ANALYZING ANURAN DIVERSITY IN URBAN AND RURAL WETLANDS USING FROG CALL CITIZEN SCIENCE DATA

By

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A Thesis Submitted to the Faculty of the University of
Tennessee at Chattanooga in Partial
Fulfillment of the Requirements of the Degree of
Master of Science: Environmental Science

The University of Tennessee at Chattanooga
Chattanooga, Tennessee

August 2024

ABSTRACT

Amphibians are currently experiencing a crisis of high extinction rates because of habitat destruction, and climate change. Citizen science, the incorporation of citizens in data collection or management of scientific research, has become necessary to monitor populations widely. The Tennessee Amphibian Monitoring Program is a citizen science program, at the state-level that conducts manual calling surveys in Tennessee. The TAMP dataset was analyzed to see if there was a difference in diversity and abundance of anurans in urban wetlands compared to rural wetlands in the Ridge and Valley ecoregion. A Hutchenson's t-test and a Mann-Whitney U test found that the diversity scores of the two groups were not equal, with the rural wetlands having significantly higher diversity than the urban wetlands. Occupancy modeling found that rural wetlands appear to contain more sensitive and rarer anurans. The TAMP data can further facilitate our understanding of land use impacts on amphibian conservation.

DEDICATION

I dedicate this work to the memory of my cousin, Jordan Hartsell, who was a loyal friend. I also dedicate this to the memory of my grandmother, June Mills Hartsell, a great travel companion and friend.

ACKNOWLEDGEMENTS

This research was made possible by so many people that I would like to thank for their assistance, support, and encouragement throughout this process.

First and foremost, I would like to thank my advisor, Dr. Thomas Wilson, whose unwavering support and expertise have been a guiding force behind this thesis. Dr. Wilson has been a mentor in the truest sense, and I am very grateful to have had him as an advisor. I would also like to thank Dr. DeAnna Beasley for sticking with this project and being a part of my committee. Thank you to Nyssa Hunt for your GIS wizardry and being a committee member.

This project would not have been possible without Bob English, the Tennessee Amphibian Monitoring Program, and the Tennessee Wildlife Resources Agency. Thank you for collaborating and sharing your data with me.

Thank you to the late Dr. George Cline at Jacksonville State University for teaching me about the world of herpetology. Thank you to Eric Cline for taking me out in the field and showing me how to flip a log over correctly.

Thank you to my family for supporting me emotionally and financially as I continued my academic journey at UTC. My friends for cheering me on as I continued my education. To my fiancé, Shauni Barrier, thank you for your encouragement and for putting up with me talking about frogs.

Finally, I would like to recognize all the friends I made at UTC. Thanks to Megan Bertoli, Caleb Gruber, and Erin Lienhard for the countless hours studying together, your moral support, and for putting up with my harmless pranks. To William Hanson-Regan and Hunter Smith, it’s hard to put into words how much your friendship and support has meant to me during graduate school. I am incredibly grateful to have you both as friends.

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LIST OF ABBREVIATIONS

CBC, Christmas Bird Count

CCS, Cowardin Classification System

DAPTF, Declining Amphibian Population Task Force

FWS, Fish and Wildlife Service

HUC-12, Hydrologic Unit Code 12

IUCN, International Union of Conservation and Nature

MCS, Manual Calling Survey

MRLC, Multi-Resolution Land Characteristics

NAAMP, North American Amphibian Monitoring Program

NLCD, National Land Cover Data

NWI, National Wetland Inventory

TAMP, Tennessee Amphibian Monitoring Program

TWRA, Tennessee Wildlife Resources Agency

USGS, United States Geological Survey

CHAPTER I

INTRODUCTION

Citizen Science

Citizen science is when the public participates in scientific research being conducted (Heigl et al. 2019). The level of participation of citizens varies across scientific projects, but they are most often involved in the data collection (Heigl et al. 2019). Examples of citizen science could involve non-invasive efforts where participants take pictures of plants or identify bird calls (Losey et al. 2022; McCaffery 2005). Historically, citizen science has been utilized in large-scale projects. The Christmas Bird Count (CBC) has been a citizen science program for over 120 years, recruiting volunteers to identify birds within defined routes (Dunn et al. 2005). The robust data from the CBC has been used in a wide variety of scientific research (Echeverry-Galvis et al. 2023; Evans et al. 2023). The incorporation of citizen science in scientific research has been rapidly growing (Burgess et al. 2023; Silvertown 2009).

Possible explanations of this increased use of citizen science are likely due to advancements in technology and more awareness of environmental issues. Scientists might also apply citizen science as a free source of labor (McCaffrey 2005). Volunteers donate their valuable time and money in order to contribute. This time and money are valuable when sampling a large area or when sampling is under temporal constraints. The CBC, for example, monitors for a few weeks in the winter with close to 2,000 routes in North America (Dunn et al. 2005). These are possible reasons why large-scale programs might employ citizen science in their projects.

Manual Calling Surveys

Calling surveys have been shown as an effective way to measure species presence, as seen with the CBC, using auditory detections to monitor the presence of birds (Dunn et al. 2005). Auditory monitoring through calling surveys can be an effective method to study animals who communicate through vocalizing. Anurans are unique amphibians due to their ability to communicate through vocalizing. Researchers have taken advantage of this behavior as a way to conduct surveys in a given area. Manual calling surveys (MCS) were created as a way to study anuran populations at a large scale. MCS consists of an observer listening to calling anurans at a particular location. At each stop, the observer identifies the anurans vocalization and calling intensity. Local and regional monitoring programs have implemented roadside surveys as a way to effectively travel and monitor calling anurans (Dodd 2009). The North American Amphibian Monitoring Program (NAAMP) was created in 1997 by the Declining Amphibian Populations Task Force (DAPTF) in response to global amphibian declines (Weir and Mossman 2005). NAAMP created its monitoring protocol modeled after the Wisconsin Frog and Toad Survey and the North American Breeding Bird Survey (Weir and Mossman 2005). The overall goal of NAAMP was to provide scientifically accurate data for calling anuran populations. In 2015, the program was discontinued but its impact is evident through the volunteers and other programs it inspired.

The Tennessee Amphibian Monitoring Program (TAMP) was established in 2004 as a state-wide NAAMP program, partnering with the Tennessee Wildlife Resources Agency (TWRA) and Middle Tennessee State University. TAMP standardized four different observation windows for sampling, beginning in late winter and ending in summer. Its overall goal is to monitor the distribution and abundance of the native anurans of Tennessee. The data collected by TAMP has only been used in TWRA Wildlife Action Plans and by approved researchers. Tennessee’s Wildlife Action Plan focuses on understanding what species endemic to the state are of conservation concern.

Importance of Declining Wetlands

Wetlands are highly diverse ecosystems due to the different geographies and climates they can be found in (Batzer and Baldwin 2012). The definition of a wetland is ambiguous and controversial due to policymakers. Some individuals describe wetlands as ecosystems that can be defined by having permanent or seasonal flooding of water that covers the naturally occurring soils (Batzer and Baldwin 2012). The Fish and Wildlife Service (FWS) defines wetlands as having one of the following criteria: if the land predominantly supports hydrophytes, if the soils are primarily undrained hydric soils, or if the land is saturated with water temporarily, seasonally, or year-round (Cowardin and Golet 1995). Wetlands are ecologically significant habitats, playing an important role in the carbon cycle, acting as sinks and sources for sequestered carbon (Kayranli et al. 2010). Wetlands are also involved in the nitrogen cycle, with denitrification occurring in the inundated soil (Martinez-Espinosa et al. 2021). Disruptions or changes to these cycles can negatively affect anuran communities, as alterations in the water's physical or chemical properties, where amphibians spend most of all of their lives and can impact their survival, growth, and development (Dodd 2009). Studies have found that changes in the nutrient chemistry of wetlands can alter the development of aquatic juveniles transitioning to terrestrial adults (Knapp et al. 2021). These landscapes also play a role in natural filtration and flood control (Aziz and Cappellen 2021; Pattison-Williams et al. 2018). By acting as flood control and filtering out particulate matter from polluted water, wetlands increase the quality of the water present. Since anurans are highly susceptible to pollution, healthy wetland increases their richness and range in that area (Ficken and Byrne 2013).

Despite all the benefits that wetlands provide, they are declining. In 1780, it was estimated that the 48 continental states contained approximately 221 million acres of wetlands but, in 1980, there were only approximately 104 million acres (Dahl 1990). A study done by Gibbs found that wetlands in northeastern United States shifted from being clustered to being more isolated, because of urban development destroying wetlands (Gibbs 2000). The TWRA found that over 90% of Tennessee's historic wetlands have been destroyed (TWRA 2014). Some studies have even found that wetlands in protected areas, like national parks, have also experienced declines (McMenamin et al. 2008). This dramatic loss is primarily due to anthropogenic effects (Hu et al. 2017; Saha and Pal 2019).

Another ecological benefit wetlands provide is that they are habitats for a wide variety of species. Frogs and toads are species that commonly use wetlands as habitats for living or breeding. Wetlands contain water seasonally or year-round, which is essential for anurans since they deposit their egg masses in water but also need water for osmoregulation. The scientific literature has shown that there is a positive correlation between frog communities and conditions of wetlands (Jansen and Healey 2003). Anuran populations have a negative relationship with the presence of urban land and poor water quality (Knutson et al. 1999; Jansen and Healey 2003). Given the destruction of wetlands for urban areas, this creates less viable habitat for anurans, thus lowering populations.

Global Amphibian Crisis

Earth is currently experiencing extinction rates similar to previous mass extinction events (Barnosky et al. 2011). Several factors have been identified as determinants in these population declines, like habitat destruction, climate change, disease, and pollution (Barnosky et al. 2011; Beebee and Griffiths 2005; Boyle and Grow 2008). All species of animals have experienced declines; however, amphibians are declining at a more rapid pace than any other taxonomic group (Stuart et al. 2004). In 2004, approximately 32.5% of amphibian species were listed as globally threatened by the International Union for Conservation of Nature (IUCN) (Stuart et al. 2004). Many amphibians have been poorly studied, with gaps in our scientific literature on how these anthropogenic activities have affected their population dynamics. Some amphibians might be poorly studied due to their unique life histories, smaller body sizes, or small geographic ranges. These are factors that could make them more susceptible to direct or indirect human activity, which could cause extinction. This is supported by the fact that approximately 80% of the potentially threatened amphibian species on the IUCN database are unlisted under the

Endangered Species Act (Harris et al. 2012). Without some conservation efforts, Earth will continue into the 6th mass extinction event.

Within the class Amphibia, there are three orders recognized, with the largest and most diverse being Anura (frogs and toads) (Wake and Vredenburg 2008). There are approximately over 6200 species of anurans found in the world with about 116 found within the United States (Vitt and Caldwell 2014, Amphibiaweb.org). They have diverse life histories with being aquatic, semi-aquatic, or terrestrial species (Dodd 2009). Anurans have become a group of interest due to population declines because of anthropogenic activities. Their declines are important because of their ecological significance. Anurans are biological indicator species; their presence can communicate information about the environmental quality of an area (Pyke 2008; Xie et al. 2018). Anuran's unique life history and sensitive skin make them susceptible to unstable environments (Wyman 1990). Their presence could indicate to researchers if human activity is altering the environmental quality of a habitat.

Anuran Vocalization

Vocalization is a form of communication that anurans depend on (Gerhardt 1994). Anurans demonstrate this behavior when fighting over territories, being seized by predators, in response to weather, and most often for mating (Xie et al. 2018). Males will attempt to attract females to mate with advertisement calls (Bosch and De la Riva 2004). Females evaluate calling males, analyzing their call frequency and intensity to find the best mate (Gerhardt 1982; Gerhardt 1994). Size has also been shown to be a factor that influences mating in some species (Gerhardt 1982). Changes in temperature and precipitation are signals for anuran reproduction to begin (Blaustein et al. 2001). Reproduction can start as early as January and can end in late August. This is why calling programs like NAAMP run during breeding seasons when calling is at its peak.

Anuran Conservation in Tennessee

Amphibian population declines are a global issue. Anurans play an essential role in our ecosystems. While issues like habitat destruction, climate change, disease, and pollution are global issues, conservation efforts often begin at much smaller scales. The United States Geological Survey (USGS) recognizes 22 species of anurans that are native to Tennessee, which can be found all over the state. Tennessee has diverse geological features like mountains, plains, plateaus, valleys, and ridges (Mahalder et al. 2018). This wide range of physiological and geological diversity in Tennessee creates different ecosystems for native anurans. Tennessee also contains 3 major rivers that flow through the state, with some of the wetlands being connected to the watersheds of the rivers. These diverse geological and biological features of Tennessee make up distinct ecoregions. Within Tennessee there at 8 major ecoregions recognized by the USGS (Griffith et al. 1997). The Ridge and Valley ecoregion begins in New York and runs parallel to the Appalachian Mountains down to eastern Tennessee and northeast Alabama. The Ridge and Valley ecoregion is the second largest ecoregion in the state, making up approximately 18.2% of Tennessee (Tennessee Department of Environment and Conservation 2000). Forests cover about 50% of the region and the region has a high diversity of aquatic habitats (Tennessee Department of Environment and Conservation 2000). Stressors like urbanization and habitat destruction are causing changes in land use and land cover. This is why there have been transformations in the Tennessee landscape but also declines in many different populations. The Ridge and Valley ecoregion has high diversity and many different urbanized areas, making it an interesting area to study how land use affects populations. Scientists and land managers need to understand these changes and how they affect the wildlife of Tennessee.

This is one of the focuses of the TWRA’s Wildlife Action Plan, to identify species of conservation need and to keep common species common. The Wildlife Action Plans are reported every ten years, with the most recent one published in 2015. Within this report, 26 amphibians were evaluated to determine their conservation needs. Six of the 26 listed amphibians are anurans. Because of the previously mentioned anthropogenic effects causing population changes, it is likely that this number of anurans in conservation need could increase by the next Wildlife Action Plan in 2025.

Research Questions

1. Is there a difference in anuran abundance and diversity in urban wetlands compared to rural wetlands located in the Ridge and Valley region of Tennessee?
2. How does time, phenology, and observer affect the diversity scores of TAMP routes?
3. Are there any differences in Tennessee ecoregion's diversity scores from the TAMP dataset?
    a. Does the Ridge and Valley ecoregion have the lowest calculated diversity scores?

CHAPTER II

METHODS

Data Acquisition

The TAMP coordinator was inquired to share all current TAMP data in January of 2022. After a data sharing agreement was signed by the University, TAMP shared all current data up to that point which included presence data, calling score codes, and route information (TWRA-UTC ESM 9500109884). In October of 2023, the TAMP coordinator sent all current data up to that time, so data from 2004 to the current 2023 data was used in analysis. Some data was omitted because of insufficient collection. Route information would be inputted into a map frame in ArcGIS Pro 3.1.1, while the presence data and calling scores would be used in statistical testing.

The National Wetland Inventory (NWI) is a project that is led by the FWS. It was established to provide scientific information about the United States wetlands, so individuals have data to make informed decisions (Wilen and Bates 1995). The NWI assesses wetlands using satellite imagery and using field surveys for verification. The wetlands mapper on the NWI website is a public dataset that can be downloaded by state. A Tennessee wetland map was downloaded as a shapefile and processed in ArcGIS Pro 3.1.1 (Date accessed: 05/19/2022).

National Land Cover Data (NLCD) was acquired from the Multi-Resolution Land Characteristics (MRLC) website (Date accessed: 01/26/2023). The data was presented in 30-meter resolution which would be large enough for habitat delineation. Land cover data was downloaded for the years 2004, 2011, and 2019 as it represents a range for the TAMP data, its land cover during route creation in 2004 to its more current land cover in 2019. The classification of the land is modified from the Anderson Land Cover

Classification System. The four categories of development were used from the Anderson Land Cover Classification System to determine impervious surface area.

Anurans of Tennessee

Table 2.1 Native anurans of Tennessee

(Asterisk indicates species has not been assessed by the TWRA. LC stands for least concern. VU stands for vulnerable. S5 status means that a species is secure, while S1 means critically imperiled. USGS range maps determined how many counties in Tennessee a species was located in. Peak calling months are sourced from TAMP websites)

Species	Common Name	Global Status (IUCN)	State Rank (TWRA)	Counties found in TN (USGS)	Peak Calling Months
Anaxyrus americanus	American toad (Holbrook 1836)	LC	*	95/95	March
Anaxyrus fowleri	Fowler's toad (Hickley 1882)	LC	*	95/95	April- May
Acris crepitans	Eastern Cricket frog (Baird 1854)	LC	*	94/95	April- May
Acris gryllus	Southern Cricket frog (LeConte 1825)	LC	S4	16/95	April- May
Pseudacris feriarum	Upland Chorus frog (Baird 1854)	LC	*	95/95	March- April
Pseudacris triserita	Western Chorus frog (Wied-Neuwied 1838)	LC	*	1/95	March- April
Pseudacris crucifer	Spring peeper (Wied-Neuwied 1838)	LC	*	95/95	March- April
Pseudacris brachyphona	Mountain Chorus frog (Cope 1889)	LC	*	48/95	February- March

Hyla avivoca	Bird-voiced treefrog (Viosca 1928)	LC	S4	32/95	May- July
Hyla cinerea	Green treefrog (Schneider 1799)	LC	*	33/95	June- July
Hyla chrysoscelis	Cope's Gray treefrog (Cope 1880)	LC	*	95/95	May- July
Hyla gratiosa	Barking treefrog (LeConte 1856)	LC	S3	51/95	June- August
Hyla versicolor	Gray treefrog (LeConte 1825)	LC	S5	6/95	May- July
Scaphiopus holbrookii	Eastern spadefoot toad (Harlan 1835)	LC	*	94/95	June- August
Gastrophryne carolinensis	Eastern narrow-mouthed toad (Holbrook 1835)	LC	*	95/95	April- October
Lithobates areolatus	Crawfish frog (Baird & Girard 1852)	LC	S4	21/95	February- March
Lithobates capito	Gopher frog (LeConte 1855)	VU	S1	1/95	February- March
Lithobates catesbeianus	American bullfrog (Shaw 1802)	LC	*	95/95	March- August
Lithobates clamitans	Green frog (Latreille 1801)	LC	*	95/95	March- July
Lithobates palustris	Pickerel frog (LeConte 1825)	LC	*	95/95	April
Lithobates sphenocephalus	Southern Leopard frog (Cope 1889)	LC	*	95/95	February- March
Lithobates sylvaticus	Wood frog (LeConte 1825)	LC	*	55/95	January/February

Both gray tree frogs, *H. chrysoscelis* and *H. versicolor*, cannot be distinguished by visual inspection. They have an overlapping range in Tennessee appearing in the same communities. They can only be differentiated by genetic testing or by examination of their trills cross-checked with the individual's body temperature. TAMP created the *H. chrysoscelis/versicolor* complex as a category to represent this group.

The western chorus frog or *Pseudacris triserita*, has more recently been discovered in the state of Tennessee (Wied-Neuwied 1838; Lemmon et al. 2007). It has also been found to hybridize with *P. feriarum* (Lemmon et al. 2007). The distribution and status of *P. triserita* in the state is still unknown. TAMP was created before there was any information of the species' status in Tennessee, which is why it is excluded from the dataset.

Table 2.2 Tennessee Amphibian Monitoring Program observation window protocol

Observation Window	Time	Minimum Temp
1	East TN: January 10th – February 20th West TN: January 27th – March 9th	42 °F (5.5 °C)
2	Statewide: March 10th - April 15th	42 °F (5.5 °C)
3	Statewide: May 10th -June 15th	50 °F (10 °C)
4	Statewide: July 1st – August 9th	55 °F (12.7 °C)

The TAMP coordinator created the observation windows to reflect the different calling phenology of the anurans of Tennessee (Table 2.2). Given that temperature influences anuran breeding activity, the temperature during the route must exceed the minimum threshold for each observation window (Table 2.2). Adherence to protocol and consistency across volunteers is achieved by holding everyone to this standard. The nomenclature when discussing a stop on a TAMP route is indicated by the route name being followed by the stop number. This nomenclature is continued throughout this paper.

Spatial Analysis

Using ArcGIS Pro 3.1.1, TAMP data and NWI data were input into a map frame with WGS 1984 coordinate system. Given that each TAMP stop is spaced 0.50 miles (0.80 km) apart, buffers of 0.20 miles (0.32 km) were created around each point. This buffer encapsulates the predicted or possible habitats of anurans that were established when the routes were created. Using the Identify tool in ArcGIS Pro, wetlands within each buffer were identified and located. In addition to the above data, an ecoregion layer of Tennessee was acquired from the Environmental Protection Agency's website and then added to the map, which showed the locations of routes in each ecoregion (Date accessed: 04/20/2023). Next a Hydrologic Unit Code 12 (HUC-12) watershed layer and the NLCD data were input into ArcGIS Pro. The Extract by Mask tool was executed to extract the land cover rasters that correlate to the defined area of the HUC-12 watersheds. The rasters were then converted to polygons, allowing for the area associated with each polygon to be measured with the Summarize Within tool. The Summarize Within Analysis tool calculates the number of points and total area the points occupy in a polygon. By setting the area as the HUC-12 watersheds, ArcGIS Pro established the land cover of each watershed. In the attribute table, all four developed areas were summed, calculating the total impervious surface area of each HUC-12 watershed.

Impervious surfaces are human made structures that prevent water seepage into soil (Arnold and Gibbons 1996). Examples of impervious surfaces are asphalt and concrete. Based on the literature located in Table 2.3, it led to the conclusion that a wetland is classified as rural if the watershed contains less than 15% impervious surface area. An area was classified as urban if the watershed contained more than 15% impervious surface area.

Some wetlands are in urbanized areas due to development and urban planning. These wetlands are strategically kept because of their ability to be natural filters and flood control for urban runoff. However, some wetlands are accidentally created through urbanization like from stormwater, wastewater, or abandoned lowlands (Palta et al. 2017). Because a watershed contains all the area of land that surface waters converge to, wetlands are a part of these watersheds. The water enters the wetlands through precipitation and impervious surfaces. Watersheds as the classification as urban or rural was chosen because of how impervious surfaces connect water bodies and areas. GIS studies have found a significant negative correlation between urban land in watersheds and habitat quality in biological communities (Wang et al. 1997). So, using geospatial and ecological literature (Table 2.3), a threshold was created that would classify an area as urban or rural depending on the percentage of impervious surface area.

Table 2.3 Rural and urban classifications from impervious surface literature

Rural Classification	Urban Classification	Source
Less than 15% ISA in watershed	15% ISA in watershed	Sauer et al. 1983
Less than 5% ISA in a wetland contribution area	11-20% ISA in a wetland contribution area	Carlisle 1998
Less than 15% ISA in an area	35% ISA in an area is residential	University of Minnesota Geospatial Lab
Less than 8% in a watershed	15-30% ISA for Medium development in a watershed	Delesantro et al. 2021
Less than 10% ISA and more than 65% Forest Cover	Greater than 10% ISA and less than 65% Forest Cover	Booth et al. 2002

Using the literature in Table 2.3, it concluded that the two categorical groups of urban and rural were set at more than 15% and less than 15% respectively. Only 5 routes of TAMP were contained within the Ridge and Valley ecoregion. With each route consisting of 10 stops, 26 out of 50 stops were identified as some type of wetland suitable for this research. Then, using the previously set classifications, sixteen wetlands were classified as urban while the other ten were set as rural. The highest impervious surface was found in the Lower South Chickamauga Creek HUC-12 watershed with 72.69% impervious surface (Table 2.4). This watershed contained 5 of the 17 urban wetlands. Some routes were located in more than one HUC-12 watershed.

Table 2.4 Percent impervious surface area of Hydrological Unit Code-12 watersheds in the Ridge and Valley ecoregion with Tennessee Amphibian Monitoring Program routes

(Data is from 2011 National Land Cover Dataset)

HUC-12 Watershed	Route	Percent Imperviousness
Boone Lake	Buncombe	18.91%
Boone Lake South Fork	Buncombe	19.55%
Steele Creek Beaver Creek	Buncombe	31.9%
Coppinger Creek	Eastview	7.38%
Grasshopper Creek	Eastview	7.72%
Little Richland Creek	Eastview	22.79%
Little Chucky Creek	Hull-Mill	14.69%
Middle Lick Creek	Hull-Mill	8.77%
Lower South Chickamauga Creek	Tyner	71.85%
Ballpark Creek	Vonore	4.22%

The NLCD data was utilized to perform a similar process with the ecoregions in ArcGIS Pro 3.1.1. Using the Extract by Mask tool, the Anderson Land Cover Classification System was separated into each ecoregion. The rasters were then converted into polygons and land cover was summarized within. The land cover data for each ecoregion was then organized in an attribute table in ArcGIS Pro (Table 2.4). Because the Ridge and Valley ecoregion has the greatest percentage area of urban land and a low percentage of wetlands, it is predicted to have the lowest calculated diversity scores. It is of note that the Central Appalachian ecoregion has no active TAMP routes within it. It was excluded from statistical testing but included in the table below (Table 2.5).

Table 2.5 Land use differences in Tennessee ecoregions

Ecoregion	Total Area (SqKm)	Total Developed Percent Area	Total Crop Land Percent Area	Total Wetland Percent Area
Blue Ridge (BR)	6367.69	6.45	4.07	0.09
Central Appalachians (CA)	2302.06	3.10	1.98	0.10
Interior Plateau (IP)	40721.94	10.92	31.92	1.15
Mississippi Alluvial Plain (MP)	2212.42	3.18	52.11	27.68
Mississippi Valley Loess Plains (MVL)	11805.43	13.63	57.89	11.48
Ridge and Valley (RV)	1917.10	18.38	30.32	0.39
Southeastern Plains (SP)	13317.66	6.45	30.94	8.38
Southwestern Appalachians (SA)	12496.23	5.98	11.15	0.20

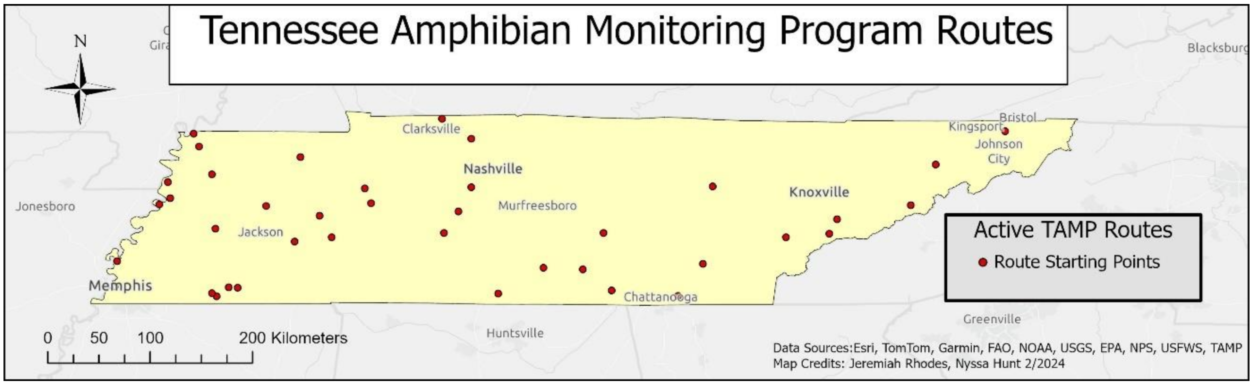


Figure 2.1 Active Tennessee Amphibian Monitoring Program routes as of 2023

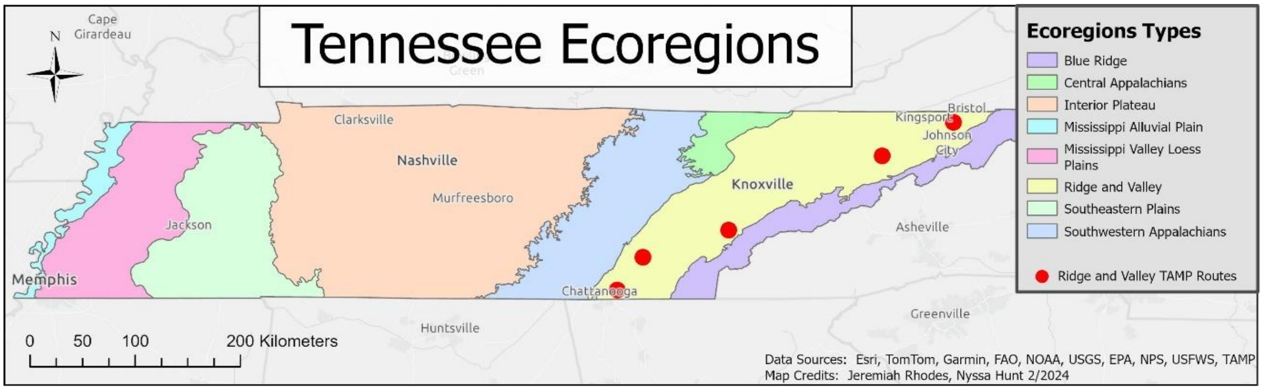


Figure 2.2 The Tennessee Amphibian Monitoring Program routes located in the Ridge and Valley ecoregion as of 2023

The NWI classified wetlands based on the Cowardin Classification System (CCS) (Cowardin and Golet 1995). This organization was created by the US FWS to systematically place wetlands into categories based on their landscape, hydrology, and vegetation. The CCS was created in order to create terminology that could be used as ecological units for comparison and as units in mapping (Cowardin and Golet 1995). The taxonomy of the CCS consists of five distinct levels: System, Subsystem, Classes, Subclasses, and Dominance Types. The System describes the overall classification of the wetland. The subsystem explains some of the hydrology of the wetland involving surface water permanence or depth of water. The class details the dominant vegetation, with the subclass and dominance type giving more detail into the vegetation. There are also special modifiers that are applied at the class level but put at the end of the CCS score for a wetland. These modifiers help describe more of the hydrology of the wetlands and are symbolized by capital letters. Utilizing the modifiers, it identifies if water is present, temporarily, seasonally, or permanently, categorizing it as a wetland according to the FWS definition.

Table 2.6 Cowardin Classification System scores of study wetlands

(Asterisks indicate space was intentionally left blank. Route and stop indicates the routes and stop number the wetland was found within. The A modifier means that the wetlands are temporarily flooded. The C modifier denotes that the wetland is seasonally flooded. F and H mean semi-permanently and permanently flooded respectively)

Route and Stop	Urban/Rural Classification	CCS-1	CCS-2	CCS-3
Buncombe-2	Urban	PFO1C	L2UBHh	*
Buncombe-3	Urban	R5UBH	PUBHh	L1UBHh
Buncombe-4	Urban	R5UBH	PUBHh	*
Buncombe-5	Urban	R5UBH	PUBHh	*
Buncombe-6	Urban	R5UBH	*	*
Buncombe-7	Urban	PEMIA	R5UBH	L1UBHh
Buncombe-8	Urban	L1UBHh	PSS1A	R5UBH
Buncombe-9	Urban	PUBFh	*	*
Eastview-2	Rural	R5UBH	PUBHh	PUBHh
Eastview-5	Rural	R5UBH	PUBHh	*
Eastview-7	Rural	PUBHh	R5UBH	*
Eastview-8	Urban	PUBHx	R5UBH	PUBHh
Eastview-10	Urban	R5UBH	PUBHx	*
Hull-Mill-2	Rural	PSS1A	R5UBH	*

Hull-Mill-4	Rural	R5UBH	PUBHh	PUBFh
Hull-Mill-6	Rural	PEM1A	PUBHh	R5UBH
Hull-Mill-7	Rural	PFO1C	R5UBH	*
Hull-Mill-8	Rural	PSS1A	R5UBH	*
Hull-Mill-9	Rural	R5UBH	PUBHh	*
Tyner-1	Urban	PSS1A	R5UBH	*
Tyner-2	Urban	PFO1A	R5UBH	PUBHh
Tyner-3	Urban	PFO1A	*	*
Tyner-4	Urban	R5UBH	*	*
Tyner-6	Urban	PFO1A	R5UBH	PUBHx
Tyner-9	Urban	PUBHh	R5UBH	*
Vonore-5	Rural	L1UBHh	R5UBH	PUBHh

The wetlands in the CCS-1 position are the wetlands of primary focus (Table 2.6). All wetlands were included in the table because of how adjacent bodies of water are connected by their hydrology and water quality (Hayashi et al. 1998). These adjacent wetlands are important to consider due to anurans possibly traveling between breeding pools.

Occupancy Modeling

The TAMP data contains presence-absence data from an amphibian calling index. There is no actual counting of individuals because it is a roadside auditory survey. Volunteers assign calling species scores based on the number of individuals heard calling. Because the goal of this study is to understand anuran populations and habitat suitability in specific areas, occupancy modeling is an effective way to answer questions about abundance. Occupancy modeling is used to estimate the occupancy of species in a sampled area while also accounting for imperfect detection and seasonality when calculating occupancy scores (MacKenzie et al. 2006). Occupancy modeling has been an effective statistical method in similar studies, which is why it was chosen for this paper (Hamer et al. 2021).

Daryll MacKenzie and colleagues created a program called PRESENCE that generates occupancy rates. This program is free to download on the USGS website (Date accessed: 02/22/2022). Using the program PRESENCE, occupancy rates were calculated for each species detected during a route in the urban or rural wetlands. A multi-seasonal occupancy model was used so all the species data could be inputted at once. A multi-seasonal model takes seasonal effects like temperature and climate into account when creating occupancy scores. Species occupancy scores were created for each species detected at a stop. Occupancy scores are calculated as percentages. These scores are compared between urban wetlands and rural wetlands. A bar graph was created to aid in the visual comparison of the occupancy scores for each species.

Statistical Testing

Using Krebs' Ecological Methodology, diversity scores were calculated from the TAMP data (Krebs 1998). The calling score code was used as a surrogate for the number of individuals. The Shannon-Wiener diversity index was used to calculate diversity scores for each wetland, and observation window, because of the familiarity with the diversity index and its widespread use in the ecological literature (Shannon and Weaver 1949). In comparison between ecoregions, a Fisher's alpha diversity index was utilized because of the larger scales of ecoregions (Fisher et al. 1943). Eighteen of the 60 routes were omitted because no data had been collected from those routes.

Diversity indices are statistical descriptions of the biodiversity of a community. These scores are comparable; however, they do not mathematically compare the data by calculating a test statistic which is then analyzed against a p-value. Hutcheson (1970) brought forth a specific t-test used to compare diversity scores between two different communities. This test would calculate if the two different scores are equal. Jerrold Zar's book Biostatistical Analysis (1984) outlines the formulas as well as other species diversity tests. Hutchenson's t-test comparing indices requires a Shannon H value for each community and the H', or variance. The null hypothesis is that the diversity scores of the urban wetlands and rural wetlands are equal. Because multiple comparisons are being made between each urban and rural wetland, a Bonferroni correction is applied to reduce type I errors (Ranstam 2016). The significance level is then set at 0.0003 from this correction. Following the formula and examples described by Zar (1984), the mathematical process was set up in Microsoft Excel to streamline the operation. Then a Mann-Whitney U test was applied to the diversity scores (Mann and Whitney 1947). This is a nonparametric test that determines if there is a significant difference between the two groups. A Mann-Whitney U test assumes that the two groups are independent and are not normally distributed (Mann and Whitney 1947). The Shannon's H diversity scores for the wetlands matched these assumptions. The alpha level for this test is set at 0.05. The program SAS 9.4 was used for statistical testing. In SAS the Mann-Whitney U test was executed using the PROC NPAR1WAY WILCOXON statement (SAS Inst 2013). The NPAR1WAY statement performs nonparametric tests. The WILCOXON procedure analysis creates Wilcoxon scores and Mann-Whitney U scores. The diversity score data of the two groups is independent, which is why a Mann-Whitney U was chosen over a Wilcoxon signed-ranked test (McDonald 2014).

To further understand the differences in diversity and composition of the different types of wetlands, a ranked abundance curve was created. A ranked abundance curve or Whittaker plot visualizes the distribution of species in a community by plotting the relative abundance of a species against its rank (Whittaker 1965). It is a useful ecological tool to analyze differences in a community structure and diversity. The rank of the species is determined by its relative abundance (Smith and Smith 2001). A species relative abundance was calculated as the summation of the species calling code scores, for each wetland type. In Microsoft Excel, the species were ranked, and a ranked-abundance curve was made. Krebs suggests using a logarithmic scale for relative abundance, so a logbase10 scale was used in all graphs (Krebs 1998). Then using Krebs' Ecological Methodology program, a theoretical curve was made from the wetlands data (Krebs 1998). A logarithmic theoretical curve was chosen because it creates a nearly straight line that represents the expected ranked abundance curve for that community (Krebs 1998: Dorigo et al. 2021). The theoretical curve is used in comparison with the calculated curve. All ranked abundance curves are compared by visual inspection. The slope of the curve indicates species evenness, and diversity is represented by the number of species on the curve.

Generalized Linear Models (GLM) are used to analyze different models of response variables (Dunteman 2006). It is a powerful statistical model that can explain the relationships between different variables. The response variable in the GLM was Shannon's H previously calculated for each observation window a route was run. The predictor variables were the observer, observation window, and year. The GLM tested to model the relationships between the response variable and the predictor variables. The null hypothesis is that the variables do not have a significant effect on the Shannon's Diversity score of the route. This assumes that the observer, sampling window, and year have no relationship with the calculated Shannon's H value. The significance level or alpha is set at 0.05. SAS 9.4 was used for statistical testing. In SAS 9.4 a GLM was run with the PROC GLM statement (SAS Inst 1989; Wolfinger et al. 1997). Normality was assessed using the PROC UNIVARIATE statement in SAS (Kolbe Ritzow 1995). A GLM was executed with all of the TAMP data to discover which predictor variables are significant. A post hoc GLM will be run to see which routes are significant. This test will again use the PROC GLM statement and will include all the data for a specific route (SAS Inst 1989).

The Fisher's alpha diversity scores were categorized by ecoregions. Subsequently, a Kruskal-Wallis test was used to determine if there was a difference between the Fisher's alpha diversity scores of each ecoregion (Kruskal and Wallis 1952). The non-parametric test was chosen because the data does not have to be normal, and variances do not have to be equal. The Fisher's alpha diversity scores for each route are independent of each other, which will not affect the outcome of the test. The null hypothesis is that the mean ranks of all the ecoregions will be the same. The alpha level is set at 0.05. Again, SAS 9.4 was used for statistical testing with the same PROC NPAR1WAY statement used for the Kruskal-Wallis test (SAS Inst 2013; Bolek and Coggins 2003). A post hoc Kruskal-Wallis will be run to evaluate the differences in diversity scores found within an ecoregion. This post hoc test will use diversity scores calculated for each year of data collected on the route. The null hypothesis is that there is no difference in the mean ranks of a route's diversity scores within an ecoregion. The alpha level is set at 0.05. This test will be carried out in SAS 9.4 with the PROC NPAR1WAY statement (SAS Inst 2013; Bolek and Coggins 2003).

CHAPTER III

RESULTS

Wetland Occupancy Scores

Occupancy scores were calculated for fifteen different species that were detected on one of the routes. The five species: *H. gratiosa*, *S. holbrookii*, *L. areolatus*, *L. capito*, and *L. sylvaticus* were not detected at any of the routes (Table 3.1). Seven different species were detected at every rural route. This was not the case with the urban routes, which had more variation in species presence temporally. A few species had little to no difference in mean and median occupancy scores in the rural or urban wetlands. These species were *A. americanus*, *A. fowerli*, and *H. chrysoscelis/versicolor* (Figure 3.1 & 3.2). Species like *Ac. crepitans*, *P. crucifer*, *P. feriarum*, and *G. carolinensis* had greater occupancy scores in rural wetlands (Figures 3.1 & 3.2). However, *L. catesbeianus*, *L. clamitans*, and *L. palustris* all had greater occupancy scores in the urban wetlands (Figures 3.1 & 3.2). The four species of *Ac. gryllus*, *H. avivoca*, *P*. *brachyphona*, and *G. carolinensis* were only detected in rural wetlands (Table 3.1). The greatest occupancy score calculated for a species was *P. feriarum*, which had an occupancy score of 0.5862 at the Eastview-2 rural wetland.

Table 3.1 Mean and median occupancy scores

(Asterisks indicate space was intentionally left blank; R.O. stands for rural occupancy while U.O. stands for urban occupancy)

Species	Mean R.O.	Mean U.O.	Median R.O.	Median U.O
A. americanus	0.1298	0.1291	0.1356	0.1429
A. fowleri	0.1061	0.0837	0.0932	0.0541
Ac. crepitans	0.2147	0.0820	0.1379	0.0345
Ac. gryllus	0.0154	*	0.0154	*
H. avivoca	0.0345	*	0.0345	*
H. chrysoscelis/versicolor	0.1722	0.1664	0.1710	0.1429
H. cinerea	0.0345	0.0762	0.0345	0.0308
H. gratiosa	*	*	*	*
P. brachyphona	0.0169	*	0.0169	*
P. feriarum	0.3276	0.2923	0.3475	0.2857
P. crucifer	0.3477	0.2584	0.3334	0.2857
G. carolinensis	0.0231	*	0.0183	*
S. holbrookii	*	*	*	*
L. areolatus	*	*	*	*
L. capito	*	*	*	*
L. catesbeianus	0.1914	0.2128	0.1610	0.2414
L. clamitans	0.2261	0.2306	0.2203	0.3243
L. palustris	0.0662	0.1710	0.0508	0.1429
L. sphenocephalus	0.1343	0.1348	0.0345	0.0690
L. sylvaticus	*	*	*	*

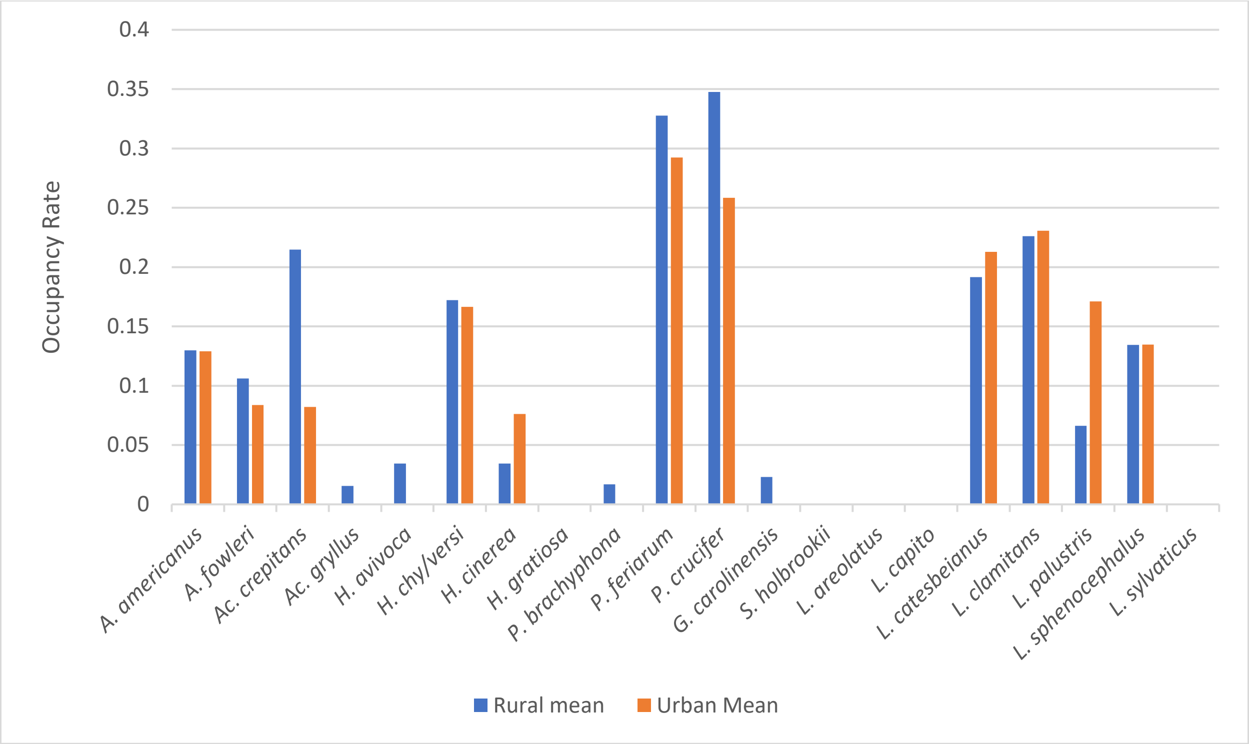


Figure 3.1 Mean comparison of occupancy scores for each species in the Ridge and Valley ecoregion of Tennessee

(Some species were not detected and were intentionally left blank)

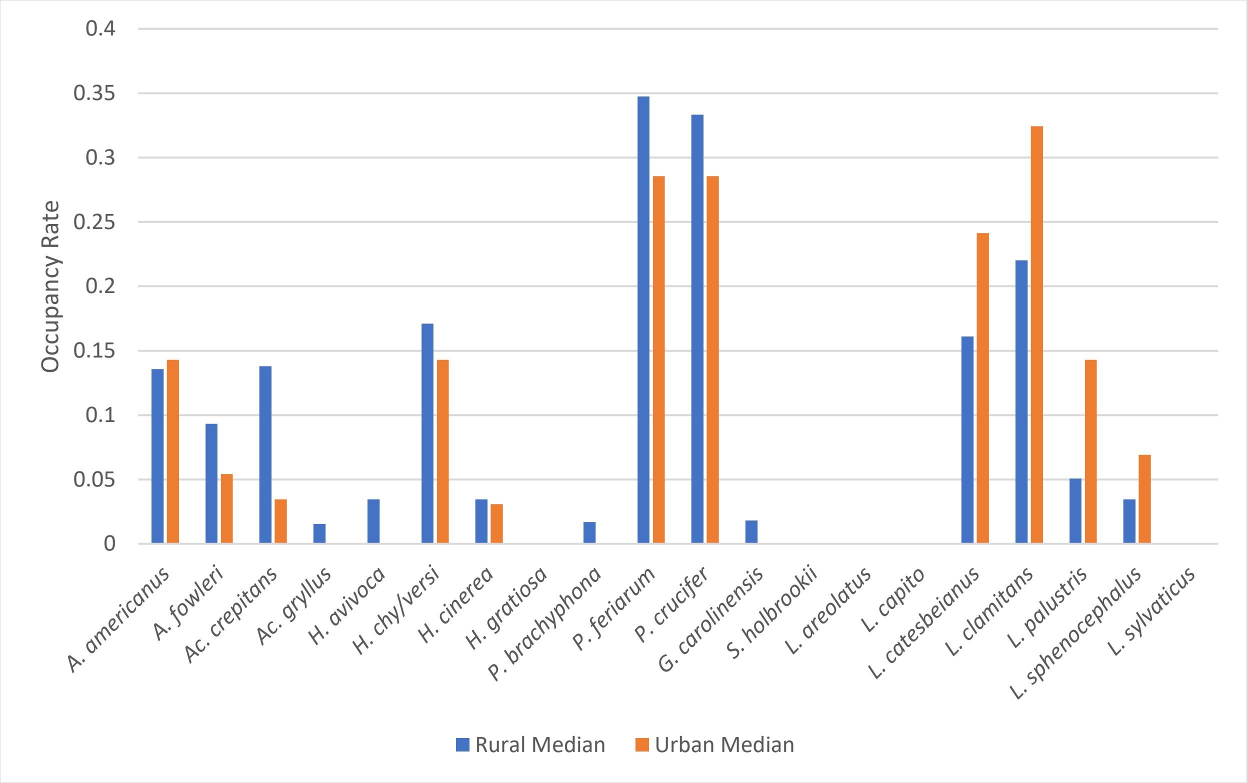


Figure 3.2 Median comparison of occupancy scores for each species in the Ridge and Valley ecoregion of Tennessee

(Some species were not detected and were intentionally left blank)

Wetland Diversity Scores

Hutchenson's t-test of comparing diversity indices was implemented because it is a comparison between two diversity scores to see if they are equal. Each urban wetland diversity score is measured against one rural wetland diversity score. In 158 comparisons of diversity scores of rural versus urban wetlands, the p-value was found to be greater than the corrected significance level of 0.0003. This did lead to the rejection of the null hypothesis, that there is no difference in diversity scores of urban and rural wetlands. However, two trials failed to reject the null hypothesis; both Vonore-5 and Tyner-9 (df=96.9, t-value= 0.38, p=0.70) and Hull-Mill-2 and Tyner-9 (df=272.7, t-value=3.22, p=0.001) had equal diversity scores. The highest diversity score calculated came from the rural wetland of Eastview-2, with a Shannon diversity score of 3.057 (Table 3.2). The lowest score came from Tyner 4 urban wetland with a score of

0.503 (Table 3.3). A Mann-Whitney U test was then conducted to assess if there was a significant difference between the two groups of urban and rural. The p-value of the Mann-Whitney U test is 0.0007. It found that the two wetland types were not equal, leading to the rejection of the null hypothesis (df=1, p-value=0.0007, n=26). The ranked-abundance curves exhibit the differences in heterogeneity of the two wetland types. The urban wetlands have greater species evenness, as seen by the gradual slope of the curve (Figure 3.3). The theoretical curve shows the expected or predicted species abundance for a calculated population. The urban slope was not as similar to the theoretical curve (Figure 3.3). Species ranked 10 and 11 exhibited higher abundance than predicted by the theoretical curve, likely due to increased evenness in urban wetlands and fewer rarer species detected (Figure 3.3). The rural wetlands exhibited greater diversity and was more similar to the theoretical curve (Figure 3.4) Species rank 10 has the greatest difference in calculated and theoretical abundance on the rural curve (Figure 3.4). After species rank 10, more rare species were detected, and the calculated curve became more similar to the theoretical curve.

Table 3.2 Diversity scores of rural wetland stops

(Route indicates the Tennessee Amphibian Monitoring Program route name while stop specifies the particular stop on the routes)

Route and Stops	Shannon’s H
Hull-Mill-2	2.709
Hull-Mill-4	2.972
Hull-Mill-6	2.557
Hull-Mill-7	2.934
Hull-Mill-8	2.781
Hull-Mill-9	2.915
Eastview-2	3.057
Eastview-5	2.897
Eastview-7	2.843
Vonore-5	2.715

Table 3.3 Diversity scores of urban wetland stops

(Route indicates the Tennessee Amphibian Monitoring Program route name while stop specifies the particular stop on the routes)

Routes and Stops	Shannon’s H
Buncombe-2	0.65
Buncombe-3	1.406
Buncombe-4	2.418
Buncombe-5	2.54
Buncombe-6	1.906
Buncombe-7	1.371
Buncombe-8	0.918
Buncombe-9	1.5
Eastview-8	3.015
Eastview-10	2.583
Tyner-1	2.059
Tyner-2	2.477
Tyner-3	2.75
Tyner-4	0.503
Tyner-6	1.956
Tyner-9	2.716

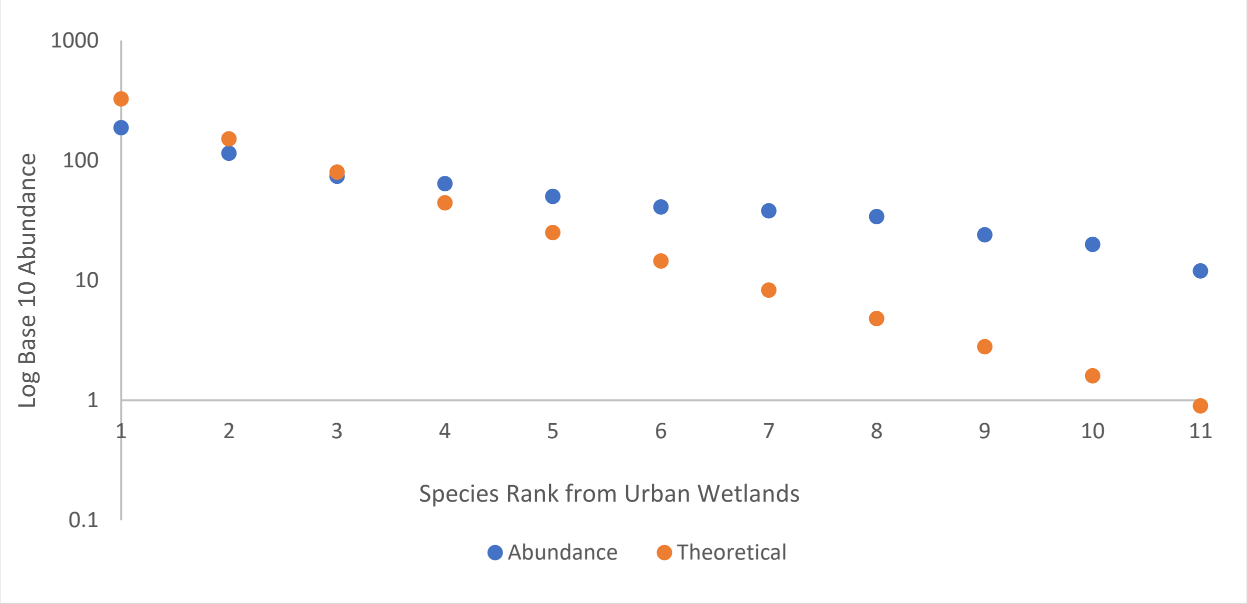


Figure 3.3 Ranked abundance curve for urban wetlands

(Theoretical curve was calculated in Krebs' Ecological Methodology program)

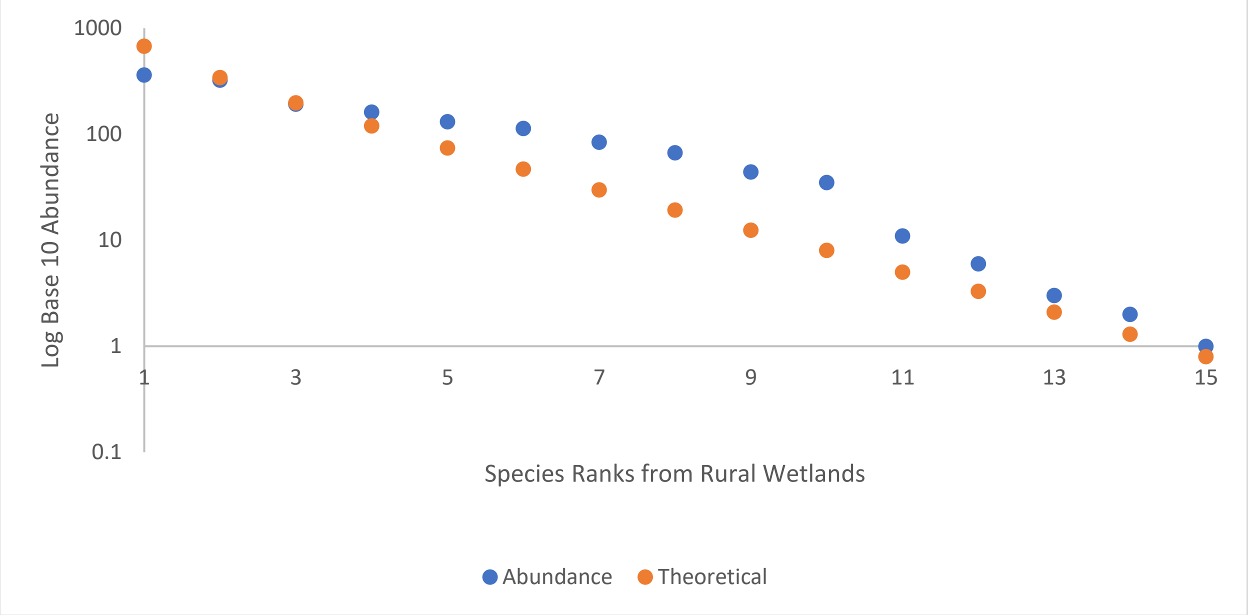


Figure 3.4 Ranked abundance curve for rural wetlands

(Theoretical curve was calculated in Krebs' Ecological Methodology program)

Table 3.4 Ranked abundance table for rural wetlands

Species	Rank
P. crucifer	1
P. feriarum	2
Ac. crepitans	3
L. clamitans	4
H. chrysocelis/ versicolor	5
L. catesbeianus	6
A. americanus	7
A. fowleri	8
L. palustris	9
L. sphenocephalus	10
G. carolinensis	11
Ac. gryllus	12
H. avivoca	13
H. cinerea	14
P. brachyphona	15

Table 3.5 Ranked abundance table for urban wetlands

Species	Rank
P. feriarum	1
P. crucifer	2
H. chrysocelis/ versicolor	3
L. catesbeianus	4
L. clamitans	5
L. sphenocephalus	6
A. americanus	7
Ac. crepitans	8
H. cinerea	9
A. fowleri	10
L. palustris	11

The urban stop Eastview-8 had the highest Shannon diversity score of all urban routes, which was 3.015 (Table 3.3). It also had the greatest number of species detected of all the urban routes. The location of the Eastview-8 stop is divided between the two HUC-12 watersheds, Little Richland Creek, and Grasshopper Creek. These watersheds had different classifications of urban and rural. The Eastview-8 stop was classified as urban because the coordinates and the closest wetland were located in the urban Little Richland Creek watershed. There are wetlands located in the Grasshopper Creek watershed that anurans could travel between for breeding. The Eastview 8 diversity score was removed, and the Mann-Whitney U test was rerun to see how it impacted the outcome. The calculated p-value after Eastview-8 was removed is 0.0001 (df=1, p-value= 0.0001, n=25). Again, this leads to the rejection of the null hypothesis.

Generalized Linear Model on Route Variables

All of the TAMP data was organized into one GLM to analyze the effect the variables year, observation window, and observer had on the diversity scores (n=863 routes run). The year variable did not have a significant effect on the response variable (F-value=1.12, p-value= 0.2893). In contrast, the observation window had the greatest statistical influence on the diversity scores (F-value=114.6, p-value < 0.0001). In addition, the observer did have a significant effect on the diversity scores, with 121 volunteers participating (F-value=13.01, p-value=0.0003). A post-hoc GLM was run to determine which routes were the most significant. Nine of the 42 routes did not create a p-value in SAS 9.4 because of insufficient data. Only three routes found that the year had a significant effect on the route’s diversity score (Table 3.6). The observation window indicated to have the greatest impact on a route’s diversity score with 21 of the 33 routes having a p-value less than the significance level set at 0.05 (Table 3.6). One route observed the volunteer to be significant in affecting the diversity scores (Table 3.6). The model was unable to calculate a p-value if only one volunteer had participated in that route.

Table 3.6 P-value table from a Generalized Linear Model of route variables

(Asterisk indicates no SAS 9.4 output was generated because of a low sample size)

Route	Year	Observation Window	Observer	Routes Run (n)
Allen	0.1305	<0.0001	0.8136	33
Bearpen Ridge	0.5821	0.9686	*	4
Buncombe	0.4699	0.0811	*	7
Cades Cove	0.1390	0.1644	0.8669	19
Carters Creek	0.3511	0.0503	0.1709	16
Como	0.1468	0.0006	0.7253	30
Cross Bridges	0.1144	0.0273	0.8835	38
Eastview	0.1381	0.0002	0.2067	29
Flewellyn	0.7247	0.0006	0.2446	51
Garret	0.7227	0.0052	0.5898	11
Gill	0.1267	0.0437	*	12
Grand Junction	0.7367	0.0064	0.4534	20
Hampton Station	0.8282	0.0369	*	11
Hickory Valley	0.0571	0.2784	0.9562	15
Hull Mill	0.0017	<0.0001	0.4092	59
JRS	0.6572	0.0046	0.4495	17
La Grange	0.4645	0.0006	0.9148	20
Linton	0.3740	0.0001	0.7497	23
Millers Cove	0.7139	<0.0001	0.0857	43
Newbern	0.0529	<0.0001	*	55
Peavine Mountain	0.5054	<0.0001	0.0041	61
Pleasant Hill	0.7082	0.9835	*	6
Shelby Forest	0.3417	0.1093	0.5169	13
Sitka	0.2280	0.0317	0.0884	11
Squeeze Bottom	0.0217	0.0692	0.1135	29
The Crossroads	0.2310	<0.0001	*	53
Towhead	0.3958	0.2552	0.4811	7
Tyner	0.8175	0.0004	0.8621	35
Upper Big Bottom	0.9045	0.0303	0.4430	20
Van Buren	0.6258	0.6252	0.4689	14
Vonore	0.0115	<0.0001	0.8642	59
Wildersville	05730	0.3125	0.4324	7
Womack	0.0843	0.4179	0.4525	12

Ecoregions

All the route data was summarized, and a Fisher's alpha diversity test was run using Krebs' Ecological Methodology program (Krebs 1998). A Kruskal-Wallis test was then executed to see if the mean ranks of the groups were the same. A p-value of 0.126 was calculated from the Kruskal-Wallis test (Figure 3.5). The null hypothesis was accepted because the p-value was greater than the alpha level set at 0.05 (df=6, p-value=0.126, n=42). There is no difference in the diversity scores between the different ecoregions.

It was predicted that the Ridge and Valley ecoregion would have the lowest calculated diversity scores. The mean and median of the diversity scores of the Ridge and Valley ecoregion were similar to other ecoregions (Figure 3.5). However, it does contain an outlier that is equivalent to the lowest whisker or box of the Blue Ridge and Mississippi Alluvial Plains ecoregions (Figure 3.5).

A post hoc Kruskal-Wallis was carried out in SAS 9.4, to evaluate the differences in diversity scores in an ecoregion. The Interior Plateau ecoregion was chosen to evaluate because it contained the most routes and data (Figure 3.6). The Kruskal-Wallis test contained diversity scores calculated for the year the routes were run. A p-value of 0.011 was calculated from the test, which is less than the alpha level set at 0.05. This results in the rejection of the null hypothesis (df=10, p-value=0.011, H=22.9). There is a difference in the calculated diversity scores found within the TAMP routes in the Interior Plateau ecoregion.

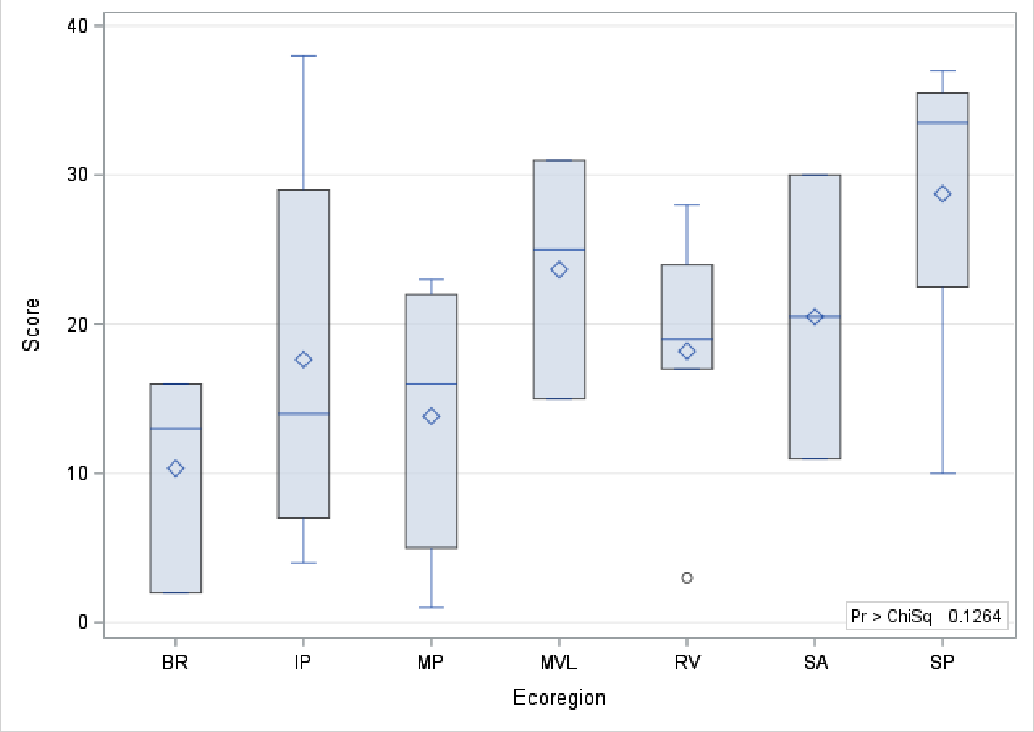
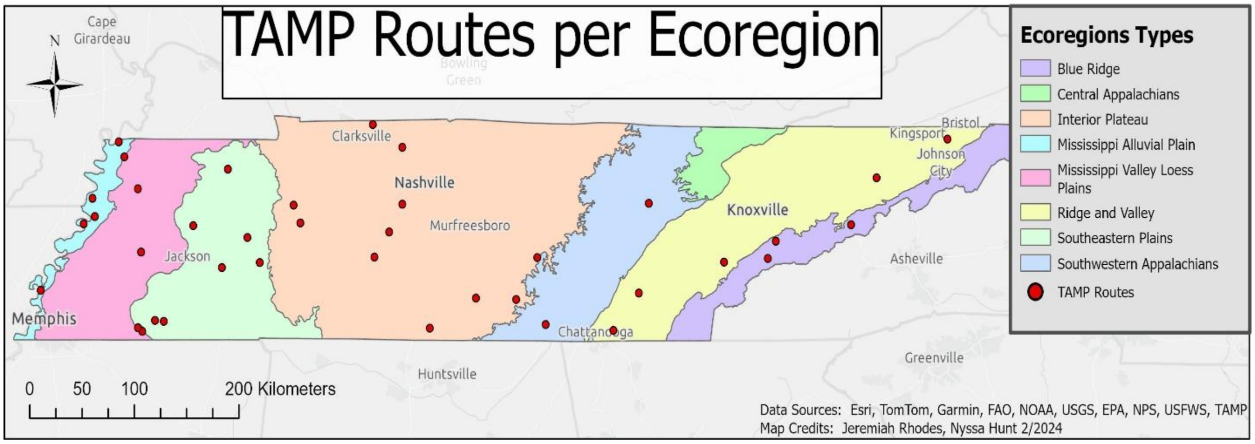


Figure 3.5 Distribution of Fisher’s alpha diversity scores of each ecoregion

(The SAS 9.4 output was unable to create error bars for some of the boxplots, this is due to some of the ecoregions having smaller sample sizes. The diamond shape on each boxplot is the mean and the line is the median)



Data Sources: Esri, TomTom, Garmin, FAO, NOAA, USGS, EPA, NPS, USFWS, TAMP
Map Credits: Jeremiah Rhodes, Nyssa Hunt 2/2024

Figure 3.6 Tennessee Amphibian Monitoring Program routes per ecoregion as of 2023

CHAPTER IV

DISCUSSION

Abundance and Diversity of Study Wetlands

Comparisons in occupancy scores found that some species had greater occupancy in rural wetlands compared to urban wetlands. Others had similar or greater occupancy in the urban wetlands compared to the rural wetlands. There appears to be a difference between most species in comparison of abundance in urban wetlands versus rural wetlands. Multiple factors can influence a species' occupancy in an area. Human development appears to be the driving factor in the change in occupancy for some species, which is important because the Ridge and Valley ecoregion has the greatest amount of human development of the ecoregions in Tennessee. These species with greater occupancy in rural wetlands can be described as sensitive species, as they are sensitive to urbanization. The calculated occupancy scores illustrate which species are more sensitive to urbanization. The species *Ac. crepitans*, *P. feriarum*, *P. crucifer,* and *G. carolinensis* all had greater occupancy in the rural wetlands, indicating that they are more sensitive to the effects of urbanization; this also appears in the scientific literature. The species. *Ac. gryllus* was only detected on two routes, both of which were rural. A study by Guzy in Florida wetlands found that *Ac. gryllus* was sensitive to urban wetlands (2012). It could be argued that this would be similar for *Ac. crepitans*, as the major morphological difference can be found in the webbing of their toes (Powell et al. 2016). Thus, it could be argued that urbanization has similar effects on both species. This may support why *Ac. crepitans* had higher occupancy in rural wetlands. Another study in wetlands surrounding the Great Lakes found that *P. crucifer* is an indicator of ecological health and was found in lower quantities in poorer wetlands (Price et al. 2007). While the Great Lakes have a different climate, hydrology, and vegetation than the Ridge and Valley region of Tennessee, that does not mean the ecological function of this species would remain consistent due to life history requirements and sensitivities. Based on the results of this research, there was a clear difference in occupancy scores of *P. crucifer* between the two different wetlands. However, it was detected more in rural wetlands, as shown by its higher occupancy score. Another species that was only detected in the rural wetlands was *G. carolinensis*. In Florida, they were found to be calling more in natural and restored wetlands but in contrast not in un-restored wetlands, which were urbanized canals reestablished into wildlife habitats (Dixon et al. 2011).

The use of diversity scores is useful in ecology because they create quantitative measures that are easily comparable between communities. They are simple to understand and communicate with the public. The decision to use diversity scores as a tool of comparison was because of their simplicity and their ability to communicate how the changes in land use can affect biodiversity. The Shannon-Wiener diversity index takes both species richness and species evenness into account when calculating an area's diversity score. The results indicate that rural wetlands have greater anuran diversity than the urban wetlands. One potential flaw in diversity indices can be found in the equal weighting of species. This can be a fault when rarer or uncommon species are detected. Ranked-abundance curves can represent heterogeneity but also how species use space in an environment (Smith and Smith 2001). This can be used to determine which species are rare. The theoretical curve on the ranked-abundance graph can also represent if an area has been adequately sampled. If a species abundance is lower than the theoretical, it was under-sampled, compared to if it's over the theoretical curve indicating the species was properly sampled; this is important when observing rarer species. The goal of wildlife agencies, like the TWRA's State Wildlife Action plan, is to "keep common species common." This goal is why it is imperative to study species of conservation concern, such as *Ac. gryllus* and *P. brachyphona*, which were documented mainly in rural routes and did not carry as much weight. The argument that rarer species carry more weight in diversity scores is because they are more sensitive, which could make them better biological indicator species. A study in Florida's cypress-dome wetlands found both *H. gratiosa* and *Ac. gryllus* to be reliable indicators for a wetland's health (Guzy et al. 2012). So even if a wetland has a higher diversity score, the species present in that wetland might also give insight into its health and structure dynamics.

It is of note that *L. capito*, and L. *areolatus* habitat range do not fall within the Ridge and Valley ecoregion, which is why they were not detected. The range of *H. gratiosa* is thought to include some of the Ridge and Valley ecoregion. The other two species that were not detected were *L. sylv*aticus which is partially located in the Ridge and Valley and *S. holbrookii* which is considered a rarer species and breeds explosively after heavy rainfall events.

Observation Window Generalized Linear Model

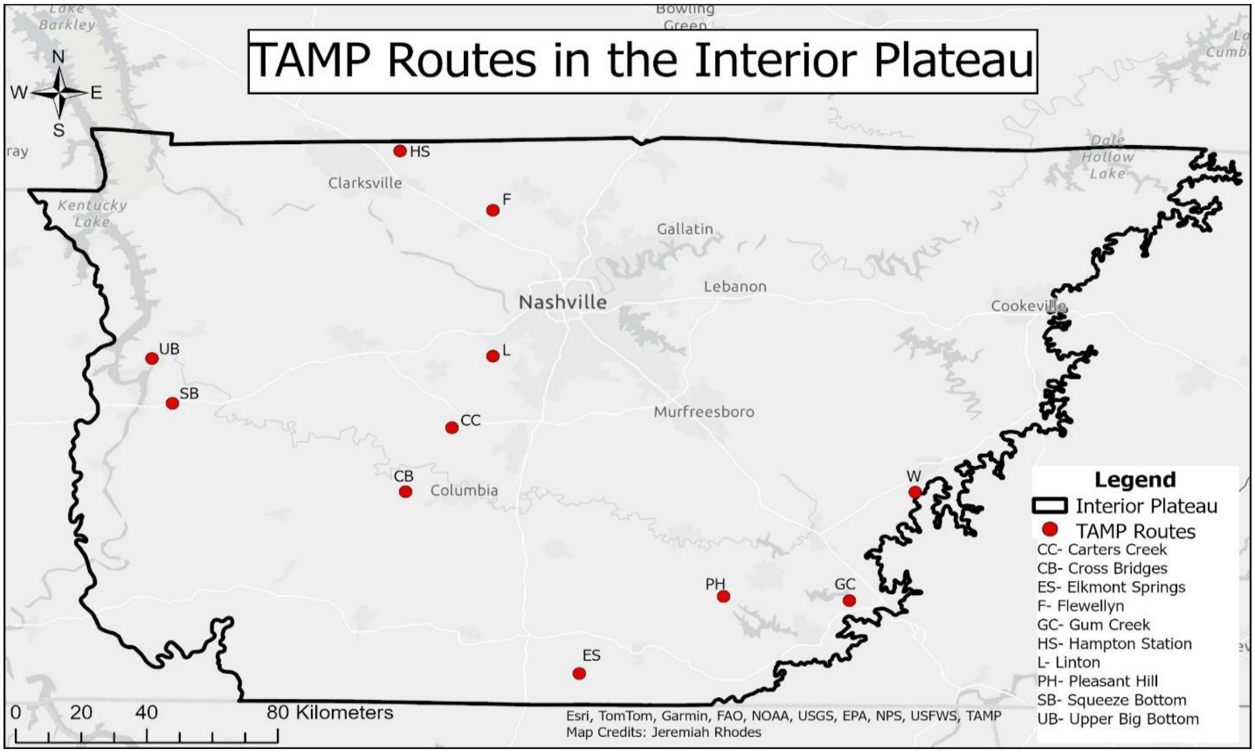
Understandably, the time of year affects species diversity due to the differences in life history and calling phenology of the species in Tennessee. NAAMP created its observation window protocol, so surveys were run during peak breeding periods for each species. This means there are four different observation windows starting in January and ending in August. State coordinators can adjust the observation windows to parallel state climate and seasonality. In Tennessee, species like *L. sylvaticus* and *P. crucifer* breed in early winter, while *H. cinerea* and *H. gratiosa* breed in the late summer; this can be seen within the TAMP data. The observation windows that had the greatest diversity scores were found in the second and third observation windows, March 10th through April 15th, and May 10th through June 15th respectively. These times have the greatest overlap in calling phenology in the state, which is why these observation windows have greater diversity. However, climate change might be transforming the calling phenology of anurans. The average global air temperature has increased over the past century, which may alter amphibians' behaviors and reproductive cues (Blaustein et al. 2001; Stocker et al. 2013). There is still some conflicting research on how much climate change has affected calling phenology (Beebee 1995; Blaustein et al. 2001).

The observer was found to have a significant effect on the TAMP dataset as a whole but during the post-hoc GLM, only one route found that the observer had a significant effect on the route's diversity scores. This suggests that the observer was found to be significant because of the differences in species distributions and the habitat of habitats between routes. TAMP has had over 121 volunteers in its 20-year history. Some routes do go through volunteers faster than others. The Peavine Mountain route, which was found to be significant, has had 13 different participants collect TAMP data. Even though this route had multiple different participants, all volunteers are trained to same way to accurately detect calling anurans species. Which is why the Peavine Mountain route yielded high diversity scores and has robust data.

Ecoregion Differences

Even though there are vast differences in land use, geography, climate, and species distribution, there was no difference found in species diversity between ecoregions. There were multiple factors considered as to why there was no difference in ecoregions. One reason is there are discrepancies found in the number of routes per ecoregion, this was likely not considered during NAAMP route creation. The Interior Plateau ecoregion contains the most routes with 11 while the Central Appalachian ecoregion does not contain a route. TAMP and TWRA should consider creating more routes in ecoregions that are deficient, to further see if there are any differences between anuran diversity and ecoregions. However, this is all dependent on finding volunteers who are willing to participate in the formation of the new routes. Another reason no difference was found in Tennessee is because it is not as diverse in anurans as other southern states like Alabama or Georgia (Mount 1975; Jensen 2008). Niemiller and Reynolds documented the amphibian diversity between ecoregions (2011). They found that the Interior plateau had the most diversity with 19 species and the Central Appalachians and Blue Ridge Mountain ecoregions had the least amount of anuran diversity with 14 species (2011). This is why some trends were noticed in differences in diversity between ecoregions, but these trends were not significant or strong enough. It is also likely that the range maps in Niemiller and Reynolds Amphibians of Tennessee are not up to date on changes in species ranges (2011).

The Interior Plateau is the largest ecoregion and has the most TAMP routes with eleven. Some routes in the Interior Plateau are more closely related in proximity (Figure 4.1). This can be seen by four volunteers participating and collecting data on two or more routes in the Interior Plateau. Even though some routes are close and have shared volunteers, there is a difference in diversity scores found within the ecoregion. One reason there could be a difference in diversity scores is because some routes have lower sample sizes. Three of the eleven routes have been active for less than 3 years. Another reason that could explain the differences in diversity scores in the ecoregion is differences in land use on the routes. Four of the eleven routes are found in or partially in watersheds that would be classified as urban. Both the routes of Linton and Hampton Station are found in the suburbs of Nashville and Clarksville respectively (Figure 4.1). The high percent area of urbanized land in those watersheds has likely affected the occupancy and diversity of the anurans on those routes. A future project could involve studying urbanization and land use changes on anurans in the TAMP dataset in the Interior Plateau ecoregion of Tennessee.



Esri, TomTom, Garmin, FAO, NOAA, USGS, EPA, NPS, USFWS, TAMP
Map Credits: Jeremiah Rhodes

Figure 4.1 The 11 Tennessee Amphibian Monitoring Program routes in the Interior Plateau as of 2023

Review of Citizen Science and TAMP's Response

Within the use of citizen science, there is criticism and backlash to the accuracy of its data. Some scientists doubt the accuracy of scientific data that was collected by non-specialist volunteers (Silvertown et al. 2013). They may also cite that volunteers are more inconsistent in identifying species due to their lack of training or they are more likely to commit sampling bias, unlike a scientific professional. These concerns are valid because of their ability to ruin data and experiments. This is why citizen science projects create measures to eliminate these concerns and biases. Volunteers of TAMP are required to take an assessment every year to test their calling identification. They may also use resources like the TAMP website or the Atlas of Tennessee Amphibians. These resources can help them practice or while they are out in the field. Volunteer training is an influential factor in increasing the quality of data (Brown and Williams 2019). The TAMP website contains an online workshop where listeners can sharpen their skills by watching informative videos. TAMP does an adequate job of preparing volunteers to record accurate scientific data.

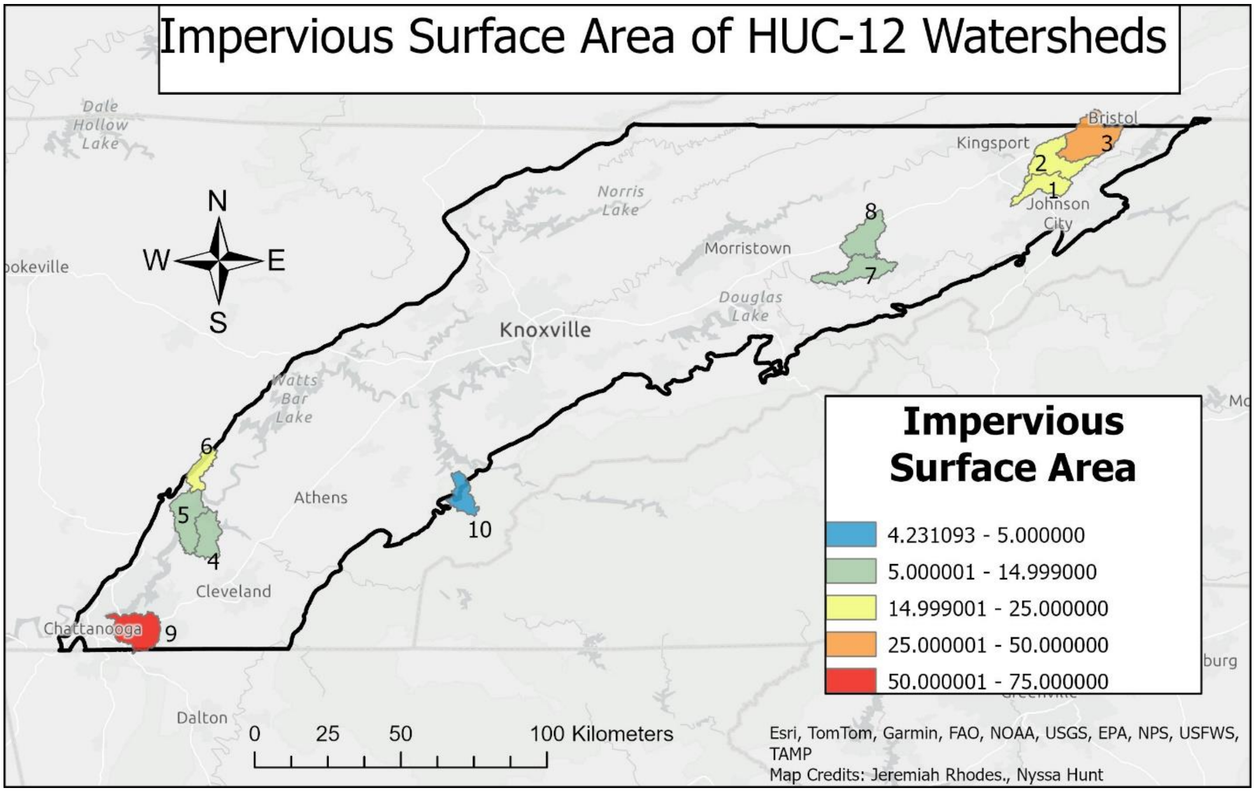
Previous studies have found that citizens can accurately detect calling anurans to a high degree. A study done by Genet and Sargent asked participants in the Michigan Frog and Toad Survey (MFTS) to identify calling species on an audio CD (2003). The majority of species were accurately recognized by over 80 percent of participants. One species was only distinguished by 60 percent of participants, which is thought to be so low because they are not found in most of Michigan (2003). As technology advances, it will be easier for citizens to practice and identify calling anurans in the field. Citizen science possesses the potential to advance scientific inquiries. It has shown that it is an effective and accurate source of data collection. As scientists look ahead, they should continue to embrace the use of citizen science as a way of engaging the community.

Impervious Surfaces and Wetland Delineation

The use of impervious surfaces as the characteristic to classify urbanization was chosen because it is a direct indicator of urbanization (Gong et al. 2019). The use of population or housing density was considered as the characteristic of urbanization. However, impervious surface was chosen because there is extensive research on how impervious surfaces categorize an area as urbanized and how impervious surfaces affect water quality (Arnold and Gibbons 1996; Lu and Weng 2006; Hall and Hossain 2020). There are quantifiable measures of impervious surfaces calculated from satellite imagery. The negative effects of impervious surfaces are well known- increased stormwater runoff, increased non-point source pollution, increased erosion, and habitat degradation (Arnold and Gibbons 1996; McDonald et al. 2008)

Impervious surfaces have negative effects on water quality. Studies in stream ecology have found that negative effects on stream health appear when imperviousness in a watershed becomes 10% (Arnold and Gibbons 1996). Once the percentage of impervious surfaces reaches 30%, the watershed is classified as fully degraded (Arnold and Gibbons 1996). A Wetland Ecological Integrity assessment from the Massachusetts Coastal Zone Management classified 10-15% impervious surface area in a watershed as detrimental. Creating changes in hydrology, biodiversity of amphibian, fish, and plant populations, and in water quality (Carlisle 1998). Impervious surfaces have a clear negative effect on watersheds and are caused by urbanization.

Previous scientific literature in stream and watershed ecology led to the classification of urban as 15% imperviousness in watersheds. In Sauer et al. study of watersheds, they used 15% impervious surfaces as their classification as urban (Sauer et al. 1983). The Massachusetts Coastal Zone management classified 11-20% imperviousness in freshwater wetlands as medium-high density in landscape (Carlisle 1998). The University of Minnesota Geospatial Lab used less than 15% imperviousness in an area to classify it as rural (University of Minnesota 2013). The locations of the routes in the watersheds that were classified as urban can also be seen as closely located to urbanized cities (Figure 4.1). Both the Tyner and Buncombe routes are located outside the Chattanooga and Johnson City areas respectively. Creating categories can be controversial, however, 15 % imperviousness appears to be a good threshold for urbanization and negatively impacts environmental factors.


Impervious Surface Area of HUC-12 Watersheds

Esri, TomTom, Garmin, FAO, NOAA, USGS, EPA, NPS, USFWS, TAMP
Map Credits: Jeremiah Rhodes., Nyssa Hunt

Figure 4.2 Impervious surface area of Hydrological Unit Code-12 watersheds of the Tennessee Amphibian Monitoring Program routes with wetlands in the Ridge and Valley ecoregion of Tennessee

(The numbers on the map correspond to Table 2.4)

Tennessee Conservation and Review of TAMP Data

Within the TAMP data, the species that were detected the most were *P. crucifer* and *P. feriarum*. The least detected species were *L. capito*, *L. areolatus*, and *S. holbrookii*. This matches the Atlas of Amphibians in Tennessee created by William H Redmond (1986). There is little record of both *L. capito* and *L. areolatus*, these species being recorded in Tennessee, they are present in small populations (https://www.apsubiology.org/tnamphibiansatlas). This has last been updated in 2019 with no new county records. Both *P. crucifer* and *P. feriarum* are found all over the state and begin breeding in early spring, which is why they have been detected had such a high rate.

During the data acquisition process, TAMP did not have all the data entered into spreadsheets. This missing data includes temperature, cloud cover, wind ratings, number of cars, and other data located on the TAMP data sheets. Some of this data was also omitted from the data sheets by volunteers. This data would have been used in the GLM to look at other variables that could have affected diversity scores. However, this was not able to be examined.

TAMP has been a citizen science project with the TWRA for 20 years. This effort could not be done without the countless hours volunteers have spent working on this project. However, TAMP is still actively seeking volunteers. Only 17 of the 37 currently active routes had volunteers participate in 2023. A common challenge of citizen science projects is keeping active volunteers. It is common for volunteers to drop out because of time constraints (Frensley et al. 2017). The TAMP coordinator also observed a drop in volunteers during the 2020 COVID-19 pandemic, which was also seen in other citizen science (Kishimoto and Kobori 2021). The reason many citizens engage in citizen science is because of their interest in the environment (Frensley et al. 2017). Studies on citizen science have found the way to keep volunteers engaged in citizen science projects is to provide feedback to participants and to encourage communication between volunteers (Mintz et al. 2023). The more experience a volunteer has in a citizen science project affects how long they participate (Frensley et al. 2017). By implementing some type of social interaction between and among participants, could help TAMP keep volunteers. The longer they stay volunteers, the more likely they will continue volunteering.

TWRA's Wildlife Action Plan, lists six species of anurans that were evaluated in 2015 as species of concern. Two of those species, *L. capito* and *L. areolatus* are species that were not detected at any of the wetlands. This is because the species ranges do not overlap with the Ridge and Valley ecoregion. One species was not detected, *H. gratiosa*, but it is located partially in the Ridge and Valley ecoregion. The other three species were *H. versicolor*, *Ac. gryllus*, and *P. brachyphona*. All three of these species had greater occupancy scores in the rural wetlands compared to the urban wetlands. Both *Ac. gryllus* and *P. brachyphona* were only detected twice at two of the rural routes. With continued land use changes, a trend might emerge of these rarer species in Tennessee becoming species of concern or going locally extinct. Understanding the changes in land use of species is one of the first steps in environmental conservation.

CHAPTER V

BROADER IMPACTS

The TWRA's goal with the Wildlife Action Plans is to keep "common species common." To accomplish this goal, the TWRA should assess all species populations, including amphibians, to better understand how anthropogenic effects like climate change and habitat destruction are negatively affecting population dynamics. This idea could be costly, as funding is an issue with the TWRA Wildlife Action Plans. So, they could create or collaborate with more citizen science programs that could help with the data collection. This would help TWRA accumulate data but also create more community involvement in state environmental issues. Citizen science programs have the ability to engage the community, raise awareness of environmental issues, and inspire leaders.

Even though Tennessee has lost over 90% of its historic wetlands, wetlands are still obtaining pushback from State Representatives. Bill HB1054/SB0631 was introduced in January of 2024, which prohibits the Tennessee Department of Environment and Conservation from classifying water as a wetland unless classified as a wetland under the Clean Water Act (HB1054/SB0631, 2024). The Tennessee Department of Environment and Conservation found that this will put more than 432,000 acres of wetlands at risk, which is approximately 55% of Tennessee wetlands (Tennessee Fiscal Review Committee, 2024). This bill was brought to committee in February of 2024 to be discussed. Due to this discouraging development, it is more important now than ever to use research like this to protect the remaining wetlands. By preserving these wetlands, it will also protect declining amphibian populations.

The future directions of this research should focus on confirming the validity of the findings in differences in urban and rural wetlands with anuran calling scores by being replicated in different ecoregions or states. The TAMP data has not been exhausted in the questions of how land use or changes in land use have affected the anuran populations. Further, many national programs or state programs still conduct MCS. The comparison of this data could help biologists, wildlife managers, and government officials understand how the changes in land use affect biodiversity and wetlands.

Manual calling surveys are a unique way to monitor a population because observers can identify species without visual or physical detection. This survey method is ideal for a citizen science project whose focus is to monitor amphibian populations over a large scale of time. However, TAMP does not have any physical count of these species detected in the wetlands. A future project could involve conducting a species inventory of each urban and rural wetland. A systematic count of individual species would occur which would put quantifiable numbers behind the differences between the two types of wetlands. Data on vegetation and water quality could also give insight into how urbanization affects each wetland. Issues could arise from landowners; however, the uniformed need to be educated on how it is our responsibility to protect and conserve biodiversity as it changes because of anthropogenic effects. May the melodious chorus of frogs echoing through our wetlands, be that call to action.

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APPENDIX A

TAMP DATASHEET

Name and Contact Information

Please complete contact information below to notify us of any changes.

Name :

Street Address:

City, State, Zip Code:

Phone: **Email:**

Instructions:

Please be sure to complete the entire datasheet.

Each datasheet represents one person's frog call observations. If you have an assistant, he/she can assist with the environmental data (e.g. air temp, count cars, etc.) but not with what frogs are heard.

Visit stops in 1-10 order. If unforeseen circumstances require you to skip a stop, write that on the datasheet.

At the start and finish of each survey record the time, wind, and sky conditions (see codes to the right).

At each stop listen for 5 minutes, then record the amphibian calling index for each species heard. Report only the species you are confident that you heard. If a species varies in calling intensity over the listening period, report the highest calling index level you heard.

At each stop, also report the environmental data requested: start time, air temperature, noise conditions, moonlight, and number of cars that passed while listening.

There are two kinds of noise disturbance questions:

- Was noise a factor? This is asking if background noise impacted your ability to hear. If yes, check the box.
- "Did you take a time out?" If an unexpected noise disturbance happens (such as a train) that lasts a minute or more, you may interrupt the 5 minute listening period to ignore the sudden disturbance. Finish up the listening time after the disturbance has passed. Do not include this type of noise in the "was noise a factor" question.

Index and Code Definitions

Amphibian Calling Index

1	Individuals can be counted; there is space between calls
2	Calls of individuals can be distinguished but there is some overlapping of calls
3	Full chorus, calls are constant, continuous and overlapping

Sky codes

0	Few clouds
1	Partly cloudy (scattered) or variable sky)
2	Cloudy or overcast
4	Fog or smoke
5	Drizzle or light rain (not affecting hearing ability)
7	Snow
8	Showers (is affecting hearing ability) do not conduct survey

Wind Codes

0	Calm (<1mph) smoke rises vertically
1	Light Air (1-3 mph) smoke drifts, weather vane inactive
2	Light Breeze (4-7 mph) leaves rustle, can feel wind on face
3	Gentle Breeze (8-12 mph) leaves and twigs move around, small flag extends
4*	Moderate Breeze (13-18 mph) moves thin branches, raises loose papers * Do not conduct survey, unless in Great Plains states
5**	Fresh Breeze (19 mph or greater) small trees begin to sway **Do not conduct survey –ALL REGIONS

Paperwork Reduction Act Statement: A Federal agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a currently valid OMB control number. Public burden for the collection of this information is estimated to average 7 hours per response. Comments regarding this collection of information should be directed to the Bureau Clearance Officer, U.S. Geological Survey, 807 National Center, Reston, Virginia 20192.
OMB NO. 1028-0078 Expiration Date: 7/31/2011

Insert sampling windows or mailing address here

Comments:

Run Information	**Start**							**Finish**						
Time (Military)														
Wind (Beaufort Scale)	0	1	2	3	4	5		0	1	2	3	4	5	
Sky (See Code Explanations)	0	1	2	4	5	7	8	0	1	2	4	5	7	8
-optional- # days since last rainfall:														

Per Stop Information

Stop #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Start Time (Military) -Optional-										
Air Temperature *Select Scale* ☐ **°C** ☐ **°F**										
Was noise a factor? (use index)										
Did you take a timeout? (check if yes)										

Species ↓ Stop # →	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**

Moon or Moon-light visible: y,n										

Check if Snow Cover: (optional)

Number of cars that passed:

If you have any additional notes, please write them in the box provided on the front of this sheet. Thank you for your participation!

VITA

Jeremiah Edward Rhodes was born in Decatur, Alabama in 1998. He grew up in Hartselle, Alabama surrounded by his loving family. He graduated from Hartselle High School in 2017. Jeremiah attended Jacksonville State University in the fall of 2017. During his time at JSU, he became interested in herpetology and worked on a research project with Dr. George Cline. In the spring of 2021, Jeremiah graduated from Jacksonville State University. Summer of 2021, he was accepted into the Environmental Science program at the University of Tennessee at Chattanooga under Dr. Thomas Wilson. Jeremiah graduates with a Master of Science degree in Environmental Science in August 2024. He hopes to continue to work in herpetology focusing on amphibian conservation.