

Genetic Determination of Sex and Diet Composition in the Hawaiian Hoary Bat

Proposal Prepared for SunEdison

Submitted: 29 June 2015

From: U. S. Geological Survey, Pacific Island Ecosystems Research Center

Principal Investigator: Frank Bonaccorso, USGS

Contact: email: fbonaccorso@usgs.gov, phone (808) 958-6444

Background

The management and conservation of the Hawaiian hoary bat depends on our knowledge and understanding of their life history processes and resource requirements. Ecological needs can vary greatly between the sexes, resulting in sex-specific habitat use, movements, and seasonal distributions in response to prey life history.

Detailed information on the insect prey taxa and relative compositions of prey within Hawaiian hoary bat diets are generally understated in previous studies concerning food habits and dietary needs for this endangered species. Past studies exploring the composition of local bat diet have relied on microscopy and dried collection comparisons to determine the taxonomic identity and general abundance of insect prey items (Jacobs 1999). These methods can limit or even bias the information gained since hard-bodied insects, such as beetles, are easier to recognize from fragments in the fecal matter than those with soft bodies, such as moths. New molecular genetics techniques are available that overcome many of the observational limitations in insect identification by using DNA barcoding (Zeale et al. 2011) and have been successfully used on several bat species around the world including tree-roosting lasurine bats (Clare et al. 2009). Specifically, the use of next-generation sequencing and metagenomic analyses have aided in detecting the diversity and quantifying the relative contributions insect taxa in bat diets across differing habitats, seasons, and between the sexes (Bohmann et al. 2011, Burger et al 2013, Clare et al. 2014, Vesterinen et al. 2013).

The factors contributing to bat collisions with wind turbines are not well understood. Effective mitigation and minimization strategies may benefit from knowing if there is a sex-specific vulnerability or seasonal pattern in wind turbine associated deaths. Sexual information from carcasses found by fatality searches can be of assistance (Cryan & Barclay 2009). Unless a bat carcass discovered under a turbine is quite fresh (1-2 days old), more often than not, the sex of decayed or scavenged carcasses are difficult or nearly impossible to identify from external observation alone, leading to a male biased dataset with females often being identified as unknown (Arnett et al. 2008, Korstian et al. 2013). Yet, a simple and reliable genetic method now exists to accurately determine the sex of bat carcasses from *Myotis* bats found under wind turbines, and has been validated for the North American hoary bat by Korstian et al. 2013.

The USGS/PIERC has already made a substantial commitment to understanding bat demographics and classification of diet and sex identification through an extensive tissue collection including samples from live captured bats and carcasses from wind facilities as well as recent work in publication on a collection of bat fecal pellets of both male and female bats from a variety of native and agriculturally dominated habitats on Hawaii, Maui, and Oahu islands (Bonaccorso et al. 2015, in review).

Funding for this proposal will 1) further the knowledge of Hawaiian hoary bat ecology 2) provide demographic information on sex of bats for application to management objectives such as REA modeling, and correct sexual identification of historical bat take at Hawaiian wind facilities, and 3) provide detailed taxonomic diet information of bats with respect to sex and habitat to

support informed host plant selection in habitat restoration. Research priorities for effective management of this bat have been recently recommended by the ESRC, USFWS and DOFAW and include specific study into foraging and demographics:

- Food habits of both male and female hoary bats at selected sites across Oahu, Maui, Kauai, and Hawaii islands, through the use of genetic bar-coding to identify insect prey taxa.
- Better understanding of the predator-prey dynamics for improvement in habitat restoration through selection and propagation of bat prey associated host plants.

Research Plan and Objectives

I. Collection of bat wing tissue and fecal pellet samples

For sex determination, we will utilize an existing collection of wing tissues at USGS taken from live captures and carcasses previously collected from wind facilities. For diet composition, we will use existing and new collections of fecal samples from at least 30 individuals across a variety of habitats on the islands of Hawaii, Maui, and Oahu. New collection of fecal pellets will be conducted by F. Bonaccorso and C. Pinzari.

II. DNA extraction and preparation

Extraction of DNA from wing tissue samples and fecal matter will be conducted at University of Hawaii (UHH) at Hilo genetics laboratory by C. Pinzari.

III. Genotype and identify sex of all bats

Genotypes will be determined from approximately 120 individuals of which more than 30 are carcasses found on wind facilities in Hawaii. This includes known (30% of carcasses) and unknown sex (70% of carcasses) following molecular Korstian et al 2013 by using PCR to amplify markers on the X and Y chromosomes of bats, gel electrophoresis, and staining to visualize resulting bands. This research will be conducted at UHH genetics laboratory by C. Pinzari.

IV. Generate library of insect DNA barcodes sequences from fecal pellets of 30 individual bats

Utilize metagenomics services at UHH Core Genomics Facility to prepare and sequence thousands of insect CO1 barcodes from each individual fecal sample using high-throughput Ion Torrent techniques.

V. Analyze data from insect DNA barcoding using a custom insect reference database and identify prey items to lowest taxonomic group possible

Utilize biocomputing and bioinformatics analysis services at UHH Core Genomics Facility to perform organization (filter, quality checks) of insect DNA barcode amplicon data into a customized CO1 database. Construct a reference database for insect CO1 barcodes that has been cross-checked with local insect distributional information and customized for Hawaiian bats. Identify sequences amplified from fecal samples to insect order or lower taxonomic group when possible.

Budget Request: \$147,824

See attached budget for breakdown

Timeline *(start time pending finalization of signed agreement and fund transfer)*

- Field collection to augment fecal pellet and insect prey base samples – ongoing
- Extract and prepare DNA from bats and feces – December 2016
- Genotype sex of all individual bats in collection and reporting – January to June 2016
- Process fecal samples and amplify insect DNA barcode sequences – January to June 2016
- Analyze insect barcode sequence data from feces – June to September 2016
- Reporting and manuscript generation – September – December 2016

Deliverables

- Progress reports delivered at 6 month intervals
- Final Report on Sex Determination: June 2016
- Final Report on Bat Diet Composition: December 2016
- Peer Reviewed USGS Manuscript on Bat Diet Composition: April 2017

Project Staff:

USGS-PIERC

Frank J. Bonaccorso, Ph.D. Bonaccorso earned his Ph.D. at the University of Florida in 1975. He has over 40 years of experience in working with bats from around the world including 11 years of intensive research on Hawaii Hoary Bats. He has published over 50 articles in peer reviewed scientific journals across the fields of community, population and behavioral ecology, population genetics, and studies of bats on wind energy facilities.

Hawaii Cooperative Studies Unit, University of Hawaii at Hilo

Corinna Pinzari is a Master's Degree candidate in Tropical Conservation Biology and Environmental Sciences at University of Hawaii at Hilo. Her thesis research focuses on the geographic structure, genetic and morphological variation in Hawaiian hoary bats across the state using conservation genetics techniques. She received her bachelor's degree of science from University of Washington in 2006 in Ecology and Evolution, with minors in Aquatic Sciences and Restoration Ecology. She has 7 years of experience working with the Hawaiian Hoary bat. Currently, she is a Research Supervisor with the Hawaii Cooperative Studies Unit, assisting USGS studies into the ecology, and behavior of bats; with emphasis on developing techniques and deploying acoustic surveys.

References:

Arnett, E., Brown, W., Erickson, W. et al. 2008. Patterns of bat fatalities at wind energy facility in North America. *Journal of Wildlife Management*. 72: 61-78.

Bohmann, K., et al. 2011. Molecular diet analysis of two African Free-Tailed bats using high throughput sequencing. *PLOS One*. 6(6) ee21441.

Bonaccorso, F., Todd, C., Bernard, R., and T. Zinn. 2015. Food Habits of the Hawaiian Hoary Bat (*Lasiurus cinereus semotus*). *In review*.

Burger, J., Murray, D., Craig, M., Haile, J., Houston, J., Stokes, V., and M. Bunce. 2013. Who's for dinner? High-throughput sequencing reveals bat dietary differentiation in a biodiversity hotspot where prey taxonomy is largely undescribed. *Molecular Ecology*. Doi: 10.1111/mec.12531

Cryan, P., Barclay, R., 2009. Causes of bat fatalities at wind turbines: hypotheses and predictions. *Journal of Mammalogy*. 90:1130-1340.

Clare, E., Fraser, E., Braid, H., Fenton, B., and P. Hebert. 2009. Species on the menu of a generalist predator, the eastern red bat (*Lasiurus borealis*): using a molecular approach to detect arthropod prey. *Molecular Ecology*. 18 2532-2542.

Clare, E., et al. 2014. The diet of *Myotis lucifugus* across Canada: assessing foraging quality and diet variability. *Molecular Ecology*. 23: 3618-3632.

Jacobs, D., 1999. The diet of the insectivorous Hawaiian hoary bat (*Lasiurus cinereus semotus*). *Canadian Journal of Zoology*. 77: 1603-1607.

Korstian, J. M., A. M. Hale, V. J. Bennett, and D. A. Williams. 2013. Advances in sex determination in bats and its utility in wind-wildlife studies. *Molecular Ecology Resources*. 13(5): 776-780.

Vesterinen, E., Lilley, T., Laine, V., and N. Wahlberg. 2013. Next generation sequencing of fecal DNA reveals the dietary diversity of the widespread insectivorous predator Daubenton's bat (*Myotis daubentonii*) in Southwestern Finland. *PLOS One*. 8(11) e82168.

Zeale, M., Butlin, R., Barker, G., Less, D. and G. Jones. 2011. Taxon-specific PCR for DNA barcoding arthropod prey in bat feces. *Molecular Ecology Resources*. 11:236-244.