ACOUSTIC SURVEY FOR RARE BAT SPECIES & ESTABLISHING A REMOTE ACOUSTIC BAT MONITORING KIOSK FOR PUBLIC OUTREACH AND EDUCATION (FINAL REPORT 2018-2020)

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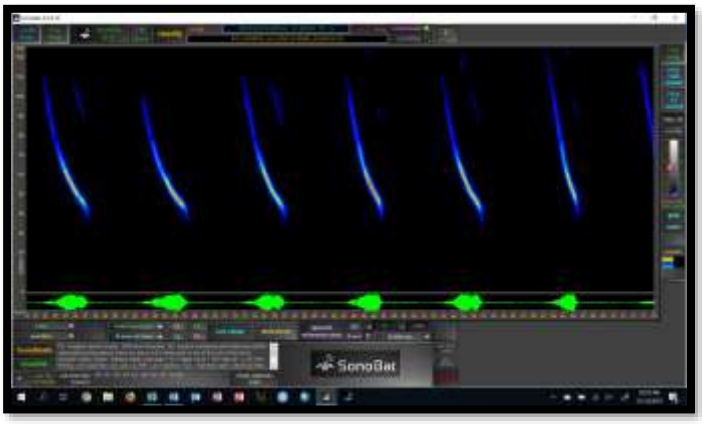
BAT CONSERVATION & MANAGEMENT



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SUMMARY ABSTRACT

Bats provide ecosystem services to human societies such as being indicators of ecosystem health and providing control over problem insect species. In the state of Pennsylvania, there are 7 species of bats that are of conservation concern, with 3 state listed as endangered and 2 species listed under the Endangered Species Act. The goal of this project was to use remote acoustic recording devices to record bat vocalizations in 6 natural areas along the Kittatinny Ridge of Pennsylvania: Cowans Gap State Park, Swatara State Park, Boyd Big Tree Preserve, Hawk Mountain Sanctuary, Lehigh Gap Nature Center and Jacobsburg Environmental Education Center. Each natural area was surveyed for bat activity using remote acoustic recording devices for 32-49 nights during the summer of 2018, and spring and summer of 2019 & 2020. Using an established bat kiosk at Hawk Mountain Sanctuary for public outreach and education, 82 nights of surveying was conducted to evaluate bat activity. We detected a total of 10 bat species along the Kittatinny Ridge of Pennsylvania: Big brown bat (*Eptesicus fuscus*), Red bat (*Lasiurus borealis*), Hoary bat (*Aeorestes cinereus*), Silver-haired bat (*Lasionycteris noctivagans*), Evening bat (*Nycticeius humeralis*), Tricolored Bat (*Perimyotis subflavus*), Eastern small-footed bat (*Myotis leibii*), Little brown bat (*Myotis lucifucgus)*, Indiana bat (*Myotis sodalist*) and Northern long-eared bat (*Myotis septentrionalis*). We found that Cowans Gap State Park and Boyd Big Tree Preserve natural area contained the greatest amount of bat activity with high species richness and species diversity. Additionally, these sites were the only two where Indiana bats and Evening bats were detected. Hawk Mountain Sanctuary also contained rare bat species including Small-footed and Northern long-eared bat. We recommend expanding remote acoustic survey efforts to additional sites within each natural area, as well as surveying more natural areas along the Kitattinny Ridge of Pennsylvania. Efforts should be taken to maintain and increase populations for these rare bat species by protecting roosting and feeding sites via forest management and hibernacula protection.

ACKNOWLEDGEMENTS & CONTACT INFORMATION

We thank the following individuals for their help and support during this project: Jeanne Ortiz, Dan Kunkle, Diane Hustic, Heather Rossell, Debee Ordway, Carter Farmer, Nick Szewczak, Laura Goodrich, Mary Linkevich, Todd Bauman, Jack Hill, Chris Houck, Chad Schwartz, Robert Neitz, Jason Baker, Emilee Boyer, Eileen Haines, George Barner and Mary-Therese Grob. We especially want to thank Kenneth Dunkelberger for setting up the remote download capability of the Hawk Mountain Bat Kiosk.

Funding for this project was financed in part by a grant from the Community Conservation Partnerships Program and the Environmental Stewardship Fund under the administration of the Pennsylvania Department of Conservation and Natural Resources, Bureau of Recreation and Conservation

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INTRODUCTION

Bat species benefit human societies and biological systems by being indicators of ecosystem health and providing biological control over problem insect species (Boyles et al. 2011; Kunz et al. 2011). Currently, there are 7 species of bats in Pennsylvania listed as species of conservation concern under the Pennsylvania Wildlife Action Plan: Big brown bat (*Eptesicus fuscus*), Tricolored bat (*Perimyotis subflavus)*, Northern long-eared bat (*Myotis septentrionali)*, Eastern small-footed bat (*Myotis leibii*), Indiana bat (*Myotis sodalis*), Little brown bat (*Myotis lucifucgus)* and Silver-haired bat (*Lasionycteris noctivagans*) (Pennsylvania Wildlife Action Plan 2015). A species of greatest conservation need is defined as a species whose populations are classified as vulnerable to extinct and for which Pennsylvania has a high responsibility for its persistence (Pennsylvania Game Commission 2015).

Ten years ago, the Little brown bat, Tricolored bat and Northern long-eared bat were common and widespread species in Pennsylvania, and the federally endangered Indiana bat was also present in Pennsylvania. In the last decade, populations of these bat species have declined dramatically with the onset and spread of the white-nose fungal disease (*Geomyces destructans*) or white-nose syndrome (WNS) (Turner et al. 2011, USFWS 2015). With the spread of WNS, Indiana bat populations were reduced a further 72% from their already diminished endangered populations (Turner et al. 2011, USFWS 2018). Population sizes for many other bat species were also reduced, some up to 98%, causing regional extinctions of local populations (Frick et al. 2010, Turner et al. 2011). These reductions have caused the Northern long-eared bat populations to be listed as federally threatened (USFWS 2015), and in summer 2019, the Little brown and Tricolored bat were state listed as endangered in Pennsylvania.

The conservation of remnant bat colonies is an increasing priority for research throughout Pennsylvania and North America (Loeb et al. 2015). Remote monitoring efforts to identify bat species presence based on their vocalizations can help reduce cost of labor in determining areas that are of importance to wild bat conservation. A bat's ability to effectively and efficiently catch prey on the wing is due to their ability to echolocate (Arnett et al. 2013). High frequency vocal signals are produced and are reflected back for interpretation when an object obstructs the bat's path, assisting in orientation and obstacle avoidance (Arnett et al. 2013). These signals can be detected by ultrasonic microphones which convert the ultrasounds to audible sounds and stores time-stamped call data on an external drive (Rydell et al. 2017). In recent years, the sophistication of new bat detector technology, as well the commercial availability of call analysis programs, have made acoustic monitoring a viable option for data collection to be used in future bat management decisions (Frick 2013).

Automated species identification software discriminates bat calls from background noise and uses a multi-variate algorithm to compare recorded bat calls to libraries of known bat species and their calls (Russo & Voigt 2016). When possible, a species-level identification is made based on maximum likelihood models (Frick 2013). The ultrasonic frequency at which bats produce their calls is fairly distinct and not used by many other organisms, offering one advantage to the use of automatic detectors for bats (Walters et al. 2013). The ability of a program to identify a bat call down to species is dependent on the quality and robustness of the library from which comparisons are being made (Walters et al. 2013, Brandes 2008, Clement et al. 2014). The

Sonobat (www.sonobat.com) and Kaleidoscope programs (Kaleidoscope Pro Analysis Software | Wildlife Acoustics) used in this study, have a robust library of bat calls to allow for consistent identification of bat audio calls.

Objectives

Much of the protected natural areas in Pennsylvania along the Kittatinny Ridge occur within state forest and state park lands, which contain acres of mature forests that could serve as habitat for many tree-roosting bat species. These areas also contain caves, abandoned stone buildings and old chimney sites that may serve as a rookery or winter sites for other rare bat species. The goals for this project were to identify areas along the Kittatinny Ridge where bat species of greatest conservation concern may still occur. Our first objective was to use remote acoustic recording devices to record bat vocalizations along the Kittatinny Ridge in Pennsylvania. The second objective was to use the Sonobat 4 and Kaleidoscope software to filter, batch and vet bat vocalizations to identify bat species activity. Our third objective was to assess species occurrence, diversity and community similarity of bat species activity between protected areas along the Kittatinny Ridge. Our last objective was to establish a permanent acoustic bat data collection kiosk at Hawk Mountain Sanctuary (HMS) to provide continuous remote collection of bat call data for long-term population monitoring (Mifusd & Vella 2019, Loeb et al. 2015). This kiosk would also provide public outreach and education about bats for visitors to HMS.

METHODS

Five temporary acoustic monitoring sites were placed in natural areas dispersed along the Kittatinny Ridge: Cowans Gap State Park, Swatara State Park, Boyd Big Tree Preserve Conservation Area, Lehigh Gap Nature Center and Jacobsburg Environmental Education Center. A continuous remote bat monitoring station was established at HMS (Figure 1).

Bat Acoustic Set-Up Protocol

Bat surveys were conducted using D500X ultrasound bat recording units (Pettersson Elektronik AB, Uppsala, Sweden) to remotely survey at two locations in each of the 5 natural areas along the Kittatinny Ridge (USFWS 2018) (Figure 1). The Pettersson D500X ultrasound detector has been used in a number of research studies around the world to record bat species in the field (Fernandez et al. 2014, Slough et al. 2014, Cox et al. 2016) (Appendix 1).

Recording devices were placed in areas most suitable for bat auditory detection free from obstruction: (a) forest-canopy openings; (b) near water sources; (c) wooded fence lines adjacent to large openings; (d) blocks of recently logged forest where potential roost trees remain; (e) road and/or stream corridors with open tree canopies; and (f) woodland edges (Britzke et al. 2010) (Figures 2-7). The GPS coordinates for each of these locations are found in the captions for Figures 2-7. The microphones were attached to the top of an extendable metal pole at least 3 meters above ground height and oriented approximately 45˚ toward possible bat flight space (USFWS 2018). This orientation served to increase quality of the recordings and the amount of airspace that was surveyed (USFWS 2018). A tree within a potential bat feeding flyway was selected and the microphone pole was attached to the trunk using zip ties so that it was greater

than three meters off of the ground (Figure 8). The microphone cord was connected to the external Pettersson recording device and stored in a waterproof tackle box tethered to the tree (Figure 8). The Pettersson units were formatted to automatically turn on and begin recording data a half an hour before sunset, until a half an hour after sunrise to record bat foraging activity (Henry 2002; Kunz 1973, USFWS 2018). Each unit was set up and allowed to remotely record for at least eight survey nights before the unit was collected and data downloaded (USFWS 2018). Our permit for conducting research on state parks can be found in Appendix 2.

Settings on the Pettersson detectors were established to increase the likelihood of collecting bat calls that are of sufficient quality for the Sonobat 4 and Kaleidoscope programs and allow identification of recorded bat calls to species. For this project, a recording length of 4 seconds and a sampling frequency of 300 kHz was used. The detector was also set to automatically record and use a high-pass filter setting to reduce abundance of low-quality calls. Also, the detector's trigger sensitivity was set to low to minimize the frequency of recording non-bat noise.

Bat Acoustic Analysis Protocol

In 2018 and 2019, all audio files recorded were downloaded and analyzed using SonoBat auto-classification software (https://sonobat.com/). SonoBat acoustic bat call analysis software has been used in a number of international bat research projects to identify bat species based on auditory recordings (Slough et al. 2014, Adams et al. 2015, Grider et al. 2016). Acoustic files were downloaded and organized based on location and date range of the collection period. The SonoBat data wizard scrubbed, batched, and parsed out files above 20 kHz that had bat tonal features. The files that did not meet this threshold were classified as noise files and deleted. Files that met the required quality parameters were attributed with location metadata, such as site name, project name, habitat, date, and the microphone model for processing. We used the SonoBat4 NE region specific package to cross reference call data for species identification. We batched bat audio files and appended each file with a species-specific identifier code based on the degree of maximum likelihood of species detection. Those files that appeared to have bat tonal features but were recorded at the edge of the microphones range, and therefore not of sufficient quality for identification, were categorized as Lofi and Hifi bat calls (Frick 2013). These files were disregarded from further analysis. We then used Sonobat to vet identified audio bat files into a spreadsheet. Bat species with < 10 identified audio files per study site were manually vetted, a process which involves a researcher looking at the individual audio file to verify species identification. Manual vetting was conducted by Dr. Aaron Haines from Millersville University and John Chenger from Bat Conservation and Management.

In 2020, we analyzed calls using the same protocol above, however, we also analyzed bat calls using the Kaleidoscope Pro program (Wildlife Acoustics 2020). The Kalediscope Program has been used in several research projects to identify bat species by vocalizations (Maine and Boyles 2015, Finch et al. 2020). Within 'Bat Analysis Mode' in Kaleidoscope, we used the Bats of North America 5.2.1 classifier and Pennsylvania as our Region for species identification. We determined species identification based on agreement of bat audio identification between the Sonobat 4.1 and the Kaleidoscope Pro Analysis software programs.

Bat Kiosk

A permanent acoustic bat kiosk was established at Hawk Mountain Sanctuary (HMS). The kiosk consisted of a data computer to operate SonoBatLIVE acoustic monitoring/auto-classification software for bat calls (Figure 9). This computer had a touch screen to allow visitors to HMS to look at bat call data. This kiosk was the first SonoBatLIVE Kiosk to be placed in the eastern United States, with the only other one of its kind being located in Yosemite National Park. The kiosk continuously monitors bat ultrasound calls from sunset to sunrise throughout the year and provides an interactive experience to HMS visitors to view bat calls calls and learn about the natural history of Pennsylvania bats. Bat audio data that was collected for each evening of kiosk operation and was stored on the computer's hard drive to be later downloaded for analysis.

Analysis of Bat Activity Data Based On Acoustic Data Analysis

After all bat data was collected, analyzed and organized, we compared community ecology data of bat activity among all 6 natural areas. Community ecology data included determination of species richness, Shannon and Simpson index of species diversity, Bray community similarity index, detrended correspondence analysis (Hill and Gauch 1980) and a species accumulation curve using the Mao Tau estimate (Colwell et al. 2012). These analyses were done using the 'Vegan' package (Oksanen 2015) in the R statistical programming language. All data analyzed was based on audio call identification, therefore we did not compare actual numbers of individuals, but rather the number of bat calls which served as an index of bat activity recorded at each of the 6 natural areas.

RESULTS & DISCUSSION

The development of user-friendly audio bat call classifiers used in software has allowed the expedient analysis of thousands of bat call files by operators with minimal experience with computer programs, bat call identification or statistical analysis (Rydell et al. 2017). However, this ease of use and efficiency fosters an environment where it is easy to passively accept results without critical evaluation (Rydell et al. 2017). Credulous acceptance of identifications can have severe negative effects on the management decisions and future research derived from erroneous conclusions. Lemen et al. (2015) called on researchers to use caution in the interpretation and use of generated identifications of bats conducted via software analysis of recorded calls. There are a number of suggestions given in the literature for prudent analysis of audio call results, such as the use of multiple software programs per data set, exclusion of calls that were recorded at a distance from the microphone (Frick 2013), manual vetting of uncommon species (Russo & Voigt 2016) and the use of algorithms that select and analyze specific call parameters (Clement et al. 2014). For our study, bat audio recording settings excluded bat calls that were a distance from the microphone based on call volume, we manually vetted uncommon species calls and we used the Sonobat 4 program and Kaleidoscope Pro Analysis software program that both use algorithms to detect specific bat call parameters for species identification.

Bat Kiosk

We were able to successfully establish a kiosk at the HMS Nature Center (Figures 5 & 9). The

microphone attached to this kiosk was able to record bat calls at night, while the SonobatLIVE program on the kiosk hard drive was able to filter and identify these bat calls to species. This was done infrequently during the spring, summer and fall of 2019. Visitors to the Nature Center were then able to access the kiosk to see what bat species were detected the previous evening. In addition, the kiosk provided natural history information on bat species found in Pennsylvania.

Hawk Mountain Sanctuary is a designated Pennsylvania Important Mammal Area (IMA #32), and the kiosk will strengthen HMS's efforts for public education about mammals and address recommendations found in this IMA's Conservation Plan. The HMS kiosk will continue to collect and record bat call data through the year, and this data will continue to be downloaded and analyzed by Millersville University.

Comparison of Bat Activity Between Natural Areas

Each natural area was surveyed for bat activity for 35-49 nights during the summer of 2018 and the spring and summer of 2019 & 2020 (Table 1). With the established bat kiosk, HMS was surveyed for 82 nights during the spring, summer and fall of 2019. We recorded the greatest number of identified bat calls per survey night at Boyd Big Tree Preserve (244), the survey location in this natural area was by far the most active site for bats (Table 1). This was followed by Swatara State Park (70) and Lehigh Gap Nature Center (63). We recorded the lowest number of bat calls per night at HMS (17). This may be due to the fact that calls at HMS were recorded using the Bat Kiosk which was set to be very conservative in identifying bat calls. The kiosk used a Binary Acoustic AR125EXT microphone, which has a more directional response compared to the D500x microphone. This means less airspace was being monitored at HMS. Of the bats that do enter the detector zone at HMS, it was more difficult to obtain longer sequences that are then accepted for ID by SonoBat.

We found that our plots of species accumulation over time reached a plateau or asymptote for each surveyed natural area after 10-22 evenings of surveying (Figures 10). For HMS, an asymptote was reached after 30 evenings (Figure 11). Therefore, we felt comfortable with the estimate of species richness for activity at each of these sites and moved forward with calculations of community comparisons between sites in each natural area. The fact that HMS site required more time to reach an asymptote is probably due to the microphone used for the kiosk, as explained above, and the location of the kiosk microphone being the location of the nature center building, not necessarily a great bat survey location. Most, if not all, sites in this study were selected based on best bat habitat in the study areas. We recorded greatest species richness at Cowans Gap State Park (8) and Boyd Big Tree Preserve (8), followed by HMS (7) (Table 2). Rare species identified at these locations were manually vetted to validate species occurrence (Appendix 3) or by agreement between the Sonobat and Kaleidoscope software.

We found that the diversity of bat activity was greatest at Cowans Gap State Park for both the Shannon and Simpson diversity indices (1.041 and 0.492) followed by Boyd Big Tree Preserve (1.010 and 0.476) (Table 2). Boyd Big Tree Preserve was also the site with the greatest mean Bray community similarity of bat activity (70%), with its number of active species detected being most similar to the other natural areas (Table 2 & 3). Cowans Gap and Boyd Big were unique sites in that they harbored a large number of recorded calls for bat species recorded infrequently at other natural sites. The correspondence analysis plot in Figure 12 shows the

outlier for the number of bat species calls recorded at Cowans Gap State Park for Evening (*Nycticeius humeralis*) and Little brown bat, and Boyd Big Tree Preserve for Silver-haired and Tricolored bats. We also found that Small-footed and Northern long-eared bats were only recorded at HMS while Evening bats were only recorded at Cowans State Park and Boyd Big Tree Preserve (Table 1).

Bat Activity Along the Kittatinny Ridge

Based on auditory surveys of bat calls, we detected call activity for a total of 10 different bat species along the Kitattinny Ridge of PA (Table 1). Since 1987, 11 bat species have been found to reside within the entire Commonwealth of Pennsylvania (Gannon and Bovard 2016). The only species we did not detect included the Seminole bat (*Lasiurus seminolus*) (Figure 1). That said, it appears more survey work needs to be done. Figure 13 outlines our species accumulation curve for the number of bat species detected when we combine all 6 natural areas. This curve did not reach a plateau or asymptote. This suggests that more study sites within additional natural areas should be surveyed in order to produce a more robust estimate of species richness for bat species activity along the Kitattinny Ridge of Pennsylvania. We recommend expanding survey efforts in each natural area to include different habitat sites (e.g., interior forest, forest edge) and identifying other natural areas along the Kittatinny ridge to survey.

Bat Management and Conservation

Because of significant declines in bat populations in Pennsylvania, it has become important to preserve habitat for remaining populations to support their continued survival. Our survey efforts within natural areas along the Kittatiny Ridge helped in identifying areas of bat activity. The data collected by the five temporary monitoring sites and the SonoBatLIVE Kiosk can be used as baseline data for future acoustic monitoring and may identify species recovery along the Kittatiny Ridge. In the meantime, a number of management recommendations should be considered at these sites as outlined in Appendix 4 for Pennsylvania bat species of greatest conservation need.

For roosting bat species of greatest conservation need along the Kittatinny Ridge of Pennsylvania, forest management is of extreme importance. The need to develop a forest matrix which includes important roost trees in mature forests juxtaposed to freshwater resources such as ponds, marshes, stream pools and rivers that provide open flight paths for foraging bats is extremely important (Bearer et al. 2016). Forest management should also work to increase diversity of native tree species, increase structural diversity of snag trees and den trees, increase age-class diversity of forest stages and increase landscape-level diversity to vary the composition of forest and freshwater resources. Bearer et al. (2016) provides a nice review forest management practices for bats by outlining forest harvest and site preparation techniques that offer potential benefits to Pennsylvania bat species.

For bats that overwinter in PA, protection of hibernacula is key. This would include mitigating activities that would continue the spread of the WNS fungus, establishing bat friendly gates in the front of caves or mines and conducting public outreach to the caving (or spelunking) community. This will also involve communicating with private landowners to work with state

agencies to avoid disturbance near hibernacula. Other practices to benefit hibernacula including retaining the openings and structural integrity of abandoned mines and provide drainage for used hibernacula to prevent flooding and mitigate drowning of hibernating bat species (Appendix 4).

CONCLUSION

We recorded bat SGCN at all surveyed natural areas along the Kittatinny Ridge. This emphasizes the importance of these sites for bat conservation. Based on our survey results, we found the Boyd Big Tree Preserve and Cowans Gap State Park contained the greatest amount of bat activity with high species richness and species diversity and a large number of recordings for unique bat species. Both Cowans Gap State Park, Boyd Big Tree Preserve and HMS contained bat species found nowhere else including the Indiana bat, Evening bat, Eastern small-footed bat and Northern long-eared bat. We recommend expanding on our survey efforts to survey more sites within each natural area, as well as surveying more natural areas along the Kitattinny Ridge of Pennsylvania. Our survey findings will be shared with the Pennsylvania Game Commission (PGC) and the Pennsylvania Department of Conservation and Natural Resources (PA DCNR) so that they can visit each natural area and confirm bat species presence. Once populations of these rare bat species have been confirmed, efforts should then be taken to maintain and increase populations by protecting roosting and feeding sites via forest management and hibernacula protection.

LITERATURE CITED

Adams, R. A., Bonaccorso, F. J., & Winkelmann, J. R. (2015). Revised distribution for Otomops martiensseni (Chiroptera: Molossidae) in southern Africa. *Global Ecology and Conservation*, *3*, 707-714.

Arnett, E.B., Hein, C.D., Schirmacher, M.R., Huso, M.M and Szewczak, J.M. (2013). Evaluating the effectiveness of an ultrasonic acoustic deterrent for reducing bat fatalities at wind turbines. *Plos one*, 8(6): 1-11.

Bearer, S., Duchamp, J and Hassinger, J. 2016. Conservation measures for bat habitats. In: The Ecology and Conservation of Pennsylvania Bats in a Time of Crisis, eds. Whidden, H. P., Reeder, D., Butchkoski, C. and Turner, G. Publication of the Pennsylvania Academy of Science, Easton PA.

Boyles, J.G., Cryan, P.M., McCracken, G.F. and Kunz, T.H. (2011) Economic importance of bats in agriculture. *Science*, 332(6025): 41-42.

Brandes, T.S. (2008). Automated sound recording and analysis techniques for bird surveys and conservation. *Bird Conservation International*, 18: 163-173.

Britzke, E.R, B.A. Slack, M.P. Armstrong, and S.C. Loeb. 2010. Effects of orientation and weatherproofing on the detection of bat echolocation calls. *Journal of Fish and Wildlife Management* 1(2):136-141.

Clement, M.J., Murray, K.L., Solick, D.I. and Gruver, J.C. (2014). The effect of call libraries and acoustic filters on the identification of bat ecolocation. *Ecology and Evolution*, 4(17): 3482-3493.

Colwell, R.K., Chao, A., Gotelli, N.J., Lin, S.Y., Mao, C.X., Chazdon, R.L. & Longino, J.T. (2012). Models and estimators linking individual-based and sample-based rarefaction, extrapolation and comparison of assemblages. *J. Plant Ecol.* 5: 3–21.

Cox, M. R., Willcox, E. V., Keyser, P. D., & Vander Yacht, A. L. (2016). Bat response to prescribed fire and overstory thinning in hardwood forest on the Cumberland Plateau, Tennessee. *Forest Ecology and Management*, *359*, 221-231.

Fernandez, A. A., Fasel, N., Knörnschild, M., & Richner, H. (2014). When bats are boxing: aggressive behaviour and communication in male Seba's short-tailed fruit bat. *Animal behaviour*, *98*, 149-156.

Finch, D., Schofield, H. and Mathews, F., (2020). Habitat Associations of Bats in an Agricultural Landscape: Linear Features Versus Open Habitats. *Animals 10*(10): 1856.

Frick, W.F., Pollock, J.F., Hicks, A.C., Langwig, K.E., Reynolds, D.S., Turner, G.G., Butchkoski, C.M. and Kunz, T.H., (2010). An emerging disease causes regional population collapse of a common North American bat species. *Science* 329: 679-682.

Frick, W.F. (2013). Acoustic monitoring of bats, considerations and options for long term monitoring. *Therya*, 4(1): 69-78.

Gannon, M.R., Bovard, B. N. (2016). The value of bats: Keystone species in the keystone state. In: The Ecology and Conservation of Pennsylvania Bats in a Time of Crisis, eds. Whidden, H. P., Reeder, D., Butchkoski, C. and Turner, G. Publication of the Pennsylvania Academy of Science, Easton PA.

Grider, J. F., Larsen, A. L., Homyack, J. A., & Kalcounis-Rueppell, M. C. (2016). Winter activity of coastal plain populations of bat species affected by white-nose syndrome and wind energy facilities. *PloS one*, *11*(11), e0166512.

Henry, M., Thomas, D. W., Vaudry, R., & Carrier, M. (2002). Foraging distances and home range of pregnant and lactating little brown bats (Myotis lucifugus). *Journal of Mammalogy*, 83(3), 767-774.

Hill, M.O. and Gauch, H.G. (1980). Detrended correspondence analysis: an improved ordination technique. *Vegetatio* 42, 47–58.

Kunz, T. H. (1973). Resource utilization: temporal and spatial components of bat activity in central Iowa. *Journal of Mammalogy*, 54(1), 14-32.

Lemen, C., Freeman, P.W., White, J.A. and Anderson, B.R. (2015) The problem of low agreement among automated identification programs for acoustical surveys of bats. *Western North American Naturalist,* 75(2): 218-245.

Loeb, S.C., Rodhouse, T.J., Ellison, L.E., Lausen, C.L., Reichard, J.D., Irvine, K.M., Ingersoll, T.E., Coleman, J.T.H., Thogmartin, W.E., Sauer, J.R., Francis, C.M., Bayless, M.L., Stanley, T.R and Johnson, D.H. (2015). A plan for the North American monitoring program (NABat). *Department of Agriculture Forest Service*, 1-112.

Maine, J.J. and Boyles, J.G., (2015). Bats initiate vital agroecological interactions in corn. *Proceedings of the National Academy of sciences*, 112(40): 12438-12443.

Mifsud, C.M. and Vella, A. (2019). Acoustic characterization of bats from Malta: setting a baseline for monitoring and conservation of bat populations. *Bioacoustics*, 28(5): 427-442.

Oksanen, J. (2015). Multivariate analysis of ecological communities in R: vegan tutorial. http://cc.oulu.fi/~jarioksa/opetus/metodi/vegantutor.pdf

Pennsylvania Game Commission. (*2015-2025) Pennsylvania Wildlife Action Plan*. Chapter 1. Retrieved from https://www.pgc.pa.gov/Wildlife/WildlifeActionPlan/Documents/SWAP-CHAPTER-1.pdf

Russo, D. and Voigt, C.C. (2016). The use of automated ID of bat ecolocation calls in acoustic monitoring: cautionary note for sound analysis. *Ecological Indicators*, 66: 598-602.

Rydell, J., Nyman, S. Eklöf, J., Jones, G. and Russo, D. (2017). Testing the performance of automated ID of bat echolocation calls: A request for prudence. *Ecological Indicators*, 78: 416-420.

Slough, B. G., Jung, T. S., & Lausen, C. L. (2014). Acoustic surveys reveal hoary bat (*Lasiurus cinereus*) and long-legged myotis (*Myotis volans*) in Yukon. *Northwestern Naturalist*, *95*(3), 176-185.

Turner, G., D.M. Reeder, and J.T.H. Coleman. (2011). A five-year assessment of mortality and geographic spread of white-nose syndrome in North American bats and a look to the future. *Bat Research News* 52:13–27.

U.S. Fish and Wildlife Service (USFWS). (1983). Recovery Plan for the Indiana Bat. U.S. Fish and Wildlife Service, Washington, D.C. 80 pp.

U.S. Fish and Wildlife Service (USFWS). (2015). Listing the Northern Long-eared Bat with Rule Under Section 4(d) of the Act. U.S. Fish and Wildlife Service, Washington, D.C. 8 pp.

United States Fish and Wildlife Service (USFWS). 2018. Range-wide Indiana bat survey guidelines. Washington, D.C. 62 pp.

Walters, C.L., Collen, A., Lucas, T., Mroz, K., Sayer, C.A. and Jones, K.E. (2013). Challenges of using bioacoustics to globally monitor bats. In R.A. Adams and S.C. Pederson (eds.), *Bat Molecular Phylogenies: Past, Present and Future Directions.* (pp. 479-499).

Wildlife Acoustics (2020). Kaleidoscope Pro Analysis Software (https://www.wildlifeacoustics.com/products/kaleidoscope-pro)

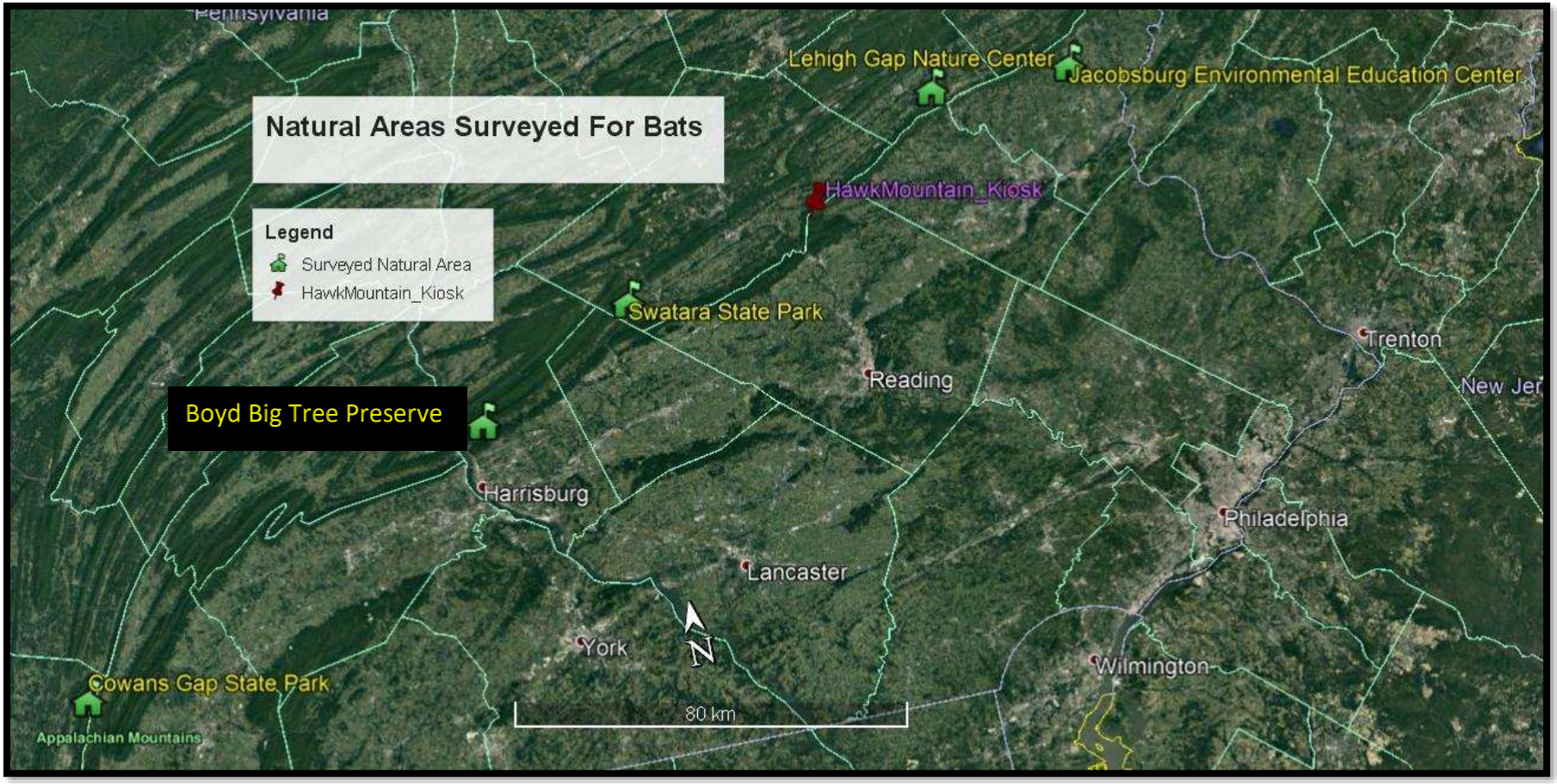


Figure 1. Six natural areas along the Kittatinny Ridge of Pennsylvania that were remotely surveyed for activity of bat species of greatest conservation concern as identified by the Pennsylvania State Wildlife Action Plan (2015). Each area contained a location where a remote bat acoustic detection device was placed during the summer of 2018 and the spring and summer of 2019. A separate location was established in each natural area during the spring and summer of 2020.

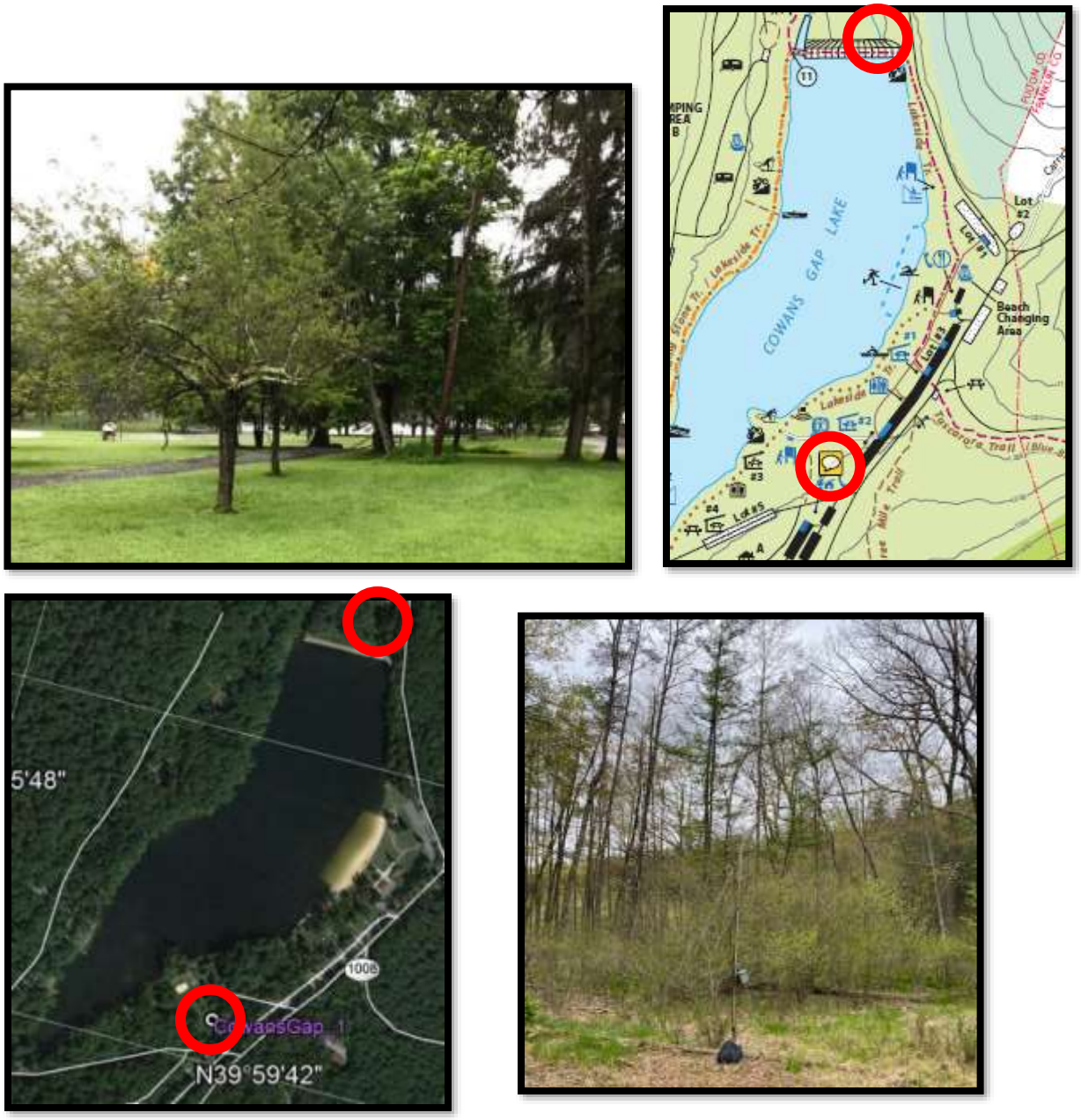


Figure 2. Acoustic bat detector locations at Cowan's GAP state park, indicated with red circles on top right and bottom left. The microphone was facing an open area leading to the Cowans Gap Lake in 2018 and 2019, which can be seen in the background of the top left picture (39°59'44" N; 77°55'28" W). In 2020, the microphone was facing a small creek at the base of the Cowans Gap Dam, as can be seen on the bottom right (39°59'57" N; 77°55'16" W).

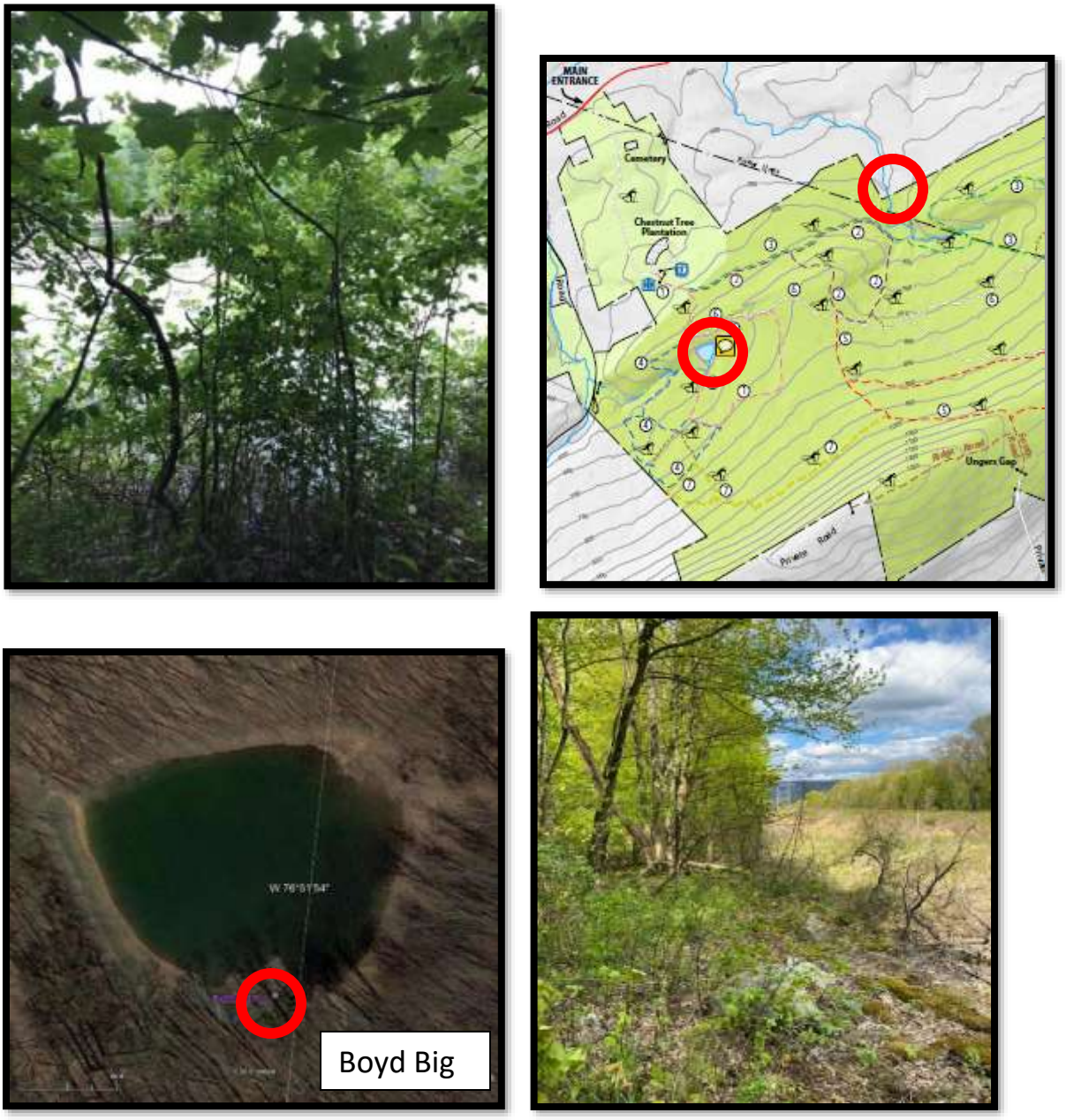


Figure 3. Acoustic bat detector locations at Boyd Big Tree Preserve, indicated with the red circles on the top right. The microphone was facing over a small pond which can be seen in the background of the top and bottom left picture in 2018 and 2019 (40°21'21" N; 76°51'36" W). In 2020, the microphone faced a powerline clearing on the edge of the forest as can be seen in bottom right (40°21'38" N; 76°51'20" W).

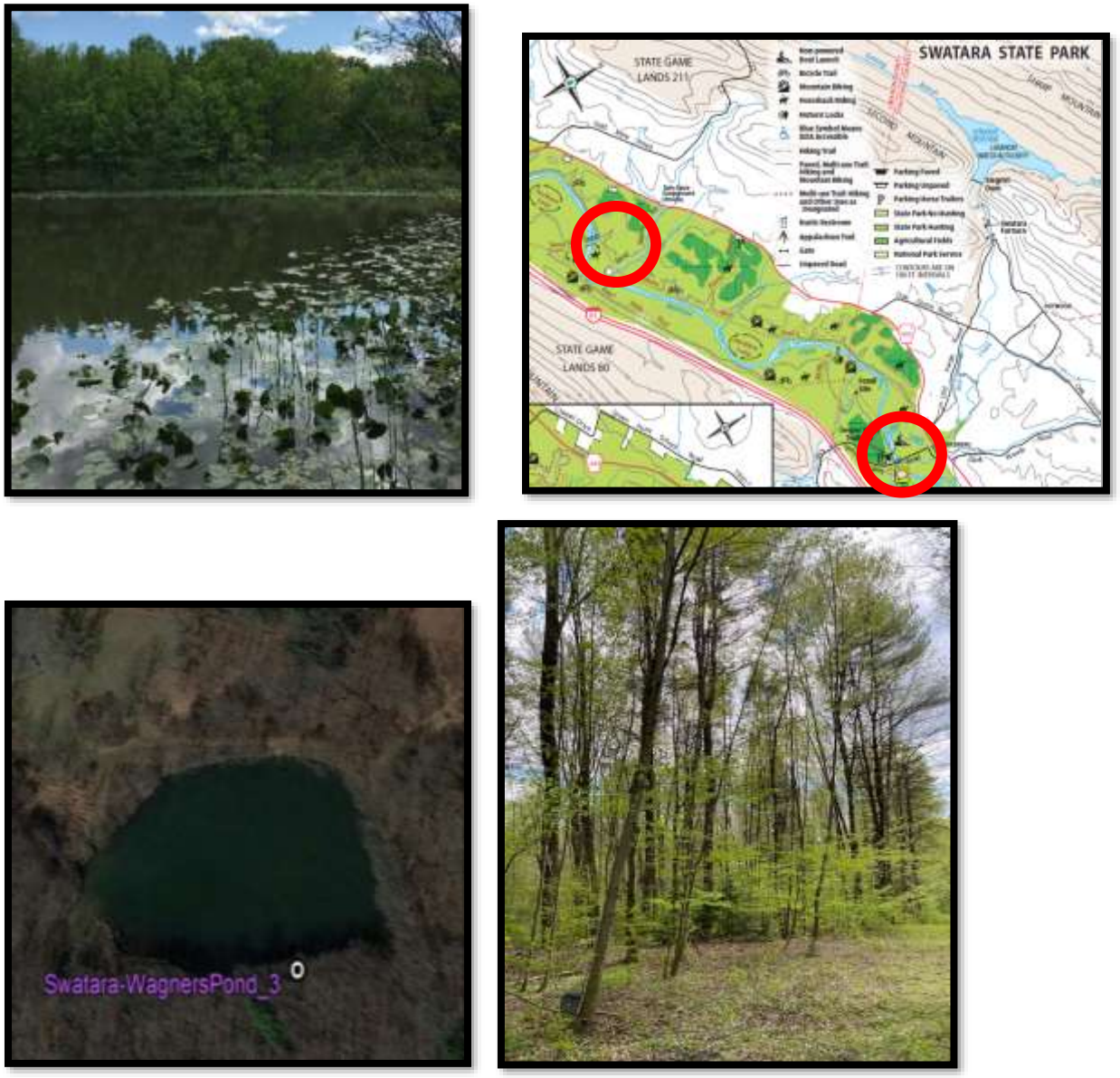


Figure 4. Acoustic bat detector location at Swatara State Park, indicated with the red circles on the top right. In 2018 and 2019, the microphone was facing over Wagner's Pond which can be seen in the bottom and top left pictures (40°30'12" N; 76°30'47" W). In 2020, the microphone faced a forest edge along a pipeline clearing close to the Swatara river as can be seen on the bottom right (40°31'17" N; 76°27'57" W).

Figure 5. Acoustic bat microphone locations at Hawk Mountain Sanctuary, indicated with the red circles. In 2018 and 2019, the microphone for the bat kiosk was located on top of the roof of the nature center facing towards the small pond in the garden area just north of the nature center. The bat kiosk was connected to the microphone but was located inside the nature center (40°38'03" N; 75°59'15" W) . In 2020, a separate microphone was facing a garden area along the fence close to the pond. Refer to the picture inset on the top right.

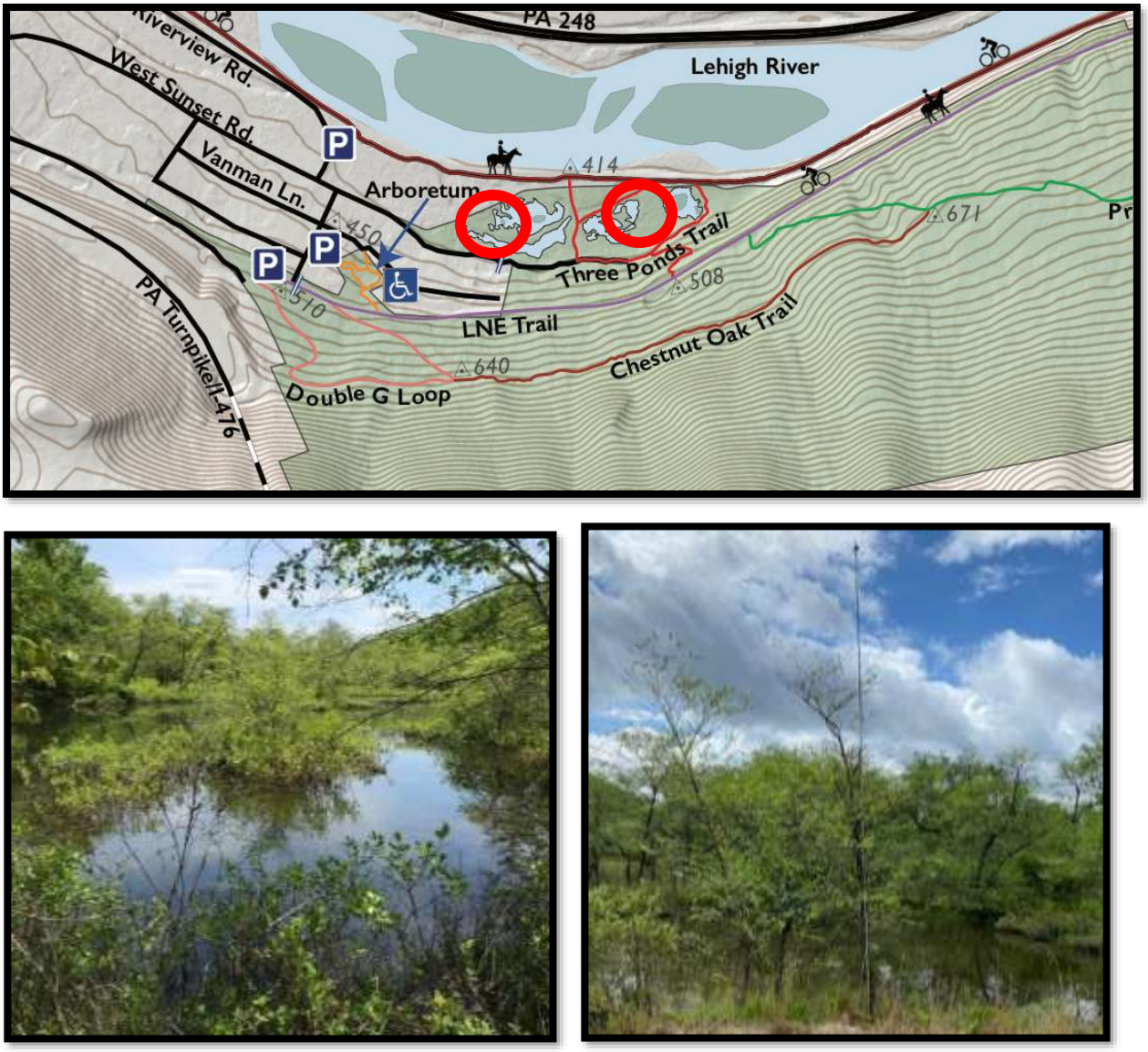


Figure 6. Acoustic bat detector locations at Lehigh Gap Nature Center, indicated within the red circles on top. In 2018 and 2019, the microphone was facing over the west pond of the Three Pond Trail area which can be seen in the bottom left picture (40°47'16" N; 75°38'51" W). In 2020, the microphone was placed over a pond on the east side of the 3 pond trail as seen on the bottom right picture (40°47'17" N; 75°38'47" W).

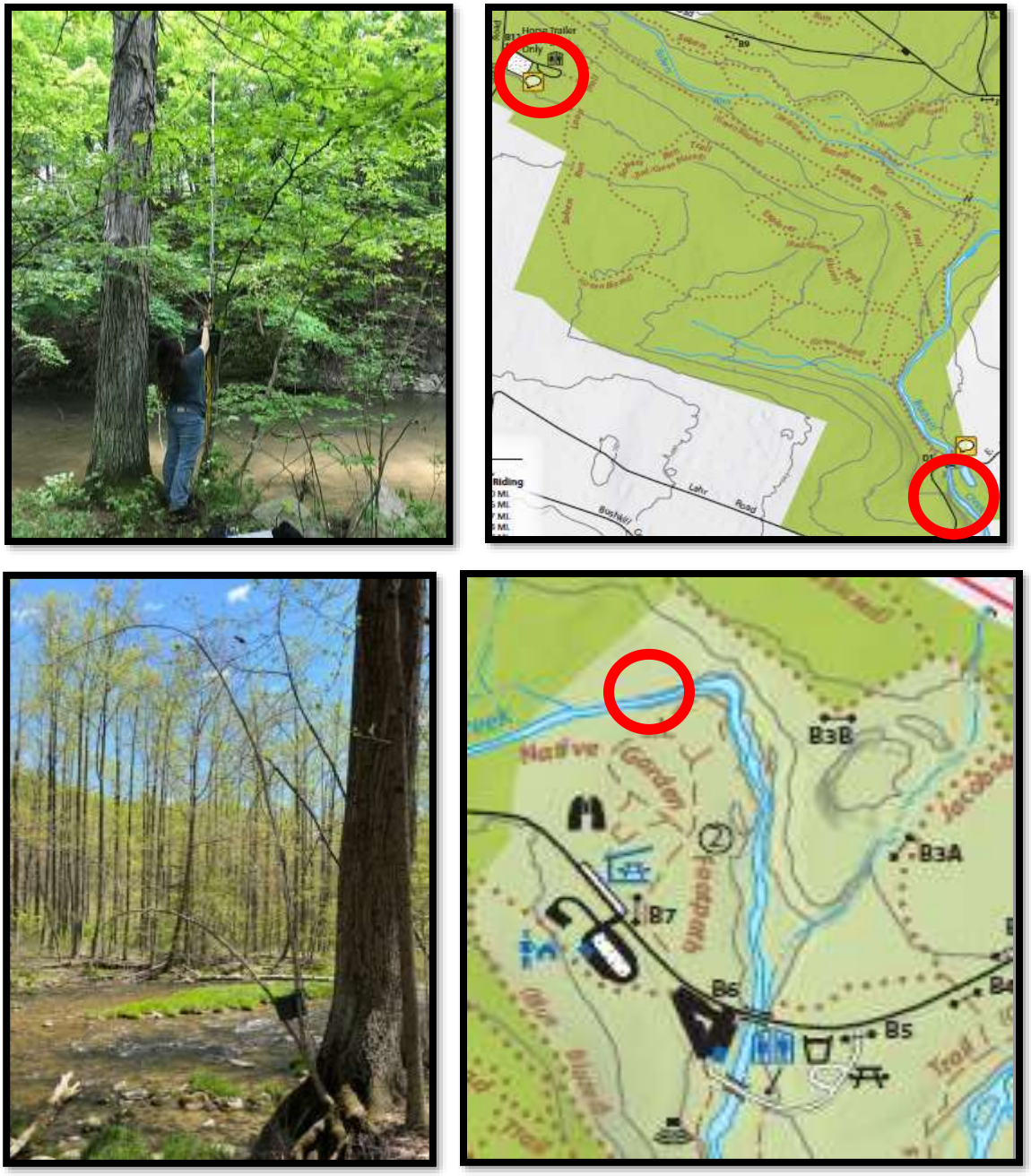


Figure 7. Acoustic bat detector locations at Jacobsburg Environmental Education Center, indicated with red circles on the top and bottom right. In 2018 and 2019, the microphone was facing over an open area of the horse trailer parking lot and then over Bushkill creek as shown in the picture above on the top left (40°46'47" N; 75°18'36" W). In 2020, the microphone faced over Bushkill creek close the Native Garden Footpath as seen on the bottom pictures (40°47'10" N; 75°17'31" W).

Figure 8. Bat acoustic set-up in the field. Microphones were attached to the top of an extendable metal pole at least 3 m above ground height and oriented approximately 45˚ toward possible bat flight space. A tree within a potential bat feeding flyway was selected and the microphone pole was attached to the trunk using zip ties so that it was greater than three meters off of the ground. The microphone cord was connected to the external Pettersson recording device and stored in a waterproof tackle box tethered to the tree.

Figure 9. Hawk Mountain Sanctuary Remote Bat Acoustic Information Kiosk.

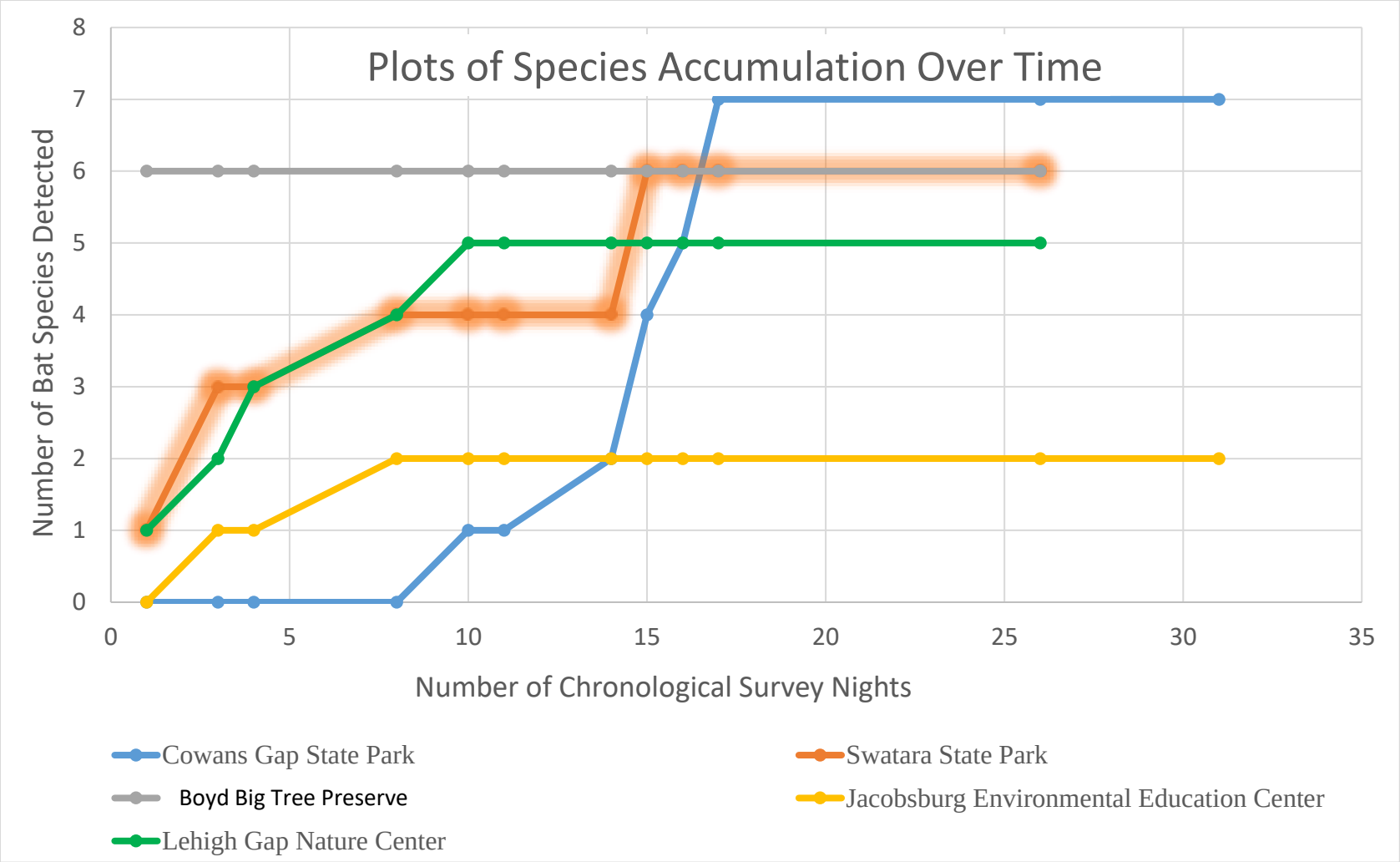


Figure 10. Plots for number of recorded active bat species detected over time for each natural area surveyed along the Kittatinny Ridge of Pennsylvania. Each survey area reached a plateau of number of bat species detected, suggesting a robust estimate of species richness for all areas.

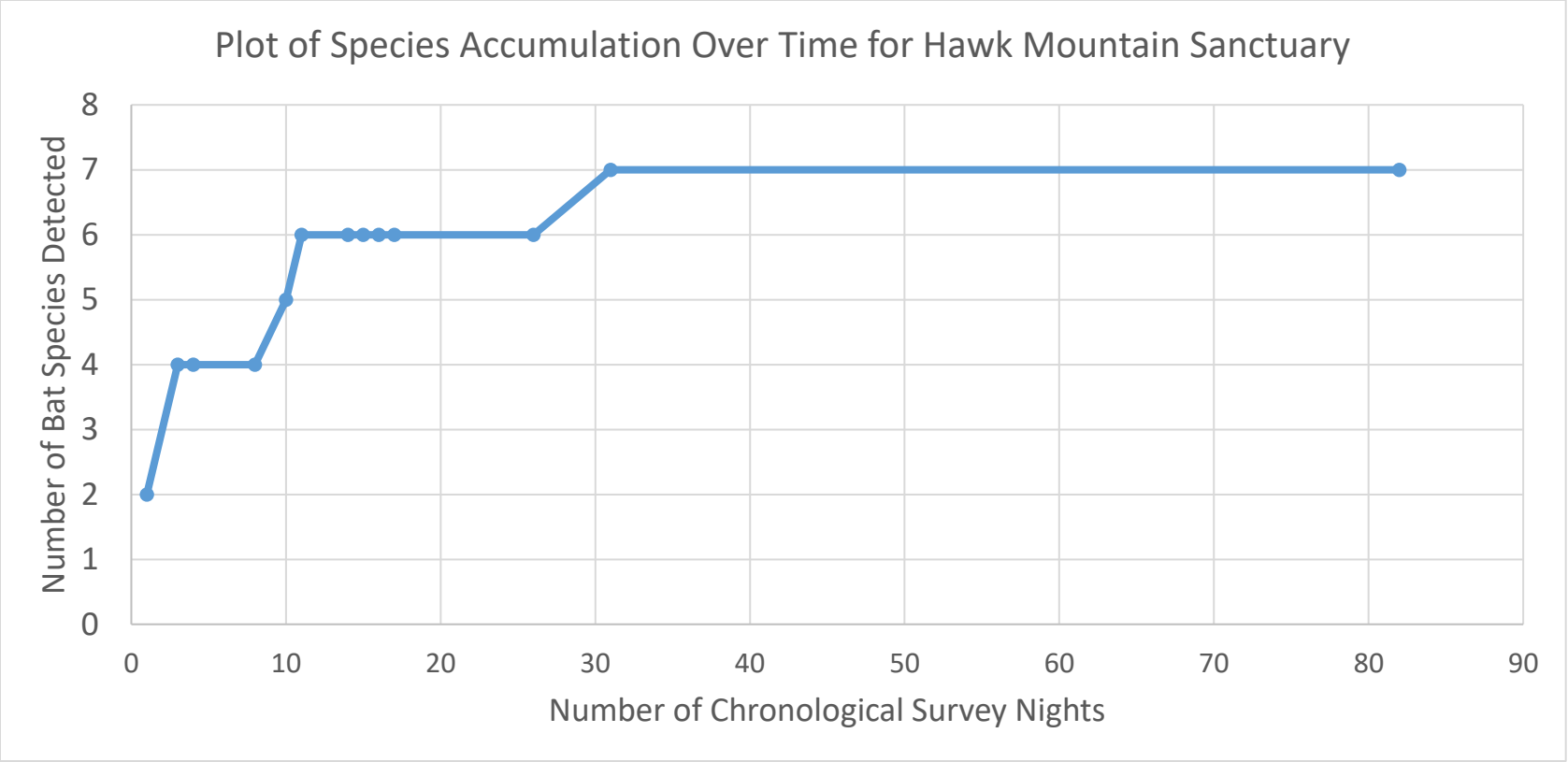


Figure 11. Plot for number of recorded active bat species detected over time for Hawk Mountain Sanctuary natural area surveyed along the Kittatinny Ridge of Pennsylvania. This plot reached a plateau or asymptote for number of bat species detected, suggesting a robust estimate of species richness for this area.

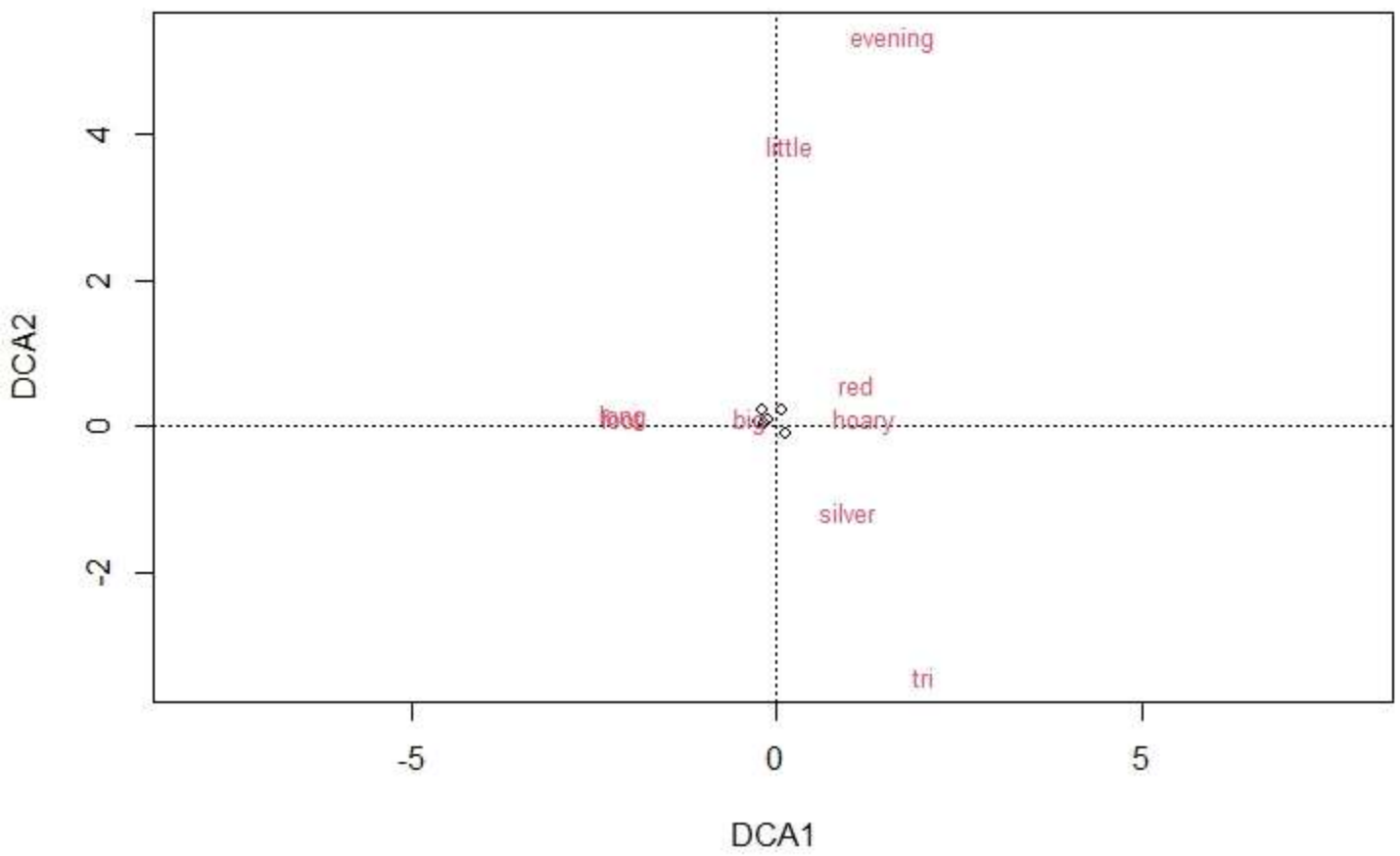


Figure 12. Plot of correspondence analysis created using the R 'vegan' package for bat species activity recorded at all 6 natural areas. Bat titles on the figure include: "big" = Big brown bat (*Eptesicus fuscus*), "red" = Red bat (*Lasiurus borealis*), "hoary" = Hoary bat (*Aeorestes cinereus*), "silver" = Silver-haired bat (*Lasionycteris noctivagans)*, "evening" = Evening bat (*Nycticeius humeralis*), "tri" = Tricolored Bat (*Perimyotis subflavus)*, "footed" = Eastern small-footed bat (*Myotis leibii*), "little" = Little brown bat (*Myotis lucifucgus)* and "long" = Northern long-eared bat (*Myotis septentrionalis)*. The plot shows the outlier for the number of bat species calls recorded at Cowans Gap State Park ("evening" and "little") and Boyd Big Tree Preserve ("silver" and "tri").

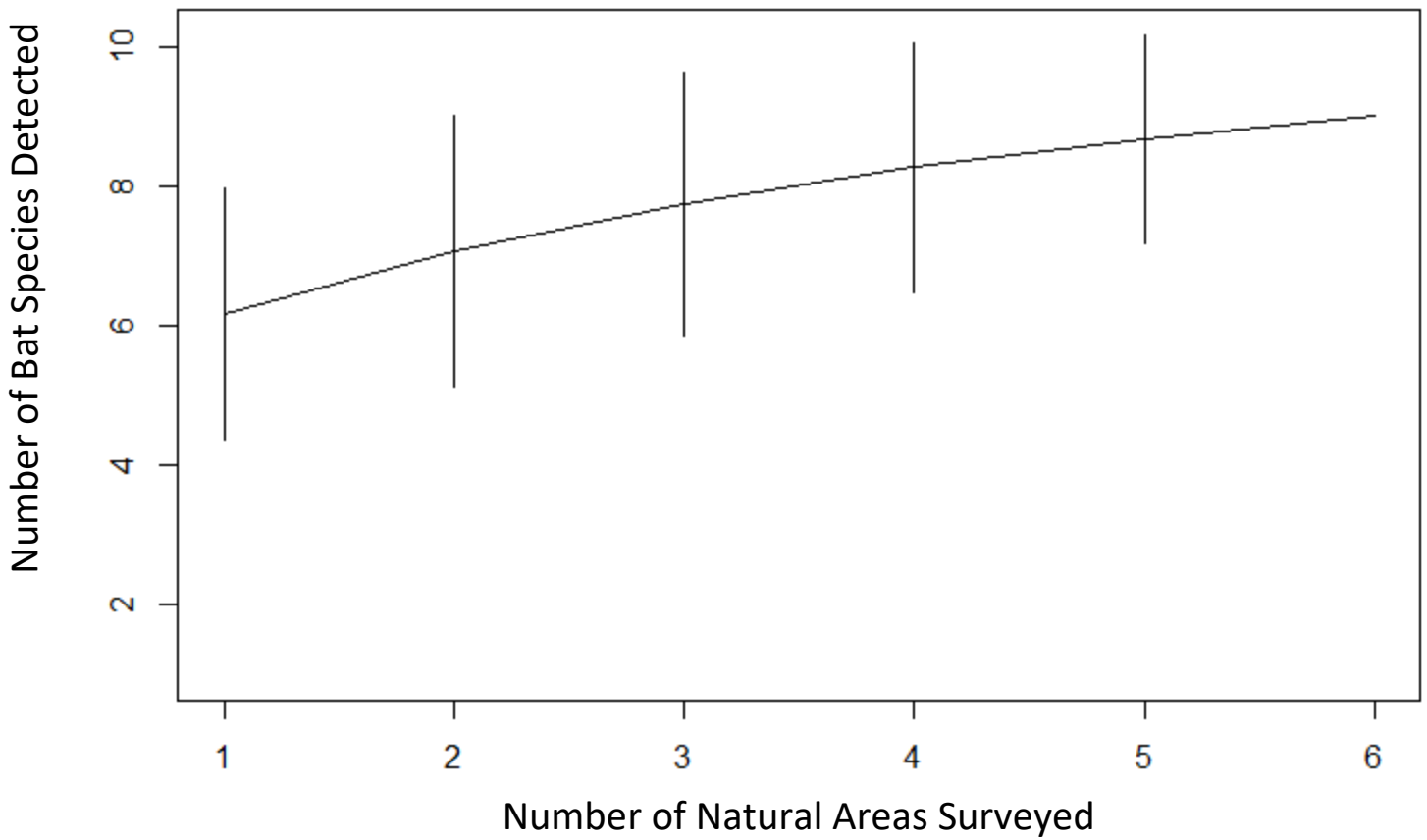


Figure 13. Exact species accumulation curve with associated variance using the Mao Tau estimate (Colwell et al. 2012) in the R 'vegan' package for recorded bat species activity at all 6 natural areas. The curve did not reached a plateau, suggesting that more study sites within more natural areas need to be surveyed for bats in order to estimate bat species richness along the Kittatinny Ridge of Pennsylvania.

Table 1. Results of an acoustic survey on protected areas located along the Kittatinny Ridge of Pennsylvania during the Spring and Summer of 2018-2020. Bat audio identification was conducted using the Sonobat 4.1 software and Manual vetting by John Chenger and Aaron Haines for 2018-2019. During the Spring and Summer of 2020, a different location was surveyed at each site and bat species determination was based on agreement of bat audio identification between the Sonobat 4.1 and the Kaleidoscope Pro Analysis software.

	Number of Calls Recorded Per Species												
Natural Area	Survey Nights*	Big brown	Red	Hoary	Silver-haired	Evening	Tri-colored	Small-footed	Little brown	Long-eared	Myotis spp.	Total Identified Calls	Bat Calls /Night
1) Cowans Gap	49	1481	243	218	113	16	5	0	60	0	3**	2139	44
2) Swatara	35	2154	50	113	122	0	5	0	0	0	2	2446	70
3) Boyd Big	45	7757	920	982	774	1	498	0	20	0	2**	10981	244
4) Jacobsburg	43	1017	84	1	2	0	6	0	31	0	0	1141	27
5) Lehigh Gap	43	2536	15	86	61	0	1	0	0	0	1	2700	63
6) Hawk Mountain* **	86	1206	141	31	58	0	7	3	0	1	0	1448	17

* Number of nights bat calls were detected.

**Identified as MYSO (Indiana bat) on both bat audio identification software for 2020.

*** Bad location in 2020.

Table 2. Bat community analysis based on auditory activity recorded at natural areas along the Kittatinny Ridge of Pennsylvania. Diversity and mean community similarity indices were calculated using the Shannon and Simpson diversity indices and the mean Bray community dissimilarity index in the R 'vegan' package.

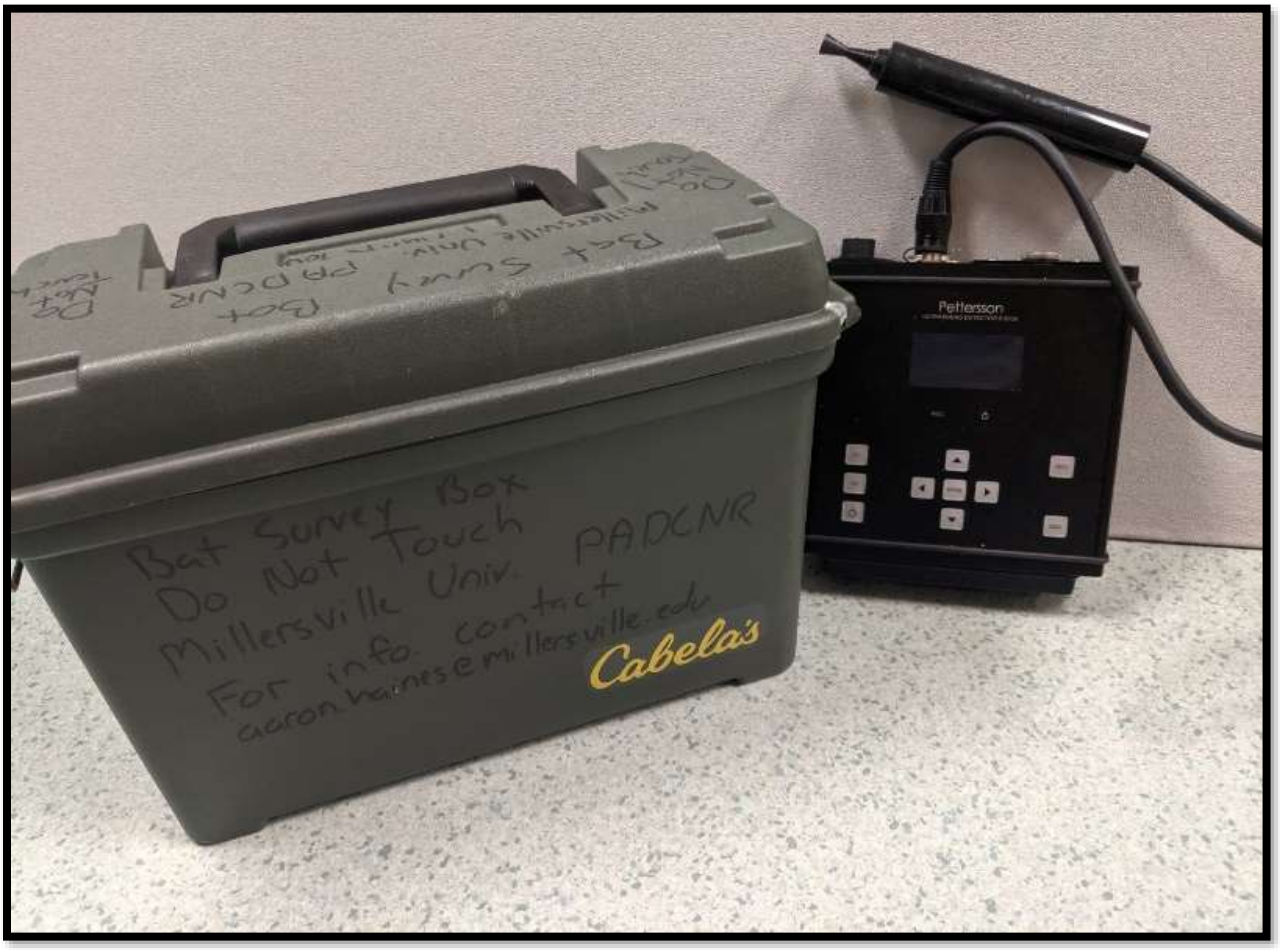
Natural Area	Species Richness	Shannon Diversity Index*	Simpson Diversity Index*	Bray Mean Community Dissimilarity Index*
1) Cowans Gap State Park	8	1.041	0.492	35%
2) Swatara State Park	5	0.495	0.218	33%
3) Boyd Big Tree Preserve	8	1.010	0.476	70%
4) Jacobsburg Environmental Education Center	6	0.437	0.199	42%
5) Lehigh Gap Nature Center	5	0.286	0.115	37%
6) Hawk Mountain Sanctuary	7	0.634	0.294	36%

*****Data for *Myostis* spp. were not included in these calculations.

Table 3. Matrix developed to calculate the mean Bray community dissimilarity index for bat community analysis based on auditory activity recorded at natural areas along the Kittatinny Ridge of Pennsylvania. Bray community dissimilarity index values were calculated using the R 'vegan' package.

Natural Areas	Cowans Gap	Swatara	Boyd Big	Jacobsburg	Lehigh Gap	Hawk Mountain
Cowans Gap		23%	68%	30%	32%	20%
Swatara	23%		64%	40%	10%	31%
Boyd Big	68%	64%		81%	60%	77%
Jacobsburg	30%	40%	81%		46%	14%
Lehigh Gap	32%	10%	60%	46%		37%
Hawk Mountain	20%	31%	77%	14%	37%	
MEAN	**35%**	**33%**	**70%**	**42%**	**37%**	**36%**

APPENDIX 1. Remote acoustic bat call detector equipment. Petterson 500x bat detector with attached weather proof microphone and protective box for hard drive storage in the field.



APPENDIX 2. Research permit for Pennsylvania state parks.

Commonwealth of Pennsylvania
Department of Conservation and Natural Resources
Bureau of State Parks
Permit for Research and/or Collection of State Park Resources

Complete form and mail/e-mail to:
Jack Hill
DCNR, Bureau of State Parks
Resources Management and Planning Division
P.O. Box 8551, Harrisburg, PA 17105-8551
jahill@pa.gov; 717.772.0293

Section 1 – Contact Information:

Name of Applicant
Aaron M. Haines
Street
PO Box 1002, Biology Department
City/State/ZIP
Millersville, PA, 17551
Phone/FAX
717-871-7451
Email
Aaron.haines@millersville.edu

Project Title
Surveying for Rare Bat Populations & Developing the First East Coast Remote Bat Monitoring Station for Public Outreach and Education.
Park Intended for Study
Jacobsburg Environmental Education Center, Swatara State Park, Big Boyd Tree Preserve Conservation area, Cowans Gap State Park.
If Student, Name of Advisor

University/Affiliation
Funding Source (If Any)
$500, 2 proposals pending

Section 2 – Collection Information:

Species to be Collected
No collection of species
Method of Collection/Capture
Remote acoustic monitoring

Total Number to be Collected
No species will be collected
Number of Collections
No species will be collected

Section 3 – Terms and conditions:

- Upon approval by bureau staff, this document serves as a permit for collection, study and monitoring activities in PA State Parks. It is required that this permit be carried at all times while conducting research activity on state park land.
- Collection and monitoring shall occur in areas inconspicuous to park visitors.
- The park manager must approve all marking, tagging and other materials used upon state park land. The permittee is responsible for removal of all such objects upon the completion of the study.
- The Bureau of State Parks reserves the right to withdrawal this permit should it determine that the interests of the Commonwealth or the Bureau of State Parks are no longer being served.
- Whenever possible, flora and fauna shall be observed and studied in the field without collection. All collections shall comply with existing laws and regulations set forth by the Pennsylvania Department of Conservation and Natural Resources, the Pennsylvania Game Commission, the Pennsylvania Fish and Boat Commission, and the U.S. Fish and Wildlife Service. If fish or other Herpetofauna are collected, a fishing license Type 1 Scientific Collectors Permit (issued by the Pennsylvania Fish and Boat Commission) must accompany this permit. All vertebrates approved for collection shall be returned to original collection area. Many types of mammalian and ornithological research require a Special Use permit (issued by the Pennsylvania Game Commission). If such a permit is required, the PGC Permit number must be submitted with this application. Disposition of preserved specimens shall be coordinated with the Bureau of State Parks at the conclusion of the study.

Additional permit requirements:

- Permittee shall inform park manager of sampling dates in advance.
- When conducting fieldwork, permittee shall check-in at the park office prior to beginning work.
- Sampling equipment shall be tagged "Research" and include name, project, and contract information.
- The Bureau of State Parks is not responsible for theft or damage to deployed equipment.
- Copies of field data and reports must be submitted to the Resources Management & Planning Division (at the address above), both in hard copy and electronically, within 60 days of the termination date of this permit, or at the end of each year's work. Failure to do so will result in future permit applications being denied.

Section 4 – For Bureau of State Parks Use Only:

Reviewed by Resources Management Section- _____ Signature _____ Date

Permit Approval- _____ Director _____ Date

Permit Number: [ ] **Issue Date:** [ ] **Termination Date:** [ ]

cc: File
Email: Park Region(s), state park(s)

pennsylvania
DEPARTMENT OF CONSERVATION AND NATURAL RESOURCES

Commonwealth of Pennsylvania
Department of Conservation and Natural Resources
Bureau of State Parks

Application/Permit
For
Research and/or Collection of State Park Resources

Name of Applicant
Dr. Aaron Haines

Collection Permit Number

Project Title
Surveying for Rare Bat Populations & Developing the First East Coast Remote Bat Monitoring Station for Public Outreach and Education.

Section 5 – Project Description/Proposal (To be completed by all applicants):

Include: List of park(s) for collection/study, start and end dates, collection/study methods, species to be collected/studied, and time(s) of collection/study. Attach additional pages as needed.

In the state of Pennsylvania, 7 species of bats are listed as species of greatest conservation need as identified in the Pennsylvania State Wildlife Action Plan (Big Brown Bat, Tricolored Bat, Northern Long-eared bat, Eastern small-footed bat, Indiana bat, Little brown bat and Silver-haired bat). In addition, the Northern long-eared bat is listed as federally threatened and the Indiana bat is listed as federally endangered. Conservation of remnant colonies for these species are of high conservation priority. The objectives for this research project are to 1) conduct active monitoring surveys for rare bat species within the Kittatinny Ridge of central Pennsylvania and 2) establish a publicly accessible remote acoustic bat kiosk at Hawk Mountain Sanctuary to monitor bat species and educate the public on bat conservation.

We will conduct bat surveys using stationary remote acoustic recording devices during the summer and fall of 2018 & 2019 at 5 natural areas along the Kittatinny Ridge (Cowans Gap, Swatara State Park, Boyd Big Tree Preserve Conservation Area, Lehigh Gap and Jacobsburg Environmental Education Center). All survey locations will be mapped and locations of confirmed bat presence will be recorded. Through shared data and reports, survey efforts will support professional conservationists and park managers identifying remaining populations of these rare bats so they can be protected and maintained. All acoustic recordings will be analyzed using the SonoBat software for bat call analysis to identify different bat species.

In addition to the five temporary acoustic monitoring sites, a permanent site will be established at Hawk Mountain Sanctuary using the publicly accessible remote acoustic bat kiosk. This SonoBatLIVE Kiosk will record bat ultrasound calls on an on-going basis during seasons of bat activity in Pennsylvania using SonoBatLIVE acoustic monitoring/auto-classification software. As an on-site kiosk, visitors to Hawk Mountain Sanctuary may view bat calls in real-time or they may view calls recorded from the night before and, in turn, will engage in bat research methods, education, and outreach. All acoustic data collected by the SonoBatLIVE Kiosk will be analyzed in the same manner as the data collected at the five temporary sites.

These data will be useful in assessing bat diversity and potentially density over short-term and long-term monitoring periods and contribute to conservation efforts for bat species of greatest conservation need.

Specific locations for placement of the remote acoustic bat recording devices at the temporary natural areas managed by the Pennsylvania Department of Conservation and Natural Resources will include the following: 1) Cowans State Park, behind the Park Office facing Cowans Gap Lake. 2) Jacobsburg Environmental Education Center, pond off Bushkill Run near gate D1. 3) Swatara State Park, Wagners Pond and 4) Boyd Big Tree Preserve Conservation Area, off the pond loop trail facing the pond.

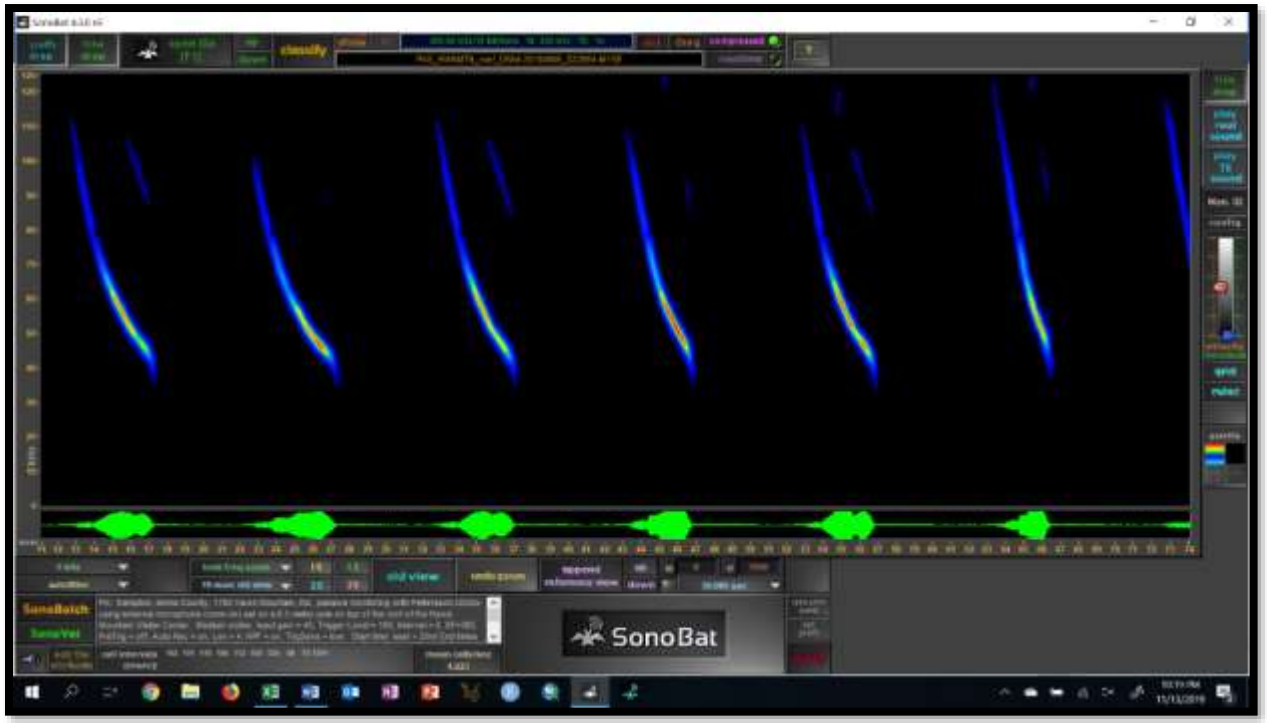
APPENDIX 3. Manually vetted bat calls accepted for species identification for analysis of bat community structure of 6 natural areas along the Kittatinny Ridge of Pennsylvania.

1) Sonobat = Tricolored bat at Cowans Gap State Park – August 4th 2018.

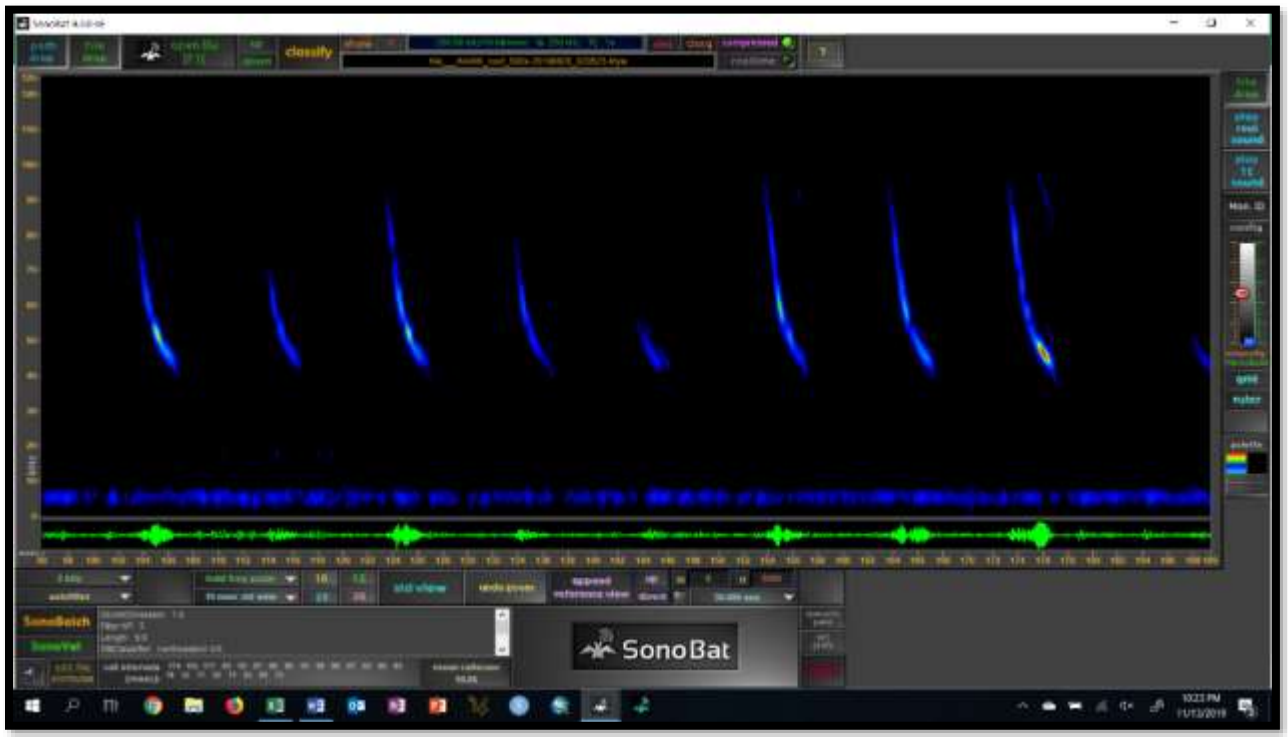
2) Sonobat = Tricolored bat at Swatara State Park – May 26th, 2019.

3) Sonobat = Tricolored bat at Hawk Mountain Nature Center – August 6th, 2018.

4) Sonobat = Northern long-eared bat at Hawk Mountain Nature Center – August 5th, 2018.



5) Sonobat = Eastern small-footed bat at Hawk Mountain Nature Center – August 20th, 2019.



APPENDIX 4. Habitat requirements and management recommendations for Pennsylvania bat species of greatest conservation need as outlined by the Pennsylvania State Wildlife Action Plan of 2015.

Bat Species	**State Conservation Status**	**Habitat Requirements**	**Management Recommendations**
Big brown	High concern	Hollow spaces in human structures and trees for breeding. Human structures, caves, mines and tunnels for wintering.	Education for proper venting and exclusion of bats from human structures. Develop treatment options to reduce white-nose syndrome (WNS) infections.
Silver-haired	Very high concern	Deciduous forest with adjacent agricultural fields or high deciduous uplands. Conifers and mixed forests adjacent to wetlands and open bodies of water. In winter migrates to various habitats.	Work with landowners to protect existing roost trees that have sloughing bark or crevices (e.g., shagbark hickory).
Tri-colored	Very high concern	Human structures, trees and cavities for breeding. Warmer locales for winter.	Education for proper venting and exclusion of bats from human structures. Enforce protections at hibernation sites through bat friendly gates in front of mines and caves, and expand discussions with the caving community. Retain openings and structural integrity of abandoned mines. Develop treatment options to reduce white-nose syndrome infections. Provide drainage for used hibernacula to prevent flooding and promote use of artificial structures and creation/retention of wildlife trees for roosting sites.
Eastern small-footed	Very high concern	Deciduous/mixed forested areas containing rock outcrops and talus. For winter species need caves, mines, rock outcrops and talus.	Limit access to caves to reduce spread of WNS. Establish forest management practices that protect forested areas with abundant rocky outcrops and loose rocks. Limit recreational activity at known summer roosts that could disturb rocks and cause landslides. Identify road sections with high bat mortality and add vegetation or barriers to direct bats over cars.
Little brown	Very high concern	Forested and human settings. For winter, species need caves mines, rock fissures	Limit access to caves to reduce spread of WNS. Work with private landowners to minimize disturbance of

		and human structures with internal temp of 40-50F.	caves. Enforce protections at hibernation sites through bat friendly gates in front of mines and caves, and expand discussions with the caving community to minimize the negative impacts of caving. Retain openings and structural integrity of abandoned mines. Promote use of artificial roosting structures with creation/retention of wildlife trees during forest management projects. Avoid forest removal. Provide drainage for used hibernacula to prevent flooding. Remove predators from caves. Identify road sections with high bat mortality and add vegetation or barriers to direct bats over cars.
Indiana	Very high concern	Riparian, bottomland or upland forests, old field and pastures. Shagbark and shell-bark Hickory trees for roosting. Winters with caves and mines at temperatures from 42-51 F.	Limit access to caves to reduce spread of WNS. Enforce protections at hibernation sites through bat friendly gates. Enforce protections at hibernation sites through bat friendly gates in front of mines and caves, and expand discussions with the caving community. Retain openings and structural integrity of abandoned mines. Promote the use of artificial structures and creation/retention of wildlife trees during forest management projects. Develop treatment options to reduce white-nose syndrome (WNS) infections. Provide drainage for used hibernacula to prevent flooding. Remove predators from caves. Work with private landowners to minimize disturbance of caves. Identify road sections with high bat mortality and add vegetation or barriers to direct bats over cars.
Northern long-eared	Very high concern	Deciduous/mixed forested areas containing mature trees with exfoliating bark/snags and human structures for summer. Caves and mines for winter.	Limit access to caves to reduce spread of WNS. Enforce protections at hibernation sites through bat friendly gates. Maintain mature interior forest habitat and creation/retention of wildlife trees. Provide drainage for used hibernacula to prevent flooding.