

**Surveillance, Monitoring, and Natural History  
Investigations of Bats Related to White-nose  
Syndrome within the Colorado River Valley Field  
Office: 2017 - 2019**

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## Introduction

Bat populations in the western portion of the US are threatened by the rapid westward expansion of White-nose Syndrome (WNS), a disease implicated in the loss of over a million bats since 2006. *Pseudogymnoascus destructans* (*Pd*), the fungus believed responsible for WNS, has been confirmed in southeastern Wyoming, southcentral Kansas, western Oklahoma and the Texas panhandle, potentially placing at least 13 of the 18 bat species native to Colorado at risk for significant population-level declines. The continued westward movement of WNS emphasizes the need for improved information on the status of bats in Colorado, a systematic and thorough survey and assessment of the importance of caves and abandoned mines to Colorado's bat populations, and a coordinated effort to monitoring for WNS in the state. This grant has assisted in funding various projects addressing these issues.

Colorado Parks and Wildlife (CPW) has developed a statewide White-nose Syndrome in Bats Response Plan (2011) and a Bat "White-nose Syndrome" Surveillance Plan and Protocols (2011). These plans are currently being updated to reflect new information gained in recent years. CPW began surveillance work in the winter of 2010-2011 with the collection of baseline acoustic data at important bat hibernation sites around the state, along with external and internal surveys of other known and possible roost sites, including the collection of microclimate data. Other important aspects of the work being undertaken by CPW include public outreach on the importance of bats and the threat posed by WNS, and coordination with the various agencies and individuals potentially impacted by WNS, such as federal land management agencies, recreational caving organizations and private land owners.

In Colorado, little is known about the historical use of caves by bats, as well as the movement of bats between different types of roost environments throughout the year but especially for hibernacula. Few museum records exist that document historical colonies in winter and anecdotal information is also rare. In a few cases, historical roost locations are known, but it is largely unknown if these sites continue to support viable colonies or are suitable for bat use. An effort to survey caves for bat use was initiated by the then Colorado Division of Wildlife (now CPW) in 2001, but was discontinued after one year due to the lack of funding (Siemers 2002). Recent survey efforts by the White River National Forest and the Colorado River Field Office of the BLM have provided additional insight into many of our caves and led to a new understanding of bat use within them. The threat of WNS in Colorado has highlighted this lack of knowledge on the status of Colorado's bats.

Caves and abandoned mines are currently believed to play an important role for many bat species in Colorado but information about cave use, particularly as hibernation sites, was largely lacking until recently. The CPW Bats Inactive Mines Program has documented mines being used by bats to varying degrees, with an emphasis put on species of concern, such as the Townsend's big-eared bats (*Corynorhinus townsendii*; Hayes et al. 2011, Ingersoll et al. 2010). Suitable

hibernation sites may come in other forms such as rock crevices for bats in Colorado as found for big brown bats (*Eptesicus fuscus*; Neubaum et al. 2006). Efforts supported by the CRVFO found little brown myotis (*Myotis lucifugus*) using talus slopes as potential hibernacula, suggesting that rock crevice resources may be used by multiple species as the primary winter roosting sources (Neubaum 2018). An examination of cave and mine use during winter in this study found that *Myotis* species, those most vulnerable to WNS, were only confirmed during 35% of the surveys in small numbers (<5) and the Townsend's big-eared bat accounted for 97% of bat records in these sites. Information on other types of roosts used by many bat species in the state, such as maternity roosts and swarming sites, is needed before various management scenarios can be fully addressed, particularly those related to WNS. For example, fall swarming by bats has been documented at caves on the White River National Forest (Navo et. al. 2002; Ingersoll et al. 2010) but in few other locations around the state. This activity could serve multiple social purposes, including mating and orientation of young bats with potential hibernacula or preparation for migration (Fenton 1969, Davis and Hitchcock 1965). Swarming sites may also play a significant negative role in the spread of WNS, but fall surveys and evaluations for swarming bats is largely absent for most of Colorado's caves and will need to be examined.

Among the specific bat species proposed to be looked at in a more in depth manner for this project, Townsend's big-eared bat is listed as a species of State Special Concern and BLM sensitive species in Colorado. The species is also designated as an endangered or sensitive species in several western states. The little brown bat, once one of the most common small mammals in North America, has now been proposed for federal listing under the endangered species act (Kunz and Reichard 2010). Consequently, filling in some of the basic gaps in these two species natural history stands to inform management decisions surrounding WNS issues greatly.

The expected spread of WNS to Colorado in the coming years will only exacerbate concerns over the status of these bat species and the impacts of the disease on population trends. With the confirmed presence of the fungus *Pd* in Wyoming less than 200 miles from Colorado, the window to collect information on bats in a pre-WNS environment is quickly closing. By collecting this information now, we will have a better understanding of how WNS interacts in the natural environment and how to best manage roost sites, bats and WNS in the future. An adaptive approach for such efforts are described by the Bureau of Land Management's White-nose Syndrome adaptive management strategy (BLM 2014).

## **Objective**

To facilitate a coordinated multi-agency surveillance effort to effectively monitor, detect and combat White-nose Syndrome (WNS) introduction in caves, mines, and rock crevices under the jurisdiction of Bureau of Land Management in the State of Colorado.

### ***WNS Monitoring***

Use of acoustic detectors has emerged as a useful tool over the last decade to monitor bat activity. CPW identified this tool as one method of collecting baseline data at important bat hibernation sites before the arrival of WNS to the state so that differences in activity can be identified when/if they occur. Acoustic monitoring at Anvil Points Mine and Claystone Cave was initiated in January of 2012 and is ongoing at the cave. Some hibernacula in the state are also utilized by bats during the summer and fall seasons as well. It was determined that the relatively safe locations of the Anvil Points Mine and Claystone Cave offered good sites to analyze use by bats on a year-round basis. Acoustic data from these two sites which are located within the Colorado River Valley Field Office (CRVFO) Resource Boundary has been processed and summarized.

In the first year of this project (2011-2012), we attempted to conduct internal surveys of as many caves as possible and external surveys of all mine sites listed in the BLM CRVFO Resource Management Plan (Table 1) to determine presence or absence of bats at the sites, as well as degree and seasonality of the use if it is documented. Locating many of the caves listed in Table 1 provided by CRVFO to conduct internal or external surveys were contingent upon assistance from the Colorado Cave Survey and volunteer cavers. Levels of technical skills required to navigate to or through some caves dictated if sites were surveyed. Site visits preferably occur during winter months but are limited by accessibility of the site or availability of caver assistance and consequently will be completed as soon as is safely feasible. Temperature/humidity data loggers were placed in a subset of caves near areas used by hibernating bats to determine if these sites provided adequate climatic conditions for *Pd* to establish and persist (addressed in previous reports). BLM and USFS biologists have been available when needed to provide assistance. Work in future years will continue to address remaining questions about the possible impact WNS will have on the bat species of Colorado. As results become available, we will work to implement them in the current Colorado WNS Response Plan and through coordinated management. Finally, digital maps in the raster format were provided to the CRVFO to assist with future management of bat species.

**Table 1.** Caves and mines identified in the BLM Colorado River Valley Field Office Resource Management Plan and considered for surveys to document potential bat use and to monitor for presence of White-nosed syndrome.

Caves	Mines
Canyon Creek area	
Panorama Cave	
Spectre Cave	
Windsong Blowing Cave	
Deep Creek area	
Big Ass Disappointment (BAD) Cave	
Cattleguard Cave	
Cave of the Studs	
Doomed Cave	
Echo Dome Cave	
Good Earth Cave	
Lasunder Cave (gated)	
Pendulum Cave	
Spinsters Cave	
Twenty Pound Tick Cave	
Glenwood Canyon area	
Amphitheater Cave	
Creaking Tree Cave	
Drapery Den Cave	
Mabel's Room Cave	
Serendipity Cave	
Shield Cave	
Other areas	
Anvil Points Claystone Cave	Anvil Points Mine adits (gated)
Dirty Pool Cave	Elephant Mountain Mine (gated)
Hack Lake Sinks	Lady Belle Mine 5 (gated)
	Sunshine Mine 2 (gated)
	Sunshine Mine 4 (gated)

## Methods

### *WNS Monitoring*

Ultrasonic acoustic detectors (Model SM2, Wildlife Acoustics, Inc., Concord, MA) were utilized to record bat vocalizations at known or suspected hibernacula to gather baseline activity levels which could be used as a reference if WNS were to reach Colorado. Deployment of the detectors and analysis of the data followed protocols developed by CPW as part of its statewide WNS surveillance efforts (Appendix A). The goal of acoustic data is to determine baseline activity levels, with an emphasis on winter months, which can act as an alarm for unusual behavior such as activity during daylight hours when it would not be expected to occur. Recordings were analyzed using SonoBat 4.3.0 (SonoBat, Arcata, CA) and vocalizations were compared to reference bat calls from the Northern Arizona bat call library (SonoBat, Arcata, CA). Recordings were initially scrubbed at a medium setting which retains all calls except those with poor quality that are difficult to discern from noise.

Call analysis parameters were set to use a discriminant probability threshold of 0.9 and an acceptable call quality of 0.8 with a maximum of 32 calls. The discriminant probability refers to the probability of a call sequence falling within the centroid of the multi-dimensional data space for reference calls for a species. Two outputs result from the analysis for assessing the likelihood of a call sequence matching reference calls from a particular species. The “sequence classification by vote” identifies the species by requiring that the species with the greatest number of calls classified be at least twice as prevalent as the sum of the 2<sup>nd</sup> and 3<sup>rd</sup> most abundant species classifications. The second output, the “mean sequence classification”, is based on the mean parameter values of the most prevalent classification group then uses these mean values (minimum of 2 calls) through a decision tree engine. If the values fall below the minimum threshold for a classification group, the call is not attributed to that group, but instead is displayed with the species groups that sum to the thresholds for the last decision tree step attained. A consensus value is also generated, which indicates the species designation if determined by both methods. I report species determinations when possible based on this consensus value. If a consensus value is not attained, I report the call as a general classification of high frequency or low frequency species. Graphs are based on any calls considered to be emitted by a bat, regardless of species or group classification so as to represent the overall bat activity at the site.

Locations where internal cave surveys were conducted were considered Tier 1 sites in the CPW Bat “White-nose Syndrome” Surveillance Plan & Protocols (2011). Survey activities at Tier 1 sites consist of entering the cave to gather presence/absence data on bats, estimate number and species of bats, gather soil samples for *Pd* testing, and collect microclimate data. Temperature/humidity data was collected opportunistically using hand held laser temp guns and temp/humidity units (Kestrel 3000 Pocket Weather Meter, Birmingham, Michigan). Internal



survey data was collected only when activities did not pose a threat to hibernating bats. Time spent in the cave or mine was kept to a minimum. To minimize disturbance to roosting colonies, known hibernacula were entered every other year when feasible. Scientific, common, and abbreviated names of bats that may be encountered during such surveys are provided in Table 2. Tier 2 sites utilized external monitoring using acoustic survey techniques previously described. Work conducted at Tier 1 and Tier 3 sites may also occur at these sites depending on the internal access of a given feature. Sites were designated as Tier 3 according to CPW Bat “White-nose Syndrome” Surveillance Plan & Protocols (2011) if they only have external access (no door in existing mine gate). Survey activities at these sites consist of surveying the cave or mine opening for presence of bats and searching for signs of bat mortalities.

Probability of occurrence maps were developed in MaxEnt (ver. 3.3.3; Phillips and others 2006) to guide conservation and management of bat species that occur in Colorado. Location data for bat species was compiled from capture events and surveys conducted at foraging and roosting sites used across the state from 1906 – 2018. Datasets included the Colorado Parks and Wildlife Bat and Abandoned Mine databases, and scientific collection reports along with historic records compiled in Armstrong et al. 1994. Records from the health department and rehabilitation centers were not included as the original collection locations could not be verified. Records with a location accuracy greater than the Section (T.R.S.) or coordinate level were removed to ensure location accuracy. Conversely, records that occurred within a 25 m buffer were also removed to prevent over representation of the same location. A total of 20,478 locations were used in the model development. Data was modeled as presence-only to account for the subset of records that represented roost sites with known species use. The analysis also incorporated a digital elevation map (DEM, 30 m resolution), landcover (Basinwide, reclassified into 11 Ecoclasses based on Armstrong et al. 2011), a terrain roughness index (TRI, developed by M. Flenner with Colorado Parks and Wildlife GIS), and a continuous longitudinal layer. These variables have been considered ecologically relevant for delineating bat species occurrences by previous investigations (Duff and Morell 2007; Barnhart and Gillam 2017).

Locations were divided into test and training datasets for each species with response curves and a jackknife analysis used to measure variable importance as delineated by the “autofeatures” function in MaxEnt. Three model replications were averaged to create the final probability of occurrence map. The default settings in MaxEnt were used with 500 iterations, a convergence threshold of 0.00001, and a default prevalence of 0.5. We modified the resolution setting by placing it at 50 m. Models were developed for all Colorado bat species with the exception of the tricolored bat (*Perimoyotis subflavus*) and the Allen’s big-eared bat (*Idionycteris phyllotis*) for which we did not have an adequate number of sample locations (minimum of 25 records). Maps were clipped to the CRVFO for species occurring within the Resource Area boundary which encompasses landownership by the Bureau of Land Management as well as other federal and private lands.

**Table 2.** Scientific, common and abbreviated names of bat species that occur in Northwest Colorado.

Species	Common name	Abbrev
<i>Antrozous pallidus</i>	Pallid bat	ANPA
<i>Corynorhinus townsendii</i>	Townsend’s big-eared bat	COTO
<i>Eptesicus fuscus</i>	Big brown bat	EPFU
<i>Euderma maculatum</i>	Spotted bat	EUMA
<i>Lasionycteris noctivagans</i>	Silver-haired bat	LANO
<i>Lasiurus cinereus</i>	Hoary bat	LACI
<i>Idionycteris phyllotis</i>	Allen’s big-eared bat	IDPH
<i>Myotis californicus</i>	California myotis	MYCA
<i>Myotis ciliolabrum</i>	Western small-footed myotis	MYCI
<i>Myotis evotis</i>	Long-eared myotis	MYEV
<i>Myotis lucifugus</i>	Little brown myotis	MYLU
<i>Myotis thysanodes</i>	Fringed myotis	MYTH
<i>Myotis volans</i>	Long-legged myotis	MYVO
<i>Myotis yumanensis</i>	Yuma myotis	MYYU
<i>Nyctinomops macrotis</i>	Big free-tailed bat	NYMA
<i>Parastrellus hesperus</i>	Canyon bat	PAHE
<i>Tadarida brasiliensis</i>	Brazilian free-tailed bat	TABR

## Results & Recommendations

### *Baseline Activity from Acoustic Detectors*

Acoustic detectors were initially deployed at the Anvil Points Claystone Cave and Mine on December 15, 2011. At the cave, the detector was placed on the hillside approximately 20 feet due south of the skylight that opens from the Lower Big Room as indicated on the cartography in Reames (2011). At the Anvil Points Mine the detector was placed 15 to 20 feet out from the gate on Adit 3. Detectors were programmed to begin recording on January 1<sup>st</sup> and run continuously, 24 hours a day. Since the Anvil Points Claystone Cave is a relatively safe location the detector was left in place and has run continuously since its deployment so that baseline levels of summer activity could be monitored for the site as well. The detector at the

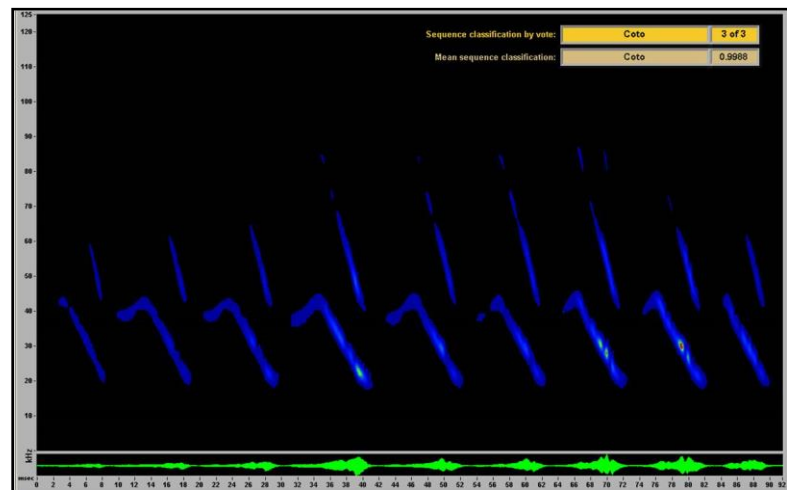


**Acoustic detector deployed at Anvil Points Claystone Cave.**

mine was taken down each year in May until the spring of 2017 when maintenance on the access road was discontinued due to relocation of the radio towers to a lower location. The detector setup at the mine was permanently removed at that time.

### Anvil Points Claystone Cave Acoustic Data

A total of 833 calls were considered attributable to bats at Anvil Points Claystone Cave from January 1<sup>st</sup> to March 15<sup>th</sup>, 2017 (Table 3). Calls were predominantly emitted by high frequency species (HiF = 473 vs LoF = 155). It should be noted that the number of calls does not equate directly to numbers of bats as one individual may circle in front of a gate or portal opening repeatedly (Gannon et al. 2003). Totals from the same time window in 2018 were over twice as high with 1,714 calls recorded. Of these recordings, 844 were high frequency and 381 low frequency calls (Table 3). Activity levels during the winter of 2017/2018 were similar to those collected in 2014/2015 and 2015/2016. Recording efforts in 2016/2017 may have been lower due to a damaged microphone that was replaced. Modest spikes in activity (<100 calls) recorded mid-winter were again the trend as with previous winters with one larger bump in activity occurring the first or second week in March when emergence from the hibernacula would be expected (Figure 1A and 2A). Activity at the site continues to be normal with calls by time collected over the winter occurring during hours after dark when bat activity would be expected (Figures 1B and 2B). Low but consistent call activity at this site during the winter months supports internal survey findings that suggest this site continues to be used as a hibernaculum by modest numbers of bats (Neubaum 2012, 2013, 2014, 2015, 2016). Calls were classified to the species level for 4 species (Table 4). The classification for the hoary bat (*Lasiurus cinereus*) is unexpected in winter as this species is thought to be migratory. Although the call was vetted by hand and does resemble examples from Sonobats call library for this species, it is similar to those for the big brown bat and the silver-haired bat (*Lasionycteris noctivagons*) and should be considered questionable. Both of the former species were classified for this site during winter as well, making them more probable contributors for the unusual call (Table 4). In general, larger numbers of calls were associated with high frequency bat species such as those in the *Myotis* genus. Many of these calls are likely to be from bats roosting in the surrounding terrain and not at the cave itself. This assumption is based on observations of only Townsends big-eared bats



**Sonogram of *Corynorhinus townsendii* such as those noted at Anvil Points Claystone Cave.**

collected during internal surveys during winter (see *Internal Cave Surveys* below). The absence of recordings for Townsend's big-eared bats from the data may be accounted for by the fact that this species is difficult to record acoustic calls from as they emit soft or low amplitude calls that are often missed by microphones or washed out by louder calls of other species.

In addition to winter surveillance, acoustic detectors were maintained continuously through the summers of 2017 and 2018 at Anvil Points Claystone Cave to examine activity levels during the reproductive period. The cave was confirmed as a maternity colony for *C. townsendii* during the summer of 2014 (Neubaum 2014). A total of 14,793 calls attributed to bats were recorded during the summer maternity season of May 15 to August 15, 2017 (Table 3). Numbers in 2018 were higher with 20,211 calls recorded during the same timeframe. Call activity during summer months reached its peak during two periods, one in mid-June and another in late July to early August (Figures 3A and 4A). These peaks would correspond with females making repeated visits to the roost soon after the birth of new pups in June and for dates expected for juvenile volancy later in the summer. As noted in previous reports, bats using the cave as a maternity colony are more likely to be exiting out of the upper portal on the cave given the internal location of the colony cluster. However, the lower portions of the cave and arch above are likely being used as night roosts by both the maternity colony and other bats found in the area as 11 species were classified in both 2017 and 2018 (Table 4).

### **Anvil Points Mine Acoustic Data**

In 2017, calls recorded at Anvil Points Mine in the winter were slightly higher than those recorded in 2016 (Figure 5A) with no unusual activity outside of night hours (Figure 5B). A total of 7,655 calls were recorded from January 1<sup>st</sup> to March 15<sup>th</sup>, 2017 with 180 attributed to high frequency and 12 to low frequency bats (Table 3). Only three species were confirmed via consensus by Sonobat with the California myotis (*Myotis californicus*) as being notable in that it was not recorded nearby at the cave (Table 4). The low call activity recorded throughout the winter at this site continues to suggest that modest numbers of bats are likely to be hibernating at the mine. It should be noted that monitoring Anvil Points Mine for winter use is difficult in that multiple portals, most connected internally, provide bats multiple exit points while recordings were limited to only one.

In 2017, summer recordings were not collected at Anvil Points Mine as the setup was removed after the winter season as previously noted. Investigating the potential movements of bats between Anvil Points Mine and the Cave would be valuable to understand in the event that WNS is introduced at either of these sites. The two sites are likely to see regular movements between one another given Townsend's big-eared bats propensity to shift sites (Sherwin et al. 2003).

**Table 3.** Acoustic totals for Anvil Points Claystone Cave and Mine collected during the winter (January 1 - March 15) and summer (May 15 – August 31) of 2017 and 2018. Total passes reflect calls from bats with suitable quality to be assigned to a high or low frequency grouping (High Freq, Low Freq). Possible high frequency (40–50 kHz) species include: MYCA, MYCI, MYEV, MYLU, MYVO, MYYU, and PAHE; possible low frequency (10–30 kHz) species include: ANPA, COTO, EPFU, EUMA, LACI, LANO, MYTH, NYMA, and TABR.

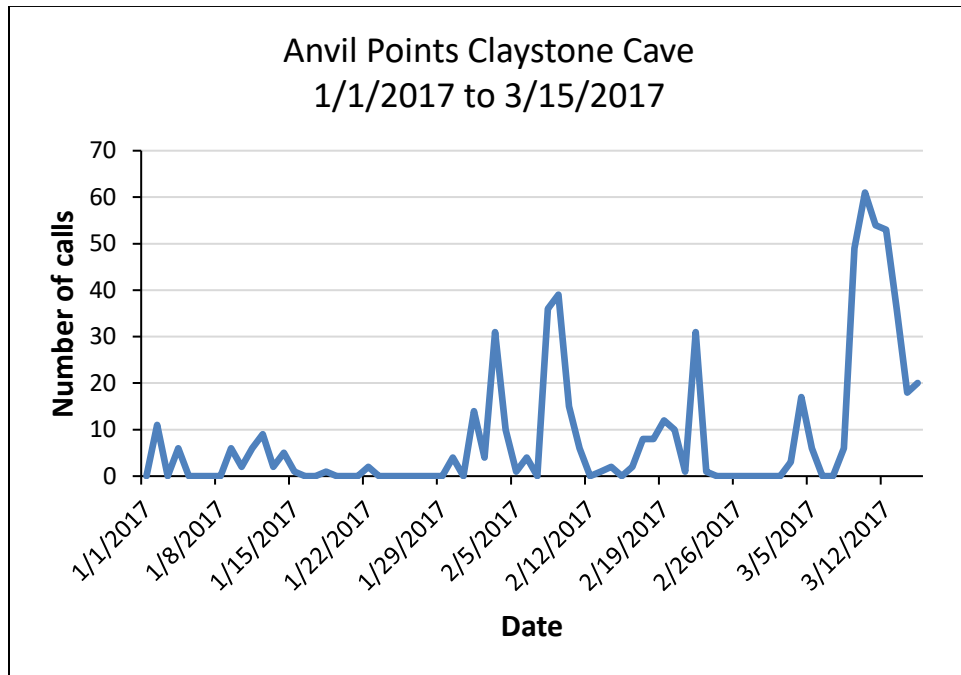
Site	Season	Year	Total	Total	High Freq	Low Freq
			Recordings	Passes		
Anvil Points Cave	Winter	2017	833	628	473	155
	Summer	2017	14,793	12,001	8,730	3,271
	Winter	2018	1,714	1,179	844	381
	Summer	2018	20,211	16,517	11,654	4,863
Anvil Points Mine	Winter	2017	7,655	187	180	12

**Table 4.** Acoustic calls that reached consensus and were classified to species by year collected at Anvil Points Claystone Cave and Mine during 2017 and 2018. Totals are presented as winter (W) and summer (S) calls for each species by year. See Table 3 for season dates.

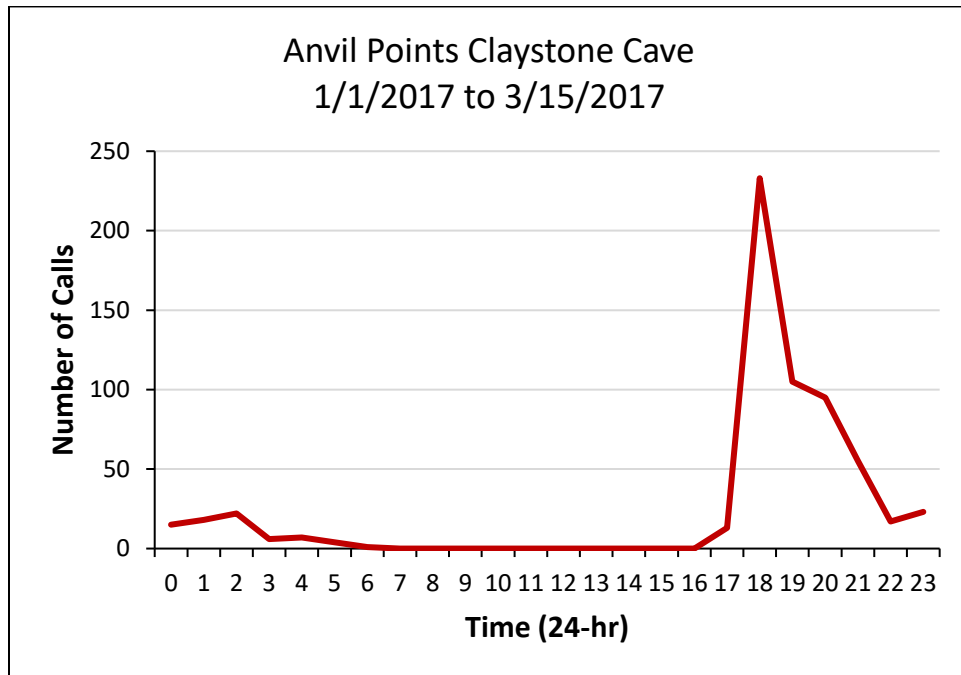
Species	Anvil Points Cave				Anvil Points Mine			
	2017		2018		2017		2018	
	W	S	W	S	W	S	W	S
Pallid bat		X		X		--	--	--
Townsend’s big-eared bat		X		X		--	--	--
Big brown bat	X	X	X	X	X	--	--	--
Spotted bat						--	--	--
Silver-haired bat	X	X	X	X	X	--	--	--
Hoary bat		X	X	X		--	--	--
Allen’s big-eared bat						--	--	--
California myotis		X			X	--	--	--
Western small-footed myotis						--	--	--
Long-eared myotis		X		X		--	--	--
Little brown myotis		X		X		--	--	--
Fringed myotis				X		--	--	--
Long-legged myotis						--	--	--
Yuma myotis						--	--	--
Big free-tailed bat		X		X		--	--	--
Canyon bat	X	X	X	X	X	--	--	--
Brazilian free-tailed bat	X	X	X	X		--	--	--

**Figure 1.** Acoustic activity levels during the hibernation season by date (A) and time (B) for Anvil Points Claystone Cave, Garfield County from January 1<sup>st</sup>, to March 15<sup>th</sup>, 2017.

A)

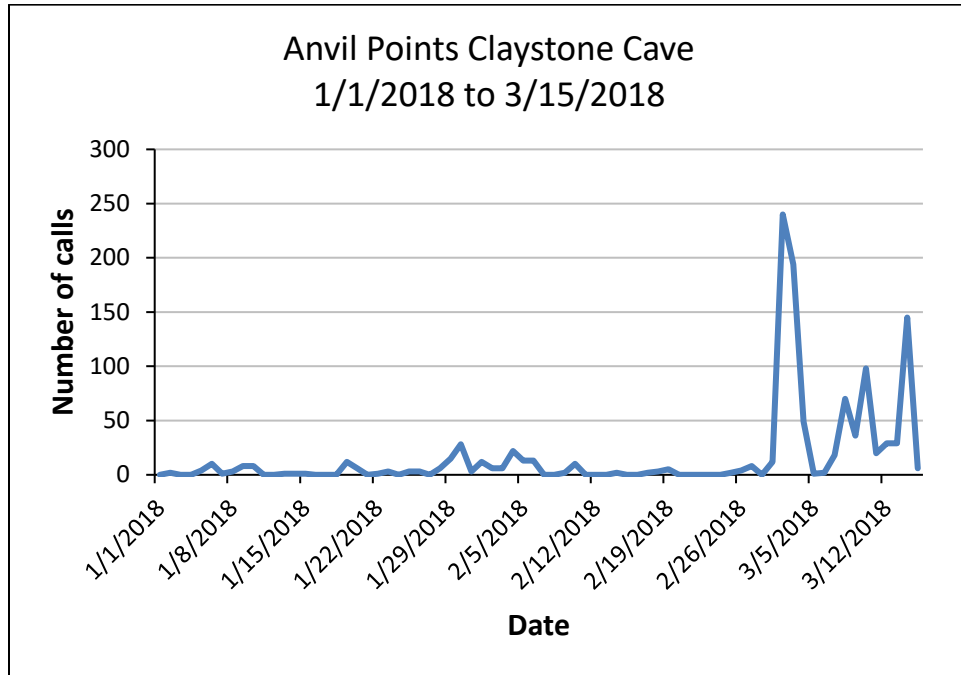


B)

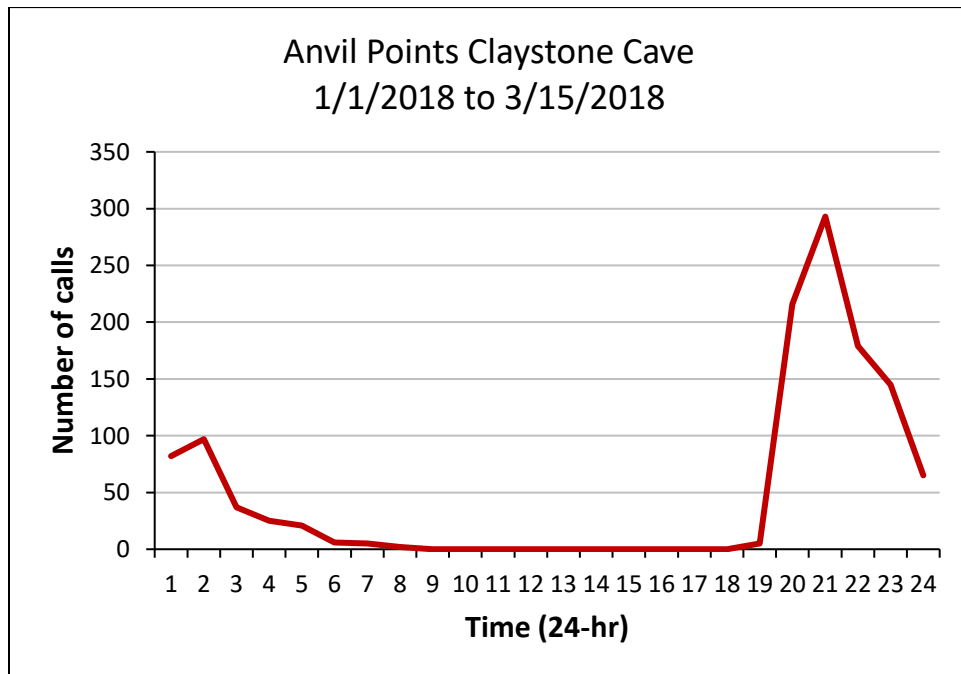


**Figure 2.** Acoustic activity levels during the hibernation season by date (A) and time (B) for Anvil Points Claystone Cave, Garfield County from January 1<sup>st</sup>, to March 15<sup>th</sup>, 2018.

A)

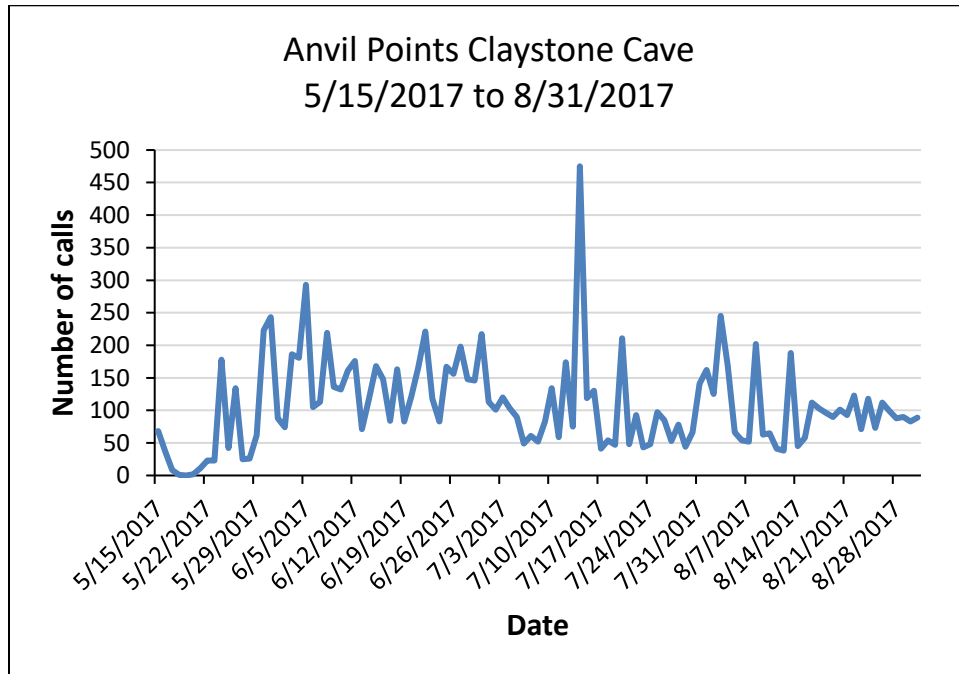


B)

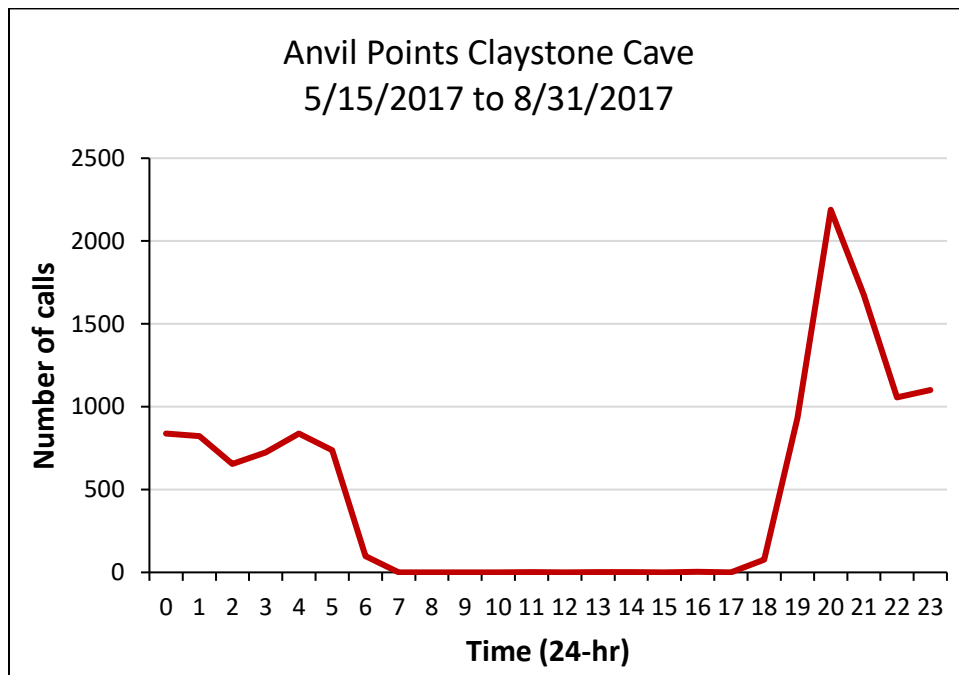


**Figure 3.** Acoustic activity levels during the maternity season by date (A) and time (B) for Anvil Points Claystone Cave, Garfield County from May 15<sup>th</sup> to August 31<sup>st</sup>, 2017.

A)



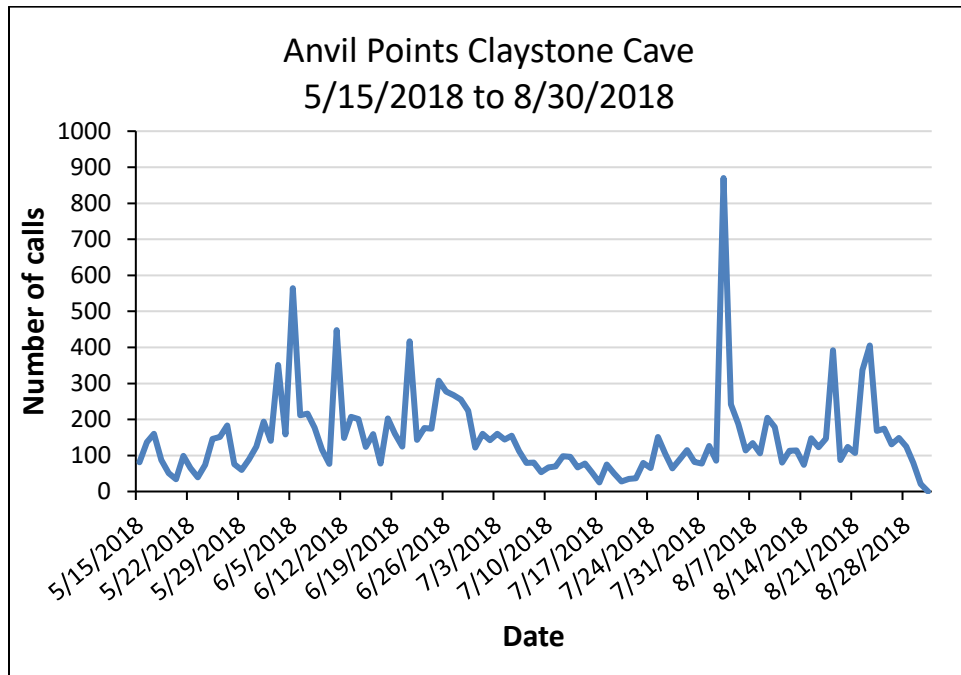
B)



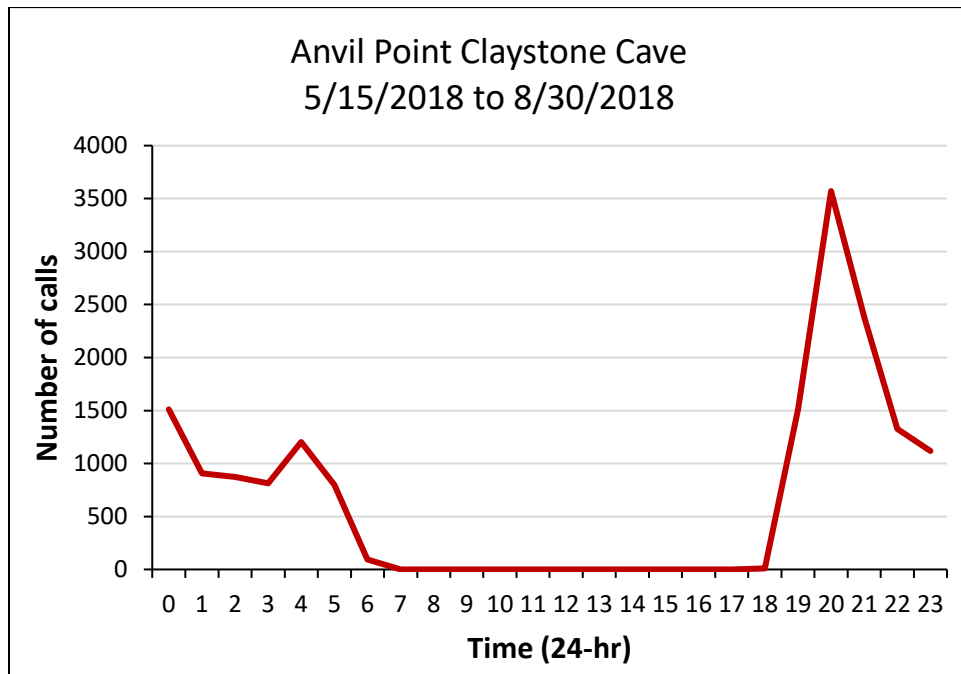


**Figure 4.** Acoustic activity levels during the maternity season by date (A) and time (B) for Anvil Points Claystone Cave, Garfield County from May 15<sup>th</sup> to August 30<sup>th</sup>, 2018.

A)

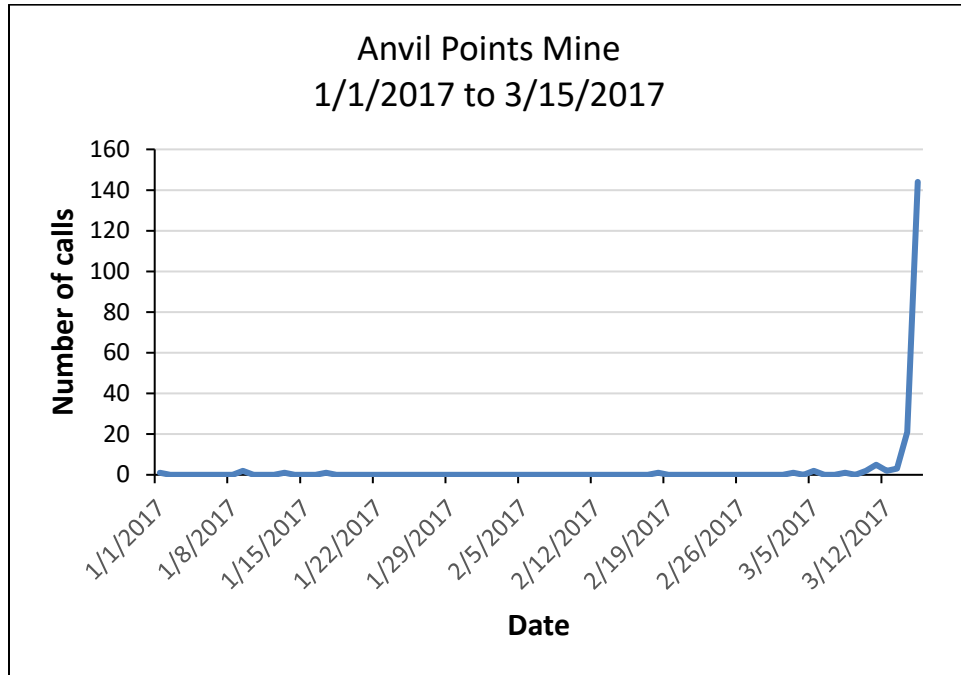


B)

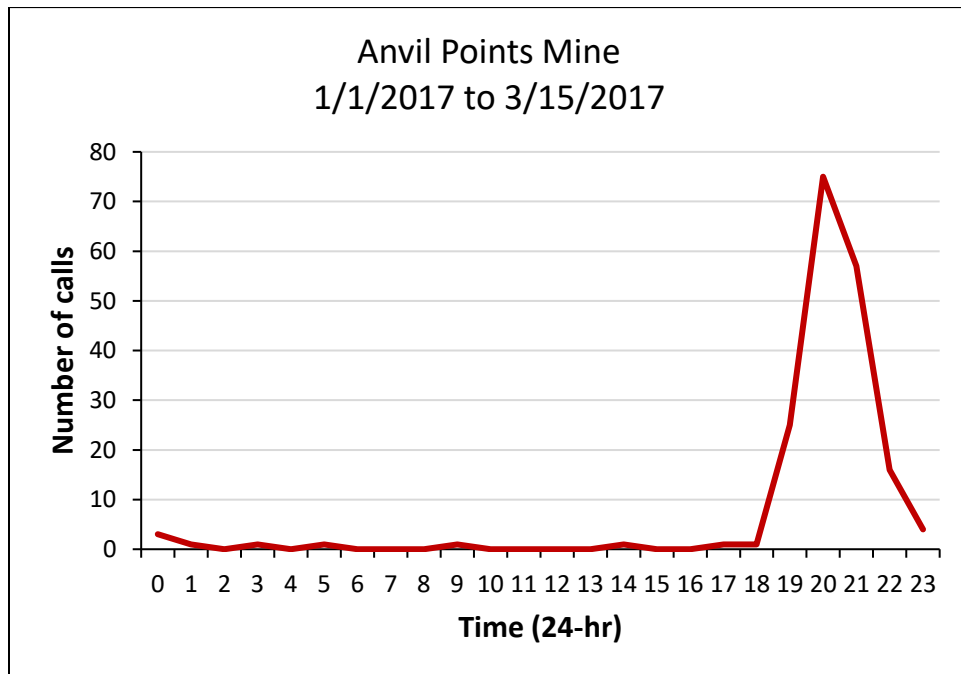


**Figure 5.** Acoustic activity levels during the hibernation season by date (A) and time (B) for Anvil Points Mine, Garfield County from January 1<sup>st</sup>, to March 15<sup>th</sup>, 2017.

A)



B)



### ***Internal Cave Surveys***

A total of 32 internal surveys have been conducted at 19 sites between 2011 and the winter of 2019 to investigate bat use (Table 5). Surveys of six caves, Anvil Points Claystone Cave, Big Entrance Cave, Cattleguard Cave, Fountainhead Cave, Surprise Pit, and Twenty-Pound Tick Cave were planned for 2018 on the CRVFO. Efforts to conduct winter surveys at Cattleguard Cave was canceled due to heavy snowfall that created adverse avalanche conditions. A winter visit of this cave will be attempted in 2019. Site visits to Twenty-Pound Tick Cave during summer, Fountainhead Cave and Surprise Pit in the autumn, Big Entrance Cave in spring, and Anvil Points Claystone Cave in winter were conducted from 2018 – 2019 (Table 5). Individual site reports that provide more detail for each site are provided under the ***Cave Visit Accounts***. Information related to the locality of a cave up Elk Creek that has had bat use reported at it is being pursued for 2019. Several caves in lower Deep Creek with vague locations placing them close to LaSunder Cave will be visited in 2019 (Table 6). An effort to reach Mabel’s Cave may be attempted during the summer of 2019 depending on access availability, time constraints and funds. A table summarizing use at all features visited between 2011 and 2019 follows the ***External Mine Surveys & Other Anthropogenic Structures*** section (Table 8).

**Table 5.** Caves, mines, and other structures on or adjacent to the Colorado River Valley Field Office surveyed internally for bat use from 2011–2019.

<b>Site Name</b>	<b>Survey Date</b>	<b>Bat Use</b>	<b>Temp Data</b>
Amphitheater Cave	3/24/12	No	No
Anvil Points Claystone Cave	3/31/11, 12/19/11, 5/7/12, 5/24/13, 6/13/14, 11/28/16, 2/26/19	Yes	Yes
BAD Cave	9/11/12, 10/2/13	Yes	Yes
Big Entrance Cave	4/12/18	No	No
Cave of the Clouds	3/24/12, 3/4/13, 7/16/14	Yes	Yes
Dirty Pool Cave	3/31/11	No	No
Drapery Den	3/4/13	Yes	No
Echo Dome Cave	9/11/12	Unknown	No
Fountainhead Cave	10/14/18	Yes	No
LaSunder Cave	5/7/11, 11/20/12, 10/2/13	Yes	Yes
Mabel’s Room Cave	8/18/15	Unknown	No
Not Spinsters Cave	10/2/13	Unknown	No
Shield Cave	8/3/16	Yes	No
Spectre Cave	7/2/11	Yes	No
Spinsters Cave	9/11/12, 10/2/13	Yes	Yes
Surprise Pit	10/14/18	Yes	No
The Tomb	9/20/13, 7/16/15	Yes	No
Twenty Pound Tick Cave	6/19/18	Yes	No
Wind Tunnel Cave	7/2/11	No	No

**Table 6.** Caves on the Colorado River Valley Field Office with special survey requirements or lacking accurate location data.

<b>Site Name</b>	<b>Special Requirements</b>
Cave of the Studs	Unknown location.
Creaking Tree Cave	General vicinity only for location
Devils Hole	General vicinity only for location
Doomed Cave	General vicinity only for location
Good Earth Cave	Survey planned for summer 2019.
Hack Lake Sinks	General vicinity only for location
Juniper Cave	Survey planned for summer 2019.
Panorama Cave	General vicinity only for location
Pendulum Cave	Technical rappel required for entry into cave
Serendipity Cave	Unknown location.
Windsong Blowing Cave	General vicinity only for location

*Cave Visit Accounts*

**Anvil Points Claystone Cave**

Location Information: Bureau of Land Management, Colorado River Valley District, Garfield County; 6,000 feet.

2016-2019 Survey Efforts: External surveys of the lower entrance and skylight to the Lower Big Room were conducted at Anvil Points Claystone Cave on 8 visits between 2016 - 2019 (11/27/16, 1/27/17, 6/7/17, 12/6/17, 2/1/18, 5/12/18, 8/30/18, and 2/26/19) while checking the acoustic detector setup. No signs of abnormal bat activity or mortalities were noted during these external visits near these exits. Educational and decontamination signs developed by the BLM were deployed on February 1<sup>st</sup>, 2018 just within the lower entrance so that anyone entering the cave would see them but hikers attention outside would not be drawn to the entrance. A full internal survey was conducted on February 26<sup>th</sup>, 2019 to look for hibernating bats and detect Pd/WNS if present.

Temperatures within the Lower Big Room were well below freezing on this visit which supports why no bats have been confirmed in this room during mid-winter (Table 7; locations as mapped in Reames 2011). One torpid Townsend’s big-eared bat was noted in the main passage between the Lower and Upper Big Rooms at a location where a torpid individual has reliably been noted during past winter surveys (see



**WNS signs deployed at the lower entrance of Anvil Points Claystone Cave.**

Neubaum 2012, 2013). This individual did not exhibit any outward signs of WNS such as skin lesions or presence of *Pd*.

**Table 7.** Observations and map notations for Anvil Points Claystone Cave collected in February, 2019 as depicted by cartography from Reames (2011).

Observations	Picture
<u>February 26, 2019 Survey</u>	
Signs still intact at the lower entrance (-1.0°C Surface, 10.6°C Ambient, 45.6% Relative Humidity)	
Lower Big Room (-8.2°C Surface, -6.8°C Ambient, 31.5% RH)	Yes
One torpid COTO hibernating in tall passage past Lower Big Room (-5.1°C Surface, 4.2°C Ambient, 57.4% RH)	Yes
Upper Big Room (1.5°C Surface, 10.5°C Ambient, 49.6% RH)	
In passage climbing uphill into a dead end room due north out of the Upper Big Room no bats were noted with temperatures at the upper end of winter suitability for bats (8.0°C surface, 15.1°C ambient, 44.2% RH)	



**Skylight in the Lower Big Room of Anvil Points Claystone Cave.**

Use Comments: No abnormal reports or findings of dead bats, such as those previously reported by local NSS grotto members were noted (Neubaum 2016). A summer maternity colony and small numbers of hibernating Townsend’s big-eared bats continue to use the cave during summer and winter.

Recommendations: Anvil Points Claystone Cave is used by several species of bats during all seasons of the year with an emphasis on the summer maternity and winter hibernacula seasons. Maintaining and updating a cave management plan that tracks visitation and requires affiliated recreational cavers to follow the most current decontamination protocols will be important in the coming years as WNS moves into Colorado. Visitation to the cave by users not affiliated with a grotto and possibly uneducated to decontamination requirements may be of concern related to spread of WNS. The discretely placed educational signs just inside the lower entrance of the cave will hopefully reduce chances of accidental spread of WNS. If WNS is documented in the state, the idea of using a trail camera placed at the lower entrance to determine number of visitations over the course of the year is highly encouraged. An idea of how many

unaffiliated public novice cavers are utilizing this site could be gained by comparing these numbers with those of affiliated cavers who have checked in with the BLM before a visit. A determination could then be made as to whether or not visitations may be causing enough of a disturbance to reduce numbers of bats using the cave. Periodic surveys of this cave should continue to be made to look for dead bats and cavers should be encouraged to continue reporting those when found. Recently diseased individuals will provide better necropsy results than mummified individuals such as those submitted in 2017.

### **Big Entrance Cave**

Location Information: Private, Garfield County; 6,570 feet.

2018 Survey Effort: Big Entrance Cave was visited in April 12<sup>th</sup>, 2018 by CPW. The approach to the cave was the same as that used for Cave of the Clouds. The feature is a large shelter cave that does not go beyond the twilight zone. Two locations in the cave have walls constructed of rock and wood. These structures were likely built by a homeless man who went by the name Paul McCartney and lived in this cave as well Cave of the Clouds intermittently for years. Large amounts of trash remain at the site today despite efforts by the caving community to haul much of it out not long after the homeless man's death. No signs of bat use were noted in the cave with the only likely area having heavy soot deposits from fires.



**Wood wall structure at Big Entrance Cave.**

Use Comments: The cave may be used by bats during the summer as a night roost but evidence such as scattered droppings were not confirmed. Occasional day roosting may occur but not in numbers that suggest use by a maternity colony as no concentrated guano piles were identified.

Recommendations: Based on this survey, it does not appear that Big Entrance Cave is used by bats for critical roosting opportunities including as a maternity colony or hibernaculum. Bats may use this shelter cave for night and transitional day roosting in small numbers. Given the aspect and shallow nature of the cave, its use by bats is unlikely to change notably. Consequently, future monitoring is not necessary at this site.

## **Fountainhead Cave**

Location Information: Bureau of Land Management, Colorado River Valley District, Garfield County; 7,700 feet.

2018 Survey Effort: Fountainhead Cave was visited on October 14<sup>th</sup>, 2018 by D. Neubaum (CPW) and S. McCollum (Caver). The cave is situated on the same ridge as Surprise Pit but over a 1,000 feet higher. The cave has one short passage that is narrow but high and runs mostly straight back into the hillside away from the cliff entrance.

Use Comments: This cave appears to be used as a night roost or occasional day roost for small numbers of bats based on scattered droppings that were found on the walls and floor, mostly within the first half of the passage from the entrance. Although no bats were noted during the full survey of the cave a few portions of the top of the passage could not be examined. Temperatures were suitable for use by bats as a hibernaculum although the shallow nature of the cave may not promote such use. Presence of some formations, such as moon milk, was noted in the back portion of the cave.

Recommendations: While this cave is relatively small it did show signs of some bat use and exhibits temperatures that would allow bats to overwinter. This cave is closely situated to the proposed Mid Continent Quarry expansion and should be considered for protection when the environmental impact statement is being developed for this resource extraction.

## **Surprise Pit**

Location Information: Bureau of Land Management, Colorado River Valley District, Garfield County; 6,647 feet.

2018 Survey Effort: D. Neubaum (CPW), and cavers S. McCollum, R. McFarland, K. Headrick, and M. Frazier surveyed Surprise Pit October 14<sup>th</sup>, 2018, the same day of the Fountainhead Cave visit. The cave is nestled discretely under several juniper trees and consists of a pit entrance about 2 m wide that drops about 3 m down to a landing. The cave slopes south, parallel to the hillside, declining for 20-30 m before pinching out. Evidence of some digging was noted at the pinch point. Ceiling height decreases from 1.5 to 1 m as it runs downhill. Ambient temperatures during our visit, a cold and snowy day, were right at freezing by the portal (range 7.0-8.9°C) internally.



**Entrance to Surprise Pit.**

Use Comments: The cave contains notable amounts of packrat droppings throughout its single passage. Temperatures suggest that the cave could be used as a hibernaculum but no bats were noted during the survey. Given that the visit occurred during the transition season a repeat visit during the middle of winter may be warranted to look for presence of torpid bats and see if the microclimate remains suitable. No evidence of use by a large maternity colony was noted despite a south aspect which may allow the cave to warm up in summer. Due to the small size and shallow nature of this pit cave, it is likely that temperatures may fluctuate, rising above and dropping below levels preferred by hibernating bats during winter.

Recommendations: Surprise Pit may provide limited day and night roosting opportunities for small numbers of bats. This cave is closely situated to the proposed Mid Continent Quarry expansion and should be considered for protection when the environmental impact statement is being developed for this resource extraction.

### **Twenty-Pound Tick Cave**

Location Information: Bureau of Land Management, Colorado River Valley District, Garfield County; 7,179 feet.

2018 Survey Effort: Twenty-Pound Tick Cave was visited on June 19<sup>th</sup>, 2018 by D. Neubaum of CPW, K. Levy and P. Fowler of the Colorado Cave Survey (CCS), and H. Boyd of the BLM. This cave has a resurgence stream that creates a sump not far past the entrance. Since its discovery in 1970, the sump and water temperatures in the low 40's (°F) greatly limited the number of visitors to this cave to a couple handfuls of cavers with dive experience. A blowhole in the same room as the sump was widened in 2017 and circumnavigates the flooded passage, effectively opening access to long stretches of additional passage by users without dive skills. The CCS and BLM are considering placing a gate on the new blowhole passage access due to concerns related to increased airflows altering humidity levels in the cave.

Use Comments: Temperatures within the first room were only a few degrees above freezing despite the survey occurring during the summer. A strong breeze ranging from 5-10 mph was recorded at the newly enlarged blowhole passage. No notable accumulations of guano were documented. Based on cold temperatures the cave may be used by bats, such as adult males, that are seeking to use torpor during summer. It is possible the cave could also be used as a hibernaculum if temperatures in the first room of the cave remain stable. Caver T.



**Blasting cap drill marks on walls of the new blow hole passage at Twenty-Pound Tick Cave.**



Shirrell reported seeing two *C. townsendii* at the cave on January 29, 1999, presumably prior to the sump. If the entrance to the cave gets covered by snow during years of heavy accumulation, this blockage may provide a buffer against unsuitable temperatures. However, bats tend to spend winters at sites with microclimates that are reliably stable across years. Access to passages beyond the blowhole are likely limited due to the high airflow there but not impossible.

Recommendations: Bats are not likely to use this cave in notable numbers due to its limited passage preceding the flooded sump and the high airflow at the blowhole. If a gate is placed on the blowhole access point it should be constructed in a manner similar to those used on mine and cave entrances using the ladder design. If blocking air flow is a concern, incorporating small gaps around the gates perimeter would allow bats to exit the feature in the event that they have crawled through the passage further into the cave prior to construction. Future cavers permitted to use the cave by the BLM should be encouraged to report any bat sightings.

### ***External Mine Surveys & Other Anthropogenic Structures***

External Tier 3 surveys were made at all mines listed on Table 1 between 2011 and 2019. No suspicious findings were made during these visits. In addition to the external visit to the mine gate, acoustic and video recordings were made in two cases to further investigate bat use at these sites. Anvil Points Mine was monitored continuously from 2013 to 2017 using acoustic detectors as part of the statewide WNS efforts (see ***Baseline Activity from Acoustic Detectors*** section). The Sunshine Mines (2 and 4) were monitored using an acoustic detector to follow up on use of the site by bats since no comparable efforts have been made at the site post gating. The entrance gates for Lady Belle Mines (1, 2, 4, and 6) were visited at least once from 2011 to 2012 on the CRVFO as part of the Tier 3 surveillance effort (see CPW 2011, Bat “White-nose Syndrome” Surveillance Plans and Protocols). Monitoring is continuing at Elephant Mountain Mine as part of a long-term survival study (Siemers and Neubaum 2019).

### **Anvil Points Mine**

Location Information: Colorado River Valley Field Office, Garfield County, 6,900 feet.

2017 Survey Effort: Anvil Points Mine portals, including Adit 3 where acoustic monitoring was conducted, were surveyed externally on December 2<sup>nd</sup>, 2016, and January 27<sup>th</sup>, April 11<sup>th</sup>, and June 6<sup>th</sup>, 2017. No dead bats or unusual behavior were noted during these visits.

Use Comments: This mine has a history of use by bats based on anecdotal reports from miners. An internal survey by Spears (2008) of URS Corporation was conducted in November of 2008 and noted possible maternity use. Acoustic monitoring in 2016 and 2017 indicates continued use by bats in summer and winter (see ***Baseline Activity from Acoustic Detectors***).

Recommendations: With the removal of the communication towers from the landing by the Anvil Point Mine Adit 4, the access road to this site has been blocked and is being allowed to slowly degrade. The decision was made to stop monitoring at the site as maintenance of the acoustic setup would be unsafe to access. However, the mine could still be reached via hiking which would be warranted periodically to check the integrity of the gates and perform visual searches at the portals. The importance of these periodic checks will be amplified if WNS is detected within the state.



**Acoustic detector deployed in front of Gate 3 at Anvil Points Mine during the winter of 2017.**



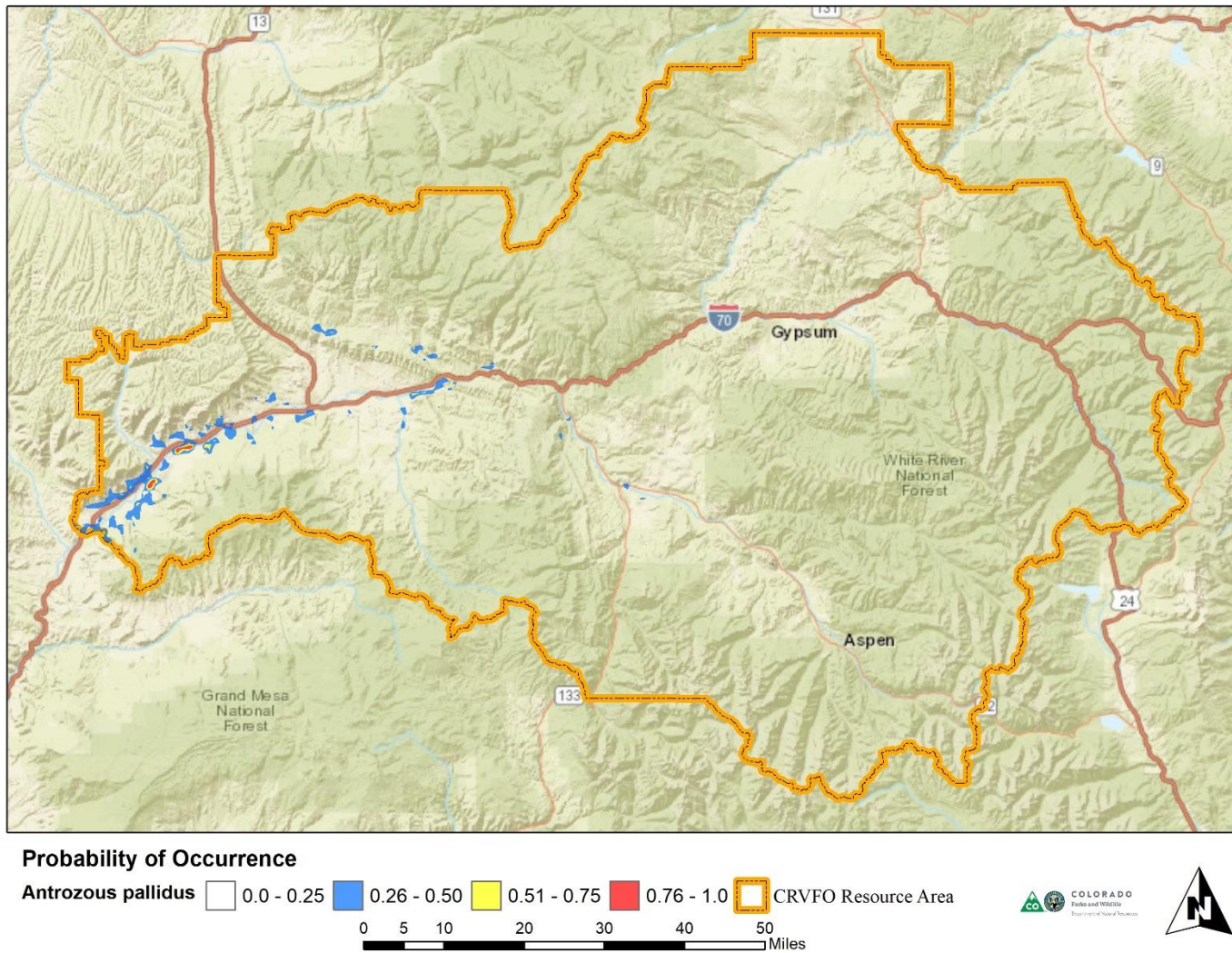
**Anvil Points Mine looking east towards the Colorado River Valley and Rifle, Colorado.**

### ***Species Range Mapping***

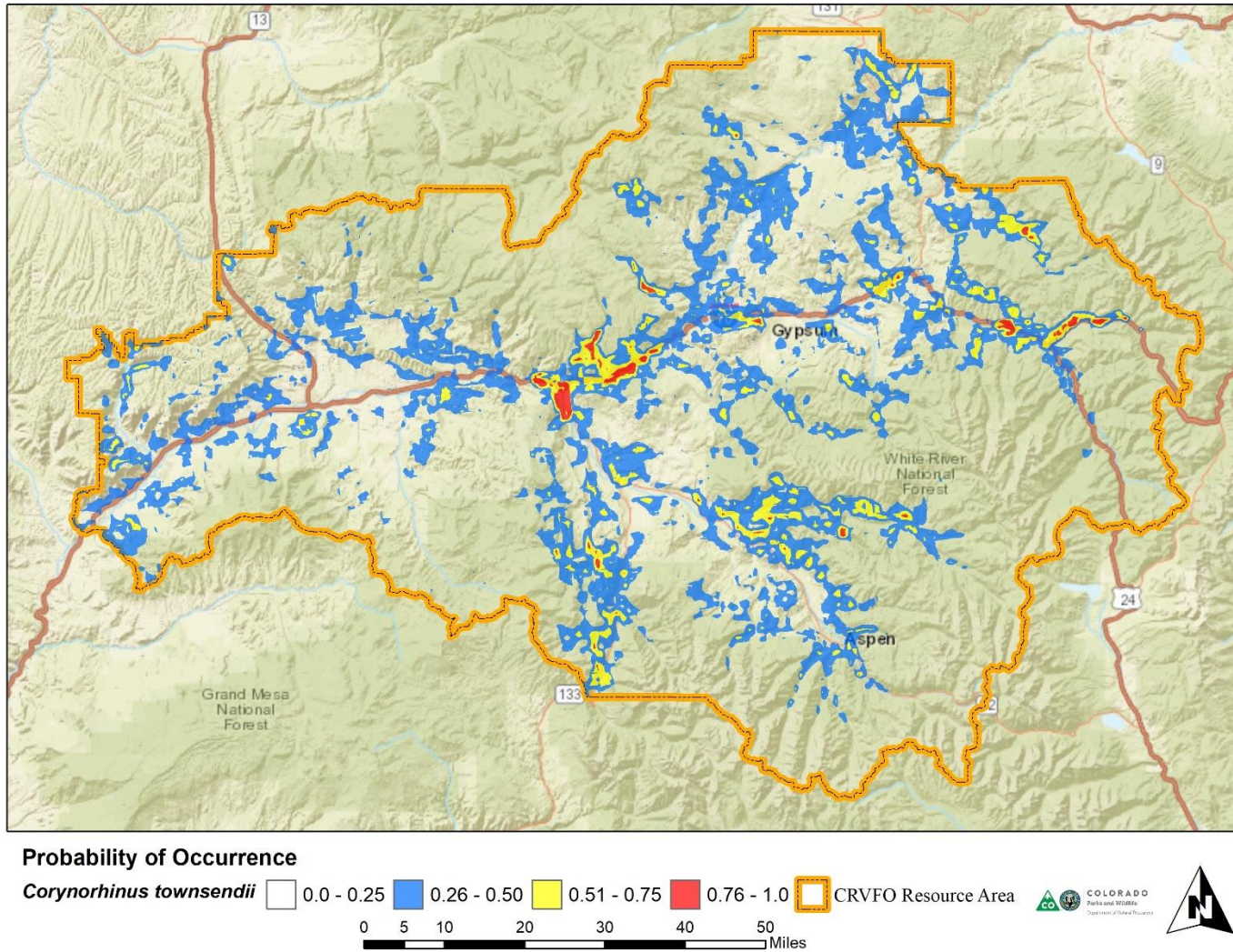
MaxEnt models depicting probability of occurrence were developed at the state level for 18 of Colorado's 19 bat species. Of these, 16 species were predicted to occur within the CRVFO field boundary. While models predict occurrence for spotted bats (*Euderma maculatum*) and big free-tailed bats (*Nyctinomops macrotis*), no physical records have been verified for these species within the CRVFO field boundary at the time of this writing. These two species are adept at avoiding mist nets which explains why they are not well represented in capture databases (e.g., CPW Bat Database). Areas with high cliff structure often in conjunction with perennial streams and rivers nearby, such as Anvil Points, the Colorado River north of Dotsero, and Deep Creek Canyon, may provide suitable habitat for these bats. Of the remaining three species of bats that occur in the state not depicted here, the Eastern red bat (*Lasiurus borealis*) and tri-colored bat (*Perimyotis subflavus*) are found from the Front Range east onto the plains and the third, the Allen's big-eared bat (*Idionycteris phyllotis*), is represented by only one record near Fruita, Colorado.

The CRVFO field boundary covers a wide range of elevations containing many diverse habitat types. This broad spectrum of niches facilitates the occurrence of a high bat species richness. Species modeled with high probabilities of occurrence within the CRVFO field boundary include Townsends big-eared bats, big brown bats, Hoary bats, silver-haired bats, Western small-footed myotis (*Myotis ciliolabrum*), big-eared myotis (*Myotis evotis*), little brown myotis, long-legged myotis (*Myotis volans*), and Brazilian free-tailed bats (*Tadarida brasiliensis*). Bat species with lower probabilities and covering less of the field office include the pallid bat (*Antrozous pallidus*), California myotis, fringed myotis (*Myotis thysanodes*), Yuma myotis (*Myotis yumanensis*), and canyon bat (*Parastrellus hesperus*). Of this later group, most of these species occur within the lower elevations of the western portions of the field office along the Colorado River Valley and its main tributaries. Exceptions to this generalization may occur related to specific behaviors such as swarming. Such events may draw species outside of the ranges they are typically associated with throughout the majority of the year to facilitate breeding opportunities (Navo et al. 2002). In addition, capture data that drives these models is severely lacking for dates that correspond with winter roosting. The Townsends big-eared bat has been shown to rely on caves and mines as hibernacula (Neubaum 2018). However, many Colorado bat species that have not been found using caves and mines in notable numbers are likely to use rock crevices similar to those found for little brown myotis and big brown bats (Neubaum et al. 2006, Neubaum 2018). Consequently, future work identifying winter roosts could influence these models and lead to broader distribution of the field office boundary by some species. In relation to WNS, these models suggest that seven species of myotis and the canyon bat will be of high concern for the CRVFO. Although declines in larger bodied bats from WNS has not been as dramatic, the remaining 8 species should be considered to have the potential for carrying the disease and potentially spreading it to vulnerable species.

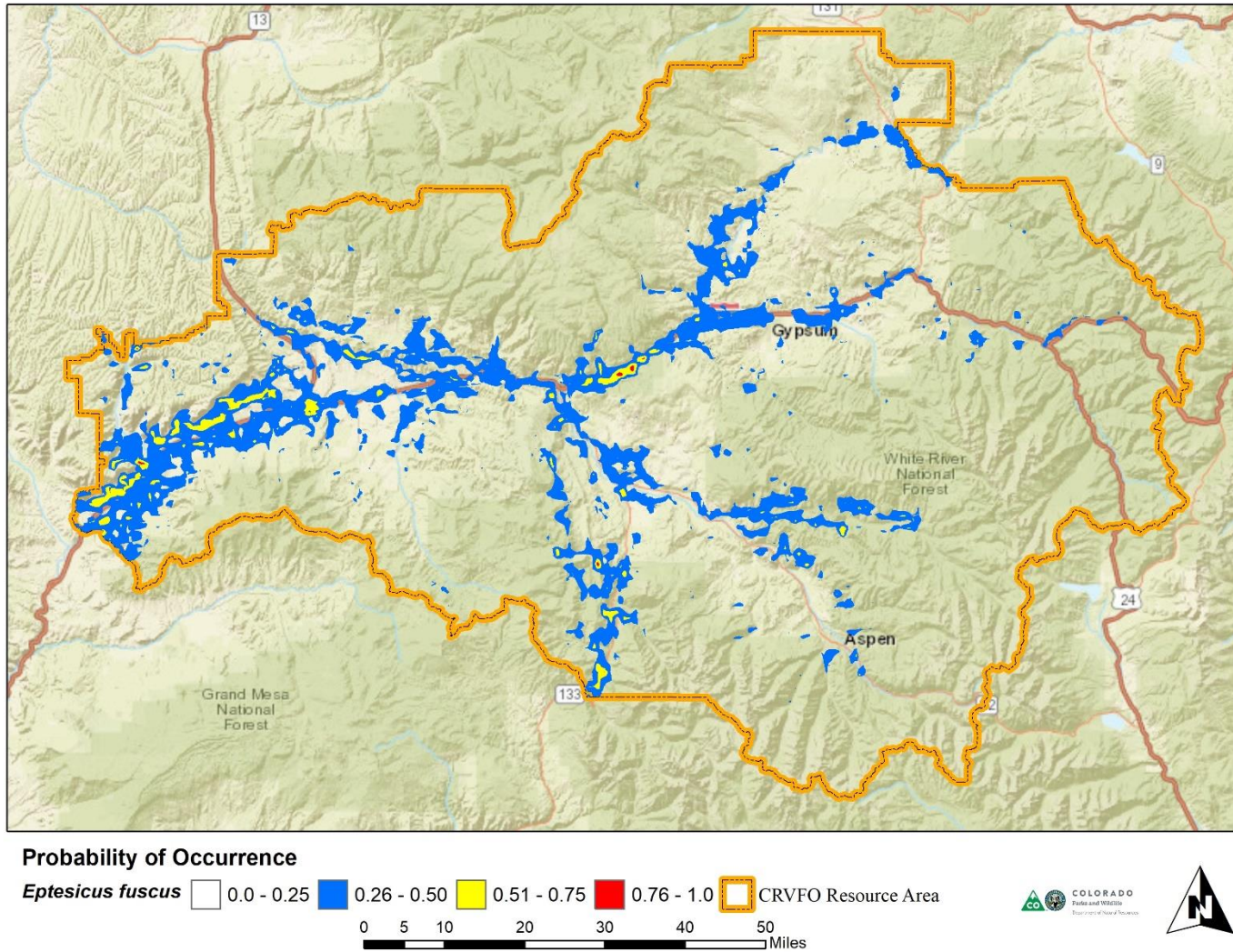
**Figure 6:** Probability of occurrence for *Antrozous pallidus* in the Colorado River Valley Field Office, Bureau of Land Management modeled using MaxEnt.



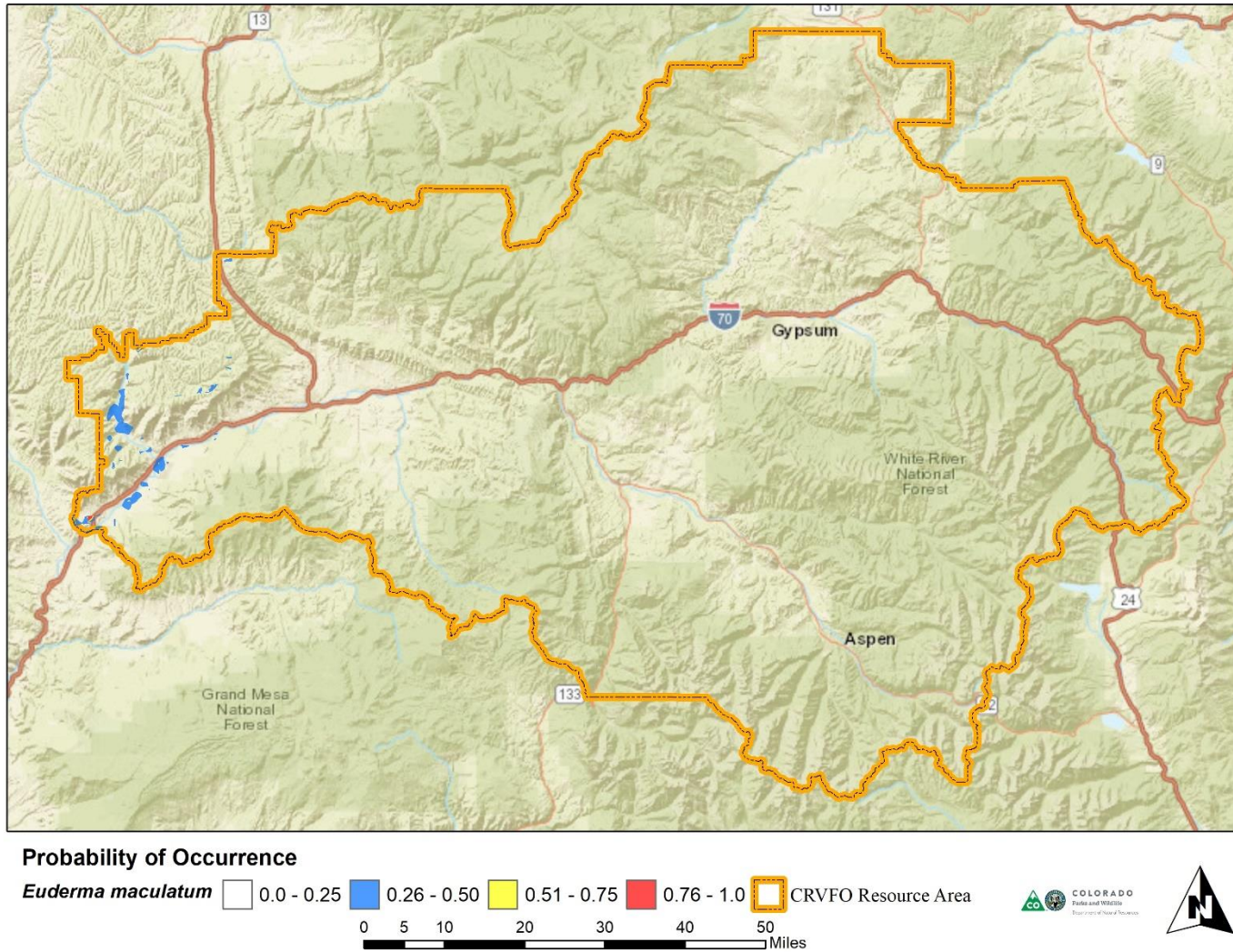
**Figure 7:** Probability of occurrence for *Corynorhinus townsendii* in the Colorado River Valley Field Office, Bureau of Land Management modeled using MaxEnt.



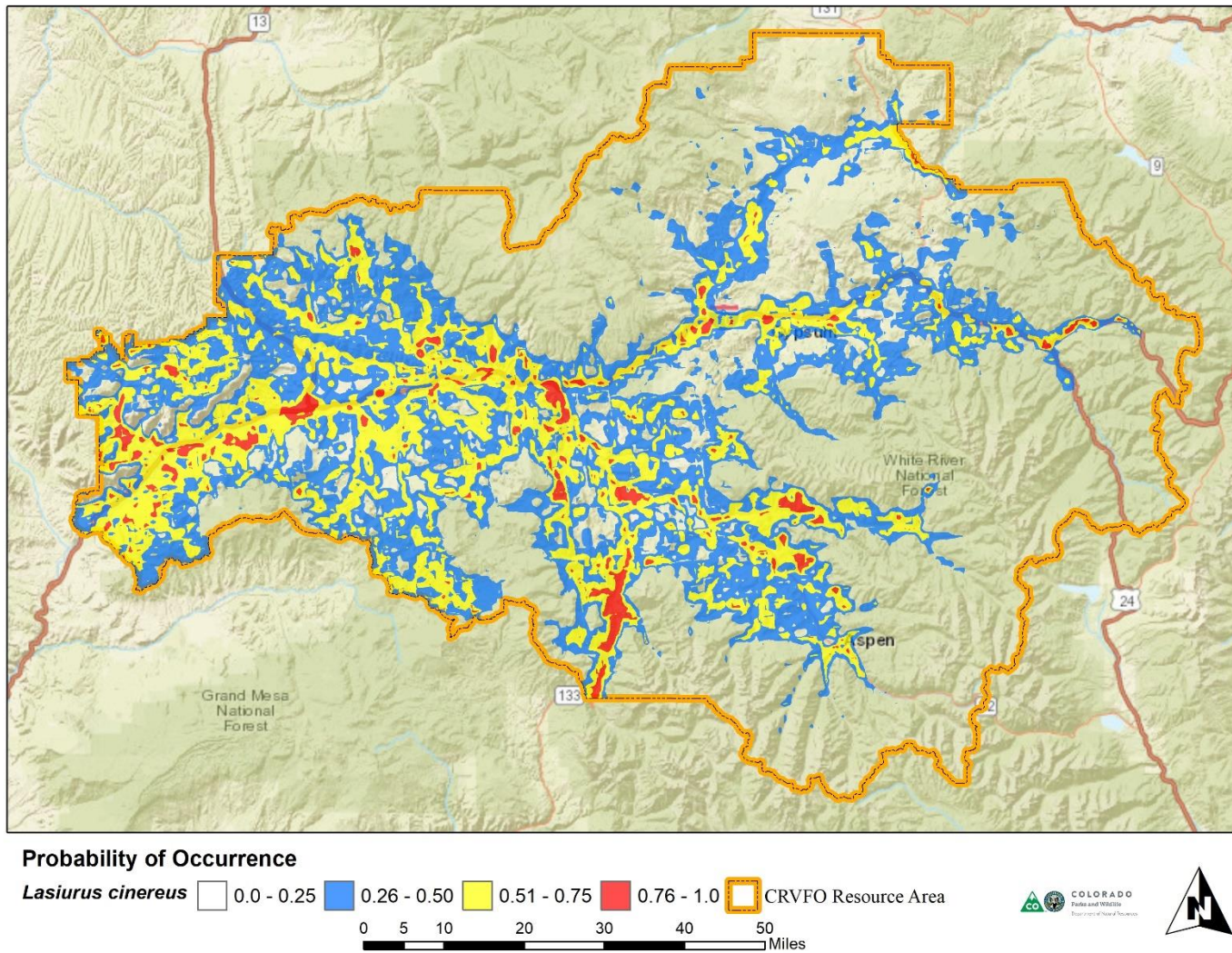
**Figure 8:** Probability of occurrence for *Eptesicus fuscus* in the Colorado River Valley Field Office, Bureau of Land Management modeled using MaxEnt.



**Figure 9:** Probability of occurrence for *Euderma maculatum* in the Colorado River Valley Field Office, Bureau of Land Management modeled using MaxEnt.

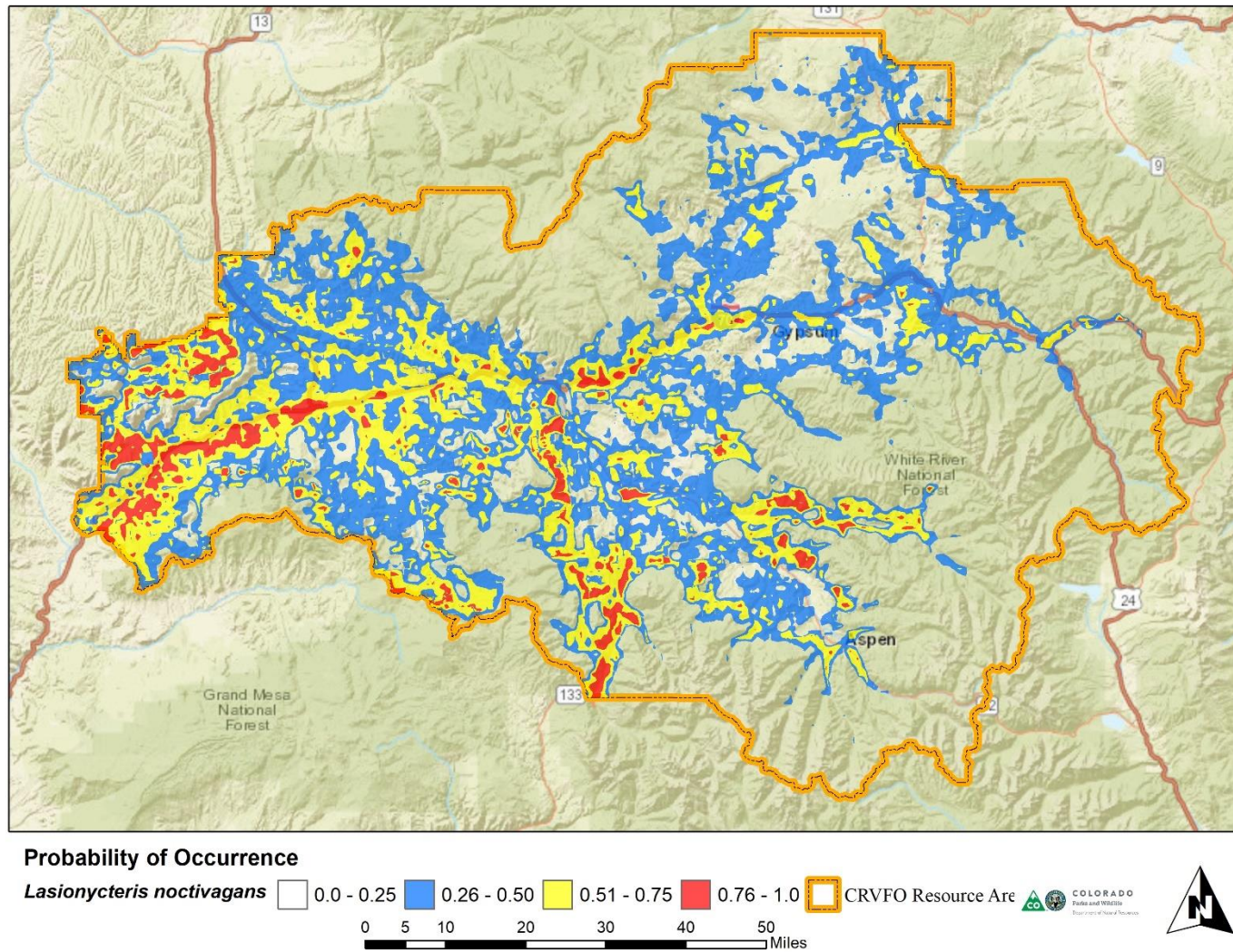


**Figure 10:** Probability of occurrence for *Lasiurus cinereus* in the Colorado River Valley Field Office, Bureau of Land Management modeled using MaxEnt.

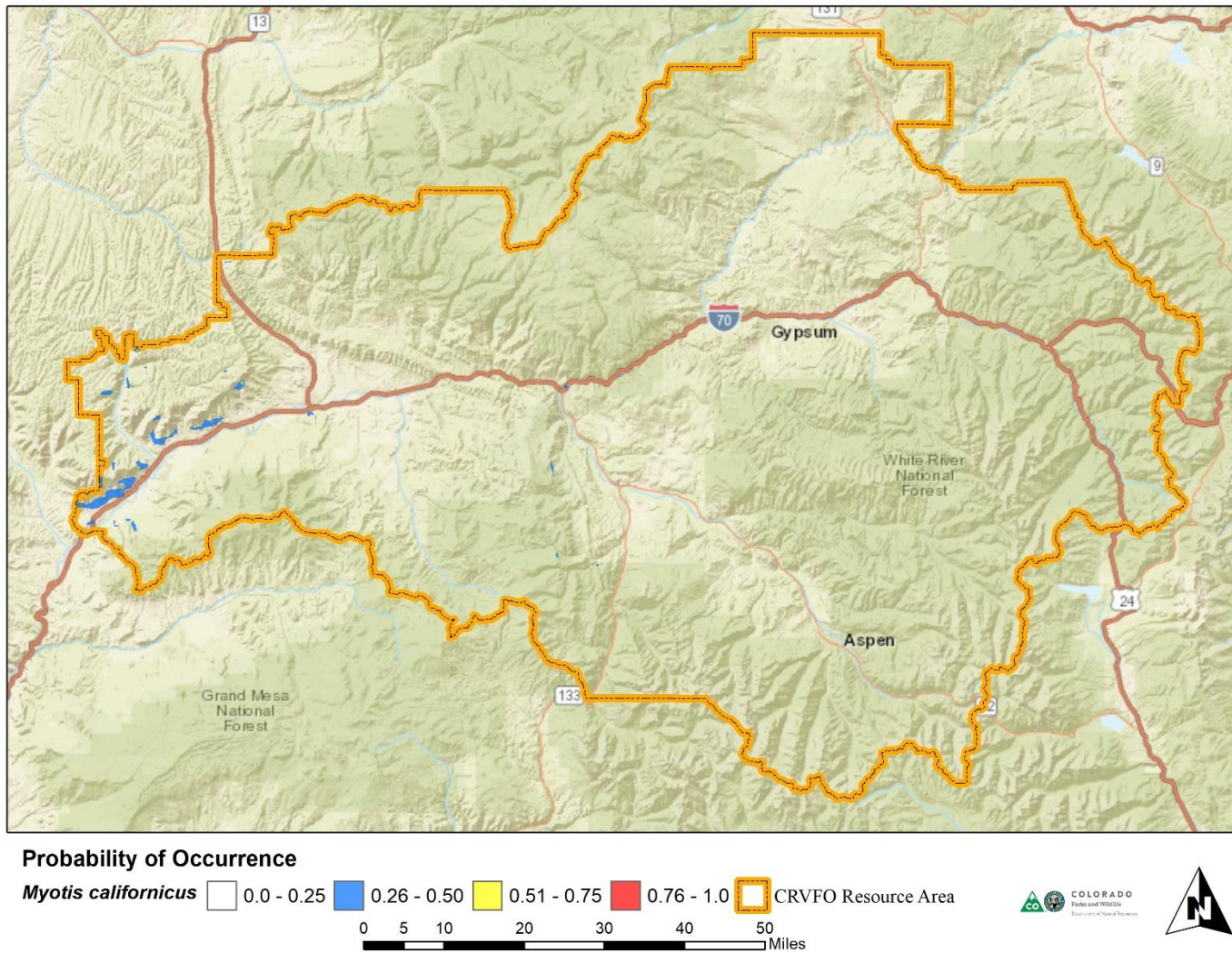




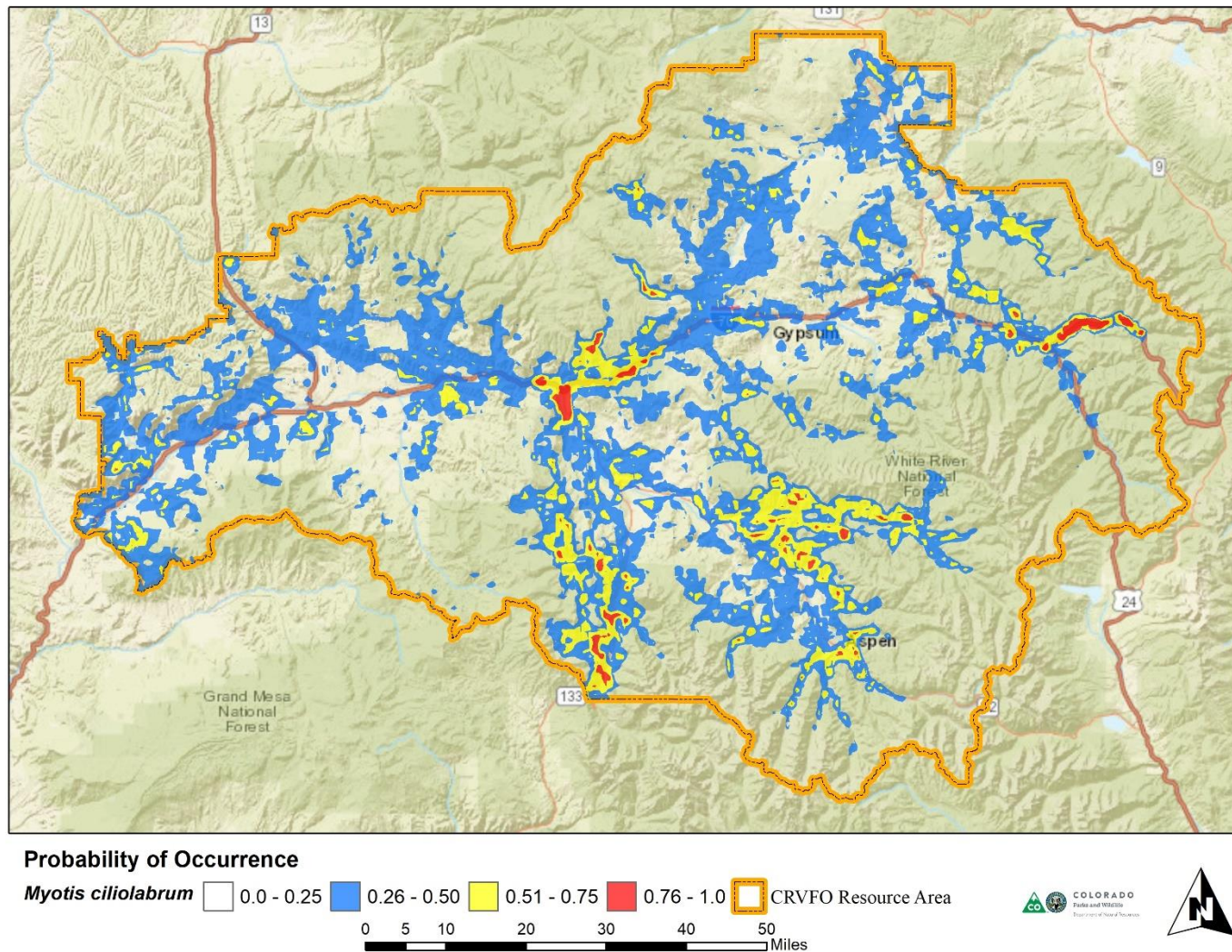
**Figure 11:** Probability of occurrence for *Lasionycteris noctivagans* in the Colorado River Valley Field Office, Bureau of Land Management modeled using MaxEnt.



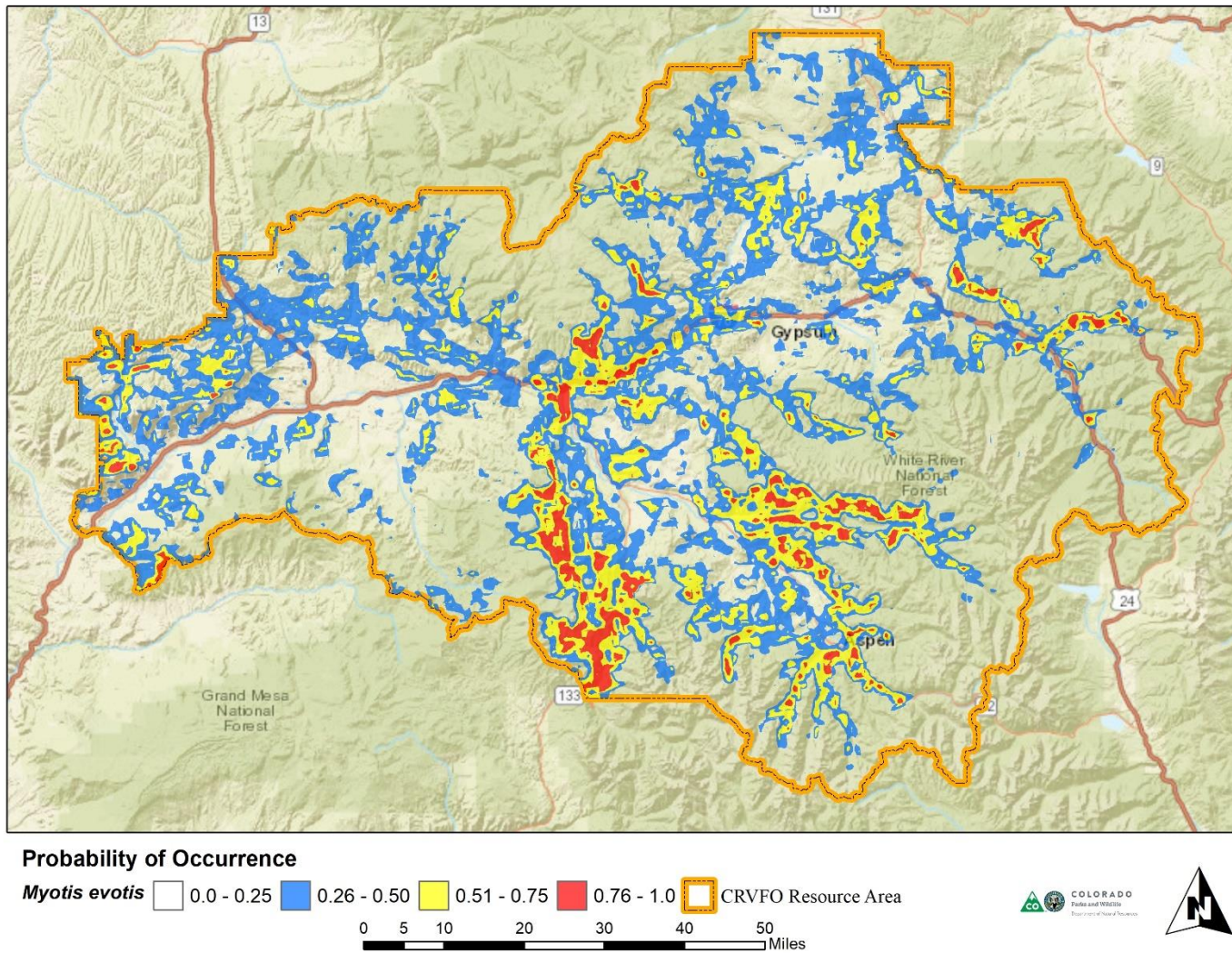
**Figure 12:** Probability of occurrence for *Myotis californicus* in the Colorado River Valley Field Office, Bureau of Land Management modeled using MaxEnt.



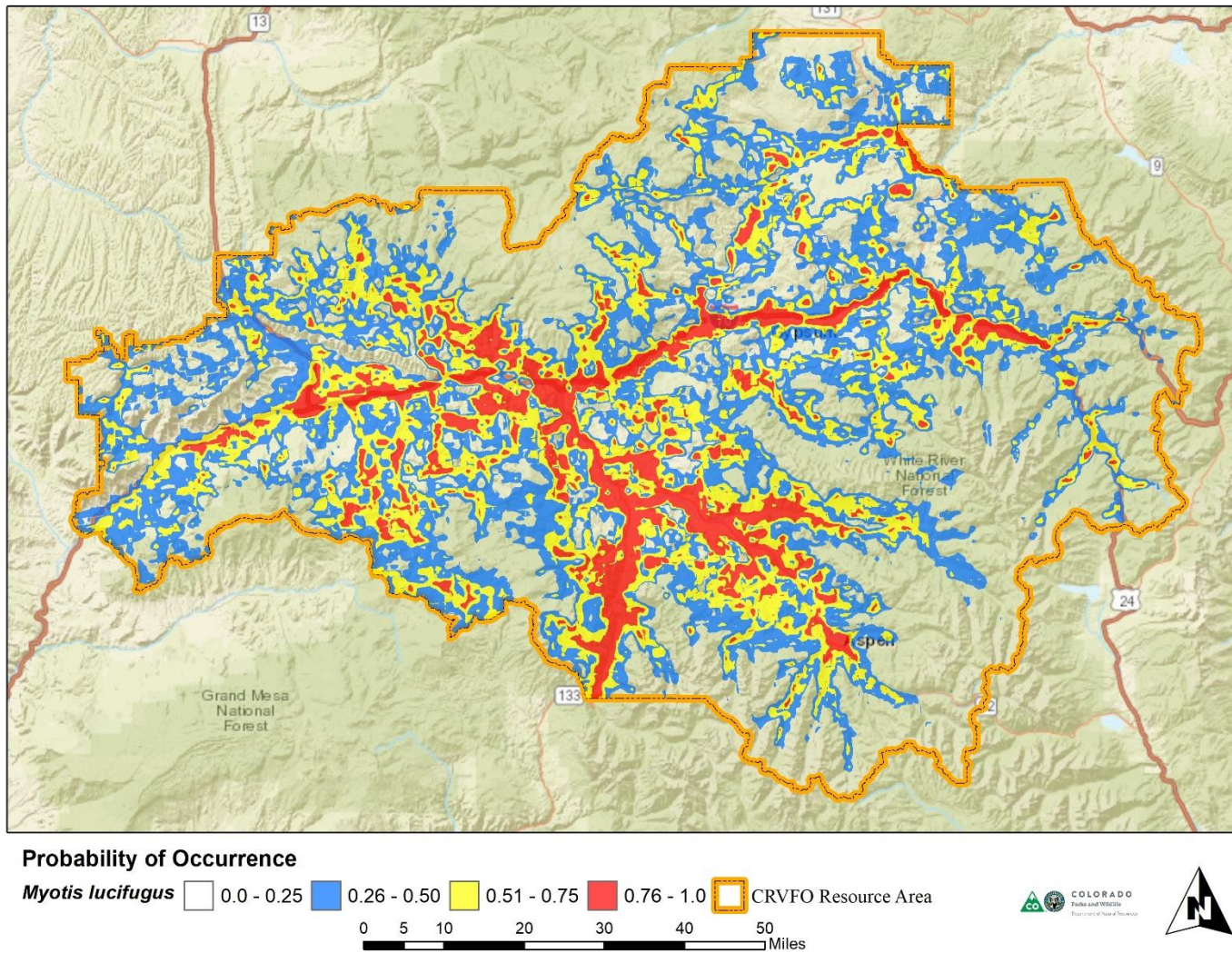
**Figure 13:** Probability of occurrence for *Myotis ciliolabrum* in the Colorado River Valley Field Office, Bureau of Land Management modeled using MaxEnt.



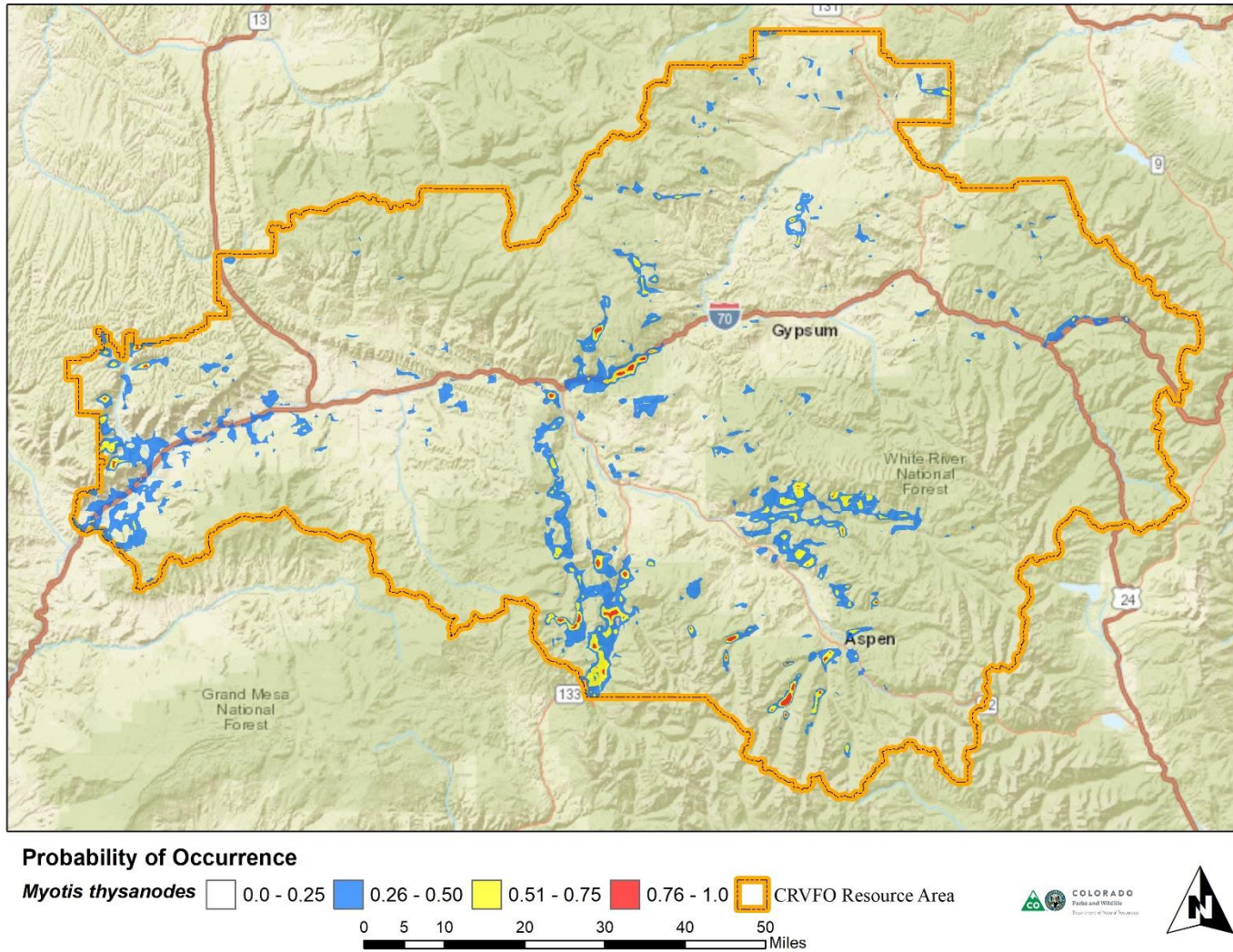
**Figure 14:** Probability of occurrence for *Myotis evotis* in the Colorado River Valley Field Office, Bureau of Land Management modeled using MaxEnt.



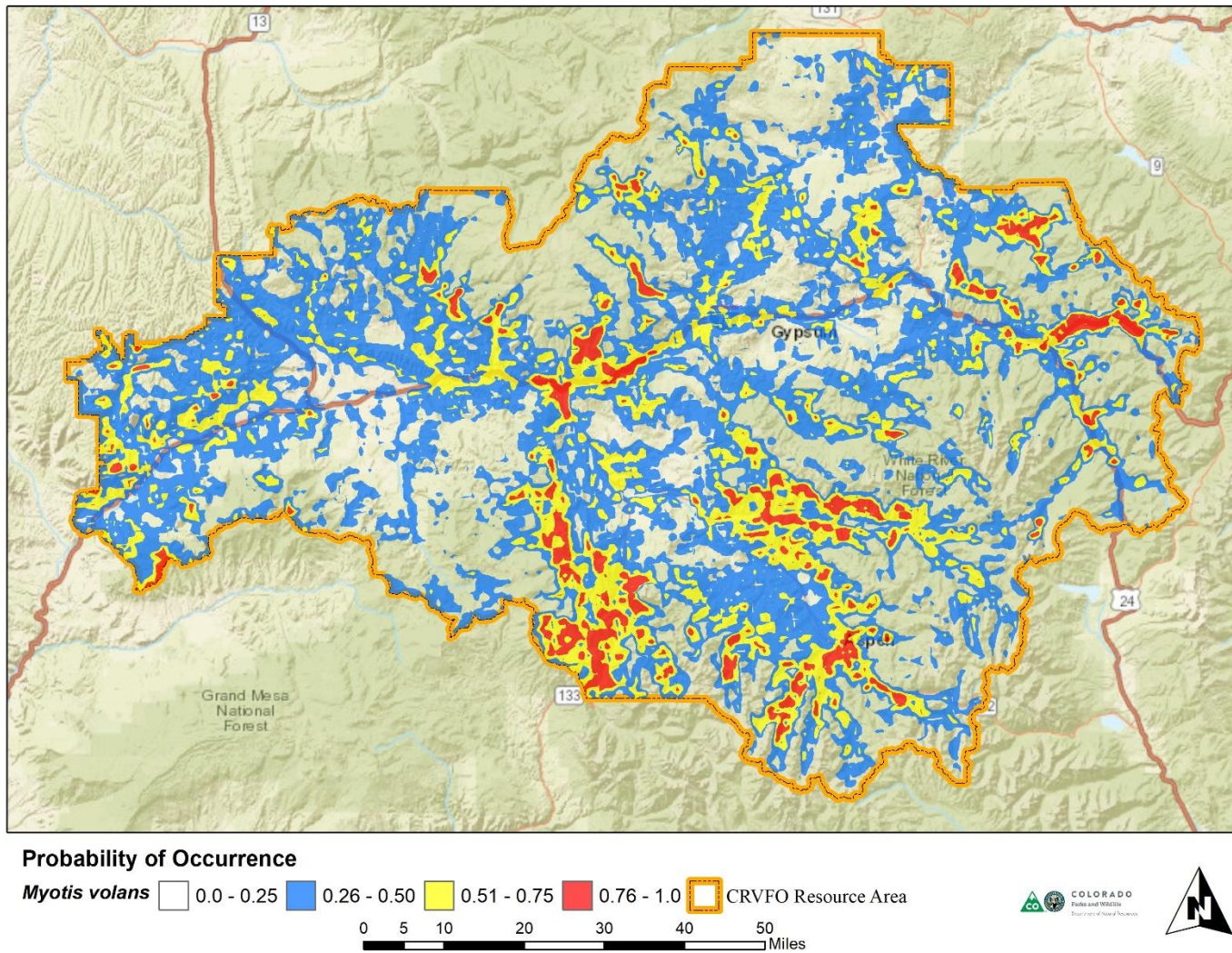
**Figure 15:** Probability of occurrence for *Myotis lucifugus* in the Colorado River Valley Field Office, Bureau of Land Management modeled using MaxEnt.



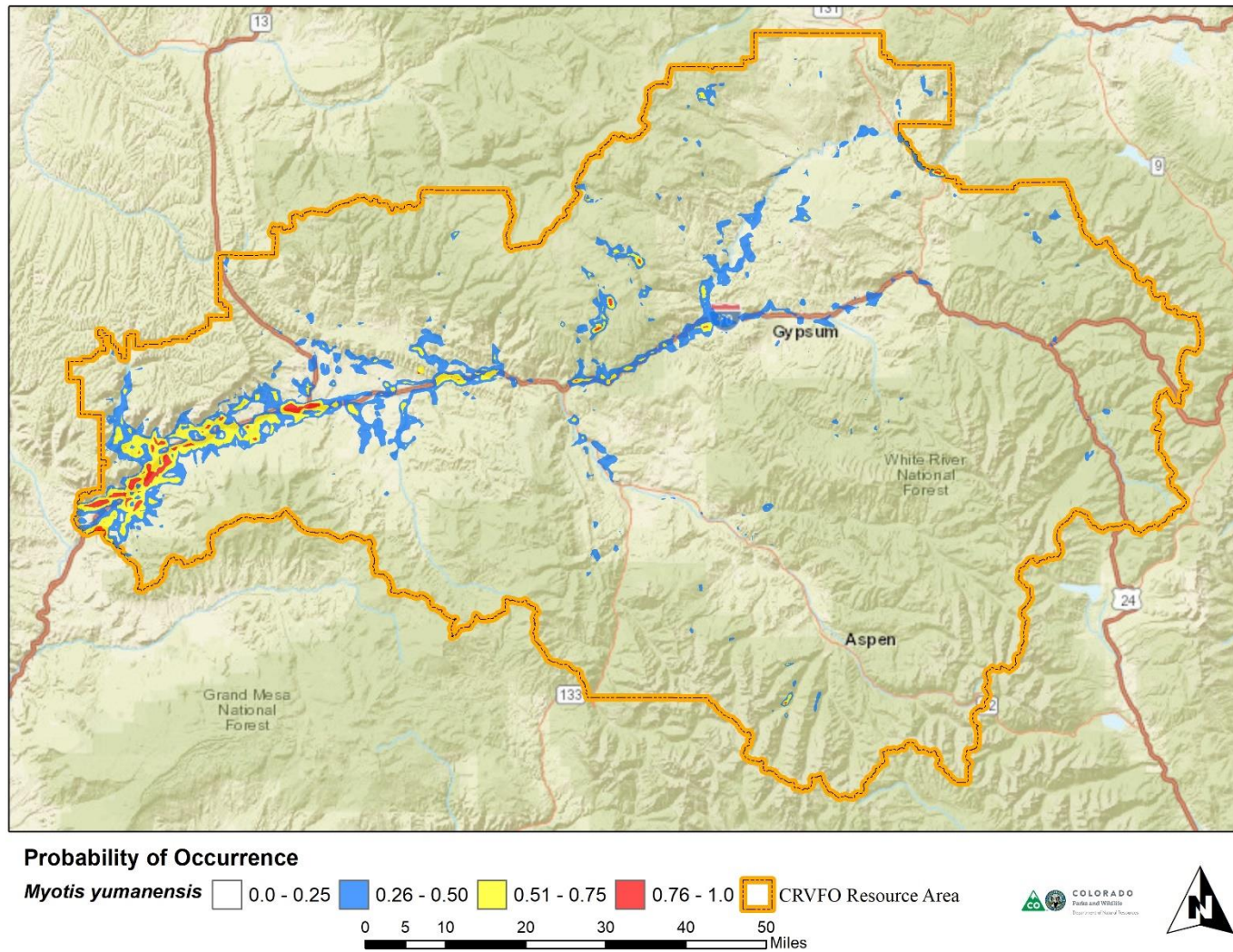
**Figure 16:** Probability of occurrence for *Myotis thysanodes* in the Colorado River Valley Field Office, Bureau of Land Management modeled using MaxEnt.



**Figure 17:** Probability of occurrence for *Myotis volans* in the Colorado River Valley Field Office, Bureau of Land Management modeled using MaxEnt.

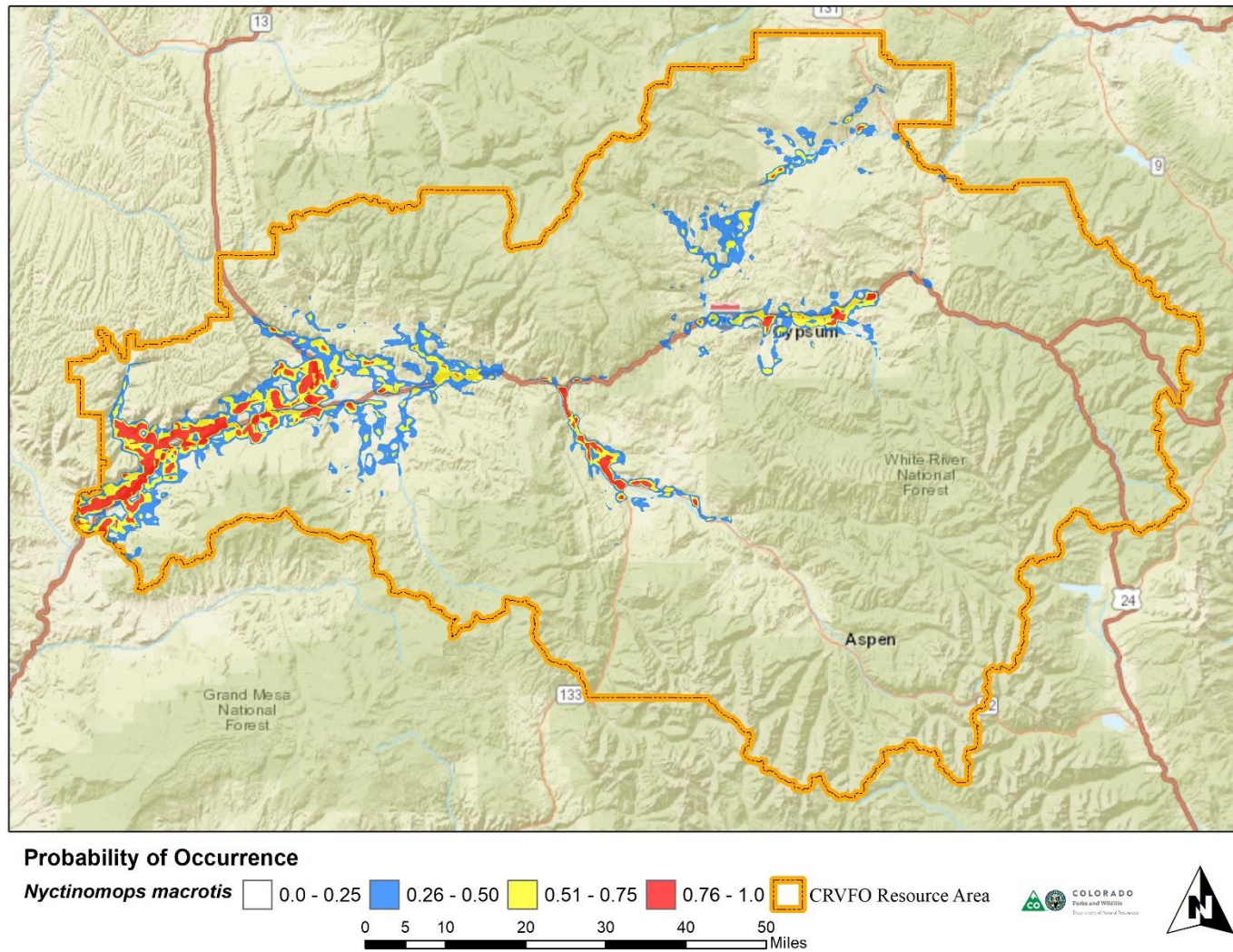


**Figure 18:** Probability of occurrence for *Myotis yumanensis* in the Colorado River Valley Field Office, Bureau of Land Management modeled using MaxEnt.

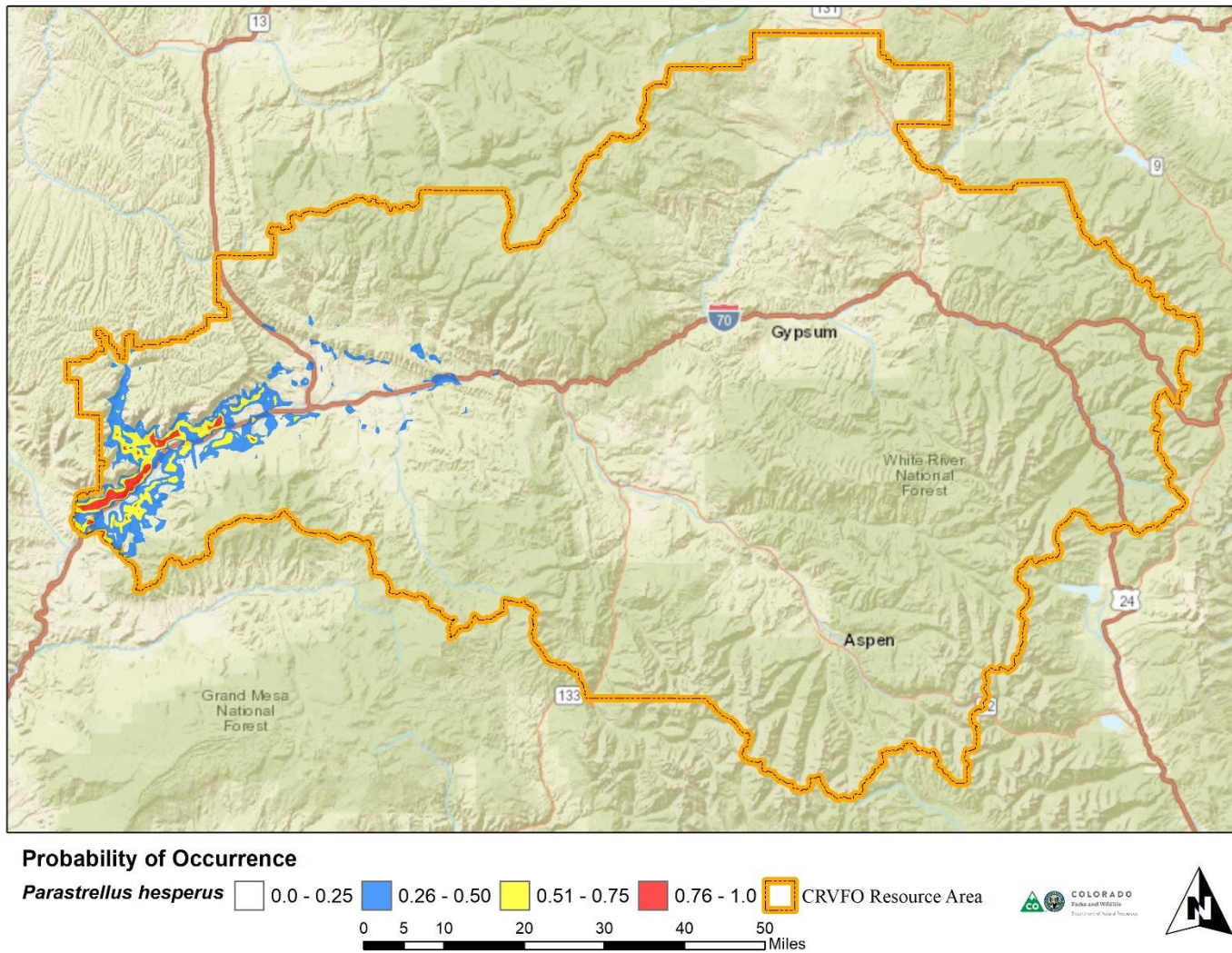




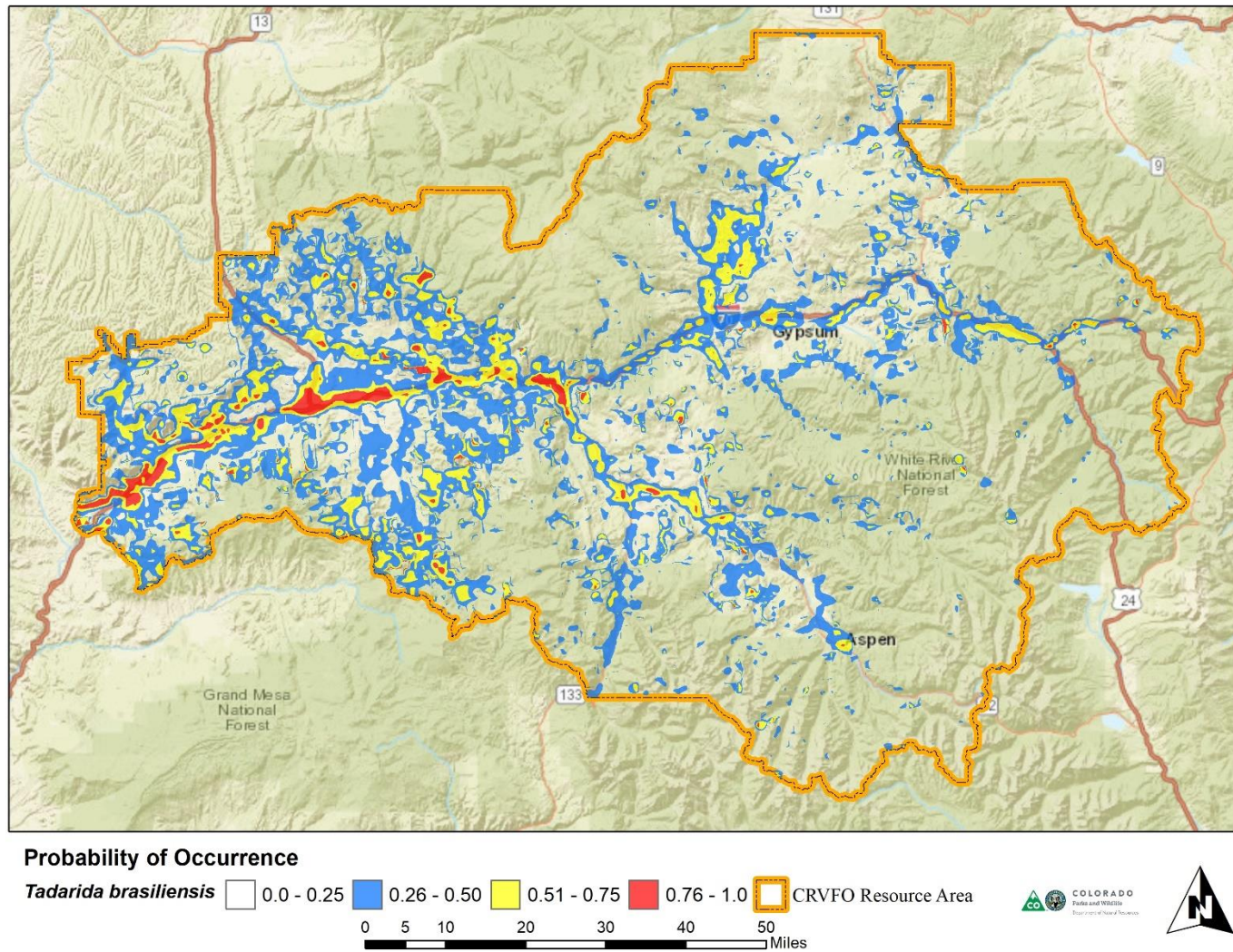
**Figure 19:** Probability of occurrence for *Nyctinomops macrotis* in the Colorado River Valley Field Office, Bureau of Land Management modeled using MaxEnt.



**Figure 20:** Probability of occurrence for *Parastrellus hesperus* in the Colorado River Valley Field Office, Bureau of Land Management modeled using MaxEnt.



**Figure 21:** Probability of occurrence for *Tadarida brasiliensis* in the Colorado River Valley Field Office, Bureau of Land Management modeled using MaxEnt.



**Table 8.** Documented use of caves and mines by bats on the Colorado River Valley Field Office or its Resource Area visited between 2011 and 2019. Site use type includes: hibernacula (Hib), transient (Trans), day, night, maternity (Mat), bachelor (Bach), and swarming (Swarm). See Neubaum et al. (2017) for roost use definitions. Use may be possible (pos), probable (prob) or confirmed (con) based on the available data. In terms of allocating agency resources, sites used as hibernaculum and maternity roosts, and for swarming activity are recommended to be given the highest priority followed by bachelor, transient, day, and night roosts.

Site	Use Type							Confirmed Species	Ownership	Year	Source
	Hib	Trans	Day	Night	Mat	Bach	Swarm				
Amphitheater Cave				pos					Private	2012	This study
Anvil Points Claystone Cave	con	con	con	con	con		con	COTO, MYsp	BLM	2008, 2009, 2012-2016, 2019	This study; caver reports 1998, 1999, 2005
Anvil Points Mine	con	con	con		con				BLM	2008, 2012-2016	This study; Spears 2008
BAD Cave		con	con						BLM	2012, 2013	This study
Big Entrance Cave				pos					Private	2018	This study
Cave of the Clouds		con	con	con	con			COTO	Private	2012, 2013, 2014	This study, private landownership confirmed
Cattleguard	pos							COTO	Private?		Caver report 1999
Dirty Pool Cave				pos					BLM	2011	This study
Drapery Den	con	pos						COTO	Private	2013	This study, private landownership confirmed
Echo Dome Cave		pos	pos	pos					BLM	2012	This study
Fountainhead Cave	pos		pos	prob					BLM	2018	This study
LaSunder Cave	con	con	prob	prob			pos	COTO, MYCI	BLM	2001, 2012, 2013	This study; Siemers 2002; Mosch et al. 2004
Not Spinsters Cave		pos	pos	pos					BLM	2013	This study
Shield Cave		pos	pos	prob					BLM	2016	This study
Spectre Cave	pos	pos	pos	con					BLM	2011	This study
Spinsters Cave		con	con	con			con	COTO, MYsp	BLM	2012, 2013	This study
Surprise Pit			pos	pos					BLM	2018	This study
The Tomb		con	con	con	con			COTO, MYEV	CDOT	2000, 2001, 2013, 2014, 2015	This study; CPW Bat Database
Twenty Pound Tick Cave	pos			pos					BLM	2018	This study; caver reports 1999
Wind Tunnel Cave				pos					BLM	2011	This study

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**Appendix A.** Results from real time PCR tests for *Pseudogymnoascus destructans* conducted by the National Wildlife Health Center on three mummified Townsend's big-eared bats and cave soil collected at Anvil Points Claystone Cave in January of 2017.



**NATIONAL WILDLIFE HEALTH CENTER**  
 6006 Schroeder Road  
 Madison, Wisconsin 53711-6223  
 608-270-2400 (FAX 608-270-2415)

**DIAGNOSTIC SERVICES CASE REPORT**

Case: 27644  
 Eptzoo:

**Final Report**

1/23/2017

Legal  Declassified  INV#:

Submitter:  
 Tina Jackson  
 Colorado Dept of Natural Resources/Wildlife/Denver  
 6060 Broadway  
 Denver, CO 80216

Date Submitted: 12/2/2016

**Specimen description/identification/Location:**

ACC	SPECIES	SPECIMEN TYPE	BAND NUMBER	SUBMITTER'S ID	COUNTY	STATE
001	Bat, Townsend's Big-eared	CARCASS	CO112816DN001		Garfield	CO
002	Bat, Townsend's Big-eared	CARCASS	CO112816DN002		Garfield	CO
003	Bat, Townsend's Big-eared	CARCASS	CO112816DN003		Garfield	CO
004	Environmental Sample	ENVIRONMENTAL SAMPLE(S)			Garfield	CO

Diagnosis:  
 Negative for *P. destructans* by PCR (Acc. 001-004)

Event History:  
 Three Townsend's Big-eared Bats were found dead and mummified 11/30/16 at Anvil Points Claystone Cave (Garfield Co., CO). They were found hanging from the ceiling, not lying on the floor. A maternity colony uses this exact spot at this cave in the summer. The submitter suspects they may have died from something like rabies or some other unknown cause. A sample of white fungus was also collected from the rocks and soil located below the dead bats. Because of the mummified state of the carcasses, only Pd surveillance testing will be performed.

Comment:  
 Combined wing/muzzle swabs collected from all 3 bats and 1 environmental sample from this location tested negative for *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome (WNS), by real-time PCR.\*

\*Note the lack of a positive result by PCR does not definitively indicate the absence of the organism. PCR may not detect the organism if it is at very low abundance in the sample.

