Utilization of Transportation Structures by Bats in Wyoming: A comprehensive investigation

WYDOT Project Champions
Dr. R. Scott Gamo
Environmental Services Manager

Jeff Booher
Assistant State Bridge Engineer Design
5300 Bishop Blvd.
Cheyenne, WY 82009

Principal Investigators
Dr. Riley Fehr Bernard
Assistant Professor
Department of Zoology and Physiology
University of Wyoming
Laramie, WY 82070

Heather O'Brien
Non-Game Mammal Biologist
Wyoming Game and Fish Department
Casper, WY

Laura Beard
Bat Biologist
Wyoming Game and Fish Department
Lander, WY

Submitted to:
Wyoming Department of Transportation
Programming Research Unit
5300 Bishop Blvd.
Cheyenne, WY 82009

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Problem Statement and Background

It is now widely accepted that bats use non-traditional roosts such as bridges and culverts throughout North America (Keeley and Tuttle 1999), however little is known about the use of these structures in Wyoming (Hendricks 1999). Since 2003, biologists in Montana, South Dakota and Iowa have been surveying bridges specifically to identify use by bats (Hendricks et al. 2005*b*, *a*, Bektas et al. 2018, Bachen and Mcewan 2019, Bachen et al. 2019, 2020). Acoustic surveys were conducted in South Dakota and confirmed the presence of six bat species near a bridge (Bachen et al. 2020). A more comprehensive study in Iowa aimed to identify the types of bridges most likely to serve as roosts (Bektas et al. 2018) and found that bridge structure, land cover distribution and bat species distribution increased the probability of bridge use in the state. Finally, the most systematic and long-term bridge study in the region occurred in Montana. First initiated in 2003 (and completed in 2019), over 2,200 bridges maintained by the Montana Department of Transportation and U.S. Forest Service were surveyed for the presence of bats (Hendricks et al. 2005*b*, Bachen and Mcewan 2019, Bachen et al. 2019). Of those surveyed, ~930 (~42%) were found to be used by bats.

Due to these findings and the need to improve our understanding of bats in Wyoming, the Wyoming Game and Fish Department (WGFD) has begun providing recommendations to the Wyoming Department of Transportation (WYDOT) to document the use of non-traditional roosts, such as bridges by bats. WYDOT maintains around 3,000 bridges scattered across the state and investigating all of these bridges for bats is a significant undertaking. Therefore, we propose to investigate the use of bridges as night, day, or maternity roosts, with the latter two being the most important for the persistence of bats to ultimately develop a cost-effective process for selecting bridges to focus upon. Additionally, WYDOT is interested in improving bridge roost sites and local habitat to maximize conservation outcomes for bats, as well as offset potential disturbance at known maternity roosts during scheduled maintenance. This proposed project will form the basis for establishing bats in bridge monitoring and guidance documents for WGFD and WYDOT.

Literature Search

Bats are an integral component in many ecosystems, providing valuable ecosystem services such as agricultural pest control (Boyles et al. 2011), pollination and seed dispersal, and human pest determent (Kunz et al. 2011). Currently, numerous bat species in temperate North America are suffering from declining populations due to the reduction of roost sites and foraging potential due to habitat loss, wind energy development, climate change and the introduction of disease (Frick et al. 2019). Although some information is known about critical habitats and the behavior of various bat species in the eastern region of the continent, much less is known about the general ecology and habitat needs of bats in western North America. Therefore, conservation efforts targeting bats in the West often lack the information necessary for making optimal management decisions and are typically modeled after eastern bat species.

Two primary conservation concerns for bats in the Rocky Mountain West, and specifically Wyoming, are 1) wind energy development and 2) the emergence white-nose syndrome (WNS). As our country's reliance on clean energy increases, conversion of once open landscapes to wind energy is becoming quite common (Kunz et al. 2007). The state of Wyoming has one of the highest wind power potentials in the United States (WINDExchange 2020), leading to an increased demand for the development of wind energy projects to help combat climate change. Although wind is a viable alternative to traditional fossil fuels, there are significant wildlife costs associated with its development (Conkling et al. 2020). Fatalities from barotrauma or direct collisions with wind turbines are now one of the leading causes of bat mortality world-wide (Frick et al. 2019, Rodhouse et al. 2019). In the United States and Canada, bat mortalities due to wind production can range from 2 to 50 individuals per megawatt per year, equating to 200,000 to 800,000 bats annually (Allison et al. 2019, Frick et al. 2019).

While mortality from wind production primarily effect migratory bats, WNS, caused by the fungal pathogen *Pseudogymnoascus destructans*, is a primary driver in the decline of hibernating bat species. The fungus was recently confirmed in Wyoming, however no mortalities due to manifestation of the disease have yet been observed (Abernethy et al. 2020). *Pseudogymnoascus destructans* was first discovered in New York in 2006 (Blehert et al. 2009) and has since spread throughout North America (U.S. Fish and Wildlife Service 2018). The fungus thrives in cold, humid environments (Langwig et al. 2012, Verant et al. 2012) and takes advantage of bat hosts during winter, when bats engage in hibernation, an energy saving strategy, due to low ambient temperature and scarce food resources (Geiser 2004). During extended torpor bouts, the fungus infiltrates the epidermis of the muzzle, wing and tail membranes, initiating a cascade of physiological disturbances (Verant et al. 2014). To date, it is estimated that nearly 6-million bats have succumbed to the disease (U.S. Fish and Wildlife Service 2018).

While not as great of concern with direct bat mortality as found with wind turbines and disease, research suggests roads are a potential threat to bat conservation via direct risk of collision with vehicles; habitat loss, degradation and fragmentation due to road building; as well as barriers to movement between habitats (Fensome and Mathews 2016). Based on records of bats casualties in Europe (n = 1207 records over a 50 year period), low-flying species, such as species within the Genera *Myotis*, have a higher likelihood of poor outcomes with vehicles than high-flying species, such as the migratory *Lasiurus cinereus* (hoary bat; Fensome and Mathews 2016). As discussed previously, bridges are often used by bats and are predominantly used by those classified as 'low flying species.' While the studies examined in Fensome & Mathews (2016) focused on road-based fatalities, they did find that low flying species were found to use corridors such as river bridges and underpasses rather than to cross directly over roads. It should be mentioned that the landscapes described in these studies are significantly different than what occurs in Wyoming. Environmental risk factors associated with high mortality rates were typically on roads close to, or bisected by, other linear features, such as tree lines, hedges, rivers, viaducts, forest edge, and woodland paths (Fensome and Mathews 2016). While regions in eastern Wyoming do not correspond with these specific habitat features, roads in the western third of the state may present a higher likelihood of risk for low flying bat species. Alternatively, bridges within the road system may benefit bats as they can be used by bats for roosting sites and may even provide some maternity roosting habitat. Detrimental impacts may occur when maintenance activities occur on bridges.

Due to these conservation concerns, the need to improve our understanding of the ecology and behavior of bats in Wyoming is of the utmost importance. One of the most fundamental elements of bat survival is habitat, and more specifically, roosting habitat. Bats use a diverse suite of natural and artificial (i.e., man-made) features to roost, ranging from caves, mines, trees, rock cavities and outcrops, to buildings and bridges. Use of such roosts can vary by species as well as time of year and provide refuge from predation, as well as act as a place for mating, hibernation, and rearing young. Transitory roosts are those that serve as refugia during nightly foraging, inclement weather or during migration. The roosting ecology of bats is therefore interpreted as a complex interaction of the physiological, behavior and morphological adaptations of an individual and species or group (Kunz 1982). Roost choice is often influenced by the abundance and availability of primary and alternative roosts, risk of predation, parasitism and disturbance, available food resources and social organization (Bernard et al. 2020). Not a lot is known about the roosting behavior of bats in Wyoming, nor is much known about the role of summer roosts in the spread of *P. destructans* (Carpenter et al. 2016, Ballmann et al. 2017, Abernethy et al. 2020). Due to the importance and necessity of roosts and the possibility for them to act as refugia for a fungal pathogen, Wyoming Game and Fish Department is interested in understanding how the conservation of these sites can maintain the long-term persistence of North American bat species. Given WYDOT's history of increasing wildlife habitat connectivity via corridor over- and underpasses, the Agency can conserve Wyoming's cryptic wildlife through the creation and maintenance of roosting habitat for bats.

Study Objectives

The purpose of this study is to collect bat use data on bridges to aid WYDOT and agency partners in determining what bridge types are attractive to bats and to develop means of mitigation to minimize impacts to bridge maintenance and reconstruction timelines.

We aim to address the following objectives and follow up questions:

1. Determine if bats use transportation structures, such as bridges, in Wyoming.
    a. Questions:
        i. What bridge characteristics (see Table 1) are most likely to predict used by bats?
        ii. What type of roosts are bridges being used as, i.e., day-roosts, night-roosts, maternity sites, hibernacula?
        iii. What is the timing and emergence of bats at these sites?
        iv. What bat species are predominately using bridges?
        v. Are there geographic regions or landscapes within the state that are more likely to lead to use of bridges by bats?

vi. Do bridges pose a risk to bat survival via increased likelihood of bat-vehicle collisions?

2. Determine the landscape characteristics (e.g., elevation, topography, habitat availability) that correspond with bridge use by bats?
3. Determine if bridge roosts in Wyoming are conducive to *Pseudogymnoascus destructans* proliferation.
    a. Are WNS mitigation measures feasible in bridge roosts?
    b. If so, which actions can be implemented to minimize the spread of *P. destructans* while also minimizing wear or damage on bridge structures?
4. Create a guidance document with the WGFD and WYDOT to 1) predict additional bridges that may serve as roosts, 2) determine the optimal time (i.e., season and time of day) to conduct inspections or maintenance to minimize disturbance and "take" (i.e., harassment, injury or death) of federally threatened species such as *Myotis septentrionalis*, and 3) provide guidance for how to improve or mitigate habitat at and around significant bridge roosts. Possible improvements could be installation of bat houses near construction sites or planting native vegetation near roosts to increase prey availability.

Study Approach

OBJECTIVE 1:

To achieve objective 1, we will survey bridges throughout the state of Wyoming. Given this study is the first comprehensive survey of bridges in the state, a targeted approach in Year 1 and 2 will facilitate the identification of new sites most likely to serve as roosts. In Year 3 (Spring 2023), we will 1) use data collected in Years 1 and 2 (including but not limited to bridge characteristics, habitat type, forest cover and type, and proximity to forest edge and bodies of water) to predict the location of roosts at other transportation structures within the state to survey, and 2) resurvey significant roosts identified in Year 1. A full list of Wyoming bridges maintained by WYDOT can be seen in Figure 1.

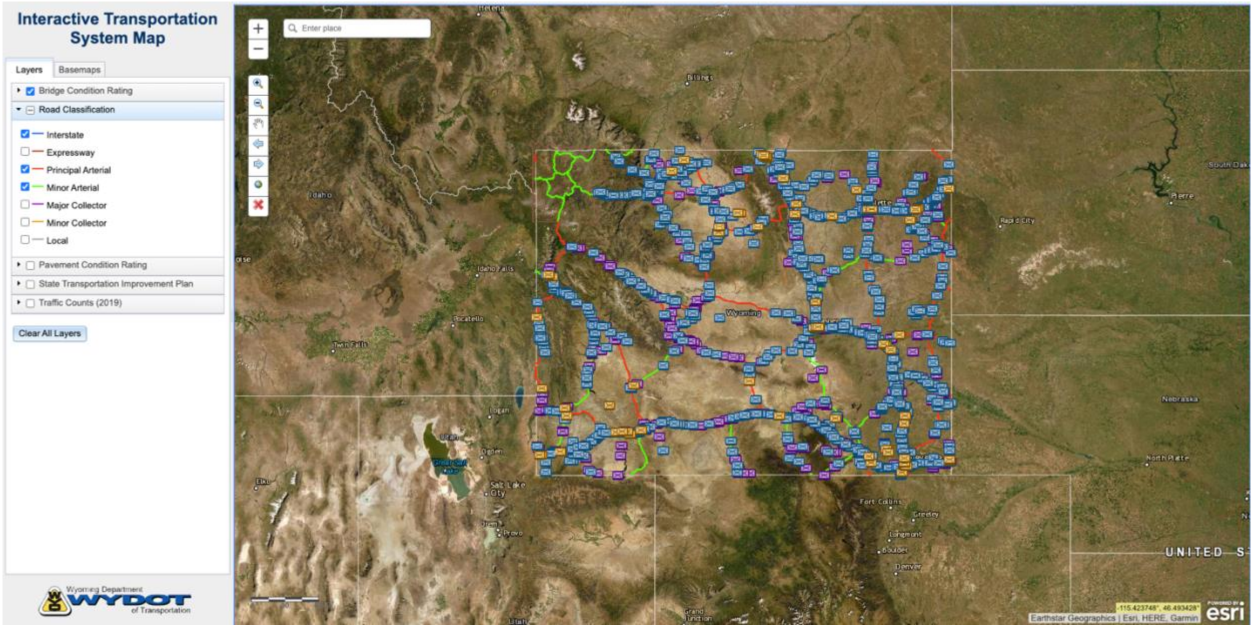

Figure 1. Map of WYDOT Managed Bridges. WYDOT managed bridges are primarily located on interstates (blue lines), principle arterial (red lines) and minor arterial (green lines) roadways. Map courtesy of WYDOT via apps.wyoroad.info/itsm/map.html.

<u>Survey Methods – Visual inspection of bridge sites</u>

Bridges will be visually inspected during daylight hours, starting in early spring. We will examine the underside of each bridge, specifically support beams, ledges, crevices, and gaps using a high-power portable spotlight, binoculars, endoscopic camera and FLIR. Evidence of use will be determined based on the presence of individuals, guano deposits and/or urine staining. We will collect a variety of bridge characteristic metrics at each structure (Table 1, based on previous bridge studies), such as construction material and design of the support structures, height above ground, feature crossed, and habitat and land-use cover types immediately surrounding and within 0.5 km surrounding the site. [See preliminary findings from year 1 in Appendix 1]

<u>Survey Methods – Roost information</u>

When bats or evidence of bat use are discovered at a site, we will attempt to identify species (via binoculars or photographs), estimate the number of adults present, look for evidence of juveniles or pups to determine the type of roost site (bachelor, maternity, or transitory roost), and measure the distance from the ground to the roost. We will search the area immediately below the roost to collect guano and carcasses (i.e., adult and juvenile), if available, to genetically identify species.

Genetic analysis: We will extract DNA from carcasses and guano samples using DNeasy Blood and Tissue Kits (Qiagen, Germantown, MD) for tissue and PowerSoil DNA Isolation Kits (Mo Bio Laboratories, Carlsbad, CA) for guano following protocols and procedures by Brown et al. (2017). We will cut tissue from wing or tail membranes from bat carcasses and store in either silica gel desiccant (4-10 mesh, Fisher Scientific, Pittsburg, PA; Wasser et al. 1997) or 20% dimethyl sulfoxide (DMSO; Worthington-Wilmer and Barratt 1996) depending on the 'freshness' of the carcass tissue. Guano samples will be stored in 2mL microtubes with silica gel desiccant (Brown et al. 2015). All samples will be frozen at -20°C within 48-hours of collect and stored until analyzed.

If bats are present at a roost during the day (i.e., day roost), we will conduct emergence counts to estimate the total number of individuals at each site. Emergence counts will occur on the first night we find the roost, or on the next optimal day (minimal wind and no rain). The count will commence 30-minutes before civil sunset and continue until an hour after sunset or until it is too dark to see emerging bats. We will only count bats that exit the roost. The emergence count will end after 15 minutes have passed since the last bat emerged or when visibility is gone.

Survey Methods – Long-term monitoring

To determine the timing, emergence, and general use of bridge roosts by bats, we will deploy acoustic detectors, microclimate iButtons and camera traps at sites with more than 10 roosting individuals. If this population number proves to be on the low end, we will adjust and choose to monitor the largest sites.

Acoustic monitoring: If at least 10 bats are found roosting at a site, one Anabat Swift, Titley Scientific, Columbia, MO, USA) will be deployed at a site to determine the timing, emergence and duration of daily and seasonal use, relative activity and species identification or classification (SonoBat 4, as some species are hard to ID via acoustics). The detector will be situated at or within 5 – 10 meters of the entrance of the structure and set to record 30-min prior to sunset until 30-minutes after sunrise. Detectors will be programed to record in full-spectrum. Depending on bat activity, the detector should run for up to 480 hours (e.g., 48 10-hour nights).

Roost microclimate: At least one Hygrochron Temperature and Humidity Data Logger (iButtonLink, LLC, Whitewater, WI, USA) will be deployed near roosting bats to record microclimate variables such as temperature and relative humidity. The datalogger will be set to record roost temperature and humidity every hour. iButtons will be attached to the bridge structure using a non-caustic material.

Roost behavior: Finally, we will deploy at least one camera trap at each significant roost site to record roost behavior, determine the number of individuals roosting at a site, and determine the timing and emergence of bats from the roosts each night. Browning Trail Cameras Dark Ops Pro XD Dual Lens BTC-6PXD has been demonstrated to be sensitive enough to capture bat activity at or near roosts (0.15 second trigger speed and 80 ft detection range).

Long-term monitoring data collection and equipment maintenance will occur at least once every 4 – 6 weeks during the active season (May – October) and may continue at larger roost sites during the winter (November – April). Visual inspection of staining will occur throughout the year to identify new sites.

Table 1. Data to be collected in the field

Bridge Details	**General Information**	**Components/Type**	**Feature Crossed**	**Material**
	Bridge ID	Superstructure type	Bare ground	Span: Concrete
	Bridge coordinates	Height above ground (ft)	Vegetation	Span: Steel
	Start time	Superstructure Depth (ft)	Flowing water	Span: Wood
	End time	Number of Spans	Standing water	Decking: Concrete
	Observer	Culvert	Railroad or roadway	Decking: Steel
		Decked	Scree or rocky substrate	Decking: Wood
	General	**Crevices**	**Road/Traffic**	**Surrounding habitat (%)**
	Date	No	Number of lanes	Coniferous forest
	Time	Abutment	Roadway material	Deciduous forest
	Cloud cover	Parallel	Est. ADT of roadway (H/M/L)	Agricultural
	Ambient temperature	Central expansion		Residential
	Northing, Easting			Riparian/Wetland

	Elevation (ft)			Grassland
	Weather			Shrub/Steppe
ROOST INFORMATION	**BAT PRESENCE**	**ROOST TYPE**	**SAMPLES TAKEN**	**DATA COLLECTED**
	Bats present (y/n)	None	Carcasses (y/n)	Photographs (y/n)
	Visual (y/n)	Night	Guano (y/n)	Endoscopic photographs (y/n)
	Guano (y/n)	Day	Environmental swabs (y/n)	FLIR photographs (y/n)
	Urine staining (y/n)	Maternity	Pd swabs (y/n)	Acoustic (y/n)
	Acoustic detection (y/n)	Bachelor	Roost temp	Roost camera (y/n)
	Species	Swarm		Roost aspect
	# of bats	Hibernation		Roost location on bridge
	# of roosts			
OTHER	**CLIFF SWALLOW NESTS**	**BARN SWALLOW NESTS**	**ROCK PIGEON**	**OTHER**
	<5	<5	Present	
	5 – 10	5 – 10	Absent	
	10 – 20	10 – 20		
	>20	>20		

OBJECTIVE 2:

To achieve objective 2, we will compare the landscape characteristics of bridges with and without bat roosts. We will survey the habitat and landscape composition at three distances: immediately adjacent to the site, within 0.5 km, and within 3.0 km around each site. The buffer distances chosen are based on the maximum distance bat's travel during foraging bouts and roost switching (see Appendix A in Bachen et al. 2019).

We will delineate the landscape of each site across all buffer distances using ArcGIS (ArcMap, ESRI, Redlands, CA, USA). Landscape composition will be determined by calculating the proportions of land cover types near each bridge site using data from the National Land Cover Database. We will also calculate the difference in landscape configuration, using total edge (km), edge density (m of edge/ha) and a contagion index (O'Neill et al. 1988), which indicates the degree of the aggregation of patches throughout the landscape. A region with a low contagion index suggests that there are numerous interspersed patches of a habitat type indicative of fragmentation (Li and Reynolds 1993, Riitters et al. 1996).

We will collect several habitat variables at both roost and non-roost sites, such as elevation of bridge, aspect of roost (compass direction), slope, habitat type, distance to major landscape features (e.g., ridge, forest, water, roads, trails). Topographic variables are often used to identify landscape features preferred or most often used by bats and represent means to measure microclimate variation within a landscape. 'Distance-to' features have been found to be useful metrics in identifying potential foraging resources and travel corridors, as well as help determine differences between roost and non-roost sites (Hammond et al. 2016).

Capture methods:

We will attempt to capture bats exiting bridge roosts using mist-nets or retrofitted butterfly nets. Upon capture, we will collect various biometrics from each individual (i.e., species, sex, reproductive condition, mass, forearm length) and visually examine flight membranes for damage associated with white-nose syndrome. We will also collect guano for diet analysis and species identification, collect epidermal swab samples to determine presence of *Pseudogymnoascus destructans*. Finally, if bat species identification is in question, we will collect a tissue sample from the wing using a biopsy tissue punch. Each bat will be held for no more than 30 minutes and released at the site of capture. All methods have been approved by the University of Wyoming Institute of Animal Care and Use Committee, Wyoming Game and Fish and the U.S. Fish and Wildlife Service. We will follow all USFWS and WGFD decontamination protocols for WNS and SARS-CoV-2 (U.S. Fish and Wildlife Service 2020).

OBJECTIVE 3:

To achieve objective 3, we will collect environmental samples following the U.S. Geological Survey National Wildlife Health Center sampling protocol (USGS-NWHC 2020) to determine if *Pseudogymnoascus destructans,* the causative agent of white-nose syndrome (WNS), can persist at bridge roosts. Environmental samples will include substrate collection from below both active and inactive roosts, sterile swab samples from roost locations (i.e., walls), and guano (Abernethy et al. 2020). Environmental samples allow for the detection of *Pseudogymnoascus destructans*, however they do not indicate that the site is WNS positive, just presence/absence and load (i.e., amount of fungus). Based on information from the endemic zone of the pathogen, *P. destructans* is typically detectable within environmental samples 1 – 3 years after detected on bats hibernating at a site (Verant et al. 2018). Here in the west, environmental samples, specifically pooled guano, have been used to confirm the presence of *P. destructans* at maternity sites in spring (Abernethy et al. 2020), as well as at bridge roosts in eastern Montana. We will collect samples from all active and inactive bat roosts three times throughout the year: spring, fall, and winter. Spring sampling will occur shortly after emergence from hibernacula, which in Wyoming is from April – June depending on elevation (Abernethy et al. 2020). Fall sampling will occur if bats use bridge roosts as swarming sites. Winter sampling will occur from mid-November to mid-March when roost temperatures are most conducive to *P. destructans* growth (0 – 10°C; Cryan et al. 2010).

Substrate samples will be collected from below active and inactive roosts (i.e., those that show signs of use, but no bats present) using sterile wooden splints (Fisher Scientific, Pittsburg, Pennsylvania, USA) and placed in sterile sampling bags with flat-wire closures (Fisher Scientific, Pittsburg, Pennsylvania, USA). To prevent contamination between samples, nitryl gloves will be replaced between sample collection at and between sites. All samples will be stored at -80°C until shipped to USGS-NWHC for analysis.

Environmental swab samples will be collected from specific roost locations, which will be determined based on the presence of bats or recent guano or urine staining. We will use a sterile polyester swab pre-moistened with sterile water. Each swab will be rolled across a ~5-cm diameter surface. The swab will then be stored in a 2mL microtube (Fisher Scientific, Pittsburg, Pennsylvania, USA) containing RNALater (Fisher Scientific, Pittsburg, Pennsylvania, USA). We will collect at least 5 samples from each bridge roost. Some bridge sites may have multiple roosts. All samples will be stored at -80°C until shipped to USGS-NWHC for analysis.

We will collect guano from below active roosts. Up to five pellets (aka., "pooled sample") will be placed in 2mL microtubes with silica gel desiccant and stored at room temperature in cardboard boxes until analyzed. We will use the methods described in Swift et al. (2018) to test in-house (in collaboration with Wyoming INBRE) for the presence of *P. destructans*.

In Year 2, we plan to swab all captured bats for the presence of *P. destructans* following methods described in Janicki et al. (2015). While in hand, we will also examine bat wings using ultraviolet light, which can be used to determine WNS manifestation (Turner et al. 2014).

If *P. destructans* is determined to persist at bridge roosts, WNS mitigation tactics will be evaluated by WGFD, WYDOT and USFWS. Currently, there are a number of treatments that are being investigated (see Bernard et al. 2019, Hoyt et al. 2019, Rocke et al. 2019), however, none have been implemented broad scale.

OBJECTIVE 4:

To achieve objective 4, we will use the information collected to address objectives 1 – 3 to create a guidance document that can be used by WYDOT, and other agencies tasked with maintaining bridge structures. This document will be collaboratively drafted by WGFD, WYDOT and the Bernard Lab at the University of Wyoming. The information gained through the evaluation and survey of bridges in Wyoming will also help inform other states within the Rocky Mountain West, including collaborative partnerships with the Montana Heritage Program and the Idaho Department of Game and Fish.

The primary objectives of this guidance document will be to 1) provide guidance for WYDOT biological consultants surveys (i.e., identify specific bridge characteristics that are sought after by bats, which will lead to cost savings on consultant fees), 2) identify the optimal time to conduct surveys and maintenance to minimize disturbance to bats while also minimizing the regulatory burden of WYDOT (e.g., proactive eviction at known day roosts). By thoroughly investigating use of bridges by bats, we will be able to provide guidance for mitigation or deterrents prior to construction, maintenance, or scheduled surveys. Finally, if WYDOT is interested in enhancing habitat at and around bridge roosts, this guidance document will provide insights as to what bridges may need onsite or near-site roost or habitat enhancement.

Analysis

We will fit logistic regression models to identify structure, landcover distribution, and bat species distribution characteristics that increase the probability of transportation structure being used by bats.

Study Benefits

Data collected during this study will be used to identify bridges and bridge types utilized by bats across Wyoming. This will provide highway engineers and wildlife managers with the information necessary to conduct bridge maintenance and reconstruction activities that minimize disturbance to bats as well as minimize the need to alter construction schedules. The study will also provide valuable information on the contribution bridge structure provide to maternity roosts for bats and general use by bats of bridges. Finally, this study will enable researchers to work closely with WYDOT engineers to identify and test mitigation measures that can be used to minimize take (i.e., acoustic deterrents, netting, eviction devices).

Output and Output Measures

Outputs of this study support WYDOT's strategic goal of exercising good stewardship of natural resources. In addition, output will support measures to maintain roadside safety and minimize costs. Output measures include valuable information about bat use to enable better development of mitigation measures and to potentially enhance bat roost and maternity site opportunities, including outside of bridges. In addition, outputs should provide mitigation approaches enabling non-interference with maintenance and re-construction activities.

Applicable Measures

Barriers could also include the ability to successfully identify bat use at bridges.

Statement of Work

We will visual inspections of bridges to determine bat use including roosting and maternal colony use. We will measure a variety of associated habitat variables along with bridge type to develop models which can be used to predict bridge use by bats. These data and analyses will inform WYDOT, WGFD, and other interests providing a basis for planning future bridge repair/construction work and minimizing impacts to bats and construction schedules. The work schedule is outlined in Table 2.

Table 2. Work schedule (grey portions indicate completed work)

Study Year	Project Component	Dates	Work plan
Year 1	Analysis of MT bridges & literature review to inform survey locations	Jan. – May 2021	Graduate student will conduct a comprehensive review of the literature to determine where bats may roost. Graduate student will also conduct a comparative analysis using data from MT Natural Heritage bridge project.
	Field surveys & sample collection	May – Aug. 2021	Graduate student will survey all bridges that meet the criteria for bridge roosts using the literature review and MTNHP bridge data (concrete & wood, with and without water). They will start in the eastern third of the state and work their way west.
	Winter survey of 'significant' roosts	Nov. 2021 – Mar. 2022	If significant sites are found summer 2022, the graduate student will resurvey the sites to determine if they are also used as overwinter roosts.
Year 2	Field surveys & sample collection	May – Aug. 2022	Graduate student and technician will survey all bridges that meet the criteria for bridge roosts using MTNHP and Year 1 bridge roost data. They will aim to survey all remaining WYDOT bridges in the state
	Analysis of samples & data	Aug. 2022 – Apr. 2023	Graduate student and technician will genetically and statistically analyze samples and data collected in the field.
	Winter survey of 'significant' roosts	Nov. – Mar. 2023	If additional sites are found summer 2023, the graduate student and technician will resurvey the sites to determine if they are also used as overwinter roosts.
Year 3	Field surveys & sample collection	Jan. – Aug. 2023	If necessary, the graduate student will finish or resurvey any bridge roosts that were not surveyed in the first two years.

	Analysis of samples & data	Jan. – Sept. 2023	Graduate student will finish analyzing samples and data collected from the field.
	Writing	Jan. – Nov. 2023	Graduate student will write and complete thesis and WYDOT & WGFD reports
	Defend & Graduate	Dec. 2023	Graduate student will defend thesis and graduate Fall 2023.

Work Plan/Scope

In-person site visits will be used to obtain bat use data on bridges. Additional data will be collected from maps and GIS files. These data will be reviewed for completeness on an ongoing basis. We will analyze these data to develop descriptive and predictive statistical models of likelihood of bat use. Statistical procedures are described in proposal methods.

Deliverables

1. Identification of bridges with bat use.
2. Identification of bridges with bat maternity roosts.
3. Development of a model to determine potential bat use.
4. Descriptions of bridge types and associated habitat types supportive of bat use.
5. Management recommendations for enhancing habitat on or near bridges.
6. Management recommendations for deterring use or providing alternate use for bats associated with bridges scheduled for repair or reconstruction.
7. Quarterly, annual, and final reports to WYDOT RAC, Environmental Services staff and District Staff.
8. Presentation of major findings to WYDOT field staff.
9. Presentations to interested members of the general public, WGFD, University of Wyoming Master's proposal and thesis defenses.
10. Publication in peer-reviewed scientific journals and presentations at scientific conferences such as regional or international meetings of The Wildlife Society, Joint North American Symposium for Bat Research and International Bat Research Conference, and the Western Bat Working Group Meeting.

Work Schedule

We anticipate the project timeline will encompass two additional years, with the first year of survey summer 2021, and an estimated completion date of December of 2023 (see table 2 for detailed schedule). All final reports will be submitted to WYDOT, WGFD and USFWS by December 2024.

Change Order Information and Agreements

We understand that any changes in the duration of the contract, work plan, scope, schedule, or costs must be submitted in writing and approved by the RAC.

Budget

We are requesting **$34,539.13** from WYDOT in this RAC proposal (Table 3). Matching funds can be found in Table 4.

Table 3. Proposed Budget for Bat and Bridge Use study.

	$ Requested	**Notes**
Direct Cost		
Total Personnel Costs	***$24,274.61***	*Salary, Student stipend, tuition & fees, fringe benefits*
Principle Investigator	$4,444.67	2-weeks PI salary
Other Personnel	$8,400.00	M.S. Graduate student stipend for 2 summers ($4,200.00 for 3 months x 2 summers)
Other Personnel	$8,400.00	Undergraduate technician for 1 season (14 weeks)
Tuition & Fees	$1,000.00	In-state tuition and fees for 2 summers ($500 x 2)
Fringe Benefits	$2,029.94	Current approved fringe rates at 36.6% for PI and 2.4% for one student & one technician
Research Travel	$4,508.00	1 vehicle rental (utility vehicle @ $512.00/mo) for 3 months = $1,536.00 Fuel (4000 miles/18miles/gal (utility vehicle)= 222 gallons x $3.25/gal = $722.00) Camping at Wyoming State Parks ($25/night x ~90 days x 1 season = $2,250.00)
Report Generation	$0.00	Electronic reporting
Equipment	$0.00	None requested
Indirect Costs	$5,756.52	Indirect costs (UW) at reduced state agency negotiated rate of 20% TDC

Requested from WYDOT	**$34,539.13**	

Table 4. Matching Funds

Source	Amount	In Hand or Requested
University of Wyoming – Faculty Start-up	$41,853.00	In Hand
University of Wyoming – Arts & Science	$3,998.00	In Hand
Wyoming Game and Fish Department	$20,000.00	In Hand
2021 Wyoming Governor's Big Game License Coalition Grant	$8,632.77	In Hand
2022 Wyoming Governor's Big Game License Coalition Grant	$28,178.88	Requested
Total Project Cost as of January 2022:	**$102,662.65**	

Implementation Process

We will work closely with WYDOT representatives and other project partners through the duration of the project to ensure our findings are relevant and useful. Our deliverables will provide valuable information and recommendations pertaining to the placement of mitigation structures and or reports discussing how to minimize disturbance to bats during critical periods (i.e., pupping season).

Technology Transfer (including Data Management Plan)

Results from this project will be shared with WYDOT staff. WYDOT Environmental Services Manager Scott Gamo will be consulted and will participate throughout the project to ensure the project meets their needs and expectations. In addition, WYDOT will receive written and/or verbal (presentations) quarterly reports over the course of the project timeframe.

Location data will be stored at the University of Wyoming. Other data and reports will be stored in electronic and paper form (when applicable) for the duration of the project and backup data files will be maintained for several years. At the conclusion of the project, we will provide a comprehensive final report, including research results, conclusions and recommendations, raw data and metadata.

Education, Outreach, and Scientific Products

1. Presentations: We will provide presentations to interested members of the general public, WYDOT and professional societies.
2. Scientific journal articles: We will prepare and submit one or more manuscripts detailing the findings of the study to appropriate scientific journals.
3. Project evaluation: We will evaluate the outputs of the education phase to ensure project outcomes were achieved.

Personnel

Dr. Riley Bernard is an assistant professor in the Department of Zoology & Physiology at the University of Wyoming. She received her PhD in Ecology & Evolutionary Biology in 2015 from the University of Tennessee and has been working with various state and federal agencies since graduating. She has been researching bats since 2008 and works extensively with USFWS, USGS, and WGFD on conservation issues affecting bats, including but not limited to, white-nose syndrome, special species status assessments and conservation management.

Dr. Scott Gamo is the Environmental Services Manager for WYDOT. He has served as a wildlife biologist and rangeland manager for over 32 years. Most of his research has involved wildlife habitats and populations. He has worked cooperatively with agencies on evaluating impacts to wildlife and implementing mitigation.

Jeff Booher is the Assistant State Bridge Engineer Design for WYDOT.

Heather O'Brien is the Non-game Mammal Biologist with WGFD.

Laura Beard is the Bat Biologist for Wyoming Game and Fish. She has worked with bats since 2006, in both Alaska and Wyoming, and has been in her current position at WGFD since December 2014. She coordinates and conducts population, WNS, and acoustic monitoring for bats throughout the state. Other areas of interest have included myotid roost selection in both the summer and winter season. Most recently, she has conducted research into the hibernation behavior of little brown myotis in Wyoming by carrying out the only known fall tracking study of this species in the state, which is due to conclude in 2021.

Principal Investigator Roles

There are 4 principal investigators (PI's) identified for this project. The University of Wyoming PI will be responsible for hiring personnel, IACUC and permit compliance, study methodologies and analysis of data as well as day to day oversight of the project including tracking of time and billing. They will be responsible for all contractual obligations tied to the grant and will ensure that deadlines for quarterly, interim, and final reports are met. The WYDOT PI's will help with project logistics, ROW access and related safety and wildlife data. The WGFD PI's will be responsible for assisting with permits and access.

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APPENDIX 1:

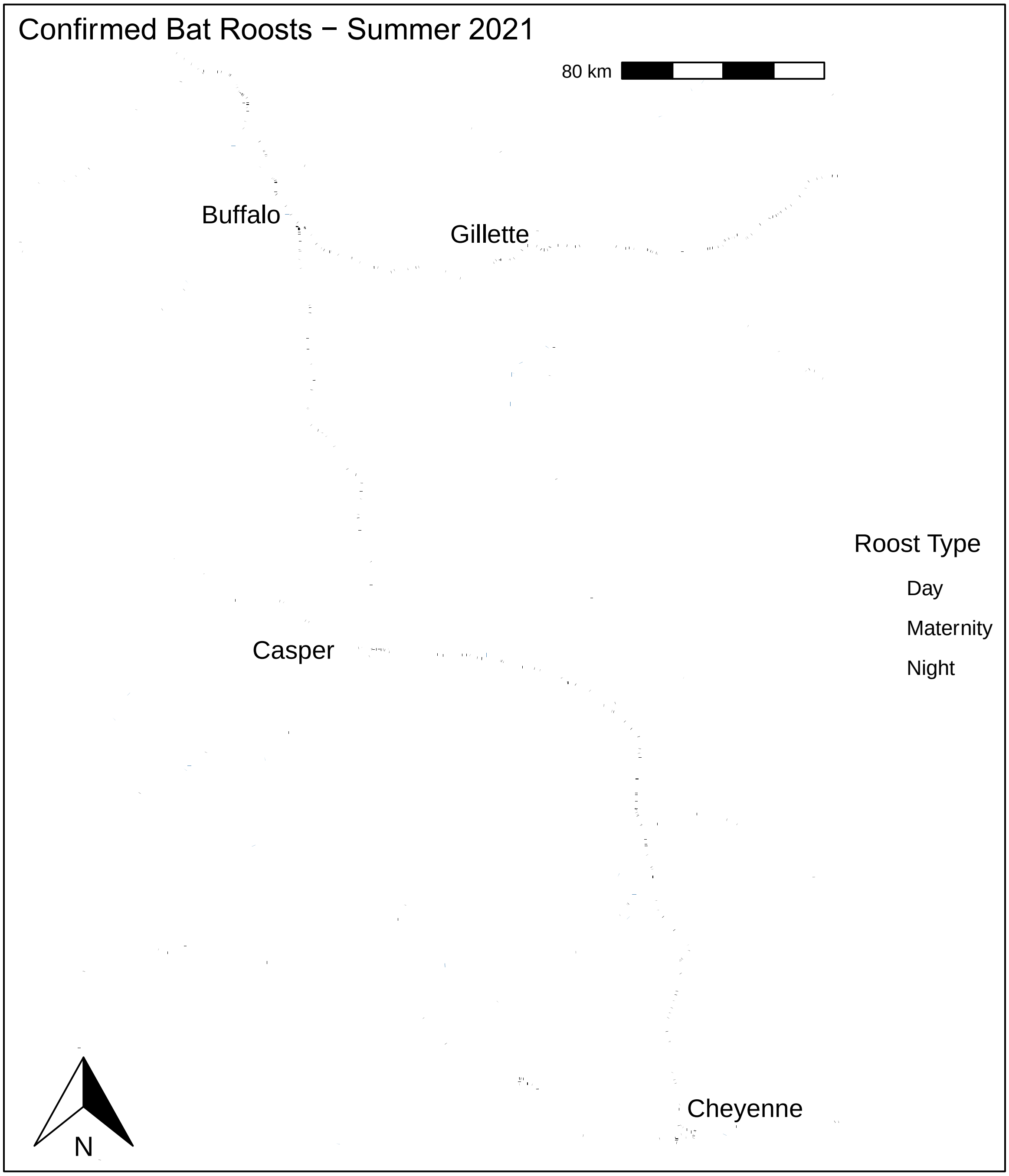

Figure 2. 196 bridge roosts were identified between May 21 and August 20, 2021. Night roosts are used by bats at night to digest food or avoid poor weather and were confirmed by the presence of guano and urine staining. Day roosts were confirmed by the presence of bats. Maternity roosts were determined by the presence of bats of different age classes (determined primarily by size and position of smaller individuals).

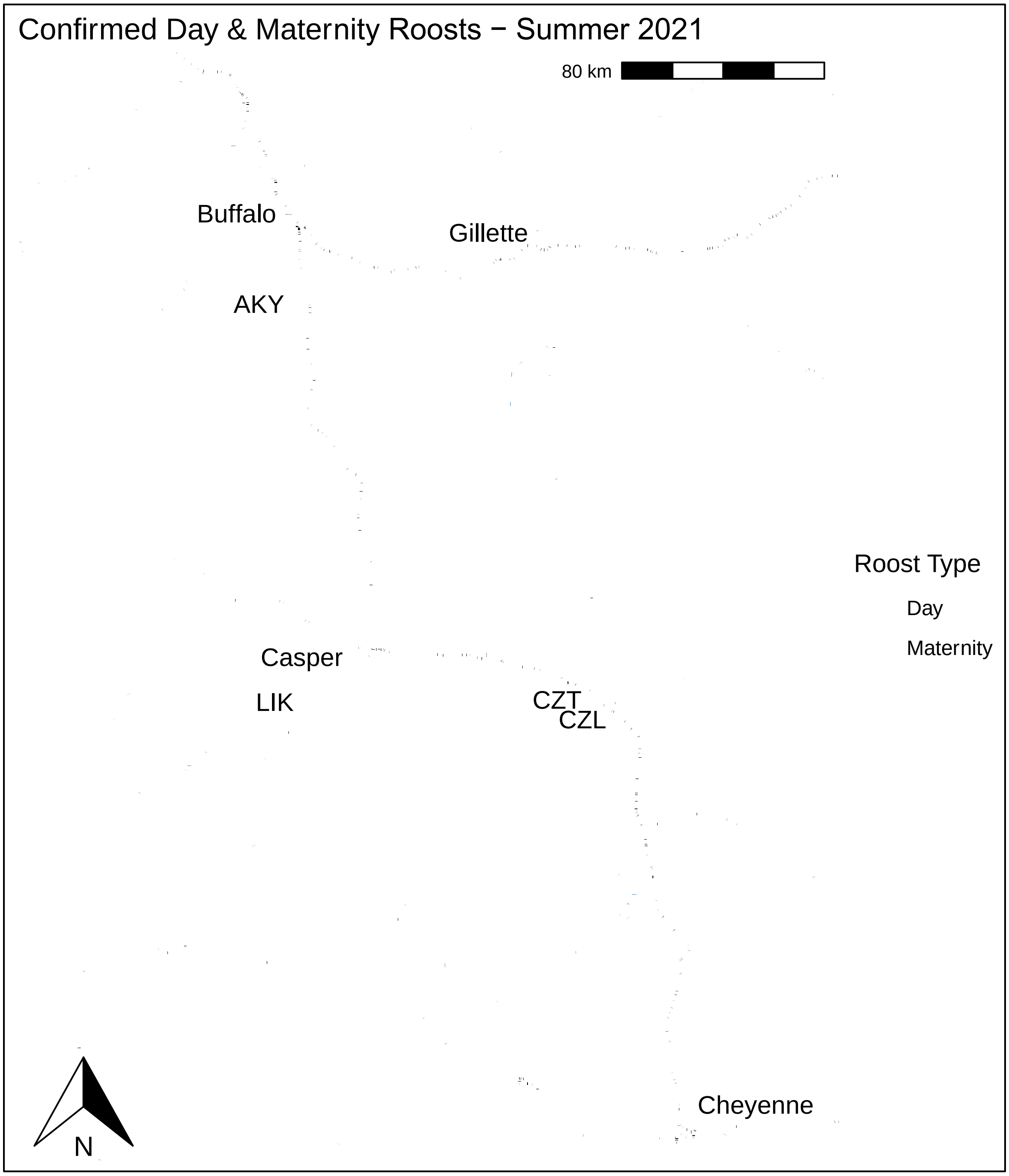

Figure 3. Day and Maternity roosts identified at bridges between May 21 and August 20, 2021. Day roosts were confirmed by the presence of bats, while maternity roosts were determined by the presence of bats of different age classes (determined primarily by size and position of smaller individuals). Of the 196 bridges determined to be used by bats, 5 were determined to be day roosts and 3 were identified as maternity roosts.