MODELING SUITABLE HABITAT FOR THE COMMON MUDPUPPY (*NECTURUS MACULOSUS MACULOSUS*) IN INDIANA, USA UTILIZING REGIONAL DATA AND ENVIRONMENTAL DNA

Payton Nicole Fischer

Submitted to the faculty of the University Graduate School in partial fulfillment of the requirements for the degree Master of Science in the Department of Geography, Indiana University

May 2020
Accepted by the Graduate Faculty of Indiana University, in partial fulfillment of the requirements for the degree of Master of Science.

Master's Thesis Committee

____________________________________
Vijay Lulla, Ph.D., Chair

____________________________________
Jeffrey S. Wilson, Ph.D.

____________________________________
Aniruddha Banerjee, Ph.D.,
AKNOWLEDGEMENTS

I would like to thank the IUPUI Geography Department and members of my thesis committee, Vijay Lulla, Ph.D., Jeffrey Wilson, Ph.D., and Aniruddha Banerjee, Ph.D., for their assistance and guidance throughout this project. Eric McCluskey, Ph.D. provided guidance and advice on creating the model for this project.

Emily McCallen, Ph.D. helped get my thoughts organized on how to go about creating this thesis project. She pointed me in the direction of many good articles and contacts that helped shape this project. Data contributors were The Williams’ Lab, HerpMapper, Indiana Heritage Database, Obed Hernandez-Gomez, Ph.D., Greg Lipps (OSU), Megan Gramhofer, and Nathan Engbrecht. Dr. Robert Brodman was a sounding board for my ideas and organized my presentation at the MWPARC meeting in Iowa. Before entering graduate school, he instilled a foundation of research and an overall passion for amphibians and reptiles.

My husband was a great support system as we got married and had our first child all while working on this thesis. I could not have done this without his calming guidance and encouragement.
MODELING SUITABLE HABITAT OF THE COMMON MUDPUPPY (*NECTURUS MACULOSUS MACULOSUS*) IN INDIANA, USA UTILIZING REGIONAL DATA AND ENVIRONMENTAL DNA

The distribution of the Common Mudpuppy (*Necturus maculosus maculosus*) is widespread but greatly understood. It is assumed that mudpuppy populations are declining due to poor habitat quality. However, there is not enough data to support this claim. The distribution of the mudpuppy is throughout the entire state, but only 43 of the 92 counties in Indiana have records. This project utilized habitat suitability modeling, focused on Indiana, to gain a better understanding of their distribution within the state. Data from Ohio and the Salamander Mussel (*Simpsonais ambigua*) were included to bolster the dataset. Environmental DNA was included to validate the model. Variables used in this analysis were Strahler Stream Order, distance to forest, percent agriculture, and tree canopy cover. Results showed that stream orders 4 to 6, a shorter distance to forest, less agriculture, and 30 to 40% of tree canopy cover was what contributed to suitable habitat. Stream order was the variable that contributed to the model the most. The areas of suitable habitat found were the HUC08 sub-watersheds in the northeastern and southwestern corners of the state. These areas included 19 counties were there were no previous records of mudpuppies. Environmental DNA showed that the negative samples were not found in suitable habitat. Further supporting the predicted area of suitable habitat. It is recommended that conservation efforts focus on the northeastern and southwestern regions. Interpreting this data to align with the regions set by the Indiana State Wildlife Action Plan shows that conservation should focus in the Great
Lakes, Interior Plateau, and Valley and Hills area. It is recommended that more environmental data be conducted and that proactive conservation measures are implemented.

Vijay Lulla, Ph.D., Chair
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<table>
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<tbody>
<tr>
<td>AICc</td>
<td>Akaike Information Criterion</td>
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<td>AUC</td>
<td>Area Under the Curve</td>
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<td>DEM</td>
<td>Digital Elevation Model</td>
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<td>eDNA</td>
<td>Environmental DNA</td>
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<td>ESM</td>
<td>Ensembles of Small Models</td>
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<td>HSM</td>
<td>Habitat Suitability Modeling</td>
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<tr>
<td>HUC</td>
<td>Hydrologic Unit Code</td>
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<td>IDNR</td>
<td>Indiana Department of Natural Resources</td>
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<tr>
<td>LULC</td>
<td>Land Use and Land Cover</td>
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<tr>
<td>MRLC</td>
<td>Multi-Resolution Land Characteristic Consortium</td>
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<tr>
<td>mSSS</td>
<td>Maximum Sum of Sensitivity and Specificity</td>
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<tr>
<td>NFHD</td>
<td>National Fish Habitat Database</td>
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<tr>
<td>NHDPlusV2</td>
<td>National Hydrography Dataset Plus Version 2</td>
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<tr>
<td>NRCS</td>
<td>Natural Resources Conservation Service</td>
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<tr>
<td>QHEI</td>
<td>Qualitative Habitat Evaluation Index</td>
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<tr>
<td>ROC</td>
<td>Receiver Operating Characteristic</td>
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<td>SDM</td>
<td>Species Distribution Models</td>
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<td>SGCN</td>
<td>Species of Greatest Conservation Need</td>
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<td>SWAP</td>
<td>State Wildlife Action Plan</td>
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<tr>
<td>UAV</td>
<td>Unmanned Aerial Vehicles</td>
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<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
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<td>USDOI</td>
<td>United States Department of the Interior</td>
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</table>
USFWS – United States Fish and Wildlife Service

USGS – United States Geological Survey
GLOSSARY

Commensal – a relationship between two organisms where one positively benefits and the other gains no benefit and are not harmed from the relationship

eDNA – (Environmental DNA) DNA that is found in the environment by means of water, soil, or general contact; the DNA is extracted from the environment without direct contact from the host organism

ENMeval – ENMeval is an R package that performs automated runs and evaluations of ecological niche models, and currently only implements MaxEnt

Jackknife – resampling technique especially useful for variance and bias estimation

Legacy effect – environmental changes resulting from antecedent human disturbances

MaxEnt – program that performs maximum entropy modelling; predicts species occurrences by finding the distribution that is most spread out, or closest to uniform, while taking into account the limits of environmental variables of known locations

Niche – position or role taken by a kind of organism within its community

Occupancy modeling – model used to account for imperfect detection of organisms in surveys and to determine the probability of the true presence or absence of a species at a site

Trophic structure – partitioning of biomass between trophic levels based on the amount of energy the organism consumes
Introduction

The Common Mudpuppy (*Necturus maculosus maculosus*) is a widespread species that inhabits the eastern United States. They are a fully aquatic salamander that resides in the streams and lakes of Indiana (Minton, 2001). Populations of mudpuppies are declining throughout their range, but the extent of this decline is unknown because they are greatly understudied. Not much is known about their movement patterns, habitat selection, or population structure (Anton, 1999; Matson, 1990). A large reason why there is an information gap is because mudpuppies are a cryptic species that are difficult to catch (Matson, 1990). There have not been official, large-scale sampling efforts of mudpuppies in the upper Midwest (Lambert, Hinz Jr & Cao, 2016). In Indiana, the Common Mudpuppy is considered a Species of Special Concern. The state of Indiana’s Department of Natural Resources defines a Species of Special Concern as, “Any animal species requiring monitoring because of known/suspected limited abundance or distribution or because of a recent change in legal status or required habitat” (Resources, 2018).

Mudpuppies are at risk because of numerous threats to their habitat. These threats are primarily caused by humans. The problems include urbanization, agriculture, and pollution caused by chemicals (Mackey & Goforth, 2005). Mudpuppies are also commonly harvested in the upper Midwest for biological supply companies. These companies sell mudpuppies for educational purposes for biology classes. Fisherman commonly catch mudpuppies in the winter due to higher activity and are known to kill them based on lore stating they are harmful to fish populations (Holman, 2012; Matson, 2005).
The preservation of this species is necessary because they are considered a bioindicator. A bioindicator species refers to a species that can inform scientists about the condition of the environment (Landres, Verner & Thomas, 1988; Noss, 1990). The animals’ reactions to the changes around them is a direct indicator to what is happening in the environment whether it is positive or negative. The abundance of bioindicators is directly correlated to high biodiversity within an ecosystem (Hager, 1998).

A keystone species is where the community structure depends on their presence and competition within the ecosystem (Mills, Soulé & Doak, 1993). If a keystone species is removed from the ecosystem, then there could be radical changes within the community structure (Hager, 1997). Mudpuppies are also considered to be an umbrella species. Umbrella species are considered species where other species in the environment benefit from their conservation (Wilcox, 1984; Wilcox, Murphy, Ehrlich & Austin, 1986).

The mudpuppy is a host for the Salamander Mussel (*Simpsonias ambigua*) which is also a Species of Special Concern in Indiana. Salamander Mussels persist in streams only if there are mudpuppies present. The Salamander Mussel needs high water quality with low siltation to persist. Since the mussels are dependent on the mudpuppy, any habitat threats to the mudpuppy are also threats to them (Watson, Metcalfe-Smith & Di Maio, 2001). The umbrella created by the mudpuppy’s conservation also spreads to other vertebrate and invertebrate species. Any individual species that benefits from the same habitat parameters of the mudpuppy will benefit from their conservation.

Since the Common Mudpuppy is a cryptic species, other scientific measures have been used to detect their presence in waterbodies. Within recent years, environmental
DNA (eDNA) has been used to extract DNA from water and soil samples. The samples are collected and filtered, trapping any DNA for further testing (Turner, 2015). Water bodies where there were records of Salamander Mussels but no mudpuppies present were sampled and analyzed for mudpuppy eDNA. This data is planned to be used to detect mudpuppies in the environment and supplement conservation plans for the Indiana Department of Natural Resources (IDNR) (Hernandez-Gomez, 2018a). The DNA of the mudpuppy has been observed traveling downstream 30 meters (Spear, 2017). This method of monitoring is useful as it indicates that there is a mudpuppy nearby. The problem with the 30-meter buffer from the individual is that if you are not close enough, you might not detect their presence in the stream. However, timing collections with breeding seasons can increase the probability of getting accurate samples (Collins, 2017).

Habitat suitability modeling (HSM) is a frequently used practice used to predict suitable habitat areas for a species to inhabit. The model includes habitat parameters that are considered necessary for that species to survive (Merow, Smith & Silander Jr, 2013). Point data is used to develop the model by analyzing the habitat around that point. When a model is being created, most researchers use presence-absence data. Presence-absence based HSM is when researchers survey an area and determine if there are individuals present. If there are no individuals present, this is represented as an absence and the model determines that the variables in this area are not conducive to supporting that species. This study used presence only point-data of mudpuppies and salamander mussels to generate a habitat model where mudpuppies could persist. This is because there is only occurrence data documented for these species. Mudpuppy populations have not been properly studied because they are difficult to find and lack funds for research. Data from
Ohio was used to bolster the dataset because it is a state that borders Indiana. This was used to create a more accurate model by including a larger area and more data points.

Modeling mudpuppy habitat mirrored HSM and occupancy modeling of the hellbender (*Cryptobranchus alleganiensis*). The hellbender is a closely related, fully aquatic salamander species that has similar habitat parameters (Minton, 2001). They are less tolerant to pollutants and low-quality habitat than mudpuppies. There have been many studies modeling suitable habitat and occupancy of hellbenders because they are a species in peril. The Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) has a small population in Southern Indiana, and usually researchers find mudpuppies when they search for hellbenders (McCallen, 2017). These studies will serve as a framework for modeling habitats for mudpuppies. HSM studies for hellbender and mudpuppies differ because the hellbender research was completed using presence-absence data while there is just presence only data for mudpuppies.

Habitat was analyzed on a watershed scale within Indiana. The United States Geological Survey (USGS) has created a Hydrologic Unit Code (HUC) that designates drainage basins throughout the United States by assigning a numeric digit to the basin. This is designated by a “HUC” and a number value that indicates how many numbers are in the identification code. (Ex. HUC8, HUC10, HUC12, etc.). More numbers within the code means the basin area gets smaller and more precise. In Lambert et al. (2016), they analyzed region-wide species data based on the HUC08 regions. They recommend and encourage analysis by HUC10 regions or stream reaches.

Nine sub-watersheds (HUC08) were identified as areas for possible conservation need for mudpuppies in Indiana. It is hypothesized that these areas are identified as
suitable habitat in this model. Ohio is also included in this study to create a larger dataset to build a more accurate model. In addition to this analysis, the eDNA results were used as a test dataset to determine confidence and accuracy within the model.
Background

Life History

The Common Mudpuppy is a large, fully aquatic salamander. They have external gills in their larval and adult life stages. Adult mudpuppies grow up to 16 inches long with their tail being 30 percent of their total body length. Mudpuppies are brown in coloration with dark stripes running through their face and down their body. Younger individuals may have a white stripe instead of the darker one. The larvae are 30 to 36 millimeters in size and have lighter markings (Minton, 2001). They are a long-lived species that can have a lifespan of over 30 years (Matson, 2005). They are found to be most active at night in the fall and winter months when feeding and reproduction takes place (Harding, 1997; Hecht & Walters, 1955; Holman, 2012; Neill, 1963). Sexual maturity in mudpuppies begins when they are about 5 to 10 years old (Matson, 2005; Minton, 2001).

Breeding occurs during late fall and eggs are laid in the following spring. The eggs are attached to the underside of rocks, logs, and other large refugia. Each egg is attached by a short stalk to the cover object. The eggs are individually laid in a cluster of 50-100 eggs (Matson, 2005; Minton, 2001; Petranka, 1998). Females will stay with the nest and guard it from potential predators (Gendron, 2000). Most of the mating and laying of eggs happens in shallow water due to the colder temperature in the winter and spring months (Harding, 1997; Holman, 2012; Matson, 2005; Petranka, 1998). Craig, Mifsud, Briggs, Boase & Kennedy (2012) conducted research on mudpuppy distribution and breeding by collecting information from fisheries bycatch in the Detroit River. They found eggs on a buoy that was 9.8 meters (32 feet) below surface level. It is widely
accepted that quality mudpuppy breeding habitat is in shallow waters, but this finding shows that breeding locations could be more variable than previously thought.

Mudpuppies have a variable diet consisting of fish, invertebrates, amphibians, and carrion (Harding, 1997; Holman, 2012; Lagler & Goellner, 1941; Petranka, 1998). Mudpuppies have the ability to locate decaying creatures because of their well-developed sense of smell (Harding, 1997). The diets of mudpuppies have been reported to vary based on their size and use of microhabitat (Bart Jr & Holzenthal, 1985; Braswell & Ashton, 1985). Researchers have found mudpuppies feeding on the invasive Round Goby (Negobius melanostomus) and using Zebra Mussel (Dreissena polymorpha) colonies as refugia (Craig et al., 2012).

Beattie, Whiles & Willink (2017) conducted a feeding study on mudpuppies in Wolf Lake, a lake that crosses the border of Illinois and Indiana. Their study focused on feeding preferences within designated age groups based on size of the salamanders. Results showed that there was variability in diet across age ranges. Only larger individuals fed on crayfish, and only juveniles, the smallest individuals, fed on leeches. They also found that the mudpuppies were feeding on invasive species, the first official recording of this occurrence. They concluded that their findings support the hypothesis that mudpuppies have variable diets according to their sizes or ages. This is beneficial because the older and larger individuals are not competing for food with the younger and smaller individuals.

Habitat and Distribution

Mudpuppies inhabit streams, rivers, lakes, and ponds throughout their range (Harding, 1997; Matson, 2005; Minton, 2001; Petranka, 1998). Individuals can be found
in depths of water from 3 to 90 feet deep (Hacker, 1957; Minton, 2001; Sajdak, 1982). Mudpuppies typically dwell among the bottom of lakes and streams (Minton, 2001). Mudpuppies have been found to occupy shallower water in the winter months when the water temperature is cooler (Harding, 1997; Holman, 2012). The streams that mudpuppies typically occupy have rocky bottoms with some siltation (Minton, 2001). They require rocks, logs, and other refugia for feeding, breeding, and predator avoidance (Harding, 1997; Hoffman, Robb & E. Fisher, 2014; Holman, 2012; Petranka, 1998).

Mudpuppies are found throughout the eastern United States and southern portions of eastern Canada (Figure 1) (Minton, 2001). Historically, they were found abundantly throughout the Great Lakes Region. This species has the largest distribution of a fully aquatic salamander in the United States (Petranka, 1998). The distribution of mudpuppies in Indiana has not been fully described. Hoffman et al. (2014) compiled mudpuppy records from anglers, museums, and the state’s current database to create a comprehensive distribution map (Figure 2). Through this research, they added 13 new county records since Minton (2001). The last extensive population survey prior to Hoffman et al. (2014) was in Lake Maxinkuckee, in Marshall County, Indiana by Evermann & Clark (1916), over 100 years ago. Even with the addition of the new county records, there is still not enough information to have an accurate distribution within Indiana (Hoffman et al., 2014). This thesis aims to fill the gaps where there are no known locations of mudpuppies. The compilation of data and habitat modeling will give a better understanding of their possible distribution within the state.

There are still approximately 10 counties where Salamander Mussels are found where there are no records of mudpuppies present (Figure 3) (Hoffman et al., 2014).
Many of the counties have a small number of records. There was an environmental DNA (eDNA) study conducted in partnership with the IDNR and Purdue to analyze the Salamander Mussels’ habitat for the presence of mudpuppies. Organisms are continually shedding skin cells into their environment around them leaving traces of DNA. The DNA available in the water is from cells released by skin, saliva, and fecal material. Analysis of the water sample is done by extracting DNA and testing by using quantitative PCR (qPCR) to see if there are traces of mudpuppy DNA (Jerde, Mahon, Chadderton & Lodge, 2011). Using eDNA to study a cryptic species is helpful because you do not need to find individuals to determine presence in an ecosystem. Collecting eDNA reduces human impact on an ecosystem and is more cost effective compared to manually searching (Thomsen & Willerslev, 2015). The eDNA results generated by Obed Hernandez-Gomez were analyzed by using amplicon sequencing on an Illumina machine which differs from qPCR analysis (Hernandez-Gomez, 2018b).

The most recent systematic search of mudpuppies was conducted by Hoffman et al. (2014). Trapping occurred in Jennings and Ripley Co, Indiana in three different rivers. They also located mudpuppies through visual surveys that consisted of overturning large rocks to look for individuals and nests. Multiple adult salamanders were found in all of the rivers sampled, as well as some nests. They recommend using artificial nest boxes to create suitable habitat for nesting and easy monitoring. They suggested that the Upper Muscatatuck River Watershed could be a key area for conservation of this species. This watershed was formally known as the Muscatatuck Watershed sub-basin (HUC08) (Survey, 2011).
Evermann & Clark (1916) is one of the few known systematic surveys of mudpuppies in Indiana. They observed populations of mudpuppies in Lake Maxinkuckee and noted seasonal behaviors and habitat use. They observed that populations in lakes tend to stay near the shoreline in shallower water. No individual mudpuppies were found deeper than 35 feet. The mudpuppies were found to make burrows into the muddy bottoms of lakes. The burrows were observed to have what seemed to be an entrance and exit. When further examined, it appeared that only one mudpuppy would occupy a burrow at a time. These burrows were not observed to be used for breeding. Individuals were commonly seen at the entrance of the burrow predating small vertebrates and invertebrates as they passed by.

Shoop & Gunning (1967) monitored the movement patterns in the Common Mudpuppy and the Gulf Coast Waterdog (*Necturus belyeri*). They found that the Common Mudpuppy moved, on average, 77 meters from their previous capture site. Even though there were movements from capture location, they suggest that the *Necturus* species stay in specific parts of the streams. They found that individuals that are displaced around 210 meters downstream can return to their original niche. This suggests that there may be a homing capability that is representative of habitat familiarity. Their conclusions suggest that mudpuppies have a small home range and exhibit high site fidelity.

Many factors pose a threat to suitable habitat for mudpuppies. Since the mudpuppy is fully aquatic, it is not able to easily combat these threats. Most of the threats are due to anthropogenic factors. Threats to suitable mudpuppy habitat are urbanization, siltation from agriculture, exotic species, and pollutants (Mackey & Goforth, 2005; Niemi, Kelly & Danz, 2007). In the Great Lakes Region, lampricide poses a large threat
to mudpuppies due to their high sensitivity to the chemical which causes mass die-offs (Matson, 1990, 1998). In Ohio, many of the occurrence data points are recorded from mass die-offs resulting from lampricide applications (Lipps, 2018). Throughout their range, these threats have already been documented as harmful to mudpuppies and have led to a range wide decline in population (Matson, 2005).

In 2015, the IDNR created a State Wildlife Action Plan (SWAP) to assess conservation needs within the state. This document identified all of the vertebrates, invertebrates, and plants in the state that were considered state endangered or threatened (Wildlife, 2015a). These species were labeled as Species of Greatest Conservation Need (SGCN). The state was divided into six regions and listed the environmental threats to the species that occupy those areas. The mudpuppy was listed as a species present in every region across the state. Threats to the mudpuppies’ habitat are natural system modifications, agriculture, aquaculture, and residential or commercial development. These threats are present across the state and the IDNR is hoping to implement practices to minimize their impact on the environment. Unfortunately, the IDNR Division of Fish and Wildlife does not have any current monitoring efforts for the mudpuppy (Engbrecht, 2017). Researchers across the state believe that the biggest problems with monitoring mudpuppies are the lack of funds and understaffing. However, there is monitoring and conservation efforts for hellbenders in the state (Wildlife, 2015a).

The SWAP report had a habitat suitability modeling analysis conducted, but it was not included in the assessment (Wildlife, 2015b). It was felt that the modeling could not predict where the individuals were residing and was therefore excluded. The Common Mudpuppy was not included in the suitability modeling for this report.
Researchers across the state were surveyed and they commented on relevant actions and barriers to mudpuppy conservation. They stated that more baseline data is needed, water quality needs to be improved, and waterways need to be protected. The barriers that faced mudpuppy research were the cost of improving sewage treatment facilities and other research priorities.

**Species Importance**

Mudpuppies are the obligate host for the Salamander Mussel (*Simpsonaias ambigua*). The salamander mussel is a small, freshwater stream mussel that lives under flat rocks (Roe, 2003). The larval stage of the Salamander Mussel uses the mudpuppy as a host in a commensal relationship (Howard, 1951; Lagler & Goellner, 1941). This means that the larvae benefit from the parasitism, but the mudpuppy is not affected positively or negatively. This mussel is significant and unique because it is the only known mussel that utilizes a non-fish host species (Howard, 1951). The salamander mussel is found in the Ohio River Basin, Mississippi River Basin, Michigan, and Ontario, Canada (Roe, 2003). Throughout their range, they are considered endangered, threatened, or a species of concern. It is suggested that these species, along with other freshwater mussel species, are declining due to siltation. Listing under the Federal Endangered Species Act is currently under review, which seeks protection for wherever the mussel is found (Service, 2018). In the state of Indiana, they are considered a Species of Special Concern (Resources, 2018).

Mussels are important within an ecosystem because of their ability to alter it and make it beneficial for other organisms ("Importance of Mussels," 2018). Mussels filter organic matter from the water column and then excreting nutrients for plants,
invertebrates, and fish to use. This “cleans” the water through filtration and are believed to stabilize stream beds by attaching to the bottom of the streams ("Importance of Mussels," 2018). A healthy mussel population indicates a healthy ecosystem for animals and people ("Flexing the Mussels of Freshwater Rivers," 2017). A singular mussel can filter 15 gallons throughout the day (Bennett, 2014). Since the mussels have a significant effect on the environment’s health, it is important that they are preserved. For the Salamander Mussel, the mudpuppy is necessary for its survival. The health of the ecosystem is directly affected by the survival of the mudpuppy.

Identifying if there is a problem within an ecosystem can be difficult for scientists because there are many unseen variables that can affect it. To help scientists understand the problems within an ecosystem, species known as bioindicators, keystone species, and umbrella species are observed to assess the overall health. These classifications are given to a species based on how they clue scientists in to the ecosystem’s health and the health of other species utilizing the same habitat (Landres et al., 1988; Noss, 1990).

A bioindicator species is one that reacts when their habitat is altered in some way. Habitats that humans consider minimally altered may pose a drastic change for the species that utilize it. A bioindicator species is one that is area sensitive and will decline if their habitat is altered drastically (Hager, 1998). Survival within a habitat, for a bioindicator, is dependent on habitat characteristics. If the habitat does not meet the conditions for survival, the bioindicator species will decline (Freda & Dunson, 1986). The presence of a bioindicator directly correlates to higher biodiversity within an ecosystem. This is seen by influencing food webs within an ecosystem. Bioindicators can
help regulate the amount of predators within an ecosystem by providing competition for a food source (Cortwright, 1988; Fauth & Resetarits, 1991).

Amphibians are considered useful bioindicators of terrestrial and aquatic habitats. When pesticides are introduced to an area, amphibians display direct effects from the pesticides (Lambert, 1997). This is due to their semi-permeable skin absorbing water with the pesticides from aquatic and terrestrial habitats. Lambert (1997) observed the results of a large pesticide spill by monitoring amphibian and reptile populations. Amphibians were found to have skeletal deformities due to absorbing the pesticide through their skin and gills as larvae. Freda & Dunson (1986) discuss that negative effects to larval amphibians and their growth can be detrimental to their survival. Seeing abnormalities and declining populations notifies researchers of a problem within the ecosystem. Scientists are made aware of a problem and can address it before the entire ecosystem suffers.

Since amphibians are known as successful bioindicators, mudpuppies can be considered very important to the health of an ecosystem. Mudpuppies are highly sensitive to lampricides and pollutants (Minton, 2001). As a bioindicator, their reaction to these pollutants are beneficial to scientists to ensure the ecosystem is healthy. Problems like sedimentation, dredging, in-channel dams, and other anthropogenic factors may seem minimal to humans. Anthropogenic factors can affect the environment worse than is expected. As an area-sensitive species, mudpuppies can be used to help conservationists understand the impacts of disturbed areas (Hager, 1998). This is beneficial to the ecosystem because this early indication can help preserve other organisms in the stream or lake system.
A species that can drastically influence a food web is considered a ‘keystone species’ (Hager, 1997). This competition allows species lower in the trophic structure, or food web, to repopulate themselves and maintain a healthy population. Their conservation is important because their removal could cause drastic changes within an ecosystem (Hager, 1997). Food webs within an ecosystem are impacted by the presence of amphibians. Salamander larvae are carnivorous predators that can reduce the populations of many species lower in the trophic structure. If there are other predatory species within a habitat, this can be beneficial for an ecosystem. Multiple predators within an ecosystem facilitates competition resulting in predation that manages the other predators population (Cortwright, 1988). This helps balance a food web within an ecosystem and increase biodiversity. Fauth & Resetarits (1991) conducted a study on the interaction between two predators, the Lesser Siren (Siren intermedia) and the Eastern Newt (Notophthalmus viridescens). When the two predators were present in the same habitat, the lowest competitor within the food chain increased. This is because the newts were eating species that were more competitive towards them.

This example of predator-to-predator predation exemplifies how much a predator can affect the biodiversity within an ecosystem. As a top tier predator, mudpuppies contribute to balancing the trophic structure. If a keystone species was removed from the food web, then it could cause a shift in the trophic structure that could be detrimental to the ecosystem (Hager, 1997). Mudpuppies have a variable diet based on their age and life stage. Being an apex predator at all age classes greatly influences the food web (Beattie et al., 2017). If mudpuppies were removed from the food web, the trophic structure would experience a large shift because there is no longer competition from them. This species
has importance because of its significant role in aquatic food webs and its ability to create a predatory balance for other species to thrive.

An umbrella species is when the protection and conservation of one species benefits others that occupy the same space or habitat as them (Wilcox, 1984; Wilcox et al., 1986). The removal of that species may deplete the populations of other species. However, the decline of other species is not due to the removal of the umbrella species unless the umbrella species is a keystone species (Hager, 1997). Hager (1997) observed groups of species types (mammals, herps, invertebrates, etc.) to determine which groups would be considered the best umbrella species. She found that large bodied amphibians and reptiles were second most likely to capture higher proportions of other species present within their range. Larger bodied species are useful as umbrella species because they have a large geographic range. The lower trophic levels benefit from the protection of the large home range (Peterson, 1988; Wilcox, 1984).

Mudpuppies can be considered umbrella species because other species are dependent on its survival to benefit their own survival. The removal of the mudpuppy from the ecosystem will dramatically affect the composition of the species within their habitat. The survival of the Salamander Mussel is completely dependent on the survival of the mudpuppy. If the mudpuppy ceases to exist in an ecosystem, the mussels have no way to reproduce (Roe, 2003). Conserving mudpuppies will directly help the mussels persist. Mudpuppies can also be considered an umbrella species because they are a large-bodied amphibian. This means that the size of their habitat and how they interact with other species within a food web directly affects the species composition within an ecosystem.
**Habitat Suitability Modeling**

Few HSM studies have been conducted on mudpuppies. There are many studies that have analyzed suitable habitats and occupancy for hellbenders. There is a basic understanding of what types of habitats adult hellbenders prefer. Research conducted on suitable habitat for hellbenders was utilized to build a model for mudpuppies since they have similar habitat parameters. The hellbender studies differ from the models for the mudpuppy study because they had presence-absence data from localized surveys.

Many studies have been conducted on the status of hellbenders because they are also experiencing large population declines. Eastern Hellbenders were a candidate species for listing under the Federal Endangered Species Act. On April 4th, 2019, the United States Fish and Wildlife Service (USFWS) concluded that the Eastern Hellbender did not warrant range wide federal protection. The Ozark Hellbender (*Cryptobranchus alleganiensis bishopi*) is currently listed as an endangered species under the Federal Endangered Species Act. The USFWS justified protection of the Eastern Hellbender in Missouri because of the current threats to the Ozark Hellbender (Service, 2019). These animals have a similar lifestyle to mudpuppies with needing clear streams and cover objects. Hellbenders are more sensitive to their environment compared to mudpuppies and need to have better water quality (Minton, 2001). The home range of mudpuppies and hellbenders have not been described in detail, but they are limited by suitable habitat within streams.

Brand & Grant (2017) monitored semi-aquatic salamander species within streams in Maryland to determine how to monitor long-term research of salamanders. Their methods focused on monitoring four salamander species for two years. They analyzed the
habitat based on substrate, size, and order of the streams. Their results showed that the position within the stream network was more determinant of occupancy. The size of the streams and stream features also determined occupancy of salamanders within a stream. They concluded that research focused on small scale habitat preferences may not be as effective as the larger scaled research. Monitoring species at a stream network scale is better for long term conservation and conservation planning.

Land use is commonly used to assess habitats along riverine systems because it is a good indicator of what might runoff into the streams. Land use and land cover (LULC) is remotely sensed data that characterizes what the landscape is being used for. Liang, Fei, Ripy, Blandford & Grossardt (2013) found that natural conditions and land disturbance were the two primary constraints on suitable habitat for fish in stream systems.

Pugh, Hutchins, Madritch, Siefferman & Gangloff (2016) conducted a study analyzing the land-use and physical habitat of hellbenders. They manually surveyed for hellbenders in North Carolina and recorded presence-absence data. LULC data was used in their analysis to characterize the habitat. To get more accurate data along the stream, they ran the process with a 100-meter riparian zone buffer, an area between land and water interfaces, and catchments along the rivers. In both analyses they generalized the LULC data to percent forest, urban, agriculture, and grass/shrub.

Their research included LULC data at the catchment and riparian level. Through their habitat analysis, they found that the local habitat is the best factor (compared to physical and chemical habitat) to predict hellbender presence and absence. The LULC data was also a strong predictor of water quality at the catchment and riparian levels.
Their results were the first quantitative link to relate remotely sensed LULC data and the local physical habitat. They suggest that re-forestation and conservation of buffer zones are the “most promising” strategies for restoration of hellbender habitat.

The research of Bodinof Jachowski, Millspaugh & Hopkins (2016) suggests that LULC data cannot be the only variable when assessing hellbender occupancy. They included physiography as a covariate for their model. Forested habitat above a certain percentage was not included in their analysis because it was assumed suitable habitat for hellbenders which would, in turn, skew their results. Fish were found to have different assemblages of species correlate with the physiography and drainage areas (Angermeier & Winston, 1999). The physiography of the landscape can affect the geology and topography of a habitat. Bodinof Jachowski et al. (2016) hypothesized that the hellbenders occurrence within the streams are affected by the physiography. They also hypothesized that the physiography of the area would make certain reaches of the streams more susceptible to LULC change.

Their LULC data was analyzed in riparian buffer zones, catchments, and riparian zones within catchments. Their study locations were chosen by percent forest of 50-94 percent where the remaining percentage of forest was excluded from the study. This was done because it was assumed that there would not be suitable habitat for the hellbenders if there was not enough forest cover. The LULC data was then analyzed for percent urban, percent agricultural, and percent grass/shrub like Pugh et al. (2016).

Their results found that there was a stronger relationship between hellbender occupancy and physiography than LULC alone. They suggest that the occurrence of hellbenders may be a legacy affect. This means that there may be a longer time period
between land-use and how it affects the stream. The physiography may have an effect of the land use because it offers different resources for humans regarding agricultural and urban use. Despite all of this, the relationship between hellbenders and physiography is unknown. They do believe that physiography influences water quality within streams which can directly affect hellbenders. They conclude that using LULC data alone was a poor predictor of hellbender occupancy. They recommend that physiography is included in the model to better represent the landscape of the riparian and catchment zones. It was also recommended that eDNA may be an effective way to conduct presence-absence surveys to understand the distribution of rare species.

A region wide conservation plan was created by Lambert et al. (2016) for the upper Midwest. Their focus was to look at species that were specifically tied to the success of their surrounding habitat. The spatial extent of this study included Illinois, Indiana, Michigan (lower and upper peninsula), and Wisconsin. This report was created to assist states in making conservation plans for species of concern in their respective states. The mudpuppy and Salamander Mussel were included in this report. The report focused on modeling the habitat of the reproductive host of mussels. The results defined HUC08 watersheds where conservation for freshwater streams would be beneficial.

Mudpuppy data was collected and compiled from all four states. The dataset was not limited by date observed. There was no systematic approach to surveying mudpuppies in any of the states included in the study area. The observations used were presence-only data points. Due to this, they used a maximum entropy method using the programs MaxEnt and R to analyze their data. They created a 50-meter buffer around the states to include all the environmental data needed. They used human disturbance indices
to see anthropogenic effects on the waterways, made available by the National Fish Habitat Partnership.

Stream reaches were used for analysis to compensate for the many variables included in the model. The individual points were linked to the closest stream reach by snapping with ArcGIS. This was an automated process unless there were many reaches within the snapping distance (100 meters). If this was the case, the points were manually moved to the largest stream reach within the snapping distance. This was to minimize the number of false presence variables within the dataset. Distribution maps of the presence of stream species were represented by a HUC12 scale. The HUC08 basins were used to describe suitable areas because the preservation of a stream requires conservationists to look at the larger picture. Watersheds and basins are critical to the habitat quality of a stream.

Lambert et al. (2016) encourages others to do analysis at a fine scale using HUC10, HUC12, or stream reaches. If this is done, they recommend adjusting their methods to better suit the analysis. Their research identifies areas within Indiana that are beneficial for freshwater mussel conservation. This conservation will directly impact mudpuppy population due to their similar habitat needs. They identified the Lower Wabash, Lower White, Highland - Pigeon, Tippecanoe, Middle Wabash-Deer, and Flotrack-Haw sub-basins (HUC08) as areas for rich conservation potential. However, there is an error in the names of one of the sub-basins, Flotrack-Haw is presumably meant to be Flatrock-Haw. There are no basins in Indiana designated as “Flotrack-Haw” (Survey, 2011). Other watersheds that may be of interest are the Middle Wabash-
Busseron and the Lower Ohio-Little Pigeon sub-basins due to being peripheral to the other watersheds (Lambert et al., 2016).

Sutherland, Stedman, Mifsud, Stapleton, Roseman et al. (2016) conducted an eDNA study on the Detroit River and analyzed habitat at a riparian scale using LULC. Water samples were taken to test eDNA of mudpuppies within the Detroit River to test if eDNA was an effective monitoring tool. Their eDNA results were inconclusive. Riparian zones were classified as a 100-meter buffer around the tributaries. The dataset for mudpuppies was procured from bycatch from fisheries. They found that mudpuppy occupancy was higher in upstream with riparian zones that had a higher percentage of forest cover and a lower percentage of development. They recommend that conservation efforts be focused on reforesting riparian zones to maintain suitable habitat for mudpuppies.

Collins (2017) conducted her thesis on utilizing eDNA as an effective tool for habitat modeling. Manual surveys and eDNA samples were taken at 10 sites across Southeast Ohio. The selected sites were based off historic records of mudpuppies and streams that were considered “good quality”. It was found that the eDNA detected mudpuppy presence in streams that yielded no individuals in their manual surveys and had no prior record of mudpuppies in the stream. This shows that eDNA data can help to find cryptic species in a stream system. Collins suggests that pairing eDNA and habitat modeling would help create conservation plans and further help the species.

MaxEnt is an application that is used for species distribution modeling based on presence-only data points and their relationship to environmental variables (Phillips, Anderson & Schapire, 2006). MaxEnt randomly selects 10,000 (default) background
points from the specified environmental variables (Elith, Phillips, Hastie, Dufk, Chee et al., 2011). These background points act as pseudo-absences since there are no absence records within the dataset (Liu, Newell & White, 2016). The absences are needed in the model to predict the probability of presence (Merow et al., 2013). These points are used to determine the density of covariates in the landscape and then relate them to how dense they are at the point locations of the species. The model outputs the results that satisfy the constraints of the species (Elith et al., 2011). The MaxEnt output is scale-independent and gives the user a range of zero to one (0-1) to compare different models outside of units (Phillips, Anderson, Dufk, Schapire & Blair, 2017).

Using the default settings of MaxEnt can be problematic because it can create an overfit model. This means that the model output corresponds too closely to the data, which leads to biased sampling (Merow et al., 2013; Muscarella, Galante, Soley-Guardia, Boria, Kass et al., 2014). The default settings of MaxEnt determines what variables to include based on how many occurrence points are within the dataset. This is done by regularization where the variables that are not contributing as much to the model are penalized. Users can adjust the level of regularization with the regularization multiplier based on what is needed for their study. Changing the default settings of MaxEnt based on species-specific data can greatly improve model performance (Muscarella et al., 2014). However, doing this manually is time consuming because the user is running MaxEnt for each regularization multiplier they would like to test.

To optimize the settings of MaxEnt, Muscarella et al. (2014) developed an R-package called ENMeval. This package utilizes six methods for partitioning data and creating multiple models based on settings defined by the user. The multiple outputs
allow the user to compare all the models to help them choose optimal settings for MaxEnt. Changing the default settings of MaxEnt helps to reduce overfitting and spatial autocorrelation. ENMeval is designed to quickly and efficiently determine what MaxEnt settings are best species-specific habitat suitability modeling.
Methods

The Upper Muscatatuck River Watershed, also known as the Muscatatuck Watershed, was identified by Hoffman et al. (2014) as an area for conservation need. This was based off their manual surveys and statewide data compilation. Lambert et al. (2016) identified watersheds with conservation needs through geospatial analysis from greatest need to lowest need: Lower Wabash, Highland-Pigeon, Tippecanoe, Lower White, Middle Wabash-Deer, Flatrock-Haw, Middle Wabash-Busseron, and Lower Ohio-Little Pigeon (HUC08). It is important to note that Lake Maxinkuckee, the location of the first systematic survey of mudpuppies in Indiana, is within the Tippecanoe watershed.

Since these nine HUC08 sub-basins have been previously identified as areas of conservation need for mudpuppies, it is hypothesized that these areas will appear as suitable habitat through this model (Figure 4). Analysis was done on a HUC10 scale as recommended by Lambert et al. (2016) (Table 1). Analysis at this scale provides comparison from the results of Lambert et al. (2016) that were analyzed on a HUC12 scale. Ohio was included in the analysis for suitable habitat of mudpuppies. Data from Ohio, along with Salamander Mussel data from Indiana, was used to bolster the dataset for a more accurate model.

The areas of suitable habitat were compared to the regions characterized by the IDNR in their SWAP report in 2015 (Figure 5). Table 1 shows what hypothesized watersheds are in each of the SWAP regions. This recommendation to the IDNR is to help them better understand mudpuppy distribution and possible areas of presence. Even though they did not include geospatial analysis in their report, it can still be a useful resource for future planning.
The eDNA results from Indiana were used as a test dataset for this model. The eDNA data is a presence-absence monitoring tool because the DNA is either present in the sample or absent. It is hypothesized that the test dataset will mirror the results of the model created with the presence only records. It is important to note that the accuracy of using eDNA as a monitoring method has not been established (Sutherland et al., 2016). However, there was success with eDNA sampling for mudpuppies in Ohio (Collins, 2017).

**Spatial Extent**

The spatial extent of this research is the Midwest region of the United States utilizing data from Indiana and Ohio. Spatial data for the two states was obtained from the United States Department of Agriculture (USDA) and Natural Resources Conservation Service (NRCS) National Geospatial Center of Excellence. A 1 km buffer around Indiana and Ohio was created to be used for analysis. This was done to include land data past the borders to accommodate points along the borders of the states.

**Occurrence Data**

Occurrence data from both states was compiled and organized. Each data source had a variable amount of data based on their state’s individual conservation plans (Table 2). Data from these sources were sorted through to remove duplicate data points. Data points prior to 1990 were not included due to major land changes around that time (Liang et al., 2013; McCallen, 2017) (Table 3). This point dataset differs from the dataset used in Lambert et al. (2016). They did not mention exclusion of any data points in their analysis. Any Salamander Mussel data that was the same exact point used in the eDNA data was
removed to exclude bias in the dataset. Only 100 occurrence records were used in this analysis.

Occurrences for this analysis were partitioned using the “block method” with ENMEval. The “block method” was used to reduce spatial auto correlation of the occurrence data. Partitioning the data with this method reduces redundancy and biased sampling (Muscarella et al., 2014; Muscarella, Krass & Galante, 2018). The “block method” is where points are partitioned spatially into four “bins” of equal numbers (Muscarella et al., 2018). This constrained the dataset to only 100 occurrences. There were 25 points organized in each bin region that defined northern Indiana (bin 1), southern Indiana (bin 2), southern Ohio (bin 3), and northern Ohio (bin 4) (Figure 6). The points in the northern Ohio region (bin 4) were spatially thinned by 1.2 kilometers (km) in the R-package Wallace. Points in this region were thinned due to the high density of occurrences, while the other regions did not exhibit high density of points. This distance was chosen because mudpuppies do not have a large home range and exhibit site fidelity (Shoop & Gunning, 1967). Even after the spatial thinning, there were still six (6) points that needed to be removed from the dataset to have an equal number of occurrences in each bin. To accommodate this, points were manually removed from the data set based on shortest distance to surrounding points.

Data of mudpuppies in Indiana was obtained from the Indiana Natural Heritage Database and personal contacts. The Indiana Natural Heritage Database’s data use agreement states:

This report includes data provided by the Indiana Natural Heritage Data Center. These data are not based on a comprehensive inventory of the State. The lack of data for any geographic area shall not be construed to me that no significant natural features are present. The State of Indiana is
not responsible for any inaccuracies in the data and does not necessarily endorse any interpretations or products derived from the data. (Indiana Natural Heritage Data Center, 2017)

Data from mudpuppy occurrences during hellbender surveys in Indiana was obtained from the Williams’ Lab at Purdue University. The eDNA dataset for Indiana was analyzed by Obed Hernandez-Gomez by using amplicon sequencing on an Illumina machine which differs from qPCR analysis. The samples were analyzed twice and were considered present if the results were positive on either analysis (Hernandez-Gomez, 2018b).

The Department of Natural Resources equivalent for Ohio was contacted for mudpuppy point data. Their partner, The Ohio State University, contributed the available point data for Ohio. Greg Lipps (2018) from The Ohio State University warned that the Ohio database may have some biases due to concentrated survey efforts. Efforts for mudpuppy monitoring started in the 1980’s when hellbender monitoring efforts began. There are more mudpuppy records for the Ohio dataset from mass die offs from lampricide. These datapoints have not yet been received and processed by Greg Lipps and are not included in this dataset. He notes that mudpuppies are more common in the eastern side of the state than the western portion.

HerpMapper (herpmapper.org) was contacted for data points for Indiana and Ohio. HerpMapper was the only online database utilized because it is the only one that requires accurate point location. This location is obtained through phones by the satellite connection and can only be uploaded if there is a photo voucher. Scientists and citizen scientists can also input data manually but need to have accurate location data to do so.
The data submitted to HerpMapper has the highest rate of acceptance of vouchers from museums compared to other online databases (Lipps, 2017).

The data was snapped to the nearest stream reach within 50 meters. If there were multiple stream reaches, the point was manually moved to the closest stream reach. Point data will not be released in this report due to the species risk. The data was represented as watersheds, landscapes, and counties. This is to protect the species from poaching and persecution from the public.

*Environmental Variables*

Variables for this model were determined by life history, anthropogenic factors, and variables used in other studies. The variables for this model are those used by Bodinof Jachowski et al. (2016), Pugh et al. (2016), and Lambert et al. (2016). Variables from field surveys (eg. riffle, substrate, pool size, visibility) was excluded because they cannot be obtained for this study. Other variables are excluded because they do not pertain to mudpuppy life history and habitat parameters. Mudpuppies are less sensitive to their habitat than hellbenders and can tolerate slightly poorer conditions. However, many of the habitat parameters for hellbenders coincide with those needed for mudpuppies. The variables selected included high and low development, agriculture, distance to forest, tree canopy cover, road density, and stream order. The jackknife methods in MaxEnt was used to determine which variables were contributing to the model.

The data for the environmental variables was obtained from the USDA/NRCS National Geospatial Center for Excellence, Department of Fisheries and Wildlife at Michigan State University, and the Multi-Resolution Land Characteristic Consortium (MRLC). The LULC data was categorized into three groups: high development
(highdev), low development (lowdev), and agriculture (agr) (Table 4). The LULC data was aggregated on a HUC10 scale. Meaning, the total amount of each LULC variable was taken and used at a HUC10 scale.

In Bodinof Jachowski et al. (2016), they used medium and high intensity development as their indicator for human development. Lambert et al. (2016) used developed open space and low intensity development. The difference between the development variables is that the “highdev” variable is buildings and structures that are completed. The “lowdev” variable is areas that are being prepared for or in the process of being completed. Both variables are being used in this study because both studies showed that human development had a negative impact on hellbenders. Agriculture as a variable is warranted because siltation and chemical pollutants from runoff impacts the habitat of mudpuppies. Nesting behavior and reproduction requires clearer portions of streams, rivers, and lakes. Agriculture can greatly impact the water quality which limits reproduction, recruitment, and general survival (Matson, 1990, 2005; Minton, 2001).

The stream data was obtained from National Hydrography Dataset Plus Version 2 (NHDPlusV2) dataset from Horizon Systems. This dataset included waterways that were not necessary for this analysis. Connector, pipeline, and underground conduit waterways were removed. Mudpuppies inhabit streams and ditches and do not exclusively live in these man-made structures. These structures carry water through pipes and underground where mudpuppies cannot access. Therefore, it was determined that they would not be necessary for this study. The stream data being used for this analysis is Strahler’s Stream Order. The streamlines were too small for the programs to be able to pick up the presence of the streams. To accommodate this, the stream data was expanded by one (1) cell in
ArcMap to help with the analysis. Liang et al. (2013) found that headwater streams with Strahler Rank 2 and 3 had better habitat quality. They also found that the quality of habitat decreased the larger the stream became. This variable applies to mudpuppies because they require relatively clean bodies of water to survive.

Physiography was considered this study because Bodinof Jachowski et al. (2016) state that using LULC alone is not a reliable predictor when physiography was not included. In their study, the study area was limited to the western part of Virginia. This analysis covers a much larger area. Their study utilized physiographic provinces to represent the physiography in the area. There is concern because there are three physiographic provinces within this study area and there is mudpuppy presence in all three. To understand the effects of physiography across a large area, the variables were analyzed to test their impact on suitable habitat.

Road density was calculated by finding the number of roads within a HUC10 area. The data provided by the National Fish Habitat Database (NFHD) was represented by catchments and the road density within the catchments. Lambert et al. (2016) used road density in their study to measure the impact humans have on the environment. Manipulation of the data was necessary to be able to calculate the density of roads within the HUC10 area. Road density is useful in this analysis because it represents how humans are directly interacting with riverine systems. Many studies have documented how there is a decline in stream quality with the introduction of roads (Trombulak & Frissell, 2000). Trombulak & Frissell (2000) suggest that roads can be used as indicators for how land-use in an area can change. This is because many times roads are built for logging, agriculture, mining, and other trades that can negatively affect stream communities.
Liang et al. (2013) concluded from their study on modeling habitat for habitat sensitive fish that roads negatively impacted stream quality and fish habitat.

Tree canopy cover and distance to forest were included to have a better representation of the landscape. Tree canopy cover is represented as a percentage of canopy cover per raster cell. Distance to forest was calculated within ArcMap. The term “forest” in this study is characterized as deciduous, evergreen, and mixed forest as defined in the LULC dataset. Forest parcels greater than 0.25 U.S. acres were considered as a suitable forest size. This size was determined because 0.25 U.S. acres is the recommended minimum size for riparian management zones (Agriculture). The Euclidian distance was calculated and is represented as squares measuring 30 sq. meters. The approximate distance to forest can be calculated by multiplying the value within the cell by 30.

*Experimental Design*

Since the mudpuppy is a completely aquatic species, background points were limited to aquatic habitats. Limiting background points to places the species can persist is recommended by Merow et al. (2013) when the default settings for background selection are used. Limiting the background point selection to streams lends to a better fit model because mudpuppies are not found outside of aquatic systems. Buffers around the occurrence points were created at a 5 km and 10 km distance. These buffers were used to generate the 10,000 background points. MaxEnt was ran twice (once with 5 km and once 10 km buffer) with all variables. It was determined that the 5km buffer around points would be used because MaxEnt was able to generate the 10,000 background points. This
was done using the jackknife feature to determine how much the variables contributed to the model. The jackknife analysis was done with four (4) replicates and cross-validated.

The jackknife test ran in MaxEnt showed that high and low development and road density were not considered important for modeling suitable habitat of mudpuppies and were therefore not included in the rest of the modeling. Variables used for the modeling were agriculture, stream order, tree canopy cover, and distance to forest. ENMeval was used to determine the optimal settings for MaxEnt. The variables were put into the R-code of ENMeval as well as the occurrence points with their specified bin number.

The optimal settings were selected as the lowest Akaike Information Criterion corrected (AICc) value. The AICc is correcting for small sample sizes to best depict “goodness-of-fit and complexity” (Muscarella et al., 2014). This is the measure used because the small sample size of this dataset and AICc value reported lowest omission rates when compared to the default models of MaxEnt (Muscarella et al., 2014). This leads to the least overfit model output. The optimized settings for MaxEnt are shown in Table 5. The ΔAICc is included to show that the optimized settings are considered to have “substantial support” because the ΔAICc is less than the value of two (2).

Results were output as ASCII files which were then converted into raster files through ESRI ArcMap. The results were depicted as the stream system on a scale of 0 to 1. The threshold of maximizing the sum of sensitivity and specificity (mSSS) was used to output a binary habitat map. This threshold is considered a more conservative threshold to use and is considered valid with presence-only modeling (Liu et al., 2016). To determine this threshold, the data was run through maxent again with the ENMeval
optimized settings and a cross validation of 4 (four). The average “maximum training sensitivity plus sensitivity Cloglog threshold” was the value used for the mSSS threshold.

The output of the model gave values for each pixel of the stream projection. To protect the localities of the species, the output values of the streams were averaged based on a HUC12 sub-basin level. Any HUC12 sub-basin with a value above the mSSS threshold was considered suitable habitat. The “average mSSS” of the HUC12 sub-basins was calculated by totaling all pixels within the basin and then dividing by the total amount of pixels above the mSSS threshold (# of pixels above mSSS/total pixels). This calculation was justified because it generalizes the data for the HUC12 sub-basins. Taking the “average mSSS” also creates a way to compare each sub-basin. Since each sub-basin has different numbers of pixels within them, the average is needed to understand the landscape within the basin.

Limitations

It is important to note that this analysis was limited to rivers and stream data. This is because the data points that were available were in streams and rivers. It is recognized that mudpuppies live in reservoirs, lakes, and other types of wetlands. However, since the data only represented those that lived in streams and rivers, this analysis only modeled suitable stream and river habitat.

One of the major limitations to this project was the sample size of mudpuppies and salamander mussels for Indiana. If there were more systematic surveys in the past, there would more data to better model suitable habitat. However, the lack of datapoints is why this project is necessary. Another limitation was how Kentucky had their data stored in their database. They did not have their mudpuppy data as point locations. Kentucky
was not able to be included in the model because of this. It would have been useful to have Kentucky included in this model because the Ohio River Valley is historically known for hellbender and mudpuppy populations. This would have been beneficial because it could help characterize habitat in Indiana along the Ohio River.

Illinois and Michigan where initially included in this project. However, there were problems with the study area size and the limitations of the programs being used to analyze the data. It was decided that only two states should be analyzed for mudpuppy habitat. Illinois was excluded because Ohio had more data points than Illinois. Ohio was chosen over Michigan because it spatially represents the same latitude range as Indiana. It was felt that Ohio would be a better representation Indiana’s landscape because of this. This analysis had to be coded to work with the R-package ENMeval. Other programs, like MaxEnt and the R-package Wallace, did not work for this analysis because of the large study area and resulting file sizes.

Another limiting factor was that we could not follow the methods of Bodinof Jachowski et al. (2016) and Pugh et al. (2016) due to the size of the study area. Their analysis was on a catchment and catchment-riparian zone. If we had analyzed on this level, we would get more detailed results. However, the sizes of the files would be very large, and we could not get enough computer processing power to create the catchment and catchment-riparian zones.

We were limited in the experimental design of this project by not being able to use unmanned aerial vehicles (UAVs). The UAVs were going to be used to characterize the aquatic habitat where mudpuppies were found. This was limited due to funding and an unusually warm February in 2018 that caused major flooding across the state.
Results

The MaxEnt model had an area under the curve (AUC) of 0.825 (Figure 7). The AUC is the probability that the model correctly ranked the presence locality higher than a random background point (Radosavljevic & Anderson, 2014). AUC values closer to one (1) are considered better or more suitable models. Suitable habitat was found for mudpuppies in stream orders of 4 to 6. Tree canopy cover was the most suitable at 30 to 40% and then decreased as the percentage of canopy cover increased. As the distance from the forest increased, suitable habitat decreased. This was also seen with percent agriculture. As the percentage of agriculture increased, suitable habitat decreased (Figure 8).

The MaxEnt model showed that stream order was the variable that contributed to the model the most or had the “highest gain” (Figure 9). Stream order is considered the most useful variable used by itself. Removing it from the model would decrease the gain greatly. The mSSS threshold was 0.5027. Any HUC12 with an average mSSS value above the mSSS was considered suitable habitat (Figure 10). The interval determination was decided by a general assumption of how agencies could best interpret the data. Intervals were created based on the percentage of habitat above the mSSS threshold. These intervals were classified as No Value (0), Very Low (0.000001-10%), Low (10-20%), Medium (20-50%), and High (50-100%). It was assumed that agencies would want to survey the areas with the most amount of suitable habitat to maximize their resources.

To determine the most suitable HUC08 sub-basins, the HUC12 areas were assigned the values of 0 to 4 based off the intervals of No Value to High. The values were counted and divided by the total number of HUC12 sub-watersheds to get the percentage
of sub-watershed classes within the HUC08 sub-basin. The values of 3 and 4 (medium and high) were totaled together. Medium and High habitat was chosen because this would be the most suitable area for agencies to focus their resources surveying for mudpuppies. The top ten (10) HUC08 sub-basins of medium and high habitat from greatest to least suitability were Lower East Fork White, Chicago, St. Joseph, Upper Wabash, St. Mary’s, Wildcat, Salamonie, Lower White, Patoka, and Auglaize (Figure 11). The Lower White HUC08 sub-basin was the only sub-basin that was in the hypothesized areas.

The top ten (10) counties were also determined this using these methods: Jackson, Monroe, Lawrence, Martin, Dubois, Wabash, Huntington, Floyd, Davies, and LaGrange (Figure 12). It is important to note that this method of analysis is based on the presence of medium and high areas of suitable habitat. It does not account for the HUC12 area of medium and high area within the county. The counties of Jackson, Wabash, Huntington, and Daviess are counties that do not have county records of mudpuppies. These four counties are within the HUC08 areas of most suitable habitat. There are 19 counties within the HUC08 regions that do not have records of mudpuppies (Table 6).

Relating suitable habitat to the eDNA results is a qualitative analysis. The eDNA results had showed few positive results. Positive results were found in the northeastern, west central, and southeastern areas of the state (Figure 13). The southern positives in Clark County along the Ohio River were the control samples and are not being considered in the qualitative analysis. None of the positive results were in the predicted area of suitable habitat. Many of the results were negative DNA samples. These samples were throughout areas that were not considered the most suitable habitat.
Discussion

Review of Variables and Analysis

Through analysis, it was found that physiography was not a predicting variable when used across a large landscape. This is likely because there is not much variability of physiographic provinces and there was presence in each of the provinces. Bodinof Jachowski et al. (2016) states that ignoring physiography and only using landcover with presence-only modeling would underestimate the occupancy of the Eastern Hellbender. However, it is seen that across a large landscape, physiography is not a determining factor of suitable habitat for mudpuppies.

Stream order was the variable that contributed the most to the model. Streams that were Strahler’s Stream Order of four (4) to six (6) were considered the best suitable habitat. Liang et al. (2013) as well as Sutherland, Meyer & Gardiner (2002) suggested that lower order streams (2 to 3) were the most suitable stream orders for fish species that need high quality habitat for breeding. Their studies also suggest that higher stream orders would have more sedimentation that would reduce suitable breeding habitat. Murphy, Hawkins & Anderson (1981) found that salamanders in the streams were more abundant in higher gradient streams (faster moving streams). The biomass of salamanders was also inversely related to the amount of sediment within the streams.

Mudpuppies may persist in these lower orders of streams, but the occurrence data is presumably only adult individuals. Adult individuals can move to other habitat that is not suitable for breeding. The mudpuppy is also a species that matures at a slower rate than other salamanders and fishes (Matson, 2005; Minton, 2001). Since the mudpuppy occurrence data did not specify the estimated reproductive age and is exclusively adult.
individuals this could be a reasoning why the higher order streams with potentially lower water quality were the most suitable.

The high contribution of stream order could also be due to bias from the samples. The samples from Indiana had a distribution of stream orders from one to six with somewhat even distribution across all values. The Ohio dataset also had this distribution of stream orders, but most were stream orders four and five. These were mainly seen in the northeastern portion of the state (bin 4). The datapoints in the northeastern part of the state were primarily collected during hellbender surveys (Lipps, 2018). Since MaxEnt utilizes presence-only datasets, it is important that the samples are unbiased. Most of the mudpuppy data points are reports from citizen scientists. This could also show a bias to higher order streams because they could be frequented more by the public than lower order streams.

Elith et al. (2011) suggests that the presence-only dataset be “cleaned” for duplicates and errors. They also suggest that biases in the dataset be mitigated by using background data with similar biases. This analysis in MaxEnt was believed to have reduced bias and spatial autocorrelation by using the bin method in ENMeval to optimize maxent settings (Muscarella et al., 2014; Muscarella et al., 2018). The points were also thinned in the northeastern portion of Ohio to reduce bias. To determine if there is a bias with stream order value, future analysis will need to be done. This would include running analysis on Indiana and Ohio separately to determine if the most suitable stream orders are the same values between the two states.

Tree canopy cover, distance to forest, and percentage of agriculture did not contribute as much to the model as stream order. The tree canopy cover was the most
suitable in the 30 to 40% range. The suitability of forest then decreased after 40%. Other studies characterizing occurrence of hellbenders (e.g. Bodinof Jachowski et al., 2016; Pugh et al., 2016) have assumed that higher levels of forest cover within the catchment would be considered suitable and, in turn, bias their results if included. Results of this model show that higher percentages of canopy cover are not favorable for suitable habitat. In Murphy et al. (1981), they concluded that streams with a more open canopy had higher rates of microbial respiration, species variability, and density of drifting invertebrates. These variables are indicative of a higher water quality. Their study shows that there needs to be some areas of less canopy cover to have better water quality. This could explain why there was a decrease in suitable habitat after 40% of canopy cover.

Tree canopy cover data was clipped to the streams. Doing this did not represent what the canopy cover was in the surrounding landscape. To see if the tree canopy cover is accurately representing suitable habitat it has been suggested that there be future analysis on this variable. Future analysis could include analyzing tree canopy cover within a moving window. This would help to characterize the percent of canopy cover in the surrounding landscape. The hope of this further analysis is to support or reject that suitable canopy cover is between 30 and 40% and looking at canopy cover at riverine level is acceptable.

As the distance from the forest patches increased, suitable habitat decreased. When mitigating riparian zones, most concentrate on the width of the riparian zone. This is to ensure that there is enough distance between streams and areas of possible runoff and pollution. Very rarely are length and area of riparian zones considered for mitigation and management. Jones III, Helfman, Harper & Bolstad (1999) studied the relationship of
riparian forest land length and area in comparison to fish assemblages. They found that the length of riparian zone affected habitat diversity, fish abundance, and sedimentation. A longer deforested area resulted in more sediment tolerant and invasive species. Streams with higher disturbances in the surrounding land lead to a decrease in fish assemblages that need low sedimentation for breeding (Jones III et al., 1999; Sutherland et al., 2002). Jones III et al. (1999) concludes that length and area of riparian zones need to be emphasized in conservation strategies and mitigation. Much of sedimentation is due to erosion and concentrating on length of forest patch sizes will help stabilize banks and reduce the amount of erosion.

Distance to forest is a good indicator of habitat quality and suitability because it represents how the landscape interacts with aquatic habitats. Agriculture has also been a well-acknowledged indicator of suitable habitat (eg. Bodinof-Jachowski et al. 2016, Pugh et al. 2016, Lambert et al. 2016). Concentrating on forest patch length along a stream system can reduce the amount of sedimentation and improve water quality. Riparian zones were not characterized within this analysis due to the processing power of the computers. However, the distance to forest variable can characterize that forested areas closer to stream systems are beneficial when determining suitable habitat for mudpuppies. It is important to note that Jones III et al. (1999) heavily emphasized that forest clearing and agriculture are serious disturbances to riverine systems. The loss of forested areas could pose a threat to the persistence of the species, especially in Indiana where agriculture and logging are exports.

To better this model, there is discussion of what other relevant variables to include to ensure there is not bias from the stream order variable. Variables like historical
land-use, distance from agriculture, distance from human development, and road variables have been considered. However, there was not enough time with this analysis to include them in the model. Historical land use, when applied to HSMs of fish species, proved to be less indicative of suitable habitat compared to recent LULC data. Use of this variable will need to be analyzed even further to determine if it is relevant within this study. All these variables, as well as comparing Indiana and Ohio, could potentially result in a better model for suitable habitat of the Common Mudpuppy. It is believed that this model best represents suitable habitat for the Common Mudpuppy with the best available data at this time.

Conclusions and Recommendations

Suitable habitat within the state of Indiana was found in the southcentral, southwest, and the northeast region of the state. The Lower White sub-basin is the only HUC08 sub-basin that was a hypothesized areas of conservation concern. The Lower White, Lower East Fork White, and Patoka are all sub-basins that are bordered by HUC08 sub-basins the hypothesized basins identified by Lambert et al. (2016). The four (4) basins identified by Lambert et al. (2016) may not have been identified as suitable habitat because they are located on the border of Indiana and Illinois. Since Illinois was not included in the analysis, the bordering HUC08 sub-basins may not be representative of the habitat in the entire sub-basin. However, it is believed that the identified suitable habitat is reflective of what is present in that portion of the sub-basin. Many of the counties that do not have previous records of mudpuppies are also located within the bordering sub-basins. It is recommended that the southwest portion of the state
coordinate with Illinois for conservation since many of the watersheds are located in both Indiana and Illinois.

The northeastern part of the state has multiple HUC08 sub-basins that are considered the most suitable habitat. The cluster of sub-basins Auglaize, Salamonie, Upper Wabash, and Wildcat have many counties without previous records of mudpuppies. The sub-basin between these four and St. Joseph is not predicted as most suitable habitat. However, the eDNA samples taken in that area resulted in a positive record for mudpuppies. This means that there may be mudpuppies in an area that was not previously recorded. It is recommended that agencies spend resources in the northeastern part of the state. This is because there is a large area of predicted suitable habitat and positive eDNA results.

The HUC08 sub-basins fit loosely into the SWAP planning regions. The planning regions where there were the most sub-basins were the Great Lakes, Valleys and Hills, Interior Plateau. Creating conservation efforts for these planning regions should include protection of waterways where mudpuppies could occupy. They should also include ways to protect areas where they have been or will be found. This is to ensure that their conservation is proactive. Even if there is not an effort to actively survey for mudpuppies, it is important to be able to react to possible individuals being found. Being proactive will help with future conservation efforts when they are able to be funded and acted upon.

Nine (9) of the top ten (10) counties identified mirror the identified HUC08 sub-basins. Floyd county is the only county that is not found within the most suitable HUC08 sub-basins. There are 19 counties within the identified HUC08 sub-basins without records of mudpuppies. It is recommended that agencies utilize their resources in these
counties within the medium to high HUC12 sub-watersheds. The Muscatatuck sub-basin was not identified as best suitable habitat in this analysis. However, there are many known mudpuppy records and eDNA results that shows their presence within the sub-basin. Surveying this area may be considered useful to agencies because it is known that they are present in this sub-basin and it has been identified as an area for conservation by other studies (Hoffman et al., 2014). It is recommended to conduct more eDNA surveys to find possible mudpuppy sites.

Results considered suitable habitat from MaxEnt were over the mSSS threshold (0.5027). This threshold was used because of the large area surveyed within the landscape. Some do not recommend thresholding because population density and prevalence of a species maybe unknown (Merow et al., 2013). Background points are not equivalent to absences and that a threshold may be reflective of the researchers’ assumptions and not what is represented in the species. Thresholds were deemed useful in this study because there is little known about where the species are. The mSSS threshold is used because the absolute best suitable habitat is desired to have any sort of idea of where to start looking for mudpuppies. The mSSS threshold selection is one of the best thresholding methods compared to others. It is also the only thresholding method that produces the most similar threshold value in presence-only and presence-absence studies (Liu et al., 2016).
Appendix A: Tables

<table>
<thead>
<tr>
<th>HUC08 Sub-Basin</th>
<th>HUC10 Watersheds</th>
<th>HUC12 Sub-Watersheds</th>
<th>SWAP Planning Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flatrock – Haw</td>
<td>16</td>
<td>48</td>
<td>Corn Belt</td>
</tr>
<tr>
<td>Highland – Pigeon</td>
<td>18</td>
<td>47</td>
<td>Valleys and Hills</td>
</tr>
<tr>
<td>Lower Ohio – Little Pigeon</td>
<td>23</td>
<td>68</td>
<td>Interior Plateau</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Valleys and Hills</td>
</tr>
<tr>
<td>Lower Wabash</td>
<td>23</td>
<td>58</td>
<td>Valleys and Hills</td>
</tr>
<tr>
<td>Lower White</td>
<td>33</td>
<td>124</td>
<td>Interior Plateau</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Valleys and Hills</td>
</tr>
<tr>
<td>Middle Wabash – Busseron</td>
<td>40</td>
<td>94</td>
<td>Corn Belt</td>
</tr>
<tr>
<td>Middle Wabash - Deer</td>
<td>15</td>
<td>53</td>
<td>Valleys and Hills</td>
</tr>
<tr>
<td>Muscatatuck</td>
<td>22</td>
<td>73</td>
<td>Drift Plains</td>
</tr>
<tr>
<td>Tippecanoe</td>
<td>34</td>
<td>137</td>
<td>Corn Belt</td>
</tr>
</tbody>
</table>

Table 1. HUC08 sub-basins with the total count of HUC10 and HUC12 units in Indiana. The corresponding Indiana SWAP Regions are listed for each HUC08 sub-basin. SWAP regions “Kankakee” and “Great Lakes” are not represented in the hypothesized areas.

<table>
<thead>
<tr>
<th>Source</th>
<th>State</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>HerpMapper</td>
<td>IN, OH</td>
<td>Necturus m. maculosus</td>
</tr>
<tr>
<td>Indiana Natural Heritage Database</td>
<td>IN</td>
<td>Necturus m. maculosus, Simpsonaias ambigua</td>
</tr>
<tr>
<td>Megan Gramhofer</td>
<td>IN</td>
<td>Necturus m. maculosus</td>
</tr>
<tr>
<td>Nathan Engbrecht (IDNR)</td>
<td>IN</td>
<td>Necturus m. maculosus</td>
</tr>
<tr>
<td>Obed Hernandez-Gomez</td>
<td>IN</td>
<td>Necturus m. maculosus</td>
</tr>
<tr>
<td>The Ohio State University</td>
<td>OH</td>
<td>Necturus m. maculosus</td>
</tr>
<tr>
<td>Williams’ Lab – Purdue University</td>
<td>IN</td>
<td>Necturus m. maculosus</td>
</tr>
</tbody>
</table>

Table 2. Source of Common Mudpuppy and Salamander Mussel datapoints with the state the data represented.

<table>
<thead>
<tr>
<th>State</th>
<th>Number of Records</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indiana (Necturus m. maculosus)</td>
<td>34</td>
</tr>
<tr>
<td>Indiana (Simpsonaias ambigua)</td>
<td>16</td>
</tr>
<tr>
<td>Ohio</td>
<td>50</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 3. Total number of records of Common Mudpuppy and Salamander Mussel after 1990 and 1.2 km thinning for northern Ohio. The total number of records in the dataset is 100 datapoints. The Salamander Mussel data was organized so it would not include duplicate data with the eDNA data.
Table 4. Variables used for mudpuppy habitat modeling. Descriptions of seven predictor variables obtained from MRLC, NHDPlusV2, USDA, United States Department of the Interior (USDOI), and NFHD.

<table>
<thead>
<tr>
<th>Variable Name</th>
<th>Variable Type</th>
<th>Description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>lowdev</td>
<td>Land Use – Land Cover</td>
<td>% of land developed open space and low intensity</td>
<td>MRLC</td>
</tr>
<tr>
<td>highdev</td>
<td>Land Use – Land Cover</td>
<td>% of land developed medium and high intensity</td>
<td>MRLC</td>
</tr>
<tr>
<td>agr</td>
<td>Land Use – Land Cover</td>
<td>% of land pasture, cultivated crop, and grassland</td>
<td>MRLC</td>
</tr>
<tr>
<td>str_order</td>
<td>Hydrology</td>
<td>Strahler’s Stream Order</td>
<td>NHDPlusV2</td>
</tr>
<tr>
<td>physiography</td>
<td>Geology</td>
<td>Proportion of catchment within a specific physiographic province</td>
<td>USDOI</td>
</tr>
<tr>
<td>road_dens</td>
<td>Disturbance</td>
<td>Density of roads within HUC10</td>
<td>NFHD, USDA</td>
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<tr>
<td>canopy_cover</td>
<td>Landscape</td>
<td>Percent of canopy cover</td>
<td>MRLC</td>
</tr>
<tr>
<td>dist_forest</td>
<td>Land Use – Land Cover</td>
<td>Distance from forest with area &gt; 0.25 acres</td>
<td>MRLC</td>
</tr>
</tbody>
</table>

Table 5. ENMeval results for the optimal settings of MaxEnt. The AICc highlighted was the lowest value of all the outputs. The “feature classes” and the regularization multiplier are the settings that were changed in the MaxEnt program. The ΔAICc is below the value of 2, showing that these settings have substantial support.

<table>
<thead>
<tr>
<th>Feature Classes</th>
<th>Regularization Multiplier</th>
<th>AICc</th>
<th>ΔAICc</th>
</tr>
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<tbody>
<tr>
<td>L</td>
<td>1</td>
<td>3224.245</td>
<td>102.7147</td>
</tr>
<tr>
<td>LQ</td>
<td>1</td>
<td>3121.531</td>
<td>0</td>
</tr>
<tr>
<td>H</td>
<td>1</td>
<td>3170.898</td>
<td>49.36765</td>
</tr>
<tr>
<td>L</td>
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<td>3225.994</td>
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<td>LQ</td>
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<td>H</td>
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<td>3227.819</td>
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<tr>
<td>H</td>
<td>3</td>
<td>3165.919</td>
<td>44.38856</td>
</tr>
<tr>
<td>L</td>
<td>4</td>
<td>3229.7</td>
<td>108.1694</td>
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<tr>
<td>LQ</td>
<td>4</td>
<td>3128.402</td>
<td>6.871833</td>
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<tr>
<td>H</td>
<td>4</td>
<td>3166.094</td>
<td>44.56355</td>
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<tr>
<td>L</td>
<td>5</td>
<td>3231.631</td>
<td>110.1009</td>
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<tr>
<td>LQ</td>
<td>5</td>
<td>3132.338</td>
<td>10.8076</td>
</tr>
<tr>
<td>H</td>
<td>5</td>
<td>3171.745</td>
<td>50.21432</td>
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<tr>
<td>HUC08 Region</td>
<td>County</td>
<td>Number of Top 10 Counties</td>
<td></td>
</tr>
<tr>
<td>--------------------</td>
<td>---------------------------------------------</td>
<td>---------------------------</td>
<td></td>
</tr>
<tr>
<td>Lower East Fork White</td>
<td>Daviess, Jackson, Orange, Pike</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Chicago</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>St. Joseph</td>
<td>St. Joseph</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Upper Wabash</td>
<td>Adams, Allen, Grant, Howard, Huntington, Jay, Miami, Tipton, Wabash, Wells</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>St. Mary’s</td>
<td>Adams, Allen</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Wildcat</td>
<td>Grant, Howard, Tipton,</td>
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<td></td>
</tr>
<tr>
<td>Salamonie</td>
<td>Blackford, Grant, Huntington, Jay, Wabash, Wells</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Lower White</td>
<td>Gibson, Orange, Pike</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Patoka</td>
<td>Daviess, Gibson, Owen, Pike, Sullivan</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Auglaize</td>
<td>Adams, Allen</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Top ten HUC08 sub-basins counties without records of mudpuppies. The Upper Wabash sub-basin is the basin with the most counties that do not have a previous record of mudpuppies. The Lower East Fork White and Lower White have the most top ten counties within the sub-basins.
Appendix B: Figures

Figure 1. Distribution of mudpuppies in the United States including extant and introduced populations (Group, 2015)
Figure 2. Distribution map from Hoffman et al. (2014) representing counties that mudpuppies and salamander mussels have been documented. This map shows new county records compiled since Minton’s publication in 2001.
Figure 3. Distribution map of mudpuppies and salamander mussels in Indiana at a county level. Triangular points represent eDNA locations taken for testing by Obed Hernandez-Gomez. This map includes new county occurrences since Hoffman et al. (2014).
Figure 4. HUC08 regions identified by Hoffman et al. (2014) and Lambert et al. (2016) as areas for greatest conservation need. These areas are predicted to be modeled as suitable habitat for mudpuppies.
Figure 5. Planning regions for Indiana from IDNR SWAP report, “Figure 3-1” (Wildlife, 2015a)
Figure 6. Bin regions used for spatial analysis within the R-program, ENMeval. Each bin region has 25 occurrence points.
Figure 7. Sensitivity and specificity of the model for the mudpuppy depicted by the receiver operating characteristic (ROC) curve. The maximum possible test AUC would be 0.816 rather than 1.

Figure 8. Marginal response curves representing how each variable affects the Maxent prediction. These curves show how the variable changes independently of the other variable. The variables are not correlated in this analysis.
Figure 9. Results of jackknife variable importance. This depicts that stream order highly contributes to the model followed by tree canopy cover and agriculture. Stream order appears to have the most useful information when used by itself.
Figure 10. HUC12 sub-watersheds with the percentage of area above the mSSS threshold. Trends of higher areas of suitable habitat are throughout south central and northeastern Indiana.
Figure 11. Top ten HUC08 sub-basins of medium and high suitable habitat. The Lower White sub-basin is the only sub-basin that was identified as a region to survey in previous reports.
Figure 12. Top ten counties of medium and high suitable area. Four of the counties (Jackson, Wabash, Huntington, and Daviess) do not have previous records of mudpuppies in the county.
Figure 13. Results of the eDNA data from Hernandez-Gomez (2018a). There were no positive results within the HUC08 sub-basins.
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**Curriculum Vitae**

Payton Nicole Fischer

**EDUCATION**

**INDIANA UNIVERSITY – IUPUI**
Indianapolis, IN  
*Master of Science – Geographic Information Science*  
May 2020

**SAINT JOSEPH’S COLLEGE**
Rensselaer, IN  
*Bachelor of Science in Biology, May 2016*  
- Concentration in Wildlife Ecology  
- Minor: Sustainability Studies  
- Summa Cum Laude

**EXPERIENCE**

**Indiana University – Purdue University Indianapolis**

*School of Medicine Project Intern* (May 2019 - October 2019): Created database files in excel and ArcMap for health initiative projects. Consulted on best avenues for creating geospatial databases and maps.


**Indiana Department of Natural Resources – Division of Water**

*Dams and Levees Section Program Coordinator 5* (2016-2018): Entered dam inspection data into Unity Database system. Created maps for online GIS software. Organized projects and files and created digital copies.

*Dams and Levees Section Graduate Internship* (2018): Identifying dams that may be jurisdictional based on Indiana dam safety statutes. This was done by GIS analysis using ArcHydrotools, python scripts, and modeling. I completed of the four process areas during the time of the internship that centered around hydrologic analysis.

**Envigo – Indianapolis, IN**

*Animal Care Technician* (2016): Cared for inventory mice at Envigo in Indianapolis, IN. Daily care included but not limited to feeding, cleaning enclosures, taking tissue samples, plating samples, and organizing orders.

**Columbian Park Zoo – Lafayette, IN**

*Seasonal Zookeeper* (2016): Cared for existing animals at the Columbian Park Zoo. Animal species included but not limited to small mammals, primates, anteaters, farm animals, marsupials, reptiles, amphibians, birds, small carnivores, invertebrates.
- Preparation of diets, cleaning animal enclosures, observing animal behaviors
- Assisting with enrichments, medications, and enclosure modification
- Working knowledge of zoo procedures and protocol with all animals within the collection
- Supervising volunteers

Animal Care Intern (2015): Cared for existing animals at the Columbian Park Zoo, Lafayette, IN with a concentration in small mammals, amphibians, reptiles, and invertebrates. Including responsibilities mentioned above.

Saint Joseph’s College – Rensselaer, IN
Teaching Assistant (2013-2016): Assist in lab sections and grade labs for introductory and upper level biology classes.
- Evolution and Ecology, Structure and Function, Comparative Vertebrate Anatomy
- Feed and clean enclosures of laboratory animals

Peer Learning Assistant (2013-2016): Conduct tutoring sessions for students in introductory and upper level biology classes.
- Evolution and Ecology, Structure and Function, Comparative Vertebrate Anatomy, Core 6
- Conduct individual and group tutoring sessions

- Build community and uphold college policy with residents (90 total)
- Mediate and display creative problem solving with roommate conflicts
- Create programs and campus events for students
- Working knowledge of campus resources and making referrals if needed
- QPR Suicide Prevention Certified (2015)

Summer Research Assistant (2013-2015): Assisted in summer research and educational outreach for the Saint Joseph’s College Biology Department under Dr. Robert Brozman and Dr. Timothy Rice.

ANIMAL POPULATION SURVEYS
- Skills: Pit tagging, data collection, snake fungal disease tissue collection


Long-term local surveys of Indiana amphibians and reptiles 2013-2016
RESEARCH PROJECTS
The Effects of Herbicide Application and Cover Crops on Salamander Larvae (2015)
The Effects of Herbicide Surfactants on Spotted Salamander Larvae (2015)
The effects of plastics and aquatic herbicide application on Salamander eggs and larvae (2014)
Assessment of oak barrens restoration: Response of the herpetofauna to aggressive canopy removal and fire management (2013)
Snake mark-recapture on Saint Joseph’s College Environmental Research Station (2014-2016)

POSTER ABSTRACTS AND PUBLICATIONS


Brodman, R., P. Kellenburger, H. Van Meter, and M. Gramhofer. 2014. The effects of plastics and aquatic herbicide application on salamander eggs and larvae. Poster presentation: Midwest PARC, MN; Paper presentation: JMIH, Chattanooga, TN.


Brodman, R., A. Marsh, P. Kellenburger, and M. Gramhofer. 2013. Short-term amphibian and reptile habitat use and abundance in response to oak savanna restoration. Poster presentation: Midwest PARC, Forest Beach Migratory Preserve, WI.

Cover Crops on Bird, Insect, and Amphibian Diversity. Final research report submitted to National Wildlife Federation, Washington, DC.

Fischer, P. and V. Lulla. 2019. Modeling Suitable Habitat for the Common Mudpuppy (Necturus maculosus maculosus) in Indiana, USA Utilizing Regional Data and eDNA. Poster presentation: Student Research and Engagement Day, Indiana University – Purdue University Indianapolis, Indianapolis, IN.

Fischer, P. and V. Lulla. 2019. Modeling Suitable Habitat for the Common Mudpuppy (Necturus maculosus maculosus) in Indiana, USA Utilizing Regional Data and eDNA. Poster presentation: Geography Research Day, Indiana University – Purdue University Indianapolis, Indianapolis, IN.

Fischer, P. and V. 2019. Lulla. Modeling Suitable Habitat for the Common Mudpuppy (Necturus maculosus maculosus) in Indiana, USA Utilizing Regional Data and eDNA. Poster presentation: IGIC Poster Contest, Bloomington, IN.

Fischer, P. 2019. GIS modeling of suitable habitat for Mudpuppy using regional data and eDNA. Oral presentation: Midwest PARC, Madrid, IA.


UNDERGRADUATE RESEARCH PROJECTS AND REPORTS


HONORS
Sister Dorothy Stang Outstanding Student in Wildlife Ecology Award, 2016
Douglas E. Bauer Memorial Senior Biology Student Award, 2016
Outstanding Senior Visual Presentation Award, SJC Colloquium 2016
Science Olympiad Lugosan-Kellenburger Student Award, instated February 2016
Father Urban J. Siegrist, C. PP. S. Junior Biology Student Award, 2015
Outstanding Junior Oral Presentation Award, SJC Colloquium 2015
Outstanding Junior Visual Presentation Award, SJC Colloquium 2015
12th Ecology Poster Session Award, Ecology 2013
Dean’s List: Fall 2013, Fall 2014, Spring 2014, Fall 2015, Spring 2016

ACTIVITIES
Science Club, 2012-2016 (Freshman Liaison, 2012-2013; Secretary, 2013-2014; President 2014-2015).
Green Pumas, 2012-2016.
Alpha Lambda Delta Honors Society, 2013.

ORGANIZATIONS AND MEMBERSHIPS
Midwest Partners in Amphibian and Reptile Conservation, 2013-present
  ▪ Attended regional meetings in 2013, 2014, 2017, and 2019
  ▪ Lead field trip for 2017 meeting in Brown Co., Indiana
  ▪ Presented preliminary thesis results at 2019 meeting in Iowa
Society for the Study of Amphibians and Reptiles, 2017-present
Indiana Geographic Information Counsel, 2017-present
Hoosier Herpetological Society, 2017-present

VOLUNTEER WORK
The Nature Conservancy: Kankakee Sands, Newton County, Indiana, 2015-2016
Regional Science Olympiad Tournament at Saint Joseph’s College, Rensselaer, IN, 2013-2017