominquez, California) comprising a 22-watt U-shaped fluorescent black light tube, a 30.5 cm diameter lid, and an aluminum funnel for insects to fall into a collecting bucket with ethylene glycol. Light traps, which bias for moths, will be switched on and off with photoelectric switches. Additionally we will use Flight Interception Traps (FIT) to collect beetles and nocturnal flying insects that may not be attracted to light as suggested by Christopher Todd (pers. comm.). When Malaise traps (MT) were compared with FIT, Lamarre et al. (2012) found that MT were biased against beetles, an important part of the Hawaiian hoary bat diet.

To help determine how prey availability varies spatially and temporally across the site, we will sample the availability of prey with methods modified from Todd (2012). Light traps will be switched on and off with photoelectric switches. A light trap will be deployed with each bat detector for seven consecutive nights once every other month in order to adequately sample all flying insects (Hirao *et al.*, 2008) for a trial run of one year. At the end of the first year of collecting insect data, a power analysis of the data will be performed to determine if the sampling regime is appropriate for the questions asked. Light traps will be placed between 200 - 300 m away from each bat detector to avoid attracting insects and bats to the detectors. The direction of the light trap from the bat detector will be chosen by random means. Additionally we will use Flight Interception Traps (FIT) to collect beetles and nocturnal flying insects that may not be attracted to light as suggested by Christopher Todd (pers. comm.). When Malaise traps (MT) were compared with FIT, Lamarre et al. (2012) found that MT were biased against beetles, an important part of the Hawaiian hoary bat diet. The authors found that FIT collected a significantly greater number of Coleoptera and Blattaria than the MT.

Because Hawaiian hoary bats are obligate aerial foragers, we will not use other traditional methods of insect sampling, such as sweep nets and sticky (Tanglefoot) traps that primarily collect insects from vegetation. Further, light trap sampling is relatively unbiased and more efficient than other sampling methods (Raimondo *et al.*, 2004). As suggested in Todd 2012, we believe any effects from sampling during full moons will be minimal because of the robust sample size. We plan on testing the efficacy of insect collecting equipment during our reconnaissance-level survey and begin collecting data when we capture our first bat.

3. Diet and insect barcoding. DNA will be extracted from fecal pellets using the QIAmp DNA Stool Mini prep Kit (Qiagen Ltd). An ~ 170 bp region of the mitochondrial cytochrom ε oxidase subunit 1 (COI) gene will amplified using QIAGEN multiplex PCR kids (Qiagen Ltd) and the forward primer Uni-MinibarF1described by Meusnier et al. (2008) and the reverse primer ZBJ-ArtR2c described by Zeale et al. (2011) modified using standard library prep methods for next generation sequencing. Amplified DNA will be quantified and pooled for next generation sequencing. Sequencing will occur on a Roche 454 platform. Sequenced products will be reduced to unique haplotypes, screened for contaminants and aligned using reference sequences. Sequences will be clustered into molecular operational taxonomic units using jMOTU (Jones et al. 2011) and identified using reference libraries following the protocols of Clare et al. (2009. 2011) for COI sequences and the quality score criteria of Razgour et al. (2011) for next generation sequencing data.



Ecological analyses (comparisons of niche size and overlap) will be conducted on MOTU data using the methods of Razgoru et al. (2011).

In order to identify species of insects consumed by the Hawaiian hoary bat in the study area, we will first need to build a DNA barcoding dictionary to determine insects to species. Although many beetles can be identified to species by their parts found in fecal pellets through the use of a dissecting microscope, (Johnston and Fenton 2001) moths are very difficult to identify other than to the order Lepidoptera. We will deploy an insect sampling station at each bat's capture point, in the other habitat types found within that bat's home range, and in the remaining habitats not already covered by delineated home ranges. Sampling stations will be moved from these remaining habitat types to locations within home ranges as bats are captured and additional home ranges are determined. Starting with the first bat capture, 9 insect sampling stations (6 in lower elevation habitats and 3 in upper elevation habitats) will be operating (Figure 1). Each station will include a light trap and a Flight Interceptor Trap (FIT) and collection will be for three nights every 2 months for a year. We will add insect sampling stations as needed in the second year of radiotracking to ensure each bat's home range is included for insect sampling. After bats are captured each bat will be placed in a clean bag for no more than an hour to collect fecal pellets for the guano samples. Guano will be frozen before molecular analysis.

4. Radio telemetry. We will use Holohil BD-2 radio transmitters (Holohil Systems, Ltd., Carp, Ontario, Canada), radio receivers (R-1000, Communication Specialists, Orange, California) with three-element and fiveelement Yagi antennas, mist nets of various sizes including large nets sized at 6m and 9m x 30m (Johnston 2000), Ultrasoundgate Player BL Pro broadcasters using Avisoft-SASLab software (Avisoft Bioacoustics, Glienicke, Germany), each with a laptop computer and powered by an external 12V battery. To study movements and provide automated triangulation, we will use either Sparrow System Bantam Automatic Receiving Units (ARU) that scan transmitter frequencies through 8 directional antennae while recording signal strength, date, and time or we will use the open source automated telemetry tower system developed by Sensorgnome (www.sensorgnome.org) using Motus towers (www.motus-wts.org). The Sparrow System Bantam Automatic Receiving Units provides better resolution but there may be issues relating to its availability. Recently developed open-source radio-telemetry receivers (www.sensorgnome.org), have enabled collaborative automated telemetry networks (McGuire 2015) in southern Ontario, Canada and along seacoasts in the Northeast U.S. This network now comprises over 250 towers in North America (www.motus-wts.org), each with detection range of up to about 20 km under perfect conditions. Bat biologists and ornithologists are using the same towers to track their perspective study animals because these receivers can track multiple animals on a single frequency by using a unique pulse pattern for each radio transmitter.

We plan to capture 8 – 10 bats for each of two years, totaling 16 - 20 bats, using a combination of acoustic lures (Reyes 2015) and different types of mist nets. Mist netting sites will be selected based on the bat activity as measured by the acoustic monitoring, prior experience catching hoary bats, observations of foraging hoary bats, and habitat features conducive to mist netting. Mist nets will be placed across fly-ways or over water to capture flying bats. Typically we will have two staff and two volunteers, and nets will be checked every 20 minutes. Nets



will be deployed along movement corridors such as road cuts through forests to increase the chance of capture (Palmereirim and Etheridge 1985). Dave Johnston developed portable poles for extremely large nets up to 30 feet tall by 100 feet long (Johnston 2000) that can be very effective at catching bats in situations such as is found along the primitive roads cut through tall forests in the Leeward Haleakala Watershed Restoration Partnership. More recently he developed poles that extend a spring-loaded mist net an additional 6 feet higher in less than a second, thus catching some bats that would otherwise fly up and over the net. Dave Johnston and Gabe Reyes introduced the use of acoustic lures to Hawaiian hoary bat researchers in 2013 and acoustic lures have been shown to increase capture rates of hoary bats in Hawaii (Corinna Pinzari, pers. comm.). Mist nets to be used with acoustic lures will be placed out of direct flyways, against vegetation or hidden under trees, with at least two acoustic lures. Hoary bat social calls will be broadcast until 2:00 AM on most nights., and the nets will be physically checked no less frequently than every 20 minutes.

When bats are captured we will collapse all nets to focus on data collection from our captured bat. We will record sex, age (Adult or Sub-adult, based on closure of epiphyseal growth plates of the carpals), reproductive condition, forearm length (mm), and weight (g). We will also mark bats with an identifying split wing band. We will collect 2 tissue samples from biopsy wing punches and attach a radio transmitter and micro LED to the intrascapular area of the bat's back and release it on site. We will collect guano samples left in the bag for diet analysis. All capture and telemetry methods and restrictions stated in a renewed USFWS Permit issued to H. T. Harvey & Associates # TE80538A-0 and in the DOFAW Protected Wildlife Permit No. WL15-01 as renewed to David S. Johnston will be adhered to.

The study of seasonal movements in hoary bats has been severely limited by their elusive nature, and the cost and size limitation of available technologies for following their movements. Recent technological advancements now make it possible to track large numbers of bats over great distances with automated telemetry networks, and ask a variety of questions that were previously impractical or impossible to address. In addition to conventional radiotracking methods of establishing locations of roosts and foraging using Yagi antennae and triangulation, we propose using stationary radio-telemetry receivers to record larger movements of bats.

We will use either Sparrow System Bantam Automatic Receiving Units (ARU) that scan transmitter frequencies through 8 directional antennae while recording signal strength, date, and time or we will use the open source automated telemetry tower system developed by Sensognome (www.sensorgnome.org) using Motus towers (www.sensorgnome.org). The Sparrow System Bantam Automatic Receiving Units provides better resolution but there may be issues relating to its availability. Recently developed open-source radio-telemetry receivers (www.sensorgnome.org), have enabled collaborative automated telemetry networks (McGuire 2015) in southern Ontario, Canada and along seacoasts in the Northeast U.S. This network now comprises over 250 towers in North America (www.motus-wts.org), each with detection range of up to about 20 km under perfect conditions. Bat biologists and ornithologists are using the same towers to track their perspective study animals because these receivers can track multiple animals on a single frequency by using a unique pulse pattern for



each radio transmitter. Automated telemetry networks would provide an opportunity to address many aspects of movement ecology including establishing movement corridors and sex-biased movements and timing.

i. Permits and Authorizations: We will procure all necessary Federal, State, and local permits as well as acquiring landowner permissions to access property prior to beginning field work as appropriate. Dr. Dave Johnston will submit renewal requests for state and federal permits to handle and study the Hawaiian hoary bat. Paul Conry will approach land owners and agencies for permission to access property.

| | | 2017 2018 | | | | | | | | | | | | | 2019 | | | | | | | | | | | | | | |
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| DOFAW Protected Wildlife Permit (WL15-01) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| and USFWS Threatened and Endagered | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Species Permit (TE80538A-0) to conduct | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| research on HHB. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Obtain renewed permits (TBD) | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| Acoustic Monitoring Study | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Prepare GIS map overlays for study area & | | | | | | | | | | | | | | | | | | | | | | | | | | | | $\overline{}$ | |
| random site selection, identify list | | | | | | | | | | | | | | | | | | | | | | | | | | | | , | |
| landowners to contact. | | | | | | | | | | | | | | | | | | | | | | | | | | | | , | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | + | | |
| Contact land owners & gain permission for site access. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Site visit to assess physical access | | | | | | | | | | | | | | | | | | | | | | T | | | | | | , 7 | |
| constraints and fine tune acoustic station | | | | | | | | | | | | | | | | | | | | | | | | | | | | . | |
| locations. | | | | | | | | | | | | | | | | | | | | | | | | | | | | . | |
| Acoustic data collection (five, 3-night | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| surveys/bi-monthly) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Acoustic data management & analysis (ong | oina) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Statistical analysis | J/ | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Insect Availability & Diet Study | | | | | | | | | | | | | | | | | | | | | | | | | | | - | | |
| Insect Availability & Diet Study Insect collection (3 nights/bi-monthly) | | | | | | | | | | | | | | | | | | | | | | | | | | | \vdash | | |
| Insect identification & data management (c | ngoin | a) | | | | | | | | | | | | | | | | | | | | | | | | | + | | |
| Availabile Prey DNA barcoding | ngoni | 9) | | | | | | | | | | | | | | | | | | | | | | | | | + | | |
| Prey consumed barcoding analysis | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Statistical analysis | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Statistical ariarysis | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Radio-telemetry & Diet Study | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Reconnaissance acoustic monitoring for Tele | metry | , | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mist-netting and telemetry | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Capture & telemetry data management | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Home range statistical analysis | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Report Preparation | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2016 Interim report preparation and submitte | al | | | | | | | | | | | | | | | | | | | | | | | | l | | | | |
| 2017 Report preparation and submittal | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2018-2019 Report preparation and submittal | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Data Delivery | | | | + | | | | | | | | | | | | | | | | - | | | | | | | + | | |
| Publication Preparation (One year following | nroioc | t co | mplo | tion) | | + | | | | | | | | | | | | | | | | | | | | | + | | |
| Tublication Freparation (One year following | projec | ı CO | πημε | uon) | | | | | | | | | | | | | | | | | | | | | | | لــــــــــــــــــــــــــــــــــــــ | | |

Reproductive period of the Hawaiin hoary bat

Non-reproductive period of the Hawaiin hoary bat

j. Monitoring and Evaluation

H. T. Harvey and Associates has established quality control processes including multiple layers of supervision to ensure that the project is completed in a timely manner, and that all milestones are met. Time sheets are entered daily, and progress of the budgets are completed monthly. Senior managers will be involved to be sure that any issues that arise can be quickly overcome, and we will provide quarterly reports to document the progress.

k. Organization and Key Personnel

H. T. Harvey & Associates' Company Overview

Since 1970, the highly trained ecologists and professionals at H. T. Harvey & Associates have delivered



exceptional consulting services to public agencies, private entities, and nonprofit organizations. The expertise of our staff encompasses a wide range of biological and design disciplines required to perform high-quality work on ecological projects.

For this project, we are collaborating with Dr. Elizabeth Clare to build the DNA barcode dictionary for potential prey moths and beetles on Maui and to apply that technology to determine species eaten by Hawaiian hoary bats within our study area. Dr. Clare currently teaches at

the University of London and helped pioneer DNA barcoding of insects to determine bats diets. We will also collaborate with Dr. Karl Magnacca to collect insects and Dr. Daniel Rubinoff to help identify moths and beetles to species when possible. Dr. Dave Johnston will oversee the project's day-to-day activities and Paul Conry will coordinate activities relating to land access, permits, and authorizations. An attached section provides CVs for Key Personnel.

In addition to the H. T. Harvey & Associates team members, Dr. Daniel Rubinoff, director of the University of Hawaii Insect Museum, will assist with insect identification, and Dr. Elizabeth Clare will manage DNA bar coding—related activities.

Ron Duke will serve as the principal-in-charge for the project and will provide overall supervision. Dave Johnston will be the principal investigator and will coordinate all the technical aspects of the program on a day-to-day basis. Paul Conry, manager of our Hawaii office, will assist.

Dave Johnston, Ph.D., Associate Wildlife Ecologist and Bat Biologist **H. T. Harvey & Associates**

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