SEASONAL USE OF OHIO DEPARTMENT OF TRANSPORTATION BRIDGES BY BATS

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16. Abstract

The Ohio Department of Transportation (ODOT) is required to inspect bridge structures for presence of bats prior to repair, maintenance, or removal. Many bridges inspected do not contain bats, but evidence of bat presence can result in project delays, added costs, and increased impacts to listed species. This study aimed to facilitate understanding of bat use of ODOT bridges and to develop a predictive model to improve planning efficiency. Study objectives included identifying characteristics making bridges more or less likely to contain bats and using results to create a priority ranking system for future bridges inspections to avoid negatively impacting roosting bats while potentially avoiding project delays.

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1.0 Introduction

The Ohio Department of Transportation (ODOT) is required under the Framework Biological Opinion on ODOT's Federal-aid Highway Program for the Federally Endangered Indiana Bat and Federally Threatened Northern Long-eared Bat (OHPBO) to inspect bridge structures for the presence of bats prior to repair, maintenance, or removal. Many bridges inspected do not contain bats, but evidence of bat presence often results in project delays, added costs, and increased impacts to listed species. As a result, ODOT's Office of Environmental Services (OES) seeks a tool to assist in identifying bridges serving as suitable bat roosting habitat as the first step toward developing a larger, geospatial predictive model to improve planning efficiency. Modeling objectives include:

- Identifying bridges unlikely to contain bats thus precluding examination until just prior to construction activity, if at all; and
- Providing ODOT with an early warning system so bridges likely to contain bats receive timely inspections prior to construction activities.

By focusing surveys on bridges likely to contain bats, ODOT saves time and money while attaining increased flexibility to address potential concerns with the U.S. Fish and Wildlife Service (USFWS) and the Ohio Department of Natural Resources (ODNR), Division of Wildlife when bats are found. A suitable model also provides the best available science allowing ODOT, in consultation with USFWS and ODNR, to exclude certain bridge types from future consultations. The model facilitates shifting focus from bridges where bats are unlikely to occur to target bridges where bats are most likely to occur and provides important conservation information to USFWS and ODNR.

Environmental Solutions & Innovations, Inc. (ESI) and the Lochmueller Group, Inc. (Lochmueller; jointly the ESI Team) designed the current study to facilitate understanding of bat use of ODOT bridges. The study is divided into two phases, each comprising multiple tasks.

1.1 Review of Phase I

Phase I includes a preliminary analysis comprising review of available literature, a preliminary analysis based on 21 ODOT bridges and culverts known to be used by bats and summarizing results into a "step-down" model.

The literature review identified 120 documents (112 North American, 5 European, 1 Central American, 1 South American, and 1 global) that record and describe bat use of bridges as habitat. Based on reviewed sources, a hypothesis was developed indicating ODOT bridges used by bats are large, concrete structures in rural areas providing adequate roosting space (crevices or protected open space) and crossing streams.

ODOT provided a list of 21 structures (15 bridges and six culverts) known to contain bats. Comparison of bridges on the list to other bridges in the ODOT database reveals bats are most likely found in bridges with high traffic volumes in the forested landscapes of eastern Ohio. Data from the analysis are combined with variables from the literature to create the preliminary step-down model including the following characteristics: deck area, distance to stream, amount of forest within five miles (8.05 km), primary construction material (concrete or prestressed concrete is preferred), type of structure (beam or box beam is preferred), and presence or absence of a concrete deck. Subsequently, the matrix was used to rank likelihood of all ODOT bridges to contain bats. Phase I activities were summarized in a report submitted on 4 May 2020.

1.2 Review of Phase II:

Phase II objectives include developing a comprehensive model incorporating field data collected from at least 150 bridges. The second phase consists of 1) using the step-down model (from Phase I) to create a list of 150 bridges targeted for bat occupancy studies, 2) conducting field studies to examine bridges identified on the list, 3) analyzing study results, and 4) creating an updated step-down model.

Based on the analyses completed in Phase I, a list of 150 bridges (50 high, 50 moderate, and 50 low suitability) considered target bridges, was presented to ODOT on 4 May 2020. Additional alternate bridges from each category were also included in the 4 May report to account for 1) a bridge examination precluded by safety concerns, or 2) time availability to sample additional bridges.

The current report details Phase II results: 1) report results of field studies on a subset of ODOT bridges, 2) and analyze results to create an updated step-down model. One hundred fifty Phase I target bridges and 68 alternates of high, moderate, and low suitability were surveyed between 23 June and 1 September 2020. Two hundred eighty-six non-targeted bridges of moderate suitability were additionally surveyed resulting in a total of 504 ODOT bridges surveyed during the 2020 field season. During field studies, environmental and structural traits associated with bridges were quantified and signs of bat presence/absence were recorded.

2.0 Methods

Five hundred four bridges across Ohio and under ODOT jurisdiction were identified and surveyed to determine how the presence or absence of bats varied and/or correlated with environmental and structural bridge characteristics. To help guide field efforts, a preliminary analysis was completed based on a review of the literature and 21 ODOT structures known to contain bats. Details of that analysis were contained in a report dated 4 May 2020 but also were summarized herein. A series of stepwise Binomial General Linear Models (BGLM) were used to identify those structural, environmental, and non-structural characteristics that separated the 21 structures with bats from those without known records of bats. Following the analysis, the final series of models was then used to rank all bridges within the state based on how their structural, design, and associated environmental variables compare to bridges known to contain bats. To help guide field studies, bridges were broken into three categories: 189 bridges with features considered to have a high likelihood of bats, 55 bridges considered to have a low likelihood of bats, and all other bridges considered moderate quality for bats.

2.1 Targeting Bridges for Field Surveys

The process for selecting bridges for field studies included selecting a minimum of 50 high, 50 medium, and 50 low suitability bridges for in-field assessment.

To select 50 bridges from the high category, the research team examined aerial photographs supplemented with images provided by ODOT (https://brphotos.dot.state.oh.us/. accessed on 25 to 30 April 2020) to determine if the underside could be safely accessed. Any bridge without a safe means of access was eliminated from the pool. Once the safety check was completed, the 50 highest-rated bridges in the state were recommended for inclusion. The remaining bridges were considered alternates that could be sampled in the event researchers discovered they could not safely access or assess a targeted bridge for use by bats.

An analogous approach was used to identify low-suitability bridges for study. A review of photography is used to eliminate any bridges that were too dangerous to access, and the 50 lowest-ranking bridges were selected for study. The remaining bridges were considered alternates that could be sampled in the event researchers discovered they could not safely access or assess a targeted bridge for use by bats.

Most ODOT bridges fall in between high and low quality and thus were considered moderate quality. The following matrix was used to select bridges (and alternates) for inclusion in the study:

- Moderate-quality bridges near the 10 highest rated bridges. These bridges provided geographic control to allow comparison of how geographic location interacts with other bridge characteristics associated with the 10 highest rated bridges.
- Moderate-quality bridges that met all criteria for high suitability except they are surrounded by little forest within 5 miles (8.04 km). These bridges served as a test of the importance of forest cover.

- Bridges within 5 miles (8.04 km) of one of the high- or low-quality bridges being studied and whose score was near the average for all ODOT bridges. The goal of this category was to identify the middle of the middle, which may provide insight into potential break-points in the data-set.

Selection criteria resulted in bridges with a variety of structure and design types that are spread throughout the state and include multiple alternative bridges to supplement field sampling.

2.2 Sampling Non-Target Bridges

Although the intent of the study was to focus field surveys on the 150 target bridges identified in Section 2.1 above, biologists were encouraged to sample other bridges as time and budget permitted. Three hundred fifty-four additional bridges were included in the sample comprising 68 bridges on the alternate sample list and 286 bridges based on their proximity to a targeted bridge or routes driven between target bridges and any bridges deemed high potential by biologists in the field.

2.3 Bridge Assessments

Bridges were assessed for signs of bat use during daytime. Multiple bridge features, including guard rails leading to the bridge, bridge decks, drains, abutments, and undersides were assessed for presence of roosting bats (detected through sight, sound, or smell). Assessments included searches for evidence of bat use such as staining, presence of guano, and culled prey remains. Bat species present were visually identified during surveys. In some cases, multiple visits were necessary to confirm an identification. If identification was uncertain guano samples were collected for submittal to a commercial laboratory (Pisces Molecular, LLC, Boulder Colorado). A bat detector was placed near one bridge to confirm presence of the state and federally endangered Indiana bat (*Myotis sodalis*) and state endangered little brown bat (*Myotis lucifugus*).

2.3.1 COVID-19 Protocol

Studies were completed during the global COVID-19 pandemic, caused by a coronavirus thought to have originated in Asian bats. North American bats are naïve to the virus. Protocols, to avoid inadvertently infecting bats with COVID-19, were developed in cooperation with ODNR and USFWS and face masks were worn during all assessments, thus potentially inhibiting biologists' ability to smell bats.

Initial entry into a bridge was typically made in areas where potential roosting areas were at least 12 feet (3.66 m) above the ground, providing a minimum of six feet (1.82 m) between observers and any bats present. For small structures, an initial assessment was made from a distance of at least six feet (1.82 m) and entry was aborted if bats were observed. Similarly, pole-mounted cameras were used to inspect cavities and crevices prior to verifying absence via use of flashlights. Upon observing a bat, biologists maintained a minimum six-foot (1.82-m) distance between themselves and the bat(s). No bats were handled during the study, thus limiting the ability of biologists to identify some species.

2.3.2 Bridge Assessments using UAV

As needed, an Unmanned Aerial Vehicle (UAV; “drone”), controlled by a Federal Aviation Administration (FAA)-certified commercial drone pilot, was used to search otherwise inaccessible areas of larger bridges. Bridge height, deep flowing water, or visual obstructions (bridge structures or vegetation) occasionally precluded ground-based inspections, and a drone (Parrot Anafi quadcopter with a camera mounted at 90 degrees to the horizon [e.g., looking straight up]) was used, allowing inspection of the underside of the bridge including cracks and crevices. The drone was equipped with after-market modifications such as auxiliary lighting to illuminate dark areas, propeller guards to protect bats and birds from rotors, and a GPS shield enabling operation without GPS.

Prior to each flight, the FAA’s Before You Fly (B4UFLY) application was implemented to evaluate and avoid hazards such as powerlines, towers, roads, and vegetation. Flights were conducted by an FAA-certified (Part 107) drone operator with over 400 hours of flight experience. A second observer maintained line-of-sight with the drone, watched for bat or bird activity, and helped avoid collisions. In accordance with FAA requirements, drones were not operated over moving vehicles, over people, or at night.

2.3.3 Acoustic Surveys

2.3.3.1 Type and Location of Bat Detector

Based on visual detection of likely little brown and Indiana bats, acoustic data were collected at Structure File Number 7003072 (SFN, a unique identification code associated with every ODOT bridge and culvert) from 18 August to 21 August 2020.

A Wildlife Acoustics Song Meter (SM) Mini Bat with an integrated SMM-U2 microphone was mounted to the top of 10-foot (3.04-m) pole and placed in an open area downstream of the bridge. The detector was programmed to begin recording at sunset and cease recording at sunrise.

2.3.3.2 Automated Data Analysis

Call files recorded were downloaded and processed through Kaleidoscope Pro (Kpro) software (classifier v5.1.0 Wildlife Acoustics, Concord, Massachusetts). The software extracted parameters including frequency, time, and slope components of each pulse. Each pulse was then assigned a species-level identification, with the entire sequence identified based on the species most frequently identified.

The software used maximum likelihood estimators (MLE), a multivariate statistical technique used to test the strength of a proposed relationship based on known or assumed error rates. In this case, the proposed relationship was protected bat presence identified by analytical software. The MLE accounted for the number of call sequences identified as a species and compared that to the number of call sequences identified as belonging to a similar species based on assumed error rates. Assumed error rates were obtained by testing the software packages against libraries of known calls. The goal was to provide a mechanism to eliminate errors resulting from misclassification.

Nine species with potential to occur in the immediate and surrounding areas of the Project were included in the analysis: big brown (*Eptesicus fuscus*), eastern red (*Lasiurus borealis*), hoary (*Lasiurus cinereus*), silver-haired (*Lasionycteris noctivagans*), evening (*Nycticeius humeralis*), little brown, northern long-eared (*Myotis septentrionalis*), Indiana, and tricolored bats (*Perimyotis subflavus*). The zero (balanced/neutral) sensitivity setting was used for analysis and classifier package, allowing calls to be classified to the species-level based on the greatest percentage of calls classified as a single species. Acoustic data are provided electronically upon request and are stored for five years.

2.3.3.3 Visual Data Analysis

All files recorded on nights with a significant MLE for a listed species were visually examined by an expert bat acoustic identification specialist (Mr. Patrick Moore; resume provided in Appendix A). Qualitative vetting included identification to species by focusing on morphological call characteristics such as frequency, slope, duration, and intensity.

2.3.4 DNA Analyses of Guano

Samples of guano were tested from 18 bridges by Pisces Molecular (Boulder, Colorado), a commercial laboratory that specializes in analysis of environmental DNA (eDNA). Details of the analysis are contained in Appendix B, but a simplified version of the analysis follows.

Dry guano was collected under bridges and refrigerated in individually labeled plastic bags until submission for genetic analysis. Upon arrival at the lab, each sample was dissolved and resuspended in lysis buffer (MoBio solution C1) to break down cell membranes and total genomic DNA was extracted using a Powersoil spin column procedure, per manufacturer's instructions (MoBio Inc., Carlsbad, CA).

A portion of the DNA region including the Cytochrome Oxidase I gene (CO1) was then amplified using Polymerase Chain Reactions (PCR, a technique that rapidly copies DNA). CO1 is a mitochondrial gene that is useful for identification of bat and other mammal species because the gene is well studied and variable enough to distinguish between species while not being so variable as to result in unrecognizable mutations that preclude species identification. Following initial amplification of CO1, a second round of PCR was used to incorporate a twelve-nucleotide index sequence into the

DNA for each sample. A second round of PCR amplification was completed with the goal of amplifying the CO1 gene for sequencing while retaining a tag to cross-reference which sequences are associated with which sample. Combined, these steps pulled DNA from the sample, made many copies of that DNA, and identified and copied a single, variable gene.

Following extraction, indexing, and amplification, the resulting samples were run on a two percent agarose gel to separate the targeted DNA from everything else in the pool. When an electrical current is applied, it pulls DNA through the gel at rate that is correlated with the size of the DNA fragment. As the gene is well-known, this also provided a visual indication that the amplification and tagging steps were successful.

Samples were then cleaned and sequenced using a commercially available kit (MiSeq v2 300-cylce kit, Illimina, San Diego, CA). Once sequencing was complete, two versions of a computer program (BLAST, Basic Local Area Search Tool) were used to compare the resulting sequence to archived sequences collected from bats of known identity. By aligning sequences of samples collected in this study to known bat sequences, we were able to reliably identify which bat species were present at bridge locations.

2.4 Statistical Analysis of Sampled Bridges

The SFN for each structure sampled is matched with information from ODOT's Transportation Information System (TIMS) regarding the bridge's location, age, and design.

The following categorical variables are examined using a Pierson's Chi-Square Test tested for non-random assortment among structures with bats:

- ODOT District
- Type (a description of the bridge's design)
    - Arch
    - Beam (Beam and Box Beam types were combined into single category)
    - Culvert (Culvert and Other types were combined into single category)
    - Frame
    - Girder
    - Slab
    - Truss
- Structure Material
    - Concrete (Concrete and Prestressed Concrete types were combined into single category)

    - Metal (Steel, wrought iron, and aluminum types were combined into single category)
    - Stone
    - Timber
- Description
    - Continuous
    - Deck
    - Filled
    - Other
    - Pony Truss
    - Simple
    - Thru
- Type of Service Under Structure
    - Highway, with or without pedestrian
    - Railroad/waterway
    - Waterway
- NBI Rating
    - Good
    - Fair
    - Poor
    - Unknown
- Type of Membrane
    - Built-up (Layers of fiberglass and coal tar)
    - Preformed Fabric (Type III Waterproofing)
    - Epoxy
    - Unknown
    - None or Not Applicable
    - Other

A series of Binomial General Linear Models (BGLM) were used to compare ODOT structures where bats were present to the remaining structures surveyed. An overall model comparing descriptive variables provided information on multiple structure attributes including physical structure of the bridge or culvert (type, material, and description [subcategories of bridge types], area of the deck, membrane type, and NBI rating), non-structural (ODOT district, type of service below the bridge, age of construction, traffic lanes under the structure, and average daily traffic), and environment features surrounding the bridge (amount of foraging habitat within 0.25 mile [0.40 km], amount of forest within 5.00 miles [8.05 km], amount of wetland habitat within 0.25 mile [0.40 km], and distance to streams).

A stepwise analysis was used to identify variables providing the most information within the overall model. Step-wise analyses are statistical tools that take information gained from preliminary analyses to narrow down the best descriptive model out of the potential pool of variables. Step-wise analyses build a series of models using the total variable pool, but the final model consists only of those variables with the highest influence. Each data category (physical structure, non-structural, and environmental variables) was analyzed separately using a BGLM followed by a stepwise regression to select the best available model for that category.

Finally, using each fitted BGLM (overall model, physical structure, non-structural, and environmental), we estimated predicted values of bat likelihood for all unsampled ODOT bridges. Predicted results from each model were split into three quantiles to create a categorical rank of potential suitability. Individual model ranks were then summarized across all models into five suitability categories: Highest, High, Moderate, Low, and Lowest likelihood for bats. Bridges ranked in the top quantile across all models were ranked as Highest likelihood for bats. Bridges that ranked in the bottom quantile across all models were ranked as Lowest likelihood for bats. The remaining bridges were split into High, Moderate, and Low likelihoods based on the combination of predictive values across models and split into three categories.

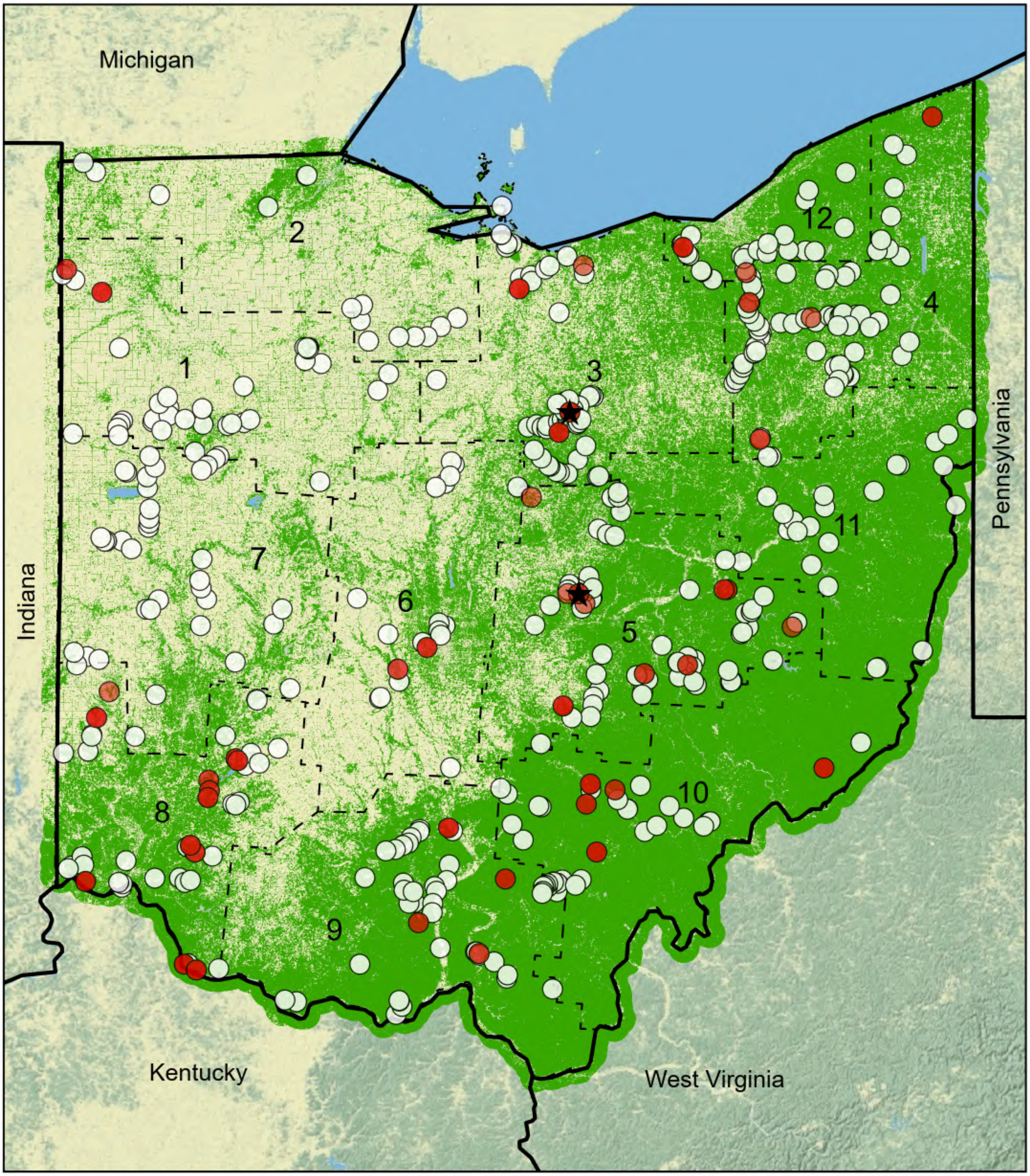
3.0 Results and Discussion

3.1 Bridges Sampled

Five hundred and four bridges were sampled (Figure 1) including 150 Phase I target bridges, 68 alternates, and 286 non-targeted bridges; eight bridges, based on size and height, were surveyed using a drone.

3.1.1 Targeted Bridges

The original intent was to survey a minimum of 150 bridges (50 each from the high, medium, and low categories identified above). Surveys were completed at 218 Phase I targeted (150) and alternate (68) bridges. Bridges initially ranked High, Moderate, and Low from the Phase I step-down model that were surveyed for bats are contained in Appendix C. Suitability categories of target bridges are discussed below to provide context regarding effectiveness of the initial ranking system.



ODOT District

Evidence of Bats
Yes
No

Bridge with Listed Bats
State Boundary
Foraging Habitat

NOTE: Bat use was evident in 46 bridges surveyed.

N
W E
S

Figure 1. Location of the 504 ODOT bridges surveyed in 2020.

Project No. 1418

0 35 70 Miles
Base Map: USGS Topographic Map

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C:\Users\nbrown\Documents\ArcGIS\Projects\ODOTbatsandbridges_1418_2\ODOTbatsandbridges_1418_2.aprx; creator: NBrown

- One hundred thirty-three high suitability bridges were surveyed and 23 (17.3%) bridges had bats or evidence of use by bats such as guano or staining. (Appendix C).
- Forty-six moderate suitability bridges were surveyed, and two (4.6%) had evidence of use by bats (Appendix C).
- Thirty-nine low suitability bridges were surveyed, and none had evidence of use by bats (Appendix C)

Based on data above, the ranking system provided by the initial step-down model proved highly effective at identifying bridges most likely to contain bats. One bridge ranked as highly suitable contained two listed species: the state and federally endangered Indiana bat and state endangered little brown bat. The bridge contained a cluster of about 24 individuals with mixed species identification (Table 1).

Table 1. Summary of bat species observed during Phase II ODOT bridge surveys.

Common Name	Scientific Name	Number of Bridges with DNA-Confirmed Records	Number of Bridges with Confirmed Sighting	Number of Bats Confirmed by Sight
big brown bat	*Eptesicus fuscus*	17	25	156
Indiana bat	*Myotis sodalis*		1	Cluster of 24 bats (both Indiana and little brown bats)
little brown bat	*Myotis lucifugus*	1	1	Cluster of 24 bats (both Indiana and little brown bats)
northern long-eared bat	*Myotis septentrionalis*		1	1
unidentified species[1]	-		2	53

[1] Visualy unidentified individuals are estimated to total 53 individuals at two bridges, SFN 2930331 and SFN 6330800. DNA analysis indicated presence of big brown bats for pellets found at both bridges.

Two bridges with evidence of bat use had received moderate suitability rankings primarily based on a relatively low amount of forest within five miles. One bridge was slightly farther from the stream compared to high suitability ranked bridges while the second bridge had only a moderate deck area. Both represented concrete or prestressed concrete box beam bridges with moderate to high amounts of foraging habitat within 0.25 mile (0.40 km).

3.1.2 Non-target Bridges Sampled

Two hundred eighty-six additional bridges were surveyed, many convenient to target bridges or other studies completed by ESI in summer 2020. Bat use was evident in 21 (7.3%) non-target bridges. Non-targeted bridges were all moderately ranked and often selected for exhibiting a wide range of structural, nonstructural, and environmental traits to clarify differences among traits. Concrete/prestressed concrete, beam/box beam, moderately sized bridges immediately surrounded by forested habitat were purposefully targeted. Bridges fitting the criteria, particularly bridges with at least eight feet (2.44-m) of clearance over water, were sampled when available. Non-target bridge SFN 4530011 contained northern long-eared bat, a listed species. One bridge with previous records of bat use (SFN 1800256) was re-surveyed and no current evidence of bat use was found.

3.2 Bat Species Encountered

Overall, bats or evidence of bats were found at 46 (9.1%) of 504 bridges (Tables 1 and 2; Figure 1). Bats were directly observed at 29 (63.0%) of 46 bridges and all but two (95.7%) were inhabited by big brown bats. A single bridge was inhabited by one northern long-eared bat (SFN 4530011), and another bridge contained little brown bats and at least one Indiana bat (SFN 7003072). All three species are considered endangered by ODNR while northern long-eared bat (threatened) and Indiana bat (endangered) are also protected by the federal Endangered Species Act (ESA). Listed species occupied two concrete or prestressed concrete, box beam bridges, each over a waterway with at least 60 acres (24.28 ha) of foraging habitat within 0.25 mile (0.40 km).

Table 2. Summary of ODOT bridges with evidence of bat use.

Structure File Number	Bat Evidence	Bat Species Identified	Threatened/Endangered Species
0433152	bats, guano, staining	big brown bat	
0502693	bats, guano	big brown bat	
1300180	guano, staining	big brown bat[1]	
1301918	bats, guano, staining	big brown bat	
1301969	bats, guano	big brown bat	
1333135	guano	big brown bat[1]	
1403524	bats, guano, staining	big brown bat	
1602578	bats, guano	big brown bat	
1634844	bats, guano	big brown bat	
1832549	guano, staining	big brown bat[1]	
1832662	bats	big brown bat	
2045400	bats, guano	big brown bat	
2203146	guano	big brown bat[1]	
2300419	bats, guano, staining	big brown bat	
2530112	bats, guano	big brown bat	
2535289	bats, guano	big brown bat	
2930331	bats, guano, staining	big brown bat[1]	
3032915	guano	big brown bat[1]	
3130000	guano	unidentified	
3700496	guano	big brown bat[1]	
3700518	guano	big brown bat[1]	
3701662	guano	big brown bat[1]	
3931412	bats, guano, staining	big brown bat	

Structure File Number	Bat Evidence	Bat Species Identified	Threatened/Endangered Species
4033957	bats, guano, staining	big brown bat	
4230086	guano	big brown bat[1]	
4500423	guano	big brown bat[1]	
4505034	guano	unidentified	
4530011	bats	northern long-eared bat	northern long-eared bat
6004105	bats, guano	big brown bat	
6004962	bats	big brown bat	
6330800	bats, guano, staining	big brown bat[1]	
6634575	guano, staining	big brown bat[1]	
6736734	guano	unidentified	
6802591	guano	big brown bat[1]	
6834116	guano	big brown bat[1]	
7002432	bats, guano, staining	big brown bat	
7003072	bats, guano, staining	Indiana bat, little brown bat[1]	Indiana bat, little brown bat
7105657	bats, guano, staining	big brown bat	
7339445	bats, guano	big brown bat	
7600100	guano	big brown bat[1]	
7745087	bats, guano	big brown bat	
8200645	bats, guano, staining	big brown bat	
8332037	bats, staining	big brown bat	
8333475	bats, guano	big brown bat	
8334617	bats, guano	big brown bat[1]	
8439303	bats, guano, staining	big brown bat	

[1] DNA from collected guano used to confirm or identify species of bat present.

3.3 Acoustic Bat Survey

3.3.1 Analysis of Call Sequences

Data files recorded totaled 5,765 with 2,962 data files identified by Kpro as potential bat call sequences. Kpro provided species-level identifications for 2,249 of these call sequences (Table 3). At this initial level of analysis, call sequences consistent with all nine species are present.

Table 3. Bat calls identified by Kaleidoscope Pro with automated call identification on ODOT bridge SFN 7003072 in ODOT District 3.

Structure File Number	Date (2020)	EPFU	LABO	LACI	LANO	MYLU	MYSE	MYSO	NYHU	PESU	Total
7003072	18 August	202	122	1	0	208	2	65	60	2	662
	19 August	119	80	3	6	85	3	53	53	4	406
	20 August	150	114	9	4	91	0	49	73	4	494
	21 August	95	82	6	12	135	0	58	108	191	687
Total/Species		**566**	**398**	**19**	**22**	**519**	**5**	**225**	**294**	**201**	**2,249**

EPFU=*Eptesicus fuscus* (big brown bat); **LABO**=*Lasiurus borealis* (eastern red bat); **LACI**=*Lasiurus cinereus* (hoary bat); **LANO**=*Lasionycteris noctivagans* (silver-haired bat); **MYLU**=*Myotis lucifugus* (little brown bat); **MYSE**=*Myotis septentrionalis* (northern long-eared bat); **MYSO**=*Myotis sodalis* (Indiana bat); **NYHU**=*Nycticeius humeralis* (evening bat); **PESU**=*Perimyotis subflavus* (tricolored bat).

3.3.2 Maximum Likelihood Estimator

Table 4 provides results of the MLE analysis, integrated into Kpro's analysis suite. MLE analysis is a goodness of fit test comparing quantity and quality of recorded calls to known libraries of call sequences and known detection error rates for each species to estimate species presence probability.

Table 4. Kaleidoscope Pro maximum likelihood estimator on ODOT bridge SFN 7003072.

Structure File Number	Date (2020)	EPFU	LABO	LACI	LANO	MYLU	MYSE	MYSO	NYHU	PESU
7003072	18 August	**0**	**0**	1	1	**0**	1	**0**	**< 0.01**	1
	19 August	**0**	**0**	1	1	**0**	0.76	**0**	**< 0.01**	0.99
	20 August	**0**	**0**	0.74	1	**0**	1	**0**	**0**	1
	21 August	**0**	**0**	0.73	1	**0**	1	**0**	**0**	**0**

EPFU=*Eptesicus fuscus* (big brown bat); **LABO**=*Lasiurus borealis* (eastern red bat); **LACI**=*Lasiurus cinereus* (hoary bat); **LANO**= *Lasionycteris noctivagans* (silvered-haired bat); **MYLU**=*Myotis lucifugus* (little brown bat); **MYSE**=*Myotis septentrionalis* (northern long-eared bat); **MYSO**=*Myotis sodalis* (Indiana bat); **NYHU**=*Nycticeius humeralis* (evening bat); **PESU**=*Perimyotis subflavus* (tricolored bat).

Scores in the table ranged from a probability (p) = 1 (indicating the species was not identified in the analysis) to p = 0 (data contain enough high-quality calls that the chance of error is minute). Presence of a species was assumed on a given night with a $p<0.05$. Thus, Kpro analysis provided evidence of big brown, eastern red, little brown, Indiana, and evening bats for all detector nights at ODOT bridge SFN 7003072. Analysis provided evidence for tricolored bats on the last detector night.

3.3.3 Visual Vetting

The MLE values reported in Table 4 provide statistical evidence for the presence of the following federal or state listed bats: Indiana, little brown, and tricolored bats. Visual vetting was done on calls and confirmed listed species presence. Note the presence is for a location near the bridge, and not the bridge itself. Based on the acoustic results, photographs, and in-field observations by biologists, Indiana and little brown bats should be considered to occupy bridge SFN 7003072.

3.4 DNA Analysis

Viable DNA was collected from 18 bridges. DNA from big brown bats was confirmed at seventeen of these sites (Table 2). Guano collected under Bridge SFN 7003072 contained only DNA associated with little brown bat. Fourteen samples provided the only means of identifying the species of bat using the bridge and, in every case, big brown bats were the only species present. For three samples, the DNA confirmed the visual identification of big brown bats. In the case of SFN 7003072 no Indiana bat was detected through DNA analysis, but this may be a result of a relatively small or isolated sample. Results of the DNA are included in Tables 1 and 2.

3.5 Bridge Features

3.5.1 Physical Structures

The 504 bridges sampled reflected a variety of different designs (Table 5). Consistent with previous studies (Keeley and Tuttle 1999, Sparks et al. 2019a; b) none of the 57 slab bridges were used by bats.

Table 5. Summary of main structural components of bridges sampled during Phase II ODOT bridge surveys.

Structure Type	Primary Material	Structure Description	# of Bridges Sampled	# of Bridges with Bats
Arch	Concrete	Deck	6	0
	Steel	Deck	1	0
	Stone	Deck	10	0
Beam	Concrete	Continuous	2	0
		Other	1	0
		Simple	13	1
	Prestressed Concrete	Continuous	15	6
		Simple	26	8
	Steel	Continuous	49	3
		Simple	31	0
Box Beam	Concrete	Simple	6	2
	Prestressed Concrete	Continuous	44	4
		Simple	169	21
Culvert	Concrete	Filled	7	0
	Steel	Filled	1	0
Frame	Concrete	Continuous	1	0
		Simple	19	1
	Timber	Continuous	1	0
Girder	Concrete	Thru	1	0
	Steel	Deck	1	0
		Other	1	0
		Thru	13	0
Other	Steel	Other	1	0
Slab	Concrete	Continuous	34	0
		Simple	16	0
	Prestressed Concrete	Simple	1	0
	Timber	Continuous	1	0
		Simple	5	0
Truss	Steel	Pony Truss	16	0
		Thru	12	0

Structure Type	Primary Material	Structure Description	# of Bridges Sampled	# of Bridges with Bats
		Grand Total	504	**46**

With one exception, a simple concrete frame bridge, all bridges with evidence of bats were beam or box beam bridges. Forty-three out of 46 bridges (93.5%) with bat presence were concrete or prestressed concrete. The remaining three bridges were primarily steel beam bridges. However, at bridge SFN 7105657 bats were roosting in crevices along the concrete portions of the bridge (Figure 2). A bat was observed flying under bridge SFN 2535289, but an exact roost was never located. Previous research also noted potential value of open-air roosting spaces such as those occurring under the deck, but between supporting structures including steel beams; the scenario for SFN 2535289. Open spaces are used by bats during both the day and night and may be used by species that typically roost in caves and trees (Keeley and Tuttle 1999, Adam and Hayes 2000, McDonnell 2001, Johnson et al. 2002, Kiser et al. 2002, Keeley and Keeley 2004, Trousdale and Beckett 2004, Bennett 2005, Bennett et al. 2008, Trousdale 2008, Trousdale et al. 2008, Willis et al. 2009, Amorim et al. 2013, Zara Environmental LLC 2013, Cervone and Yeager 2016, Sparks et al. 2019a).

Most bats were found in crevices within concrete decking where additional material was present allowing bats to grip the surface and potentially provided additional insulative value. In Figure 2, a fibrous insulation material is evident and was common in bridges with roosting bats. The material is potentially the bottom of the grouted key connection, possibly made from manilla rope.

3.5.2 Non-Physical Structures

Bridges were examined in all 12 ODOT districts and evidence of bats was found in all but Districts 2, 7, and 11 (Figure 3). Twelve bridges with bats were found in District 8. Ten new bridges with bats were found in District 5. Including previously known sites in the tally, District 5 contains 22 structures with known bat presence.

The vast majority (476 out of 504, 94.4%) of bridges surveyed are used to carry roadways across water. Despite the data-set's domination by bridges over water, evidence of bat use was found in only two bridges carrying a roadway across another roadway.

In the field, biologists noted bats were most often observed on, but not limited to, bridges with at least an 8-foot (2.44-m) clearance over water features and little evidence of flooding under the bridge deck. A single big brown bat represented the only bat found under a bridge exhibiting recent flood debris on the bottom of the deck.

Figure 2. Big brown bats (*Eptesicus fuscus*) roosting in crevices of concrete decking on a primarily steel bridge (SFN 7105657).

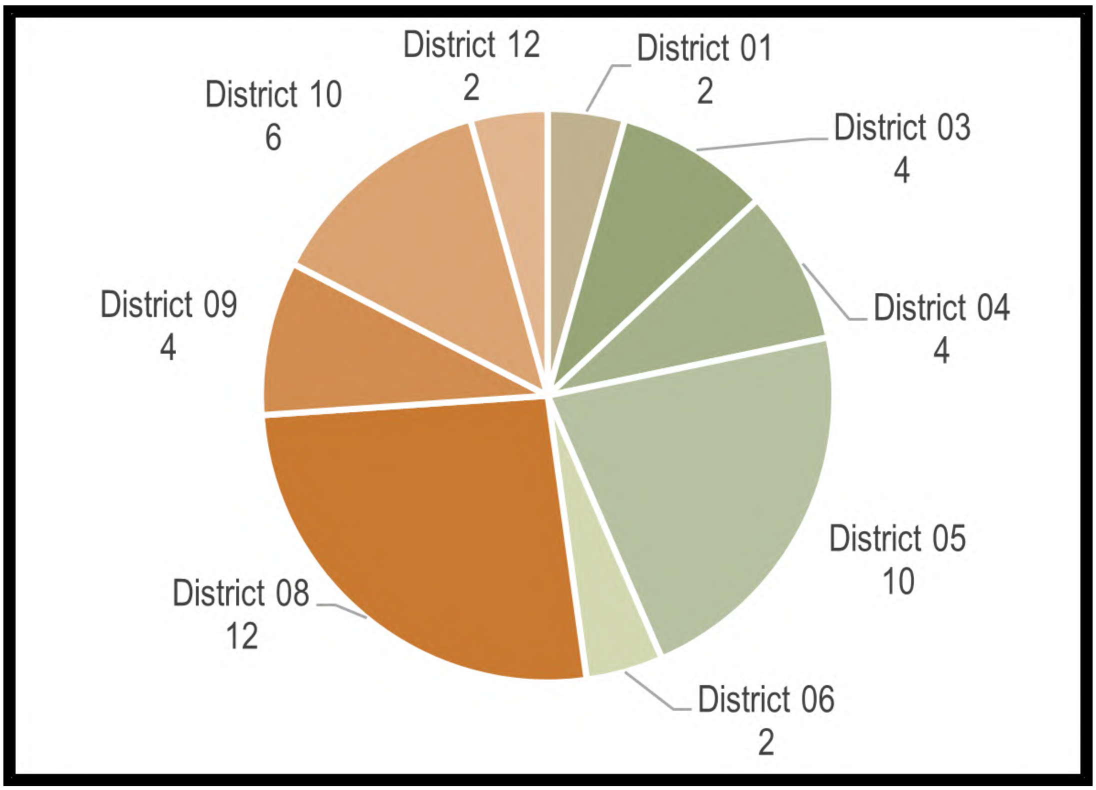

Figure 3. ODOT districts and number of bridges with bat evidence.

3.5.3 Environmental Features

Most (38 of 46, 82.6%) bridges with signs of bats were on landscapes where more than 40 percent of the landcover within 0.25 mile (0.40 km) of the bridge could be considered potential foraging habitat (NLCD landcovers: deciduous forest, mixed forest, evergreen forest, woody wetlands, herbaceous emergent wetlands, developed open space, low intensity developed land, shrub/scrub, grassland/herbaceous, and pasture/hay; Figure 4).

3.6 Statistical Analysis

As described in the methods section, statistical analyses included univariate approaches to discern patterns among occupied structures while multi-variate analyses were used to compare occupied structures to all other structures owned/controlled by ODOT.

3.6.1 Univariate Analyses

Evidence of uneven bat distribution for several categorical variables occurred (Table 6). Distribution of bat presence in bridges varied significantly among ODOT districts. Previous surveys (prior to 2020) recorded the greatest number of bats in District 5 while District 8 provided no data on bat presence. Survey results based on 2020 data demonstrated the greatest evidence of bat use of bridges in District 8 (Figure 3).

Table 6. Summary of analyses for ODOT structures used by bats.

Variable Tested	X^2 Value	Degrees of Freedom	p-value	Significant Relationship?
ODOT District	**34.31**	**11**	**< 0.01**	**Yes**
Type	**18.33**	**6**	**< 0.01**	**Yes**
Material	**11.81**	**3**	**< 0.01**	**Yes**
Description	8.96	6	0.18	No
Type of Service Under Bridge	11.03	6	0.09	Trend
NBI Rating	5.04	3	0.17	No
Type of Membrane	**23.38**	**6**	**< 0.01**	**Yes**

Bat distribution varied significantly depending on bridge type and material with most bats found in the beam and box beam types constructed of concrete and prestressed concrete materials. Membrane type also influenced uneven bat distribution. Bats were nearly randomly distributed among structures of different descriptions and National Bridge Inventory ratings (NBI rating; a descriptor of structural condition). As noted above, most structures used by bats were across water, a pattern nearing significance.

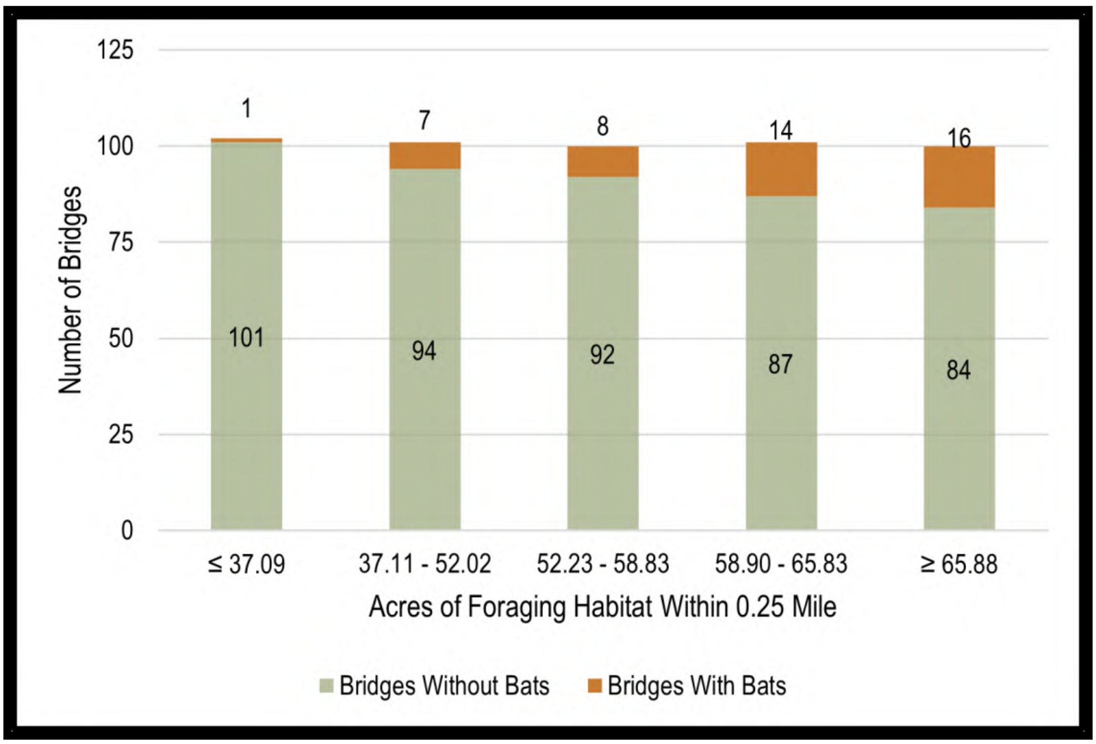

Figure 4. Bat presence at bridges sampled in 2020 associated with available foraging habitat.

3.6.2 Multivariate Analyses

A series of BGLMs were used to compare 46 structures with records of bat use to 458 structures without records of bat use. Data were entered in a stepwise regression to develop a best-fit model containing only variables that facilitate explaining the most amount of data variation.

3.6.2.1 All Variables

The first step in the analysis included creating a model examining all variables concurrently. Results of the stepwise regression best-fit BGLM are summarized in Table 7 and indicated several important predictors of bat presence. Significant predictors included larger deck area, younger bridges not made of metal (aluminum, steel, or wrought iron), and increased foraging habitat near the bridge.

Table 7. Stepwise regression results of a Binomial General Linear Model comparing ODOT structures with bats to those without bats.

Coefficient[1]	Estimate	Standard Error	P-value	Significant Effect?
Intercept	**-5.24E+01**	**1.77E+01**	**< 0.01**	**Yes**
District 2	-1.56E+01	2.21E+03	0.99	No
District 3	3.99E-02	1.01E+00	0.97	No
District 4	-7.68E-01	1.00E+00	0.44	No
District 5	5.89E-01	9.37E-01	0.53	No
District 6	-1.00E-01	1.20E+00	0.93	No
District 7	-1.53E+01	1.51E+03	0.99	No
District 8	7.92E-01	9.62E-01	0.41	No
District 9	-4.17E-01	1.04E+00	0.69	No
District 10	-2.20E-02	1.04E+00	0.98	No
District 11	-1.77E+01	1.76E+03	0.99	No
District 12	-1.28E+00	1.17E+00	0.27	No
Material: Metal[2]	**-2.24E+00**	**7.36E-01**	**< 0.01**	**Yes**
Material: Stone	-1.28E+01	3.13E+03	1.00	No
Material: Timber	-1.41E+01	3.57E+03	1.00	No
Deck Area[3]	**1.45E+00**	**2.90E-01**	**< 0.01**	**Yes**
Year Built	**1.78E-02**	**8.58E-03**	**0.04**	**Yes**
Foraging habitat within 0.25 mile	**5.48E-02**	**1.80E-02**	**< 0.01**	**Yes**

[1] Categorical coefficient testing for ODOT District and Material coefficients is calculated by comparison to the first category (District 1 and Material: Concrete). Randomized order of categories was tested and no substantial difference in results was found.
[2] Material category metal contains aluminum, steel, and wrought iron bridges
[3] Transformed as log(area+1)

3.6.2.2 Structural and Design Variables

A stepwise regression of structural and design variables included material and deck area. Significant predictors included larger deck area and bridges not made of metal (Table 8).

Table 8. Results of a stepwise Binomial General Linear Model comparing structural and design characteristics of ODOT structures with bats to those without bats.

Coefficient[1]	Estimate	Standard Error	P-value	Significant Effect?
Intercept	**-1.18E+01**	**2.02E+00**	**< 0.01**	**Yes**
Material: Metal[2]	**-2.39E+00**	**6.69E-01**	**< 0.01**	**Yes**
Material: Stone	-1.38E+01	1.22E+03	0.99	No
Material: Timber	-1.32E+01	1.48E+03	0.99	No
Deck Area[3]	**1.20E+00**	**2.38E-01**	**< 0.01**	**Yes**

[1] Categorical coefficient testing for Material coefficient is calculated by comparison to the first category (Material: Concrete). Randomized order of categories was tested and no substantial difference in results was found.
[2] Material category metal contains aluminum, steel, and wrought iron bridges
[3] Transformed as log(area+1)

3.6.2.3 Nonstructural Variables

A stepwise regression of BGLM examining nonstructural variables included the intercept, each district, and year bridge was built (Table 9). District 8 and marginally District 10 represented significant predictors of occupancy along with the year of bridge construction.

Table 9. Results of a stepwise Binomial General Linear Model comparing nonstructural characteristics of ODOT structures with bats to those without bats.

Coefficient[1]	Estimate	Standard Error	P-value	Significant Effect?
Intercept	**-4.64E+01**	**1.49E+01**	**< 0.01**	**Yes**
District 2	-1.53E+01	1.59E+03	0.99	No
District 3	4.34E-01	8.98E-01	0.63	No
District 4	2.01E-01	8.94E-01	0.82	No
District 5	1.31E+00	8.08E-01	0.10	No
District 6	9.86E-01	1.05E+00	0.35	No
District 7	-1.55E+01	1.07E+03	0.99	No
District 8	**1.97E+00**	**8.06E-01**	**0.01**	**Yes**
District 9	5.26E-01	8.97E-01	0.56	No
District 10	1.61E+00	8.64E-01	0.06	Trend
District 11	-1.54E+01	1.20E+03	0.99	No
District 12	4.66E-01	1.04E+00	0.65	No
Year Built	**2.19E-02**	**7.48E-03**	**< 0.01**	**Yes**

[1] Categorical coefficient testing for ODOT District coefficient is calculated by comparison to the first category (District 1). Randomized order of category was tested and no substantial difference in results was found.

3.6.2.4 Environmental Variables

A stepwise BGLM examining environmental variables indicated that the amount of foraging habitat within 0.25 miles (0.40 km) of the structure was the best and only significant predictor of occupancy, although distance to stream was also weakly associated (Table 10).

Table 10. Results of a stepwise Binomial General Linear Model comparing environmental variables associated with ODOT structures with bats to those without bats.

Coefficient	Estimate	Standard Error	P-value	Significant Effect?
Intercept	**-4.69E+00**	**8.45E-01**	**<0.01**	**Yes**
Distance to Stream	-1.22E-02	7.24E-03	0.09	Trend
Foraging Habitat within 0.25 mile	**4.65E-02**	**1.37E-02**	**< 0.01**	**Yes**

3.6.3 Summary of Analyses

Following multiple analyses, several variables proved important for distinguishing the 46 bridges with bats from 458 where no signs of bats were found. As noted earlier, approximately 60 percent of structures are in ODOT Districts 5, 8, and 10, within areas with extensive forest and available foraging habitat, potentially explaining the most important environmental variable, amount of foraging habitat within 0.25 mile.

Important predictors associated with the bridge itself include material and area of the deck. Area of the deck was used as an indicator of a bridge's size, an important correlate of the bridge's thermal stability. Previous studies indicated thermal stability is an important factor in predicting the value of a bridge for bat use (Keeley and Tuttle 1999, Bektas et al. 2018a, Bektas et al. 2018b, Sparks et al. 2019a; b). Bats also primarily roosted in portions of bridges made of concrete or prestressed concrete, including bridges where other materials (especially steel) were present. Concrete has higher insulative capabilities than metal and is likely more attractive to bats.

Amount of foraging habitat within 0.25 mile represented the only environmental variable significantly differing among bridges with bats and those with no signs of occupancy. All bats in Ohio regularly roost in trees and make extensive use of forests and forest edge for commuting and foraging (Brack et al. 2010). However, the big brown bat is the least forest-dependent species in the state and was also the most frequently encountered species in the current study. Notably, amount of forest at a larger scale (within five miles) was not a significant predictor, potentially indicating bridges surrounded by small, isolated patches of forest, wetlands, and grasslands play an important role in sustaining colonies of big brown and other bat species. A summary of the findings and supplemental photos can be found in Appendix D.

4.0 Conclusion

Study objectives included identifying characteristics making bridges more or less likely to contain bats and using results to focus monitoring and management actions on bridges where bats are reasonably likely to occur. Results indicated bridges lacking concrete abutments, beams, piers, or decking likely have low probability of bat occupancy. Similarly, bridges prone to flooding or in areas dominated by intense development or agriculture within 0.25 mile (0.40 km) are rarely used. Additionally, bat use evidence recorded during previous surveys does not ensure detection of bats in subsequent survey efforts.

Bridges most likely used are located near high quality foraging habitats such as forests and wetlands, provide bats with insulated gaps (such as expansion joints) in concrete, and are at least 8 feet (2.44 m) above water. Results mirror previous findings indicating bats often use protected concrete cracks (typically expansion joints) with widths roughly the dimensions of the bat species (0.5 to 1.25 inches, 1.27 to 3.18 cm) and located at least 10 feet (3.05 m) above the ground (Davis and Cockrum 1963, Hirshfeld et al. 1977, Fraze and Wilkins 1990, Keeley and Tuttle 1999, Adam and Hayes 2000, Lance et al. 2001, McDonnell 2001, Trousdale and Beckett 2002, Diamond and Diamond 2003, Feldhamer et al. 2003, Keeley and Keeley 2004, Ferrara and Leberg 2005a; b, Celuch and Sevcik 2008, Trousdale 2008, BCI 2011, Ferrarini 2012, Amorim et al. 2013, Martinez et al. 2015, Sasse 2016, Sparks et al. 2019a).

The first modeling objective was to identify bridges that are unlikely to contain bats with the intent that ODOT could either eliminate these bridges from consideration or conduct preconstruction surveys immediately prior to construction. The final step-down model identifies 1,656 bridges with the lowest likelihood of being used by bats out of a total of 44,403 unsampled ODOT bridges. There is little risk bats will use these bridges as built, but structural damage (spalling) or the addition of artificial roosts such as signs could provide roosting habitat not described by the variables used in the step-down model (see Appendix E). Lowest suitability bridges are found in all 12 ODOT districts except Districts 8 and 10. Additionally, there are 14,577 bridges with low likelihood of bat use which can be flagged for low priority examination. Low suitability bridges are found in all ODOT districts. Surveys of low suitability bridges can be completed by staff of limited technical skills. A list of all 44,403 unsampled bridges with suitability ranking and associated structural, design, and environmental features are provided as Supplementary Materials A.

The second modeling objective is to provide ODOT with an early warning system so bridges likely to contain bats that can be prioritized for early survey. The final step-down model identifies 1,663 unsampled ODOT bridges that rank highest in predictive likelihood for bat use. These bridges should be given highest priority for additional inspections for bat use. Highest suitability bridges are in ODOT Districts 3, 4, 5, 6, 8, 9, 10, and 12. Additionally, 8,153 bridges have high suitability for bat use and should be inspected well in advance of construction activity. High suitability bridges are found in all ODOT districts except District 2, 7, and 11.

With the aid of the step-down model ranking system ODOT is able to prioritize which bridges receive early, prioritized, or repeated inspections to avoid negatively impacting roosting bats while potentially avoiding project delays prior to bridge construction activities. The ranking system should be implemented with the knowledge that there is always potential for bat activity. However, limited training can provide nontechnical staff the ability to ascertain bat presence or probable absence on all bridges, thus greatly reducing costs associated with assessing bridges for bats. Results also indicate that adding bat habitat to bridges that lack cracks and crevices, but are otherwise ideal, should be considered.

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APPENDIX A
RESUME

ESI

Environmental Solutions & Innovations, Inc.

Real Science, Real Solutions

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QUALIFICATIONS AND EXPERIENCE

Mr. Moore is a Certified Wildlife Biologist® specializing in large, complex projects. For twelve years, he worked throughout Appalachia, the Ozark/Boston Mountain region, and the intermountain west, preforming all aspects of summer and winter bat surveys, migratory studies, and wildlife habitat surveys. Mr. Moore worked for numerous entities, both public and private, including the Ozark-St. Francis National Forest; Sumter National Forest; National Park Service; Arkansas Game and Fish Commission; Arkansas, Oklahoma, and Florida Departments of Transportation; and US Fish and Wildlife Refuges.

Mr. Moore is a recognized bat echolocation acoustic specialist. Following three years of experience, Mr. Moore attended a required six-day BatSense acoustic training course with Titley Scientific in 2014. In total, Mr. Moore conducted acoustic vetting and data management for nearly ten years. He is not only an expert in eastern bat call identification, but has extensive experience with western bats, neotropical bats, and niche species such as the Florida bonneted bat. Mr. Moore managed and vetted calls on acoustic projects from the east coast through the intermountain west, including experience on large-scale federal, national guard, and wind projects in Alabama, Arkansas, Illinois, Indiana, Missouri, Nevada, Montana, Oklahoma, Tennessee, Texas, West Virginia, Utah, and Virginia. Mr. Moore is approved to vet calls in high-threshold experience states such as Oklahoma. Mr. Moore conducts classes on acoustic analysis and assists in beta-testing new releases of Kaleidoscope Pro, and acoustic equipment from two companies. Mr. Moore is also one of the few qualified individuals with experience eliminating false-positive calls from southeastern myotis, often producing calls similar to federally-listed species.

PROJECT EXPERIENCE

Arkansas State University

Arkansas *Principal Investigator*

Indiana bats/bat population surveys in Ozark-St. Francis National Forest in Arkansas. Responsible for mist netting and acoustic surveys, radio-telemetry and aerial tracking of Indiana bats, banding migratory and cave bat species found in Arkansas. Also conducted spatial modeling, made recommendations on habitat and timber stand improvements, and performed agency coordination.

Technical Lead

Arkansas State University — *Co-Principal Investigator*

Aerial foraging studies of 130 foraging gray bats in Arkansas. Developed methods and executed analysis of home range, core-foraging areas, and habitat-use. Study resulted in the publication "Habitat Use of Female Gray Bats Assessed using Aerial Telemetry" in the Journal of Wildlife Management.

Arkansas Game and Fish Commission

Arkansas — *Co-Principal Investigator*

Winter hibernacula and summer maternity counts throughout the state of Arkansas. Project includes monitoring 60-160 caves per year for population counts of all known caves harboring threatened and endangered bats. Population monitoring/WNS surveys conducted on other lower use caves. Conducted acoustic monitoring at hundreds of WMA sites.

Arkansas and Oklahoma Departments of Transportation

Arkansas and Oklahoma — *Technical Lead*

Bat acoustic surveys throughout Arkansas and eastern Oklahoma, including post-processing call vetting on bridges, roads, and culverts.

Various State and Private Clients

Florida — *Technical Lead*

Acoustic analysis on various projects for the Florida bonneted bat in Collier, Charlotte, Lee, and Osceola counties.

West Virginia Department of Highways

West Virginia — *Principal Investigator*

Acoustic and foraging study on Virginia big-eared bats (*Corynorhinus townsendii virginianus*) in northeastern West Virginia.

Private Client

Virginia and West Virginia — *Co-Principal Investigator*

Conducting a three-year continuous monitoring study of landscape effects to bats using 22 acoustic detectors across the Ridge and Valley region, both inside karst features and on the landscape.

Private Clients

Southeast, Midwest, and Intermountain West — *Co-Principal Investigator*

Seasonal and year-long acoustic projects, monitoring bat activity for pre-construction monitoring phases of facility development.

Camp Atterbury

Indiana — *Co-Principal Investigator*

Presence/absence acoustic monitoring for the endangered Indiana bat and threatened northern long-eared bat on a base-wide survey.

Duke Energy

North and South Carolina — *Principal Investigator*

NABat acoustic monitoring for species-presence composition over time. Includes yearly acoustic monitoring stations over a 40-year period.

APPENDIX B
DNA ANALYSIS FROM PISCES MOLLECULAR

NGS Identification of Bat Species in Fecal Material

Test Samples:

Organization:	Environmental Solutions & Innovations
Received From:	Dale Sparks
Received Date:	10/20/20
Number of samples:	18
Type/condition of sample(s):	Fecal material, dry
Comments	

Sample Preparation:

The fecal material in each sample was resuspended in lysis buffer (MoBio solution C1) and total genomic DNA extracted using a Powersoil spin column procedure, per manufacturer's instructions (MoBio Inc., Carlsbad, CA). Six approximately 0.5 cc subsamples were taken from the very large volume sample ("Spring- lg bag") and given individual Pisces sample numbers (157575 – 157580)

Aliquots of the extracted DNA from each sample were run in a procedure to amplify and sequence a region of the bat (*Chiroptera*) Cytochrome Oxidase I gene (CO1) as follows:

Bat COI Sequencing Protocol:

A 202 bp portion of the *Chiroptera* CO1 gene was PCR amplified from each genomic DNA sample using the SFF_145f (5'-GTHACHGCYCAYGCHTTYGTAATAAT-3') and SFF_351r (5'-CTCCWGCRTGDGCWAGRTTTCC-3') primers from Walker *et al.* [1]. Each 40 µL PCR reaction was mixed according to the Promega PCR Master Mix specifications (Promega catalog # M5133, Madison, WI) which included 0.4uM of each primer and 3 µl of genomic DNA. DNA was amplified using the following PCR conditions: initial denaturation at 94°C for 1 minute, followed by 45 cycles of 30 seconds at 94°C, 30 seconds at 60°C, and 30 seconds at 72°C, then a final elongation at 72ºC for 10 minutes. 20µL of this first round PCR amplicon was cleaned using an ExoI/SAP reaction (8.85µL water, 0.023µL ExoI, 0.2275µL SAP was added to each PCR reaction and incubated 37ºC for 30 minutes, then both enzymes inactivated by incubation at 95ºC for 5 minutes.)

A second round of PCR was performed to give each sample a unique 12-nucleotide index sequence. The indexing PCR included Promega Master mix, 0.5uM of each primer and 4 µL of template DNA (cleaned amplicon from the first PCR reaction) and consisted of an initial denaturation of 95°C for 3 minutes followed by 8 cycles of 95°C for 30 sec, 55°C for 30 seconds and 72°C for 30 seconds, then a final elongation at 72ºC for 2 minutes. After Co1-specific and indexing PCR reactions, 5µl of PCR products of each sample were visualized on a 2% agarose gel.

Final indexed amplicons from each sample were cleaned and normalized using SequalPrep Normalization Plates (Life Technologies, Carlsbad, CA) prior to being pooled.

Amplicons are sequenced using an Illumina MiSeq (San Diego, CA) using the v2 300-cycle kit (cat# MS-102-2002)

The following summarizes how the CO1 amplicons were processed via a joint QIIME [2] and UPARSE [3] pipeline similar to that of Andrei et al. [4], with modification. Sequences were demultiplexed by taking advantage of Golay barcodes [5] via QIIME v1.9.1 [2]. The following options were used to output raw unfiltered fastq files for both forward and reverse reads: split_libraries_fastq.py -q 0 --max_bad_run_length 250 --min_per_read_length_fraction 0.0001 --sequence_max_n 250 --store_demultiplexed_fastq… . Primer sequences were trimmed using Cutadapt v1.8.1 [6] in 'paired-end mode' to remove the primers SFF_145f (GTHACHGCYCAYGCHTTYGTAATAAT) and SFF_351r (CTCCWGCRTGDGCWAGRTTTCC). Trimmed paired-ends where then merged by the –fastq_mergepairs option of usearch v8 [7]. From here, the general quality filtering and ESV (Exact Sequence Variant, [8]) construction was completed as per the UPARSE pipeline [3], with the following modifications: ESVs were generated by clustering the reads at 99% sequence similarity, and the ESV table was generated by mapping quality filtered reads back to the ESV seeds by setting the following parameters: -maxaccepts 128 -maxrejects 1024. These parameters help to ensure that individual reads are correctly mapped to their respective ESVs.

Taxonomy was assigned by recording the top BLAST [9,10] hit for any sequence in which the query coverage and identity exceeded 80% and 95% respectively. Any ESVs with taxonomy assignments not meeting these criteria were removed from the ESV table.

Individual sample results are shown in the attached spreadsheet file.

References:

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APPENDIX C
CHARACTERISTICS OF 504 OHIO DEPARTMENT OF TRANSPORTATION BRIDGES SURVEYED FOR EVIDENCE OF BATS IN 2020

Characteristics of 504 ODOT bridges surveyed for evidence of bats in 2020.

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present: Common Name	Bat Species Present: Scientific Name
100641	29 June	222	high			
104183	29 June	231	high			
200395	20 August	112				
200425	20 August	112				
230138	23 July	231				
232246	21 July	171	low			
232440	18 July	231				
232572	21 July	171	low			
236950	18 July	34A				
238996	21 July	34A				
239097	17 July	34A				
239143	18 July	34A				
241156	23 July	34A				
241431	21 July	553	low			
241725	23 July	553				
241792	23 July	231				
245518	21 July	231				
245623	21 July	34A				
247952	21 July	231				
249580	18 July	34A				
251550	23 July	34A				
301028	22 August	231	alternate high			
301078	19 August	195				
305464	22 August	322				

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present	
					Common Name	Scientific Name
305855	22 August	112				
306061	22 August	112				
330809	20 August	231	alternate high			
331287	24 August	231				
331449	23 August	231				
332402	22 August	231				
332739	19 August	364				
332860	19 August	321				
333018	19 August	321				
333050	19 August	231				
333077	23 August	232				
360002	25 July	364				
360171	25 July	364				
360724	25 July	553				
400173	23 July	231	alternate high			
431591	23 July	231	alternate high			
431648	23 July	231	alternate high			
433152	23 July	231	alternate high	bats, staining, guano	big brown bat	*Eptesicus fuscus*
433942	23 July	222	alternate high			
500631	8 July	322				
500666	8 July	322				
502693	8 July	232	high	bats, guano	big brown bat	*Eptesicus fuscus*
504963	8 July	322				
530001	7 July	231	high			
534536	7 July	231	high			

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present: Common Name	Bat Species Present: Scientific Name
534641	7 July	111				
535095	8 July	231	high			
542946	7 July	231	high			
544906	8 July	231	high			
600857	25 July	231				
631191	18 July	231				
631353	18 July	231				
634484	25 July	34A				
634727	25 July	34A				
636967	25 July	131				
660001	25 July	231				
661104	22 July	344	low			
661481	22 July	344	low			
661988	22 July	344	low			
700215	11 July	221	high			
700223	11 July	221	high			
700290	11 July	221	high			
733202	11 July	364	moderate			
734152	11 July	232	high			
803901	29 June	411	moderate			
1000640	25 August	321				
1000691	25 August	112				
1001477	12 July	231	high			
1030590	25 August	321				
1031589	12 July	364				

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present: Common Name	Bat Species Present: Scientific Name
1133063	18 August	231				
1160109	18 August	364	low			
1250213	25 June	364	low			
1255843	25 June	111	low			
1300180	24 June	221	alternate high	guano, staining	big brown bat[1]	*Eptesicus fuscus*
1301918	28 June	221	high	bats, staining, guano	big brown bat	*Eptesicus fuscus*
1301969	28 June	221	high	bats, staining, guano	big brown bat	*Eptesicus fuscus*
1304798	1 July	231	alternate high			
1330373	28 June	171	moderate			
1331841	24 June	111	moderate			
1332341	29 June	231	high			
1333135	24 June	221	alternate high	guano, staining	big brown bat[1]	*Eptesicus fuscus*
1333240	1 July	231	alternate high			
1333259	24 June	231	alternate high			
1333968	24 June	231	alternate high			
1359339	24 June	231	alternate high			
1403524	18 August	231		bats, staining, guano	big brown bat	*Eptesicus fuscus*
1430114	2 July	231	alternate high			
1430351	2 July	231	alternate high			
1431412	2 July	231	alternate high			
1443224	18 August	231				
1446738	18 August	231				
1503731	12 July	222	high			
1533517	12 July	231	alternate high			
1537601	12 July	231	high			

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present: Common Name	Scientific Name
1538322	12 July	231	alternate high			
1602578	20 August	231		bats, guano	big brown bat	*Eptesicus fuscus*
1602934	20 August	322				
1633538	20 August	232	alternate high			
1634844	20 August	232	alternate high	bats, guano	big brown bat	*Eptesicus fuscus*
1741519	22 August	231				
1800256	13 July	231	alternate high			
1801929	29 August	153				
1807455	30 August	232				
1830058	30 August	121				
1830643	30 August	120				
1831623	30 August	172				
1831674	29 August	232				
1832549	30 August	221		guano, staining	big brown bat[1]	*Eptesicus fuscus*
1832662	26 August	171		bats	big brown bat	*Eptesicus fuscus*
1832719	29 August	112				
1832913	13 July	232	alternate high			
1832980	13 July	232	alternate high			
1833677	29 August	153				
1833898	29 August	232				
1834770	30 August	221				
1860003	30 August	221				
1890182	30 August	231				
1890573	30 August	231				
1931431	27 June	231	moderate			

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present	
					Common Name	Scientific Name
1932713	17 August	553				
1932888	27 June	231	moderate			
1954970	22 July	121				
1955020	22 July	231				
1959581	22 July	231				
2045400	18 July	131	moderate	bats, guano	big brown bat	*Eptesicus fuscus*
2045737	18 July	171	moderate			
2045761	20 August	171	low			
2200635	29 June	112	moderate			
2200694	29 June	112	moderate			
2200724	29 June	322	moderate			
2201593	21 August	364	low			
2201623	21 August	363				
2203146	21 August	322		guano	big brown bat[1]	*Eptesicus fuscus*
2230216	21 August	112				
2230313	29 June	231				
2230615	21 August	231				
2231476	21 August	231				
2231654	21 August	231				
2231700	29 June	231	moderate			
2231867	28 June	171				
2231948	21 August	231				
2232154	21 August	221				
2300419	24 August	222		bats	big brown bat	*Eptesicus fuscus*
2330814	6 July	232	high			

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present: Common Name	Bat Species Present: Scientific Name
2331543	23 August	232	alternate high			
2516810	24 August	231				
2530112	17 July	231	moderate	bats, guano	big brown bat	*Eptesicus fuscus*
2531496	17 July	221	moderate			
2531682	17 July	122	moderate			
2534428	17 July	231	moderate			
2535289	25 August	322		bats, guano	big brown bat	*Eptesicus fuscus*
2535297	25 August	321				
2560127	24 August	553	low			
2562456	24 August	111	low			
2600439	20 August	322				
2800799	23 July	231	alternate high			
2803399	13 July	221	alternate high			
2803461	22 July	231				
2803496	22 July	231	alternate high			
2830299	13 July	232	alternate high			
2832224	23 July	232	alternate high			
2832704	23 July	232	alternate high			
2930331	18 August	231		bats, staining, guano	big brown bat[1]	*Eptesicus fuscus*
2931230	18 August	231				
2933063	18 August	231				
2935694	18 August	221				
2935716	18 August	221				
2938995	25 June	171	low			
3000230	14 July	231	alternate high			

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present: Common Name	Bat Species Present: Scientific Name
3002233	24 August	112				
3002292	24 August	112				
3003302	24 August	395				
3004023	24 August	221				
3005070	24 August	322				
3005682	24 August	112				
3030113	24 August	231				
3030709	24 August	364				
3032140	24 August	322				
3032205	24 August	231				
3032523	24 August	112				
3032915	24 August	231		guano	big brown bat[1]	*Eptesicus fuscus*
3033945	24 August	321				
3034828	14 July	232	alternate high			
3102521	26 June	344				
3102548	26 June	322				
3130000	26 June	222	alternate high	guano, staining	unidentified	
3131661	26 June	111				
3131696	26 June	122	alternate high			
3131815	26 June	231	alternate high			
3133699	24 June	231	alternate high			
3137139	23 June	322				
3165000	24 June	344	low			
3199905	24 June	344	low			
3199982	24 June	344	low			

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present	
					Common Name	Scientific Name
3201686	22 August	222				
3204812	18 July	231	moderate			
3230001	22 August	232				
3236897	18 July	231	moderate			
3260178	18 July	231	moderate			
3260180	18 July	171	low			
3260322	22 August	222				
3262219	22 August	232				
3334805	20 August	121				
3400611	12 July	231	alternate high			
3401588	21 August	111				
3430731	12 July	231	high			
3600521	29 June	232	high			
3700496	6 July	222	high	staining, guano	big brown bat[1]	*Eptesicus fuscus*
3700518	6 July	222	high	staining, guano	big brown bat[1]	*Eptesicus fuscus*
3701263	11 July	231	high			
3701336	10 July	171	moderate			
3701409	10 July	111				
3701662	8 July	121	high	guano	big brown bat[1]	*Eptesicus fuscus*
3730204	6 July	231	high			
3731731	10 July	231	alternate high			
3733181	10 July	231	high			
3830500	14 July	321				
3931412	22 August	231		bats, staining, guano	big brown bat	*Eptesicus fuscus*
3932478	21 August	231				

					Bat Species Present	
Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Common Name	Scientific Name
3938565	22 August	231				
4002237	1 July	322	moderate			
4002245	1 July	221	moderate			
4002253	1 July	221	moderate			
4002326	1 July	232	high			
4003543	1 July	112	moderate			
4030214	1 July	34A	moderate			
4032349	1 July	321	moderate			
4032357	1 July	321	moderate			
4032381	1 July	321	moderate			
4033272	1 July	321	moderate			
4033957	9 July	221	high	bats, staining, guano	big brown bat	*Eptesicus fuscus*
4037448	1 July	171	moderate			
4060172	1 July	112	moderate			
4062965	1 July	121	moderate			
4163338	12 July	231	high			
4203119	14 July	231	alternate high			
4230086	23 August	222		guano	big brown bat[1]	*Eptesicus fuscus*
4233476	14 July	231	high			
4235347	14 July	232	alternate high			
4235401	14 July	232	high			
4236327	14 July	211				
4237439	24 August	321				
4303326	23 July	232	alternate high			
4433459	9 July	232	high			

					Bat Species Present	
Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Common Name	Scientific Name
4500032	26 August	322				
4500423	26 August	231	alternate high	guano	big brown bat[1]	*Eptesicus fuscus*
4502183	23 August	231	alternate high			
4505034	26 August	231		guano	unidentified	
4505042	26 August	231				
4505085	26 August	112				
4530011	24 August, 26 August	131		bats	northern long-eared bat	*Myotis septentrionalis*
4530025	24 August	231				
4531078	25 August	321				
4536607	24 August	231				
4536649	24 August	231				
4536843	26 August	321				
4537475	26 August	321				
4537602	26 August	321				
4538854	26 August	321				
4540182	26 August	195				
4806565	20 August	231	alternate high			
4861884	21 August	153	low			
4861892	21 August	153	low			
4931351	25 August	322				
4931432	25 June	553	low			
4932501	24 August	153	low			
5001544	22 July	231	alternate high			
5048265	22 July	231	alternate high			
5050065	14 July	231	alternate high			

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present	
					Common Name	Scientific Name
5102758	16 July	231	moderate			
5136504	16 July	231	moderate			
5137993	16 July	231	moderate			
5138191	16 July	231	moderate			
5400554	25 July	231				
5400589	25 July	231				
5442540	22 July	121				
5444187	22 July	121				
5454492	22 July	231				
5501563	27 June	232	moderate			
5530393	28 June	222	moderate			
5533481	27 June	221	moderate			
5533759	19 August	131				
5536308	19 August	364	low			
5537371	19 August	171	low			
5603137	11 July	231	high			
5734150	18 August	231				
5765315	18 August	231				
5901685	23 August	322				
5901715	23 August	322				
5933188	23 August	344	low			
5933706	23 August	344				
6001653	23 August	231				
6004105	23 August	231	alternate high	bats, guano	big brown bat	*Eptesicus fuscus*
6004849	24 August	231				

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present	
					Common Name	Scientific Name
6004873	24 August	112				
6004962	23 August	231	alternate high	bats	big brown bat	*Eptesicus fuscus*
6006469	23 August	111				
6031374	24 August	321				
6032095	23 August	321				
6032281	23 August	34A				
6032370	24 August	321				
6032516	23 August	322				
6032613	23 August	34A				
6036155	14 July	222	high			
6036619	14 July	232	high			
6037526	23 August	322				
6037747	14 July	111	moderate			
6037771	23 August	164				
6048110	23 August	321				
6201644	21 August	300	low			
6330800	20 August	231		bats, staining, guano	big brown bat[1]	*Eptesicus fuscus*
6335217	20 August	364	low			
6400817	23 August	112				
6400949	23 August	231				
6402321	23 August	231	alternate high			
6403212	23 August	231				
6403271	23 August	111				
6540023	28 August	321				
6600964	29 August	322				

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present	
					Common Name	Scientific Name
6600999	29 August	322				
6602347	29 August	322				
6603173	29 August	231	alternate high			
6632602	30 June	231	high			
6632807	30 June	231	high			
6632963	30 June	231	alternate high			
6633617	29 August	321				
6634354	30 June	231	high			
6634575	29 August	231	alternate high	staining, guano	big brown bat[1]	*Eptesicus fuscus*
6635164	30 June	221	alternate high			
6700160	24 August	322				
6703402	19 July	231	alternate high			
6703879	25 August	112				
6704174	24 August	112				
6704573	19 July	231	alternate high			
6730256	24 August	321				
6730582	24 August	321				
6730868	24 August	322				
6731279	24 August	221				
6732534	24 August	112				
6733492	24 August	231				
6733662	19 July	231	alternate high			
6734006	24 August	111				
6734022	19 July	231	alternate high			
6734650	24 August	112				

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present	
					Common Name	Scientific Name
6735304	24 August	112				
6735569	25 August	171				
6736734	24 August	221		guano	unidentified	
6738575	14 July	231	alternate high			
6741096	24 August	321				
6802591	17 August	221		guano	big brown bat[1]	*Eptesicus fuscus*
6833438	27 June	131	moderate			
6834116	17 August	231		guano	big brown bat[1]	*Eptesicus fuscus*
6835686	17 August	231				
6835759	17 August	231				
6835945	17 August	131				
6841643	17 August	231				
6841686	17 August	231				
6933386	18 July	231	moderate			
7000243	24 July	344				
7001479	19 August	322				
7001657	22 August	322				
7001681	22 August	322				
7002165	25 July	553				
7002432	24 July	231	alternate high	bats, staining, guano	big brown bat	*Eptesicus fuscus*
7002653	24 July	232	alternate high			
7003005	19 August	195				
7003056	19 August	195				
7003072	25 July	232	alternate high	bats, staining, guano	Indiana bat, little brown bat[1]	*Myotis sodalis, Myotis lucifugus*
7003919	24 July	221	alternate high			

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present	
					Common Name	Scientific Name
7004834	22 August	322				
7004869	22 August	322				
7004990	24 July	232	alternate high			
7005075	23 August	121				
7006063	25 July	364				
7006152	25 July	553				
7006586	22 August	231				
7030000	24 July	34A				
7030738	22 August	111				
7032080	19 August	111				
7032439	22 August	322				
7032692	19 August	121				
7032803	19 August	111				
7032978	22 August	353				
7033206	19 August	195				
7033931	19 August	231				
7034032	24 July	231	alternate high			
7034628	19 August	231	alternate high			
7035365	22 August	232				
7035489	19 August	171				
7035616	19 August	195				
7060424	25 July	364				
7062575	25 July	553				
7066007	24 July	232	alternate high			
7100035	10 July	232	high			

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present	
					Common Name	Scientific Name
7102755	27 August	322				
7103077	27 August	232				
7103115	27 August	112				
7103182	27 August	112				
7103247	27 August	112				
7103573	27 August	322				
7105657	27 August	322		bats, staining, guano	big brown bat	*Eptesicus fuscus*
7140002	27 August	232	alternate high			
7141548	27 August	322				
7300557	30 June	232	high			
7303815	29 August	121	alternate high			
7303874	29 August	111				
7305796	29 August	322				
7326025	30 June	411	moderate			
7326068	30 June	412	moderate			
7326106	30 June	472	moderate			
7337973	29 August	231				
7338376	29 August	321				
7338740	29 August	121				
7339429	29 August	231				
7339445	29 August	231	alternate high	bats, guano	big brown bat	*Eptesicus fuscus*
7401086	22 August	553	low			
7403011	22 August	231				
7403119	22 August	231				
7403763	22 August	195				

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present: Common Name	Bat Species Present: Scientific Name
7431880	22 August	171	low			
7439288	22 August	411	low			
7445598	22 August	411	low			
7448694	22 August	153	low			
7530625	19 August	171	low			
7562039	19 August	321				
7600100	27 August	222		guano	big brown bat[1]	*Eptesicus fuscus*
7605536	25 August	112				
7605749	22 July	231	alternate high			
7605919	27 August	322				
7605943	25 August	112				
7606478	25 August	231				
7630581	25 August	321				
7633432	25 August	111				
7635621	25 August	231				
7635788	25 August	231				
7635796	27 August	322				
7636008	25 August	231				
7707037	13 July	231	alternate high			
7707509	28 August	112				
7707568	28 August	112				
7709935	26 August	322				
7730268	28 August	231				
7730411	26 August	322				
7730446	26 August	322				

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present: Common Name	Bat Species Present: Scientific Name
7730454	26 August	222				
7730462	27 July	221				
7730500	26 August	222				
7733194	29 August	322				
7734050	28 August	232				
7734069	28 August	112				
7738064	28 August	112				
7738137	28 August	112				
7738404	28 August	321				
7745060	26 August	322				
7745087	26 August	231		bats, guano	big brown bat	*Eptesicus fuscus*
7745206	13 July	232	high			
7745249	26 August	232				
7751001	26 August	231	alternate high			
7759339	24 August	112				
7762356	27 July	344	low			
7762380	27 July	344	low			
7762542	27 July	360	low			
7802234	22 July	231	alternate high			
7804571	14 July	231	alternate high			
7805713	22 July	232	alternate high			
7807678	22 July	231	alternate high			
7849206	22 July	232	alternate high			
7901348	27 August	322				
7904142	27 August	322				

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present	
					Common Name	Scientific Name
7905785	21 August	322				
7930267	21 August	34A				
7931352	21 August	322				
7934807	21 August	231				
7936451	20 August	231	alternate high			
7937369	27 August	121				
7961324	12 July	231	alternate high			
8030480	24 August	411	low			
8138257	23 July	171	low			
8140278	23 July	231				
8140413	23 July	231				
8140472	23 July	121				
8140553	23 July	231				
8200181	9 July	231	high			
8200645	8 July	231	high	bats, staining, guano	big brown bat	*Eptesicus fuscus*
8231079	9 July	232	high			
8231249	9 July	232	high			
8231397	9 July	232	high			
8332037	30 August	231		bats, staining	big brown bat	*Eptesicus fuscus*
8333475	30 August	231	alternate high	bats, guano	big brown bat	*Eptesicus fuscus*
8334617	30 August	232	alternate high	bats, guano	big brown bat[1]	*Eptesicus fuscus*
8404615	7 July	231	high			
8433119	7 July	231	high			
8435731	7 July	171	moderate			
8439303	11 July	231	high	bats, staining, guano	big brown bat	*Eptesicus fuscus*

Structure File Number	Survey Date (2020)	Main Structure Type	Initial Targeted Bridge Quality Classification	Evidence of Bat Use	Bat Species Present	
					Common Name	Scientific Name
8603219	20 August	322				
8632731	20 August	321				
8739900	22 August	34A				
8802769	22 August	322				
8834687	22 August	171	low			

[1] Species identity confirmed through DNA analysis of guano pellets.

APPENDIX D
ODOT RESEARCH PROJECT FACT SHEET: SEASONAL USE OF OHIO DOT BRIDGES BY BATS

ESI

Ohio Department of Transportation Research Project Fact Sheet

Seasonal Use of Ohio DOT Bridges by Bats

Researcher(s)	Environmental Solutions & Innovations, Inc., and Lochmueller Group, Inc.
Agency	ODOT's Office of Environmental Services
Report Date	15 July 2021
Project Number	109465

The Problem

Ohio is home to the federally endangered Indiana bat and the federally threatened northern long-eared bat. Both species along with little brown and tricolored bats are listed as endangered by the Ohio Department of Natural Resources (ODNR). All bat species in Ohio are protected by law. Bats, including endangered species, occasionally roost in bridges. Thus, in compliance with state and federal law, the Ohio Department of Transportation (ODOT) is required to inspect bridge structures for bat presence prior to repair, maintenance, or removal. Although most bridge inspections do not yield bats or evidence of bat use, finding evidence of bat use late in the planning process can lead to increased project timelines and costs, and failure to detect bats can also lead to negative impacts on the species.

Northern long-eared bat roosting in a crack surrounded by insulation.

Project objectives included producing a mathematical tool to identify bridges most likely to contain bats and a GIS mapping tool illustrating relative suitability. Tools facilitate ODOT's focus on surveying bridges with a realistic chance of being used by bats and allow ODOT to manage and protect bats using bridges in sync with ODNR and the U.S. Fish and Wildlife Service (USFWS) objectives.

Research Approach

- Literature Review: One hundred twenty available publications regarding bat use of bridges and culverts were reviewed and used to generate a list of characteristics shared by bridges used by bats. Characteristics were compared to 21 bridges and culverts where ODOT previously documented bat presence.
- Designing the Step Down Model: Characteristics optimal for predicting bat presence were used to build a statistical model for ranking suitability for bat use for all ODOT bridges. To test the model, a sampling scheme was developed for at least 300 bridges based on ranked suitability, ranging from very likely to very unlikely. As time permitted, additional bridges were added to the study.

Little brown and Indiana bats under a Box Beam Bridge.

- Field Testing the Step Down Model: 504 bridges throughout Ohio were visited during summer 2020 and a search for bats or their sign was completed at each bridge. Visual inspection, unmanned aerial vehicles (drones), binoculars, and pole and snake cameras were used to sample bridges. Guano samples were collected and submitted for DNA analysis when direct species identification was not possible. Statistical analysis and the modeling processes described above were repeated based on 2020 field study findings to identify important characteristics shared by bridges used by bats.

To access copies of the final report, visit: www.dot.state.oh.us/research

This research was sponsored by the Ohio Department of Transportation and the Federal Highway Administration.

Ohio Department of Transportation Research Project Fact Sheet

Findings

Evidence of bats was found at 46 of 504 bridges surveyed in 2020. Bats were exclusively observed using beam and box beam bridge designs. Bats were directly observed at 25 bridges, with big brown bat (a common species) observed at 23 sites. One bridge contained a northern long-eared bat and a second bridge contained both Indiana and little brown bats. Thus, 2020 field studies more than tripled the number of ODOT bridges known to contain bats.

Bats typically avoid Slab Bridges like this. Adding bat boxes would make this bridge ideal bat habitat.

Statistical analyses revealed bats in Ohio select many of the same features as bats in other states. Large bridges made of concrete or prestressed concrete providing insulated cracks (such as expansion joints) approximately one inch wide are extensively used. Under ideal conditions, cracks are positioned at least eight feet above water and on a bridge with at least 37 acres of high-quality foraging habitat (such as forests and wetlands) within 0.25 mile. Bats avoid bridges that flood or are regularly disturbed by people.

Big brown bats in a Box Beam Bridge. Note staining on concrete and insulation.

Big brown bat resting beside mud dauber nests under an I-beam Bridge.

Recommendations

Focus early sampling efforts on large box beam and beam bridges crossing streams in rural areas and immediately surrounded by forests or wetlands. Bridges fitting these criteria are most likely to contain bats, and thus most likely to impact projects. Early detections afford ODOT time to coordinate with partner agencies such as ODNR and USFWS. Bridges of other designs are unlikely to contain bats. Limited training affords nontechnical staff the ability to ascertain bat presence or probable absence, thus greatly reducing costs associated with assessing bridges for bats. In some cases, consider adding bat habitat to bridges lacking cracks and crevices, but otherwise exhibiting ideal bat use conditions.

Typical images of ODOT bridges used by bats. A box beam bridge is shown on the left; the right is an I-beam bridge; both cross streams and abundant habitat exists within the immediate area. NOTE: the added colors show places where bats (red), staining (yellow), and guano (blue) were observed.

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This research was sponsored by the Ohio Department of Transportation and the Federal Highway Administration.

APPENDIX E
BRIDGE STRUCTURE AND HABITAT SUMMARY

Bridge Structure and Habitat

Upon approach, take note of the bridge's structural components and surrounding habitat. In Ohio, bats prefer large concrete bridges at least eight feet above water and surrounded by forest or wetlands. Box beam and beam-style bridges are more often selected for use by bats particularly when bridge surroundings comprise forests or wetlands. Bridges that mimic natural habitats such as rock crevices, caves, and large hollow trees are preferred. Typical bridge designs used by bats are shown in Photos 1 and 2; colors show places where bats (red), staining (yellow), and guano (blue) were observed. Bridges that flood frequently (evinced by debris within the superstructure, Photo 3), bridges lacking protected habitat (particularly slab-style bridges), small bridges, and bridges without concrete are rarely used. Be attentive to structural damage (cracks or spaulding, Photo 4) or add-ons such as signs, cliff swallow nests, or bat boxes creating habitat on an otherwise unsuitable bridge.


1. Box Beam


2. I-Beam


3. Debris


4. Spaulding


5. Guano


6. Crevice

Use all Senses to Detect Bats or their Sign

While approaching the bridge, look for hanging bats, staining along the sides, or guano (Photo 5; bat droppings, resembling mouse droppings with shiny pieces of insect) hanging along the sides or underneath the bridge. Focus initial searches under the bridge on the closest abutment. From there, visually search the bridge in manageable pieces with additional scrutiny on any crack or crevice approximately one inch in width (Photo 6). Spotlights (400+ lumens), pole-mounted cameras, or unmanned aerial vehicles (drones) allow examination of otherwise inaccessible places. When checking cracks, try to get right under the crack and remember bats commonly roost in among debris such as wasp nests (Photo 7) and insulation (Photo 8). Closely examine areas where beams connect to abutments or the road deck as staining or guano are retained despite the absence of bats. Look on the ground for guano (Photo 5), potentially occurring as large piles or sprinkled like pepper across the ground or rip rap. Other than sight use your ears and nose, bats often chatter during the day and large concentrations of bats have a distinct, musty odor.


7. Wasp Nest

If Bats are Found

Be as quiet as possible during surveys to avoid atypical noises and disturbing bats. Disturbances can cause bats to emerge during daylight making them susceptible to daytime predation. If roosting bats are found, quietly move away and notify the Ohio Department of Transportation including bridge identification number, date, and observations.

8. Insulation