



Ohio Division of Wildlife Mobile Bat Acoustic Survey Report for 2017

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ABSTRACT

Ohio mobile bat acoustic surveys have taken place since 2011 and currently consist of 42 routes in 41 counties. Surveys began after white-nose syndrome (WNS) was discovered in a hibernaculum in Lawrence County, Ohio. This disease has killed an estimated six million bats in the United States and Canada since 2007 (USFWS, 2012). The goal of this project was to non-invasively monitor the summer bat populations in Ohio and determine the negative effects (e.g. population declines and loss of species diversity) that WNS may be having statewide. Because bats are the primary predator of night-flying insects, they are incredibly important to the ecosystem and for agriculture by reducing pesticide loads and crop damage. Bats are estimated to save around \$3.7 billion dollars each year for the agriculture industry (Boyles *et al.*, 2011). The average decline in cave-dwelling bats for states that have had WNS is 30-99% (Frick *et al.*, 2010). In Ohio, similar declines were reported by 2016 in its two largest hibernacula, the Preble and Lawrence County mines (89.56% and 99.5%, respectively). Mobile bat acoustic surveys allow the Division of Wildlife to determine if winter declines are consistent in the summer populations. Survey results are compared annually to monitor changes in bat abundance along each route. There is evidence of declines, although not statistically significant, in state bat abundance overall from 2011 to 2017. This is likely as a result of WNS in combination with other various threats to bats. However, there is a significant increase in abundance between 2014 and 2017 ($p=.014050$). Increases from 2014 could represent beginning recoveries in Ohio's bat populations or a change in species composition across the post-WNS landscape.

INTRODUCTION

There are 11 bat species in Ohio (Table 2). While a common reaction to bats is fear, most of these feelings are based on a lack of information, and bats are actually incredibly beneficial to the environment and humans. Bats are the main predator of night-flying insects. A single *Myotis lucifugus* (little brown bat) can consume up to 1,000 mosquito-sized insects in an hour

(Bekkering, 2010). This provides insect control for people in general and farmers specifically, as bats consume crop pests that can cause damage to a farmer's yield.

Bats often roost in the exfoliated bark or cavities of trees in the summer but can also be found in caves, houses, bat condos, and rock crevices. After raising pups in the summer, they migrate to areas to mate in the fall and roost for the winter. A

group of bats that are often termed cave-dwelling bats typically hibernate in caves during the winter, but some are also thought to use less traditional hibernacula sites (such as cliff crevices and trees). Cave-dwelling bats are the species that are known to be affected by white-nose syndrome (WNS). In Ohio, this includes *Eptesicus fuscus*, *Perimyotis subflavus*, *Myotis lucifugus*, *M. septentrionalis*, *M. sodalis*, and *M. leibii*. There are also some species of bats in Ohio that migrate further south and stay active for longer periods of time including *Lasiurus borealis*, *L. cinereus*, *Nycticeius humeralis*, and *Lasionycteris noctivagans*. These bats are often referred to as migratory tree bats and while they can carry fungal spores, they are not known to be impacted by WNS.

Bats have seen major declines in recent years. Development can impact local bat populations by leading to the destruction of potential bat habitat (Kunz, 2003). This includes both destroying roosts and habitat connectivity (Scott *et al.*, 2010). In addition to habitat destruction, run-off from construction sites can pollute waterways and environments elsewhere that bats may use (Wisconsin DNR, 2015). Bats have also seen declines from collisions with man-made structures such as wind turbines (Arnett *et al.*, 2008). Finally, bats have suffered major declines as a result of WNS.

White-nose syndrome is an emergent, deadly fungal infection which presents itself as fuzzy, white material on the wings and muzzles of hibernating bats. It is caused by the fungus *Psuedogymnoascus destructans* (formerly *Geomyces destructans*, Pd). The disease was first discovered in New York in the winter of 2006-7 and has since spread across the eastern portion of North America and Canada (Blehert *et al.*, 2009).

The fungus grows best in cold, humid environments like caves. It affects the bats by coating the wing membrane, leading

to irritation. The irritation causes bats to arouse from their hibernation state, known as torpor. Another factor that causes bats to arouse from torpor is an immune system response to the virus. This response causes their body temperatures to elevate in an attempt to fight off the foreign substance. This arousal expends a lot of energy because bats have to raise their body temperatures from around 15°C. The other problem with waking up when they are supposed to be hibernating is that the bats have no way to replenish their fat stores that they accumulated over the fall. Insect activity is too low for them to recover, and they eventually starve to death (USGS, 2015).

Two hibernacula, located in Preble and Lawrence County, Ohio, have been surveyed every other year for about 20 years. White-nose syndrome was found in the Lawrence County mine in 2011. As of 2018, the Lawrence county mine has seen declines averaging 99.5% (Katrina Schultes, personal communication). As of 2016, the Preble hibernaculum has seen declines averaging 89.56% (Table 1, Division of Wildlife unpublished data). These surveys are only an estimate of the winter population of cave-dwelling species because hibernacula surveyed are only those known to Division of Wildlife (DOW); there are other hibernacula throughout the state that are unknown and/or inaccessible. As a result, the DOW decided it was pertinent to create a population monitoring program for bats in the state during the summer for a further understanding of the impacts of WNS on bat populations.

Table 1: Percent change in Ohio's two largest hibernacula from 2012 to 2016 when WNS was documented.

Species	% Change from 2012-18	% Change from 2012-16
	Lawrence	Preble
Big brown bat	N/A	-88.89%
Tri-colored bat	-100%	-98.89%
Indiana bat	-100%	-68.73%
Northern long-eared bat	-100%	-92.49%
Little brown bat	-100%	-98.79%
Average	-99.5%	-89.56%

Mobile bat acoustic surveys were created by the DOW to monitor bat populations during summer months across the state. Regular mist-net capture surveys performed by the DOW and other entities during summer months are beneficial for identifying bats to species but provide minimal information across space for the extensive effort and costs it requires. For these reasons, the DOW mobile bat acoustic surveys are ideal for large data collection over space and time. Acoustic surveys began in 2011 as a citizen science program. With the help of volunteers, the acoustic surveys allow for a chance to monitor changes in bat abundance from year to year across the state in both cave-dwelling bats and migratory tree bats. Public involvement facilitates coverage of large areas with relatively minimal effort making it a suitable method for monitoring trends of bats in Ohio (Roche *et al.*, 2011). The program currently has over 100 volunteers with 42 routes in 41 counties.

METHODS

Acoustic Route Protocol

The Ohio Division of Wildlife developed a standardized protocol for a mobile acoustic route to survey bats in 2011. These protocols were modified from the 2009 Britzke and Herzog acoustic survey guidelines. Transects were approximately 30-mile loops that were driven around 15 miles per hour to increase the likelihood that individual bats were detected only once (Hayes and Hounihan, 2016). Most routes were driven three times throughout the month of July on non-consecutive nights. Surveys were conducted in temperatures above 50°F, with winds less than ten miles per hour, and with no precipitation or fog. All surveys began 30 minutes after sunset and alternated direction (original or reverse) every other survey. Due to the fact that there were three surveys, there was a bias towards recordings in the original direction. Routes were randomly created based on availability and interest from volunteers, and 42 routes were conducted in summer 2017 (Figure 1).

Nineteen out of 120 surveys did not follow standard protocol in 2017. Fifteen of these surveys were coordinated by the Wayne National Forest (Routes 1-6) and were done using the standard Forest Service acoustic monitoring protocols. The Forest Service survey protocols did not limit the surveys to the month of July but commenced in June and some ended in August. In addition, they normally ran their routes four times instead of three. Their routes followed a straight path instead of a loop and also did not alternate directions but rather continued in the same direction each time. Two other surveys (Routes 29 and 42) were not completed in July due to time constraints. Two routes were run four times instead of three (Routes 28 and 35) and one of those

surveys fell outside of the month of July (the third survey on Route 35).

Surveys that followed the standard protocol additionally had the following problems: equipment issues (12 surveys), road closures (three surveys), detours (25 surveys), stops due to lights and signs and traffic (25 surveys), and stops due to animals in the road (three surveys). Adjustments for mileage and/or time were made and accounted for in the bat detection rate.

Busy roads and highways were avoided on mobile acoustic routes for volunteer safety and unnecessary noise interference. Surveys were driven on non-consecutive nights due to the fact that bats may be affected by moon illumination (Gannon and Willig, 1997). All of the surveys were done on roadways. The habitat surrounding the road varied from agricultural lands to forests to waterways.



were run.

Each route was conducted by volunteers and/or Division employees. In order to standardize the protocol, each volunteer received training with the equipment and a step-by-step instruction binder to use as a reference when conducting the surveys.

The following data was catalogued at the beginning and end of each survey: temperature, wind speed, cloud cover, time, moon visibility, latitude, longitude, and odometer readings. The equipment for taking this data included a La Crosse Technology® anemometer and a General® probe thermometer.

Bat calls were recorded using Anabat™ Secure Digital (SD) II units (Titley Scientific, Columbia, Missouri). Prior to the survey season, SD II units were calibrated with an Anabat™ Chirper (MK II) with a jig holding system and the Anabat™ Equalizer software to reduce the microphone variability. Microphones were attached to the top of volunteers' vehicles and directed off center toward the back of the vehicle to prevent wind interference. The microphones were then connected to the Anabat system inside the vehicle. Most geographical references were recorded with a Compact Flash GPS card with the PDA kit. Five routes (7, 20, 26, 38, and 42) used GPS mouse units instead.

Data Analysis Protocol

Recorded bat calls were classified using Bat Call ID East (version 2.6a) (BCID). This is a quantitative, automated identification program that was employed to determine which acoustic sound files were bat calls and to which frequency group those bat calls belonged. Classification of species in BCID was filtered through the program with the following default alterations: *Myotis leibii* (eastern small-footed bat) was added to the species to identify, the smoothness was changed to 30, the frequency was altered to a range of 5-60, the sweep was altered to a range of 1-70, and the duration was altered to a range of 1-30. All other filters were kept with the default standards in BCID. These parameters were tailored to Ohio bat calls by DOW staff and

BCID. They were also set up to eliminate noise and interference while allowing the highest possible amount of bat calls to be identified. Bat Call ID frequency groups (Table 2) were used in classification for annual comparisons to allow for greater classification accuracy in statistical analysis.

This year, the calls were also run through a software program known as Echoclass. The species classification results from BCID were compared with Echoclass for classification agreement across softwares. Calls with different species identification between the two programs were manually vetted.

Lastly, survey effort was defined by minutes per mile, and detection rate by bat calls per minute per mile. Detection rate serves as a proxy for bat abundance and will be used interchangeably throughout.

Statistical Analysis Protocol

Once the data was sorted into species and frequency groups, statistical analyses were conducted. The mean detection rate was found by taking the total number of calls divided by the rate (minutes/mile). This mean detection rate was then compared to determine the percentage change overall since 2011 and every year in between.

Linear regressions were created to determine the significance in detection rate changes over time, between species, between frequency groups, throughout the month, with moon illumination, and with temperature. A two-tailed test was then run to determine the significance of each of these regressions.

An Unpaired Equivalence T-test was used to determine the correlation in data between regions of the state and between counties with WNS and without confirmed WNS.

Table 2: A breakdown of the frequency groups for the species of bats that are found in Ohio, excluding the Rafinesque's big-eared bat that has not been found in Ohio since 1952 in Adams County (ODNR: Division of Wildlife, 2016).

Frequency Group	Common Name	Scientific Name	Status
Low	Hoary Bat	<i>Lasiurus cinereus</i>	Species of Concern
Low	Silver-haired Bat	<i>Lasionycteris noctivagans</i>	Species of Concern
Low	Big Brown Bat	<i>Eptesicus fuscus</i>	Species of Concern
Mid	Evening Bat	<i>Nycticeius humeralis</i>	Species of Special Interest
Mid	Eastern Red Bat	<i>Lasiurus borealis</i>	Species of Concern
Mid	Tri-colored Bat	<i>Perimyotis subflavus</i>	Species of Concern
Myotis	Indiana Bat	<i>Myotis sodalis</i>	Federally Endangered
Myotis	Northern Long-eared Bat	<i>Myotis septentrionalis</i>	State and Federally Threatened
Myotis	Small-footed Bat	<i>Myotis leibii</i>	Species of Concern
Myotis	Little Brown Bat	<i>Myotis lucifugus</i>	Species of Concern

RESULTS

During the summer of 2017, 120 surveys on 42 routes were run with a total of 15,651 minutes of survey time. One new route was created this year (Route 42) bringing total surveys completed in seven years to 636 (Appendix E). Surveys have accumulated 39,090 bat call detections since 2011.

Mean detection rate in 2017 was 17.87 detections per mile. The detection rate for low, mid and *Myotis* BCID frequency groups in detections per mile are 10.56, 6.56, and 0.46 respectively.

For 2017, average detection rate was also compared between species. These were developed after comparing calls between both Echoclass and BCID and manual vetting (Figure 2).

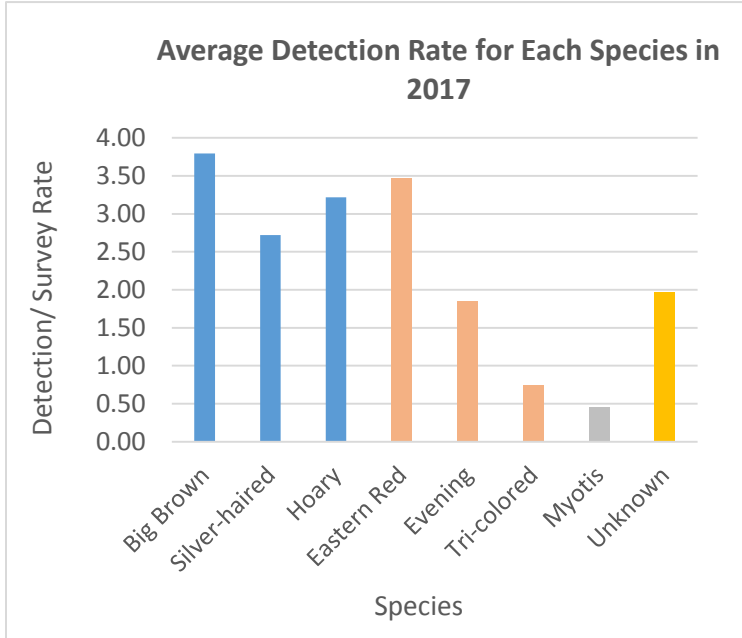


Figure 2: Average detection rate for each species among all 42 routes run in 2017.

From 2011 to 2017 there has been a 24.21% decline in overall bat abundance. This breaks down to declines of 5.62% in the low frequency bat detections, 45.48% in the mid-frequency bat detections, and 66.33% in the *Myotis* frequency bat detections ($p=0.1185$, $n=7$, Figure 3). While this trend is noticeable, it is not statistically significant.

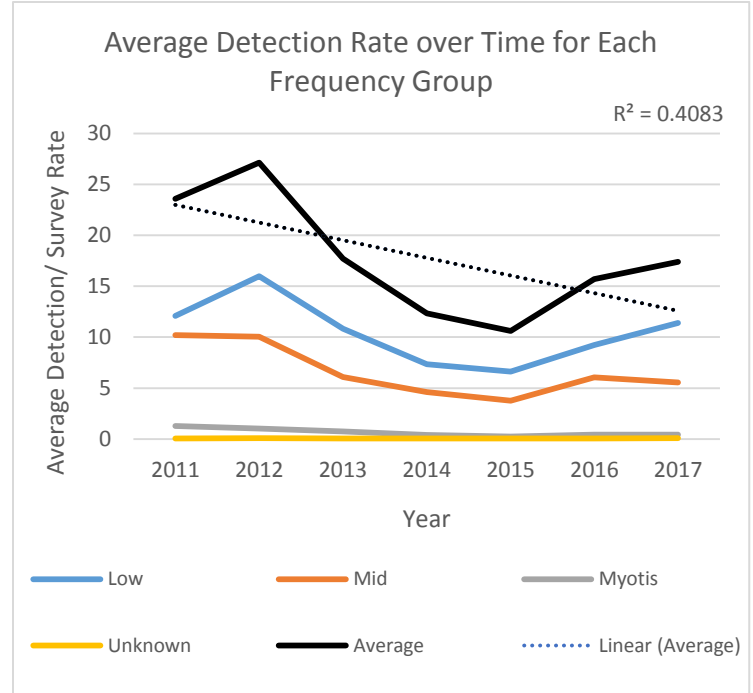


Figure 3: A comparison of the changes in the average bat abundance for each frequency group from 2011 to 2017.

Bat detection rate has increased each year since 2014 ($p=0.014$, $n=4$, Figure 4 and Table 3). This increase from 2014 to 2017 is statistically significant.

Table 3: Percentage increase of average detection rate for each year. The negative values indicate a decline.

	% Increase
2011 to 2012	9.51
2012 to 2013	-48.41
2013 to 2014	-40.07
2014 to 2015	7.01
2015 to 2016	13.90
2016 to 2017	14.15
2011 to 2017	-31.95
2014 to 2017	42.56

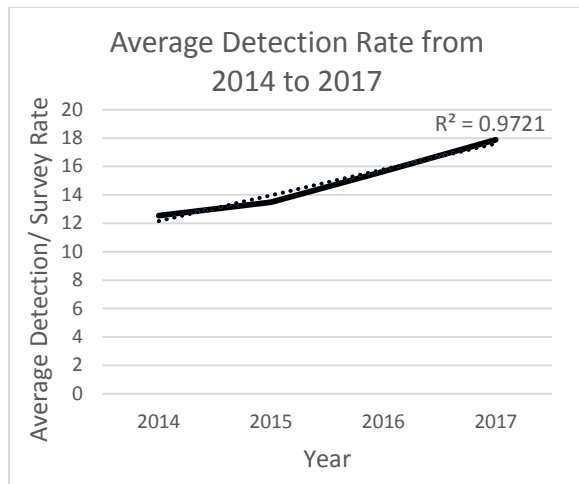


Figure 4: A comparison of total average detection rate over time from, 2014 to 2017.

When comparing the mean bat abundance of the routes in the 17 Ohio counties that are listed as having evidence of WNS and the 22 counties that are not, there is not a significant difference ($p=0.2470$).

In 2017, the route with the highest mean bat abundance was Route 17 in Vinton County (39.72 detections per mile). The route with the lowest mean bat abundance was Route 14 on Kelley's Island (2.80 detections per mile). The lowest mean detection rate on the mainland of Ohio was Route 36 in Sandusky County (7.55 detections per mile).

When looking at temporal differences in the routes, there appeared to be little to no significant difference. Average detection rate did not display any trends throughout the month of July or with moon illumination percentage (Appendix B). However, there was a significant difference in bat abundance with change in temperature ($p=0.00438$, $n=136$, Figure 5).

Thirty-three of 42 routes showed a higher proportional abundance of the low frequency calls, seven showed a higher proportional abundance of the mid-frequency calls, and the remaining three did not show a consistent pattern. In the north half of the state, 90.91% of the routes that

were run showed a higher proportional abundance of low-range frequency group calls. In the southern half of Ohio, 61.9% of the routes showed a higher proportional abundance of the low-range frequency calls and 28.57% of the routes showed a higher proportional abundance of mid-range frequency calls. This difference in frequency group composition was the only noticeable trend amongst the regional data.

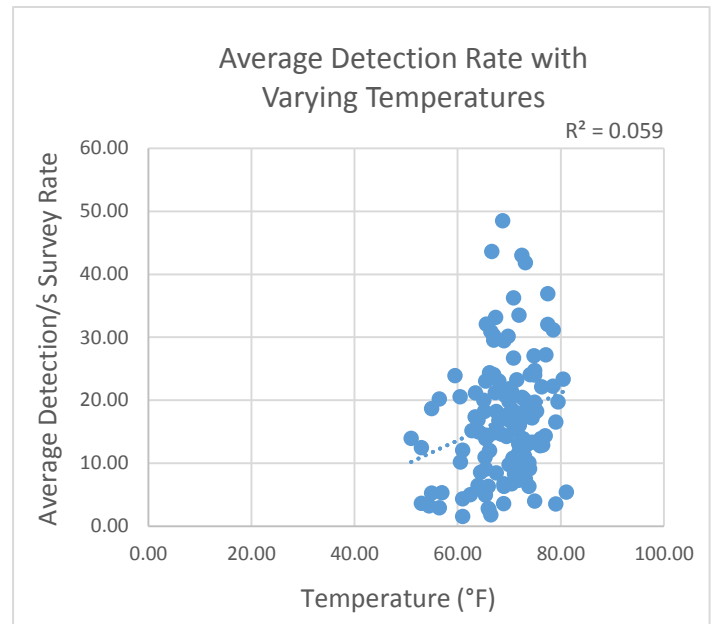


Figure 5: A model of the total average detection rate for each route over the changes in temperature for the month of July.

DISCUSSION

The goal for the project was to monitor the changes in bat abundance over time in Ohio after the discovery of WNS.

Despite the overall trend of declines since the discovery of WNS in Ohio, the decline from 2011 to 2017 was not statistically significant, and there have been recent statistically significant increases in bat abundance beginning in 2014. This could be due to changes in species composition, with certain species becoming more prominent in the area over time. Due to the fact that the multi-year analysis is limited to frequency group, it is difficult to

tell which species are seeing increases. The manual vetting process that was used in 2017 allowed for more species-specific observations. It was noted that the species with the highest average abundance on the surveys was the big brown bat followed by red bat, hoary bat, silver-haired bat, evening bat, tri-colored bat, and all of the *Myotis* species combined. This may lend more credence to the impact that WNS has had on *Myotis* species, as they were the least abundant. However, that cannot be definitively determined without comparison data.

Many of the species that were the most abundant this year can carry the fungus *Pd*, but they have not actually shown symptoms of the disease. As a result, they could potentially be making use of the habitat and prey that is not being consumed as readily by the WNS impacted species. The only species that this doesn't qualify for is the big brown bat, which has not shown declines in hibernacula to the same degree as the other cave-dwelling bats. Without comparison to previous years, particularly from before WNS or even the start of WNS, this is mostly speculation. These species may have always been more abundant on the acoustic recordings.

This recent increase could also be indicative of a recovery of bats due to some form of resistance to WNS. The state of New York has begun to see such recoveries, and more studies are being done to research if these trends occur in other states as well (Langwig *et al.*, 2016). As the mobile bat acoustic survey program continues into additional years, trends may become more apparent.

It should also be noted that the sample size for the bat abundance comparison from 2014 to 2017 was low ($n=4$). So while this data comes from an average of 496 individual data points, reducing all those data points to a four data

point comparison may lead to a stronger correlation than there actually is, as the data have been simplified. More consistent data collection to increase the sample size and additional ecological modeling could allow for further deductions on the trends in the data.

Even with recent increases in bat abundance, there is still a 31.95% decline in bat abundance from 2011 to 2017. This is not statistically significant, however, the decline represented in both the summer surveys and the winter hibernacula strongly suggests an overall decline in bat numbers since detection of WNS in Ohio.

Declines similar to that in Ohio have been seen in many states affected by WNS, and has led to the listing of additional species in various states. For example, the northern long-eared bat has been impacted to such a degree that it was listed as a federally threatened species as of April 2015 (USFWS, 2015). As this disease continues to spread west, it is likely that these declines will continue to be seen.

The strong correlation between detection rate on WNS routes and non-WNS routes also indicates that the effects to bat abundance are equivalent across the state, whether declared a WNS county or not. This suggests that WNS is indeed found throughout the state and impacts all areas equally. This data agrees with the statement by the USGS that Ohio is a WNS endemic state.

There are, however, promising studies to combat WNS. At the University of Georgia, researchers are studying *Rhodococcus rhodochrous*, a bacterium that has been shown to inhibit *Pd* spore germination in lab settings and could potentially be used in hibernacula to prevent the spores from spreading and infecting the bats (Cornelison, 2016). The biggest issue with studies on treatment is the field application. It is important to find a

treatment that can not only reduce fungal growth, but can do so without adversely affecting the bats and cave ecosystem.

Bat detection rates in 2017 were similar across the state, no matter the region of the route (e.g., southeast versus northwest). Therefore, the data can be treated as a whole in comparison with previous years. However, there was a difference in the frequency group that showed the highest abundance in 2017, with low frequency groups making up a larger percentage of the overall bat abundance in the north and mid and low frequency groups making up a roughly equal percentage of the overall bat abundance in the south. This statistic suggests a difference amongst frequency group composition between the northern and southern half of the state. When analyzing previous data, this trend has also been represented in each year since the beginning of the study. This difference in frequency group composition could be a result of habitat differences. This is difficult to determine because little is known about the specific ecology of bats in Ohio, and the data is not species-specific for the cross-year studies.

Due to the rapid change in Ohio bat populations as a result of WNS, certain parameters (e.g., most common time for bats to be detected) can only be compared within the year of study. Other limitations for analysis relate to the survey window and certain equipment malfunctions. Since most of the surveys were run in July, the bat abundance changes through seasons are not well represented. Then again, the temperature ranges, despite being limited, showed that there was a significant variation in bat activity relative to temperature. Even though the temperatures for the surveys ranged from 50° F to 85°F, there was still a significant increase in bat abundance with increasing temperature. This result may signify that average bat abundance is

dependent on temperature and would be even more apparent with wider range. Additional surveys are needed to document activity at a wide range of temperatures in order to determine if this is in fact the case.

Certain limitations to the surveys may impact the conclusions drawn from the analysis. All of the surveys are conducted on roadways. Thus, interior species, like most *Myotis*, may not be well represented (Bertinussen and Altringham, 2011). Therefore the impact on these species could be poorly estimated. Surveys from the water, in boats or canoes, have been shown to be effective in other states, and could be a potential method for future studies (Scott *et al.*, 2010).

While nine of the routes have been surveyed for all eight years, the remaining transects were added later and may only have data from two or three years. In addition, the first few years had an over-representation of routes run in the southern half of the state. For example, six of the eleven routes were in the Wayne National Forest in the year 2011. A more extensive study adding further data from all regions of the state will be needed to make more concise conclusions. This should also entail analyzing landscape-level information to determine the difference in habitat use.

Finally, the call identification software may confuse and misidentify different species due to overlaps in their call features (Adams, 2013). Therefore, the cross-year analyses in this study are limited to frequency groups, as they tend to be more accurate. In order to compare these results to other years, manual vetting would need to be done for all of the data. Calls need to be put through the analysis programs, compared, and each call that does not match needs to be looked at individually for a confirmed species identification. While reviewing the calls this year, more than half of the species identification information did not match

between the two programs. The identification software agreed far more often on the frequency group though. This information adds further emphasis to the disparity between acoustic programs and the potential inaccuracy if not analyzed by hand.

With the mixture of threats affecting bat species, there needs to be coordinated efforts put into place to mediate the danger. More research is required. Bat ecology is a poorly studied topic and has numerous gaps such as understanding migration patterns, roost site preferences, and diet composition. Resources that bats utilize need to be known if bats are to be protected.

There have also been recent studies exploring the recovery and resistance of bats to WNS. Some of these studies suggest that bats may develop a resistance over time. As certain bats that are genetically able to persist are more likely to survive long enough to pass on their genes. There has also been research suggesting roosting

adaptations in certain species, like *Myotis lucifugus*. In certain hibernacula in Pennsylvania, *M. lucifugus* have been persisting in colder portions (Johnson *et al.*, 2016). Studies like this are essential to the future of bats as they will provide the knowledge needed to properly protect bats in the United States and around the world.

Education for the public and policy makers is also crucial to the survival of bats. The more people know, the higher the chances are for the survival and persistence of bats. By increasing awareness of the positive impacts of bats, exercising proper decontamination protocols when entering and leaving a cave to decrease the potential spread of WNS, and maintaining a variety of habitats for bats allow, people can make a daily contribution to the conservation of Ohio's bat populations. It is the collective effort of all people that will allow for the continual survival of bats.

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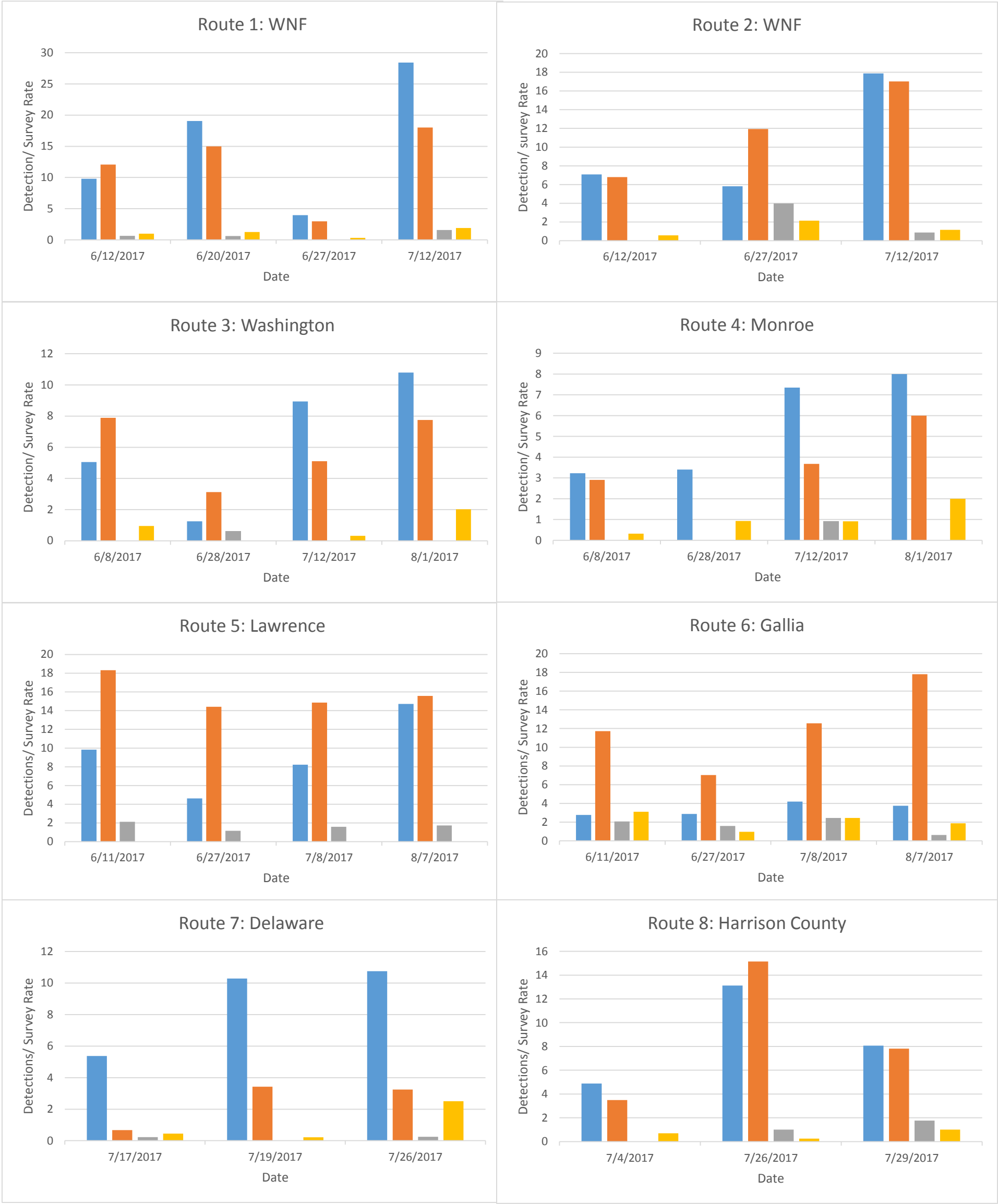
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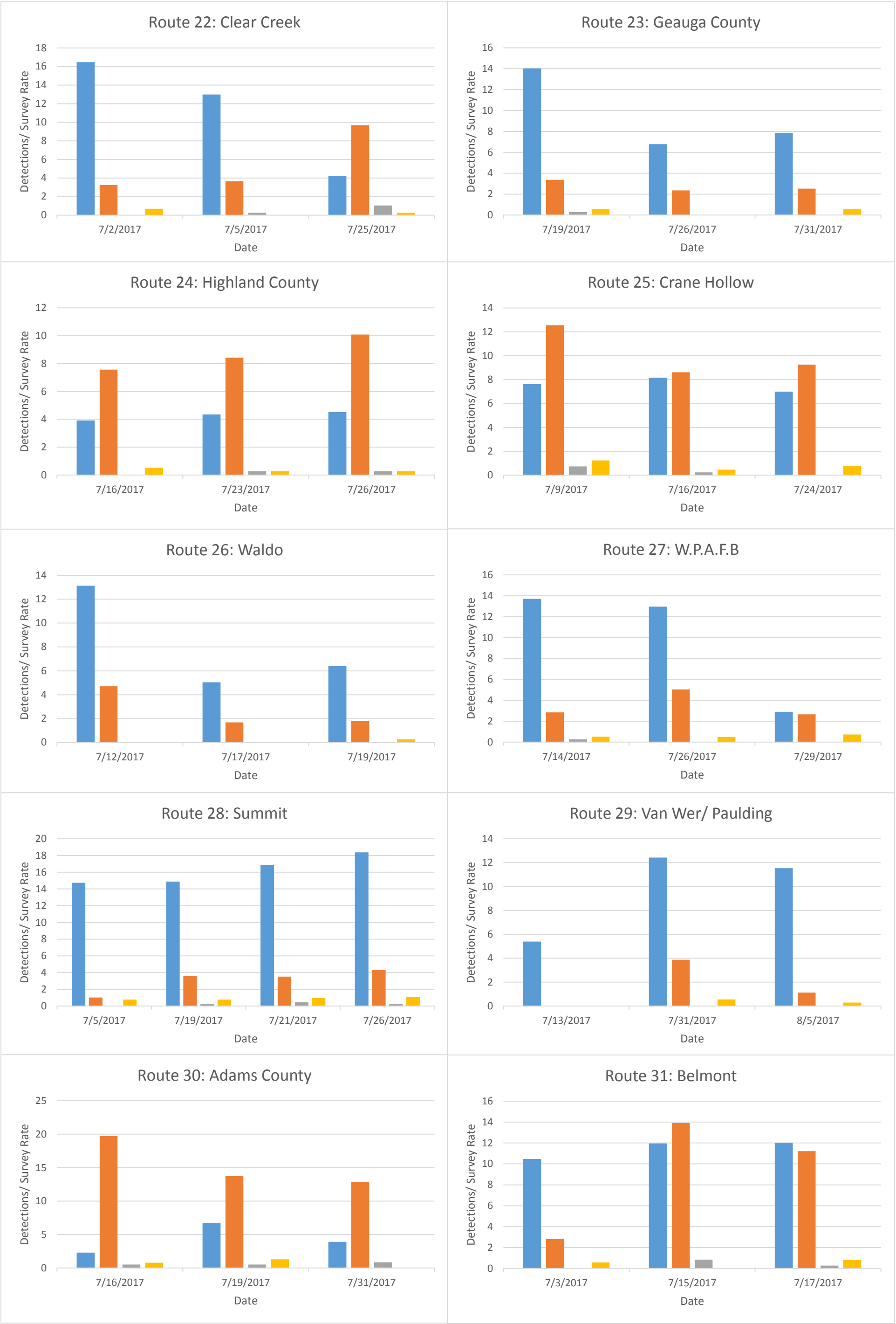
Appendices

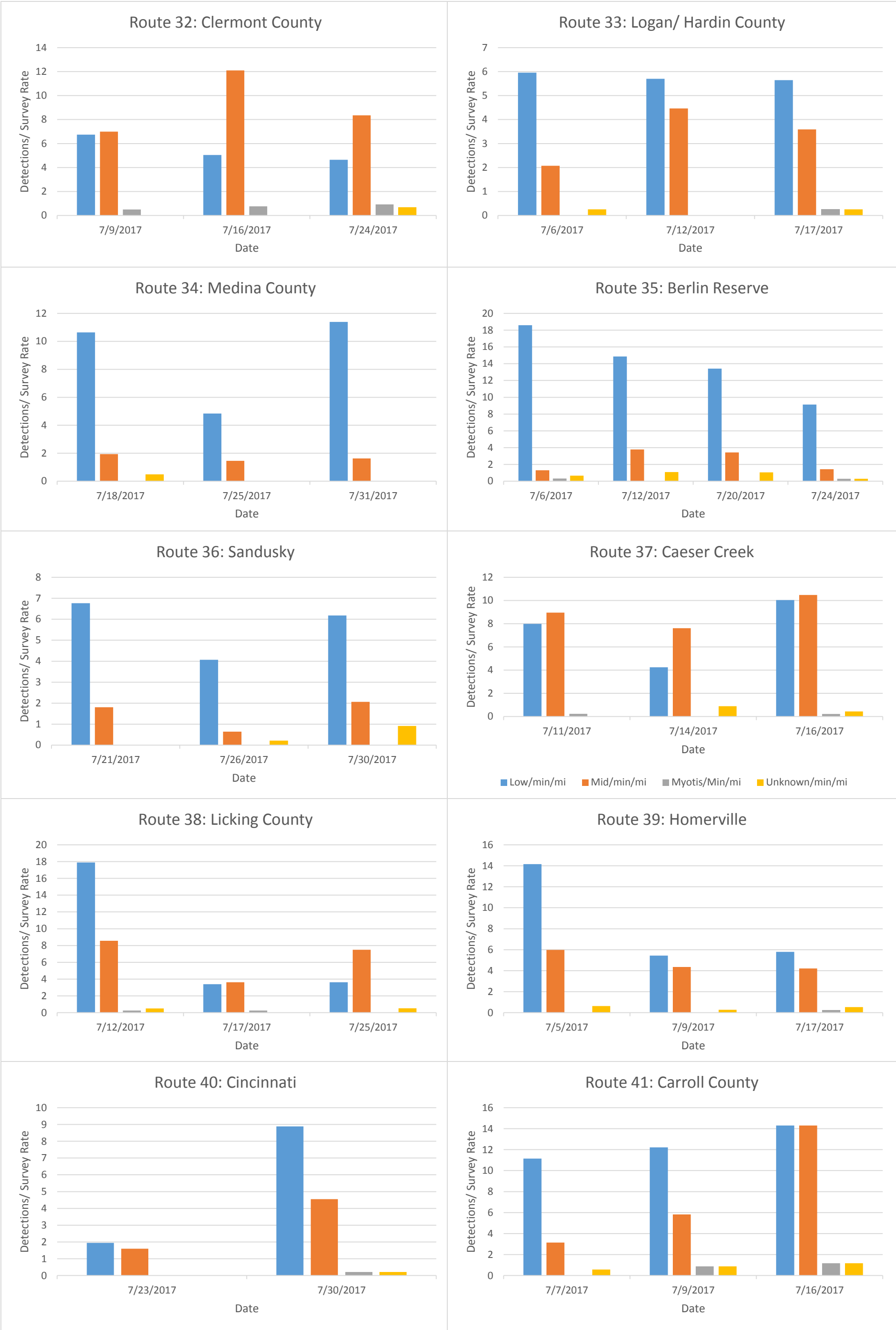
Appendix A: The breakdown of each route surveyed in 2017 into date-specific and route-specific information. All low frequency data are indicated by the blue, mid frequency data are indicated by the orange-red, *Myotis* data are indicated by the gray, and the unknown data are indicated by the yellow. Route 16 is not included as the

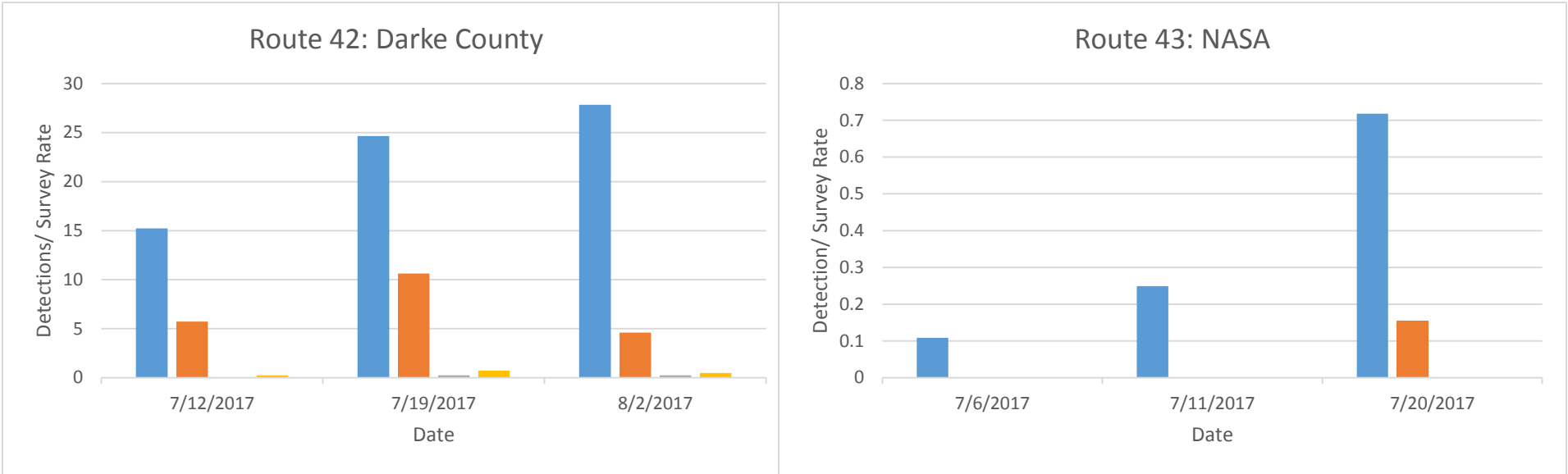


equipment did not save the information properly this year.

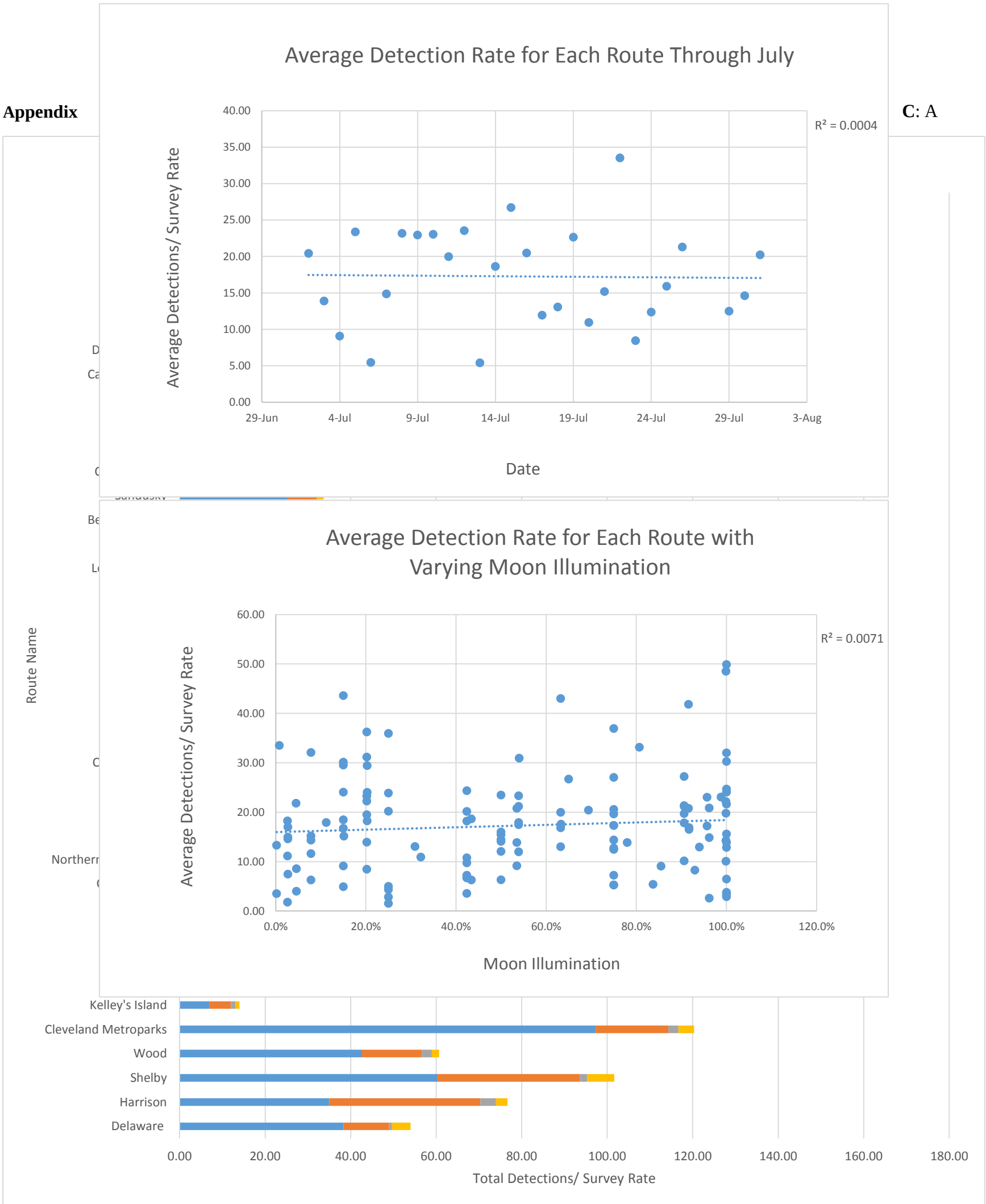






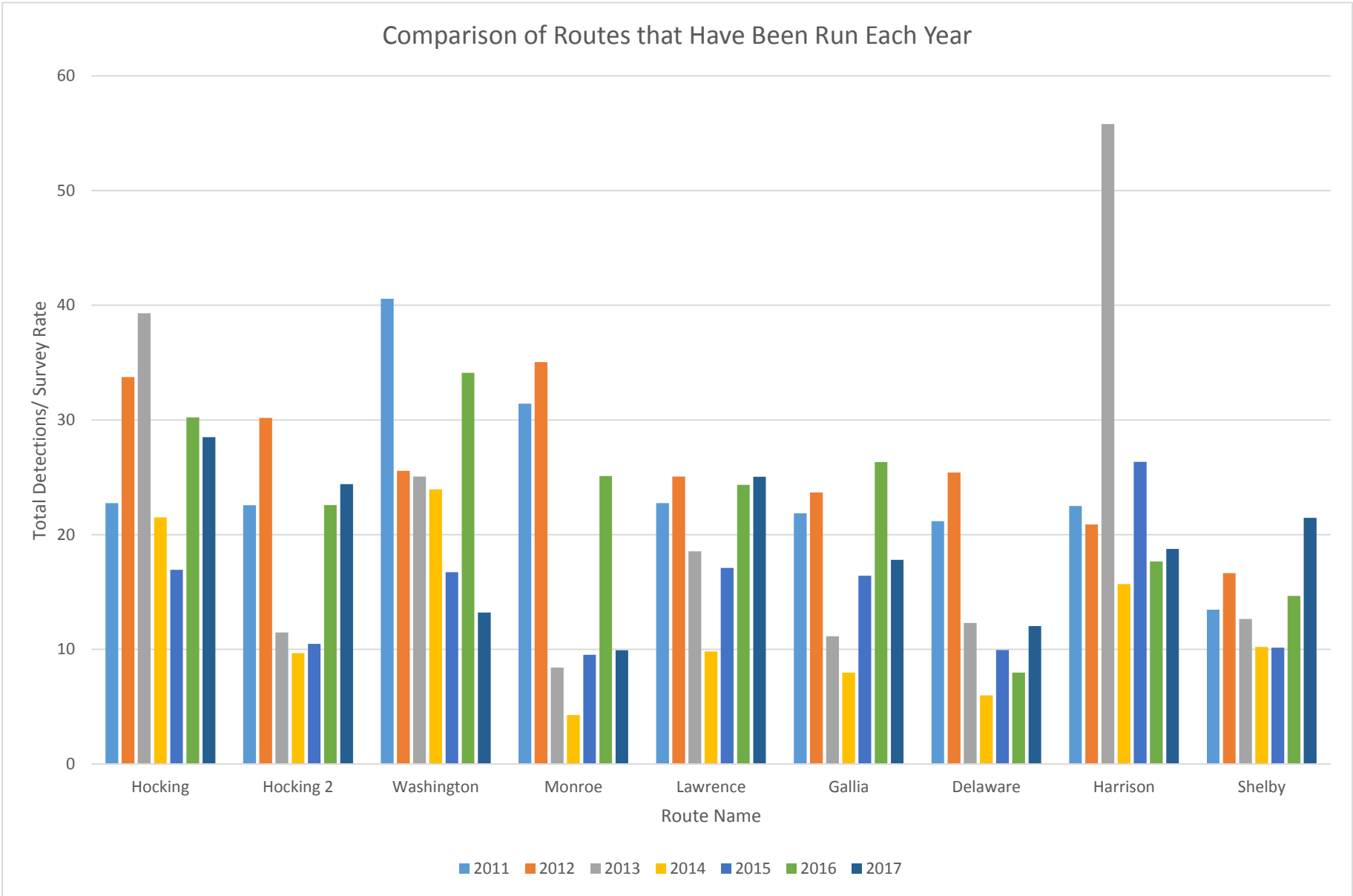


Appendix B: Models for the average detection rate for each route over time (in the month of July) and moon illumination. Neither of these relationships were significant.



comparison of the average bat detection rate for each frequency group amongst all routes run in the year 2017.

Appendix D: A comparison of the original nine routes that have been run each year between 2011 and 2017.



Appendix E:

	'11	'12	'13	'14	'15	'16	'17
Route							
1	3	3	2	3	4	4	4
2	2	3	2	3	3	3	3
3	4	3	4	4	4	3	4
4	2	3	4	3	4	3	4
5	5	4	4	3	4	4	3

6	4	4	3	3	5	4	3
7	3	3	3	3	4	3	3
8	3	3	3	3	3	3	3
9	3	3	3	3	3	4	3
10	3	3		3	4	3	3
11		2	2	3	1	3	3
12		1		3	1		
13		2	1	3	1		
14		1		3	3	3	3
15		3	3	3	4	3	3
16		3	3	3	3	3	
17		3	3	3	4	3	3
18			4	3	3	3	3
19			3	1	2	2	1
20			3	3	3	3	3
21			3	3	3	4	3
22			3	3	3	3	3
23			3	3	4	3	3
24			2	3	2	3	3
25			2	4	3	3	3
26			3	3	3	3	3
27			2	3	3	3	3
28			3	4	3	4	4
29			4	3	3	3	3
30				3	3	3	3
31				3	4	3	3
32				3	4	3	3
33				3	4	4	3
34				3	3	3	3
35				3	3	4	4
36				3	3	3	3
37				3	3	3	3
38					5	1	3
39					3	3	3
40					3	3	3
41						3	3
42							3
Total	32	47	75	112	128	122	120

