REPORT ON 2014 ISSSSP BAT DNA ANALYSIS

Myotis yumanensis (Yuma Myotis)

11/26/2014

Middle Fork Ranger District, Willamette National Forest

In 2014, the Interagency Special Status/Sensitive Species Program granted the Middle Fork Ranger District funds to conduct DNA analysis of bat guano in order to identify species utilizing newly constructed alternative bat habitat structures. The DNA analysis confirmed use by several species of *Myotis* and also confirmed the need for further monitoring.

Report on 2014 ISSSSP Bat DNA Analysis

MIDDLE FORK RANGER DISTRICT, WILLAMETTE NATIONAL FOREST

INTRODUCTION

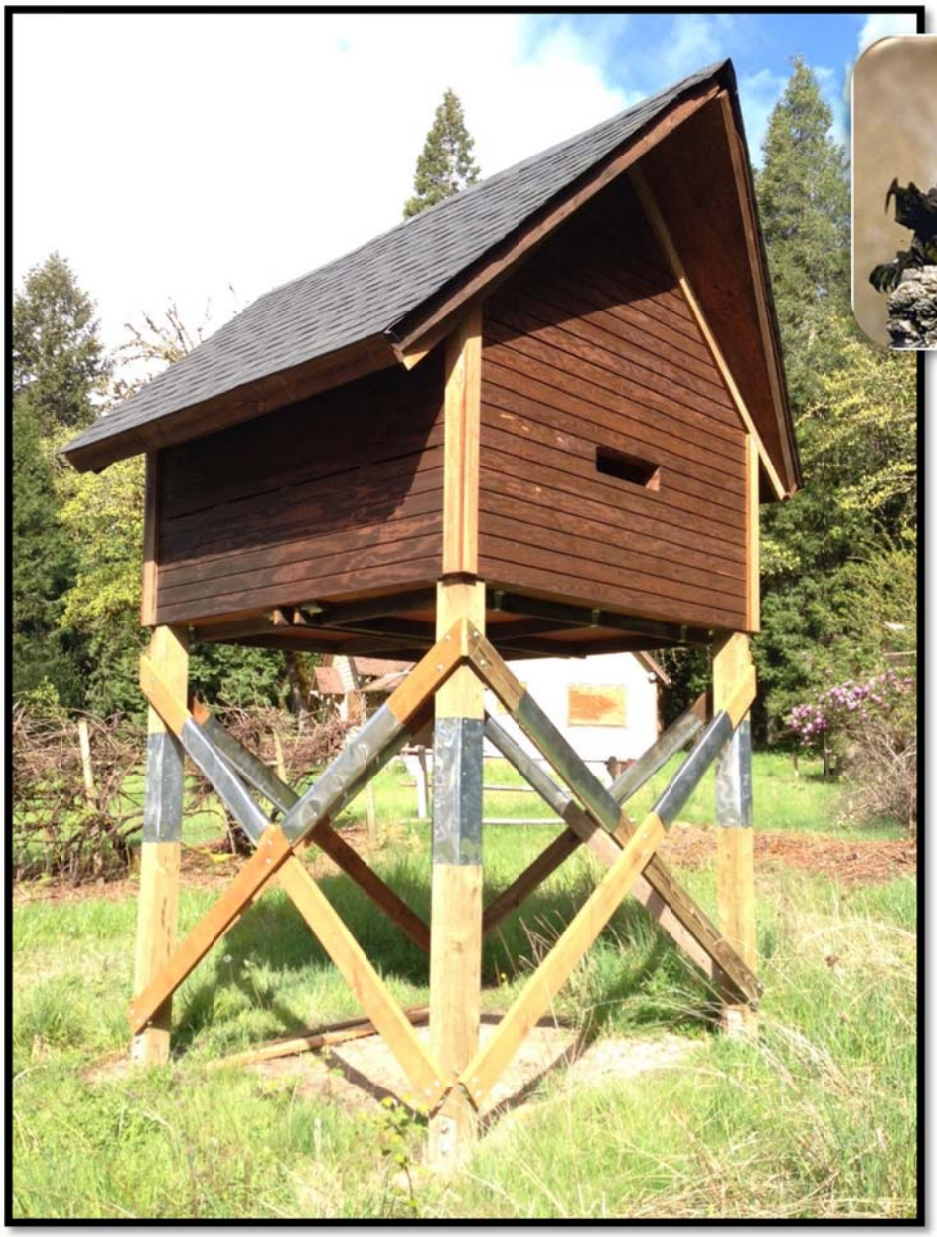
In 2014, the BLM and Forest Service Interagency Special Status/Sensitive Species Program (ISSSSP) granted the Middle Fork Ranger District $2,200 for bat guano DNA analysis. The objective of the analysis was to identify the species of bat using two new alternative habitat structures.

The new structures are located at two sites on U.S. Forest Service lands that are part of the Middle Fork Ranger District of the Willamette National Forest.

1. ***Hemlock Houses*** – *These two former government dwellings are located 0.25 miles northwest of the community of Westfir, Oregon. The project area, which includes two houses and the new alternative bat habitat structure, is contained within a 3-acre open lawn area surrounded by mature conifer forest. The bat habitat structure, known as the Hemlock Bat Bunker, was completed in September 2013. The structure was built in hopes of housing Townsend's Big-eared Bats (Corynorhinus townsendii), which currently use the old government houses. C.townsendii are a regionally sensitive species that use the historic houses year-round including a maternity colony of 30+ individuals. The houses are expensive to maintain and sustain frequent vandalism, so the bunker was built with the hope that C.townsendii would relocate to it.*

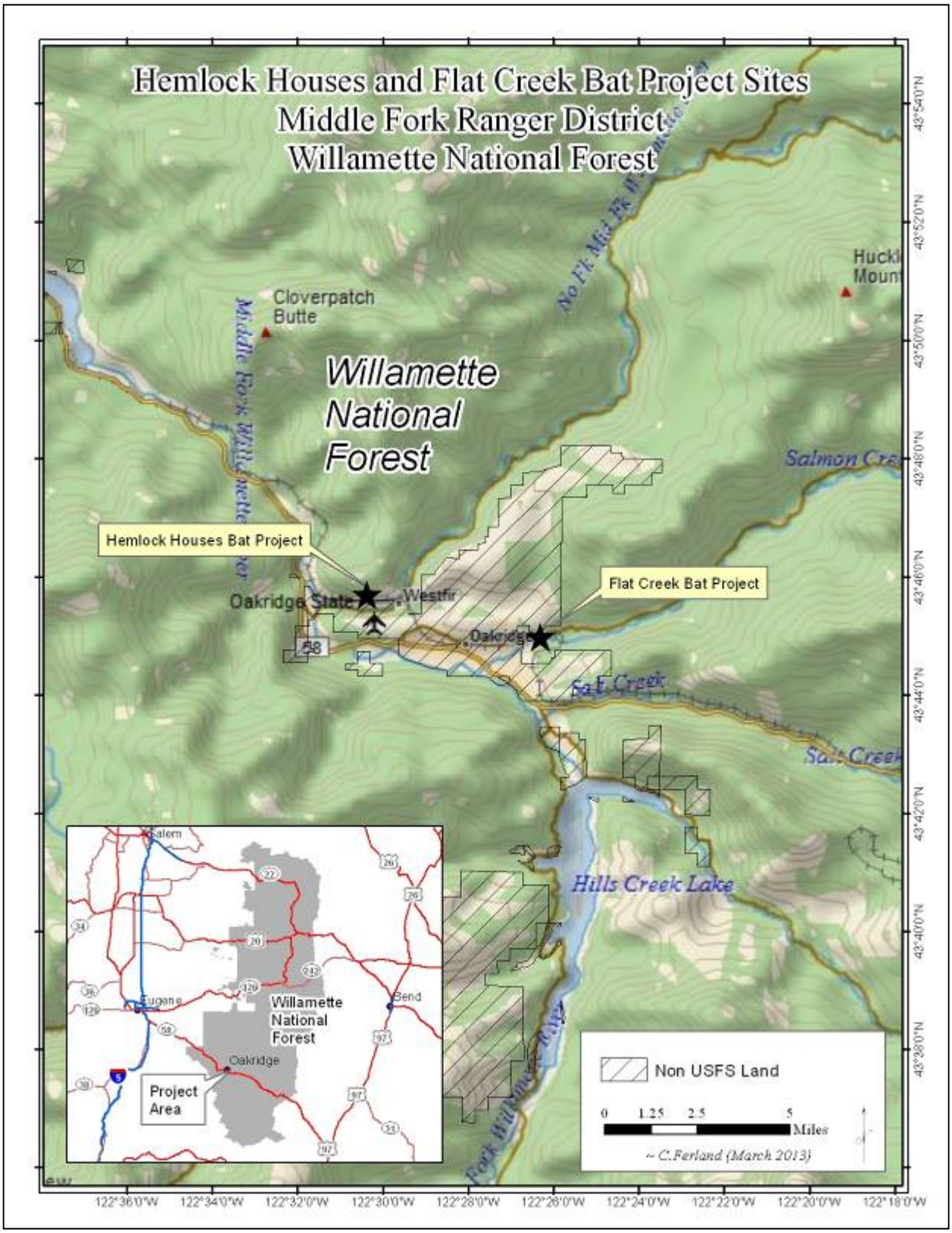
Hemlock Bat Bunker with West Hemlock House in background, which houses C. townsendii maternity colony.

2. ***Flat Creek*** *– This site is a 10-acre workcenter compound. It includes three former government quarters, several large warehouses and other storage buildings, as well as two newer bunkhouses. It is located 0.5 miles east of Oakridge, Oregon. The compound is mostly lawn and gravel drives surrounded by mature conifer and riparian forest. One of the houses has hosted a maternity colony of Long-eared Myotis (Myotis evotis) for decades. The colony is thought to be between 50 and 100 breeding females. The former government quarters are slated for demolition so the bat condo pictured below was built in hopes that the M. evotis will relocate to it. It was completed in June 2012 and there was guano present in the structure within weeks.*



Myotis evotis
(Long-eared Myotis)

Flat Creek Bat Condo with Flat Creek former quarters in the background, which housed Myotis evotis maternity colony.


Hemlock Houses and Flat Creek Bat Project Sites
Middle Fork Ranger District
Willamette National Forest
Cloverpatch Butte
Willamette National Forest
Hemlock Houses Bat Project
Flat Creek Bat Project
Oakridge State
Westfir
Oakridge
58
Hills Creek Lake
Salem
Eugene
Bend
Willamette National Forest
Oakridge
Project Area
Non USFS Land
0 1.25 2.5 5
Miles
~ C.Ferland (March 2013)
122°36'0"W
122°34'0"W
122°32'0"W
122°30'0"W
122°28'0"W
122°26'0"W
122°24'0"W
122°22'0"W
122°20'0"W
122°18'0"W
43°54'0"N
43°52'0"N
43°50'0"N
43°48'0"N
43°46'0"N
43°44'0"N
43°42'0"N
43°40'0"N
43°38'0"N

METHODS

The alternative bat habitat structures have been "open for business" for more than a year. We collected guano samples in order to identify species utilizing the structures. We collected guano in August 2014 and submitted it to Dr. Maarten Vonhof at Western Michigan University for DNA analysis. There was very little guano present in the Hemlock Bat Bunker and it appeared to be of *Myotis* origin rather than *Corynorhinus*. We submitted nine guano pellets from the bunker. Conversely the Flat Creek Bat Condo had larger quantities of guano and we submitted 22 samples. We also sampled the wedge boxes at Flat Creek which have been in place for decades. Additionally, we noticed abundant *Myotis*-like guano in the West Hemlock House (where the Townsend's maternity colony resides) and wanted to identify which species was also occupying the house. We submitted a total of 43 guano pellets for DNA analysis.

Below is the report submitted by Dr. Maarten Vonhof:

> *DNA was extracted using a Qiagen DNEasy kit with a final elution volume of 50 µl. I then used several markers to pursue species identifications. I amplified a 657 base pair (bp) fragment of the mitochondrial COI gene using primers (LCO1490 and HCO2198) and cycling conditions outlined in Hebert et al. (2003, Proceedings of the Royal Society of London B 270, 313-321). This marker is commonly used for bat species identifications based on tissues, but its use for feces is not always successful because of its relative length (DNA in feces is often degraded and consists of shorter fragments). However, I tried this marker because it contains sufficient variation to unambiguously distinguish species, it can provide valuable information when successful, and I have an extensive database of sequences from known ID bats to compare with.*
>
> *I also amplified shorter fragments of two other mitochondrial genes, the 12S (175 bp) and 16S (200 bp) ribosomal genes using primers and cycling conditions in Kitano et al. (2007, International Journal of Legal Medicine 121:423-427). These markers have greater success when used with degraded DNA, but required me to develop a database of known ID sequences. I assessed variation at each marker along, as well as with the sequences combined into to make a 375 bp fragment.*
>
> ***It is important to note that for all mitochondrial markers we cannot distinguish between several groups of Myotis, including M. californicus/ciliolabrum/leibii and M. lucifugus/evotis/keenii/thysanodes.***
>
> *Amplified product was cleaned using shrimp exonuclease (PCR-Product Pre-Sequencing Kit, Affymetrix) and sent to the Arizona Research Labs DNA Sequencing Facility for sequencing. Sequences were visualized using CodonCode Aligner software and trimmed of flanking primer sequences. The cleaned sequences were then BLAST searched to identify the most similar sequences present on the NCBI nucleotide database. In addition, for the 12S/16S combined sequences I performed a neighbor-joining analysis to visualize the relationship of the sequences from fecal pellets with known ID sequences, as concatenated sequences are not available on public sequence databases.*

RESULTS

Below are the results from Dr. Maarten Vonhof's report:

> *The amplification success and species identification of the unknown samples are outlined in Table 1, and relationships among combined 12S/16S sequences can be visualized in Figure 1. COI amplifications were successful for 17 (40%) of samples. All but one of the pellets collected at the Flat Creek Bat Condo matched Myotis lucifugus COI sequences in the NCBI database with 100%*

similarity, and a single pellet was ID'd as M. yumanensis. Pellets from Hemlock West House and Flat Creek West Box East were also ID'd as M. yumanensis based on COI.

The 12S and 16S markers were less variable overall, and the 12S marker in particular could not distinguish among species of Myotis. The 16S marker provided some resolution, and could distinguish between M. yumanensis/M. velifer and other western species. Given that M. velifer does not occur in Oregon, I feel confident that ID's of this species pair represent M. yumanensis. The two markers combined provided additional resolution, although the number of sequence differences among species of Myotis was low compared with the COI marker.

There were four fecal pellets from which we could not obtain sequences at all, and an additional pellet for which we could sequence the 12S marker only. Of the remaining pellets, the majority of pellets in the Flat Creek Bat Condo were M. lucifugus, and all of the pellets in the Hemlock Bunker and Hemlock West House were M. yumanensis based on combined 12S/16S sequences (Table 1). Pellets from both M. lucifugus and M. yumanensis were sampled from the Flat Creek West Box East and West.

Table 1. Amplification success (x denotes a successful amplification) and species ID for each marker. Samples sharing the same 12S/16S haplotype number had identical sequences. Haplotype 1 was identical to a *M. lucifugus* sample from Prince William Sound, Alaska (MYLU-UAM68933), and haplotype 2 was identical to a *M. yumanensis* sampled in North Cascades National Park, Washington (MYYU-NC34).

Sample	Location	12S	16S	12S/16S Haplotype	12S-16S Combined	16S	COI	COI	Comment
FC-BC-01	Flat Creek Bat Condo	x	x	1	*M. lucifugus*		x	*M. lucifugus*	
FC-BC-02	Flat Creek Bat Condo	x	x	2	*M. yumanensis*		x	*M. yumanensis*	
FC-BC-03	Flat Creek Bat Condo	x							Could not ID
FC-BC-04	Flat Creek Bat Condo	x	x	1	*M. lucifugus*		x	*M. lucifugus*	
FC-BC-05	Flat Creek Bat Condo	x	x	1	*M. lucifugus*		x	*M. lucifugus*	
FC-BC-06	Flat Creek Bat Condo	x	x	1	*M. lucifugus*		x	*M. lucifugus*	
FC-BC-07	Flat Creek Bat Condo	x	x	1	*M. lucifugus*		x	*M. lucifugus*	
FC-BC-08	Flat Creek Bat Condo	x	x	1	*M. lucifugus*		x	*M. lucifugus*	
FC-BC-09	Flat Creek Bat Condo	x	x	1	*M. lucifugus*		x	*M. lucifugus*	
FC-BC-10	Flat Creek Bat Condo	x	x	1	*M. lucifugus*		x	*M. lucifugus*	
FC-BC-11	Flat Creek Bat Condo								All amplifications failed
FC-BC-12	Flat Creek Bat Condo	x	x	1	*M. lucifugus*		x	*M. lucifugus*	
FC-BC-13	Flat Creek Bat Condo	x	x	1	*M. lucifugus*		x	*M. lucifugus*	
FC-BC-14	Flat Creek Bat Condo	x	x	2	*M. yumanensis*				
FC-BC-15	Flat Creek Bat Condo	x	x	1	*M. lucifugus*		x	*M. lucifugus*	
FC-BC-16	Flat Creek Bat Condo	x	x	1	*M. lucifugus*		x	*M. lucifugus*	
FC-BC-17	Flat Creek Bat Condo	x	x	3	*M. lucifugus*		x	*M. lucifugus*	
FC-BC-18	Flat Creek Bat Condo	x	x	1	*M. lucifugus*		x	*M. lucifugus*	
FC-BC-19	Flat Creek Bat Condo		x			*M. yumanensis*			
FC-BC-20	Flat Creek Bat Condo								All amplifications failed

FC-BC-21	Flat Creek Bat Condo								All amplifications failed
FC-BC-22	Flat Creek Bat Condo		x			*M. yumanensis*			
HB-01	Hemlock Bunker	x	x	2	*M. yumanensis*				
HB-02	Hemlock Bunker	x	x	2	*M. yumanensis*				
HB-03	Hemlock Bunker	x	x	2	*M. yumanensis*				
HB-04	Hemlock Bunker	x	x	2	*M. yumanensis*				
HB-05	Hemlock Bunker	x	x	2	*M. yumanensis*				
HB-06	Hemlock Bunker	x	x	2	*M. yumanensis*				
HB-07	Hemlock Bunker	x	x	2	*M. yumanensis*				
HB-08	Hemlock Bunker	x	x	2	*M. yumanensis*				
HB-09	Hemlock Bunker	x	x	2	*M. yumanensis*				
HWH-01	Hemlock West House	x	x	2	*M. yumanensis*				
HWH-02	Hemlock West House	x	x	2	*M. yumanensis*		x	*M. yumanensis*	
HWH-03	Hemlock West House	x	x	2	*M. yumanensis*				
HWH-04	Hemlock West House	x	x	4	*M. yumanensis*				
HWH-05	Hemlock West House								All amplifications failed
HWH-06	Hemlock West House	x	x	2	*M. yumanensis*				
WBE-01	Flat Creek West Box East	x	x	2	*M. yumanensis*		x	*M. yumanensis*	
WBE-02	Flat Creek West Box East		x			*M. volans, californicus, or ciliolabrum*			
WBE-03	Flat Creek West Box East	x	x	5	*M. lucifugus*				
WBW-01	Flat Creek West Box West	x	x	2	*M. yumanensis*				
WBW-02	Flat Creek West Box West	x	x	2	*M. yumanensis*				
WBW-03	Flat Creek West Box West	x	x	5	*M. lucifugus*				

DISCUSSION

Based on the DNA analysis, we were able to determine that the Myotis using the Hemlock Bat Bunker as well as the West Hemlock House were Yuma Myotis (*Myotis yumanensis*). As for the Flat Creek Condo, there was Yuma Myotis present as well as either Little Brown Bat (*Myotis lucifigus*), Long-eared Myotis (*Myotis evotis*), and/or Fringed Myotis (*Myotis thysanodes*). As Dr. Maarten Vonhof pointed out in his report –

It is important to note that for all mitochondrial markers we cannot distinguish between several groups of Myotis, including M. californicus/ciliolabrum/leibii and M. lucifugus/evotis/keenii/thysanodes.

Thus, the *M.lucifigus* results could be *M.lucifigus*, *M.evotis*, or *M.thysanodes*. *M.keenii* does not occur on the Willamette National Forest. I did not realize this limitation of mitochondrial DNA analyses for *M.evotis* until after the DNA analysis. *Lesson learned!*

Our next step will be to monitor the Flat Creek Bat Condo in June 2015 during the breeding season. We plan to net individuals in order to confirm species identification, confirm if pups are present, and also utilize acoustics for species identification. *M.evotis* CAN be positively identified both in the hand and based on vocals. We will also continue to conduct bi-annual visual monitoring of the Hemlock Houses and Bunker as well as maintain temperature data loggers in all structures at the Hemlock site. In future years, we may enlist more sophisticated and remote monitoring such as pit tagging, remote acoustic detectors, and video feeds.

CHERON FERLAND
WILDLIFE BIOLOGIST
26 NOVEMBER 2014