USING A MULTI-DIMENSIONAL APPROACH TO DETERMINE THE CONSERVATION AND TAXONOMIC STATUSES OF TWO RARE CRAYFISHES IN NORTHERN ARKANSAS

BY

KATHLEEN B. QUEBEDEAUX

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Natural Resources and Environmental Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2021

Urbana, Illinois

Master's Committee:

Dr. Christopher A. Taylor, Adviser
Assistant Professor Eric R. Larson, Adviser
Dr. Milton Tan

ABSTRACT

Crayfish are chronically understudied, despite playing important roles in our freshwater ecosystems. The goal of this study was to deepen our understanding of *Cambarus causeyi*, the Boston Mountain Crayfish, and *Cambarus hubbsi*, Hubbs' Crayfish, so they can be more effectively conserved and managed. *Cambarus causeyi* and *C. hubbsi* are classified as Species of Greatest Conservation Need in Arkansas, and they are both endemic to Ozarks of North America. The relatively small range and rarity of these species makes them vulnerable to extinction, and both have knowledge gaps that need to be addressed to facilitate conservation. For *C. hubbsi* we searched for potential cryptic diversity by analyzing both genetic and morphological characteristics. We sequenced two mitochondrial gene regions from individuals across its range and conducted Bayesian and maximum parsimony analyses on these data. Additionally, we recorded a suite of morphological measurements in order to conduct a principal coordinates analysis on the morphology of the species. We identified three unique evolutionarily significant units (ESUs) in need of separate conservation attention. However, our morphological analysis had conflicting results, and only showed one of the recovered clades to be unique. Further genetic data should be analyzed in the future to fill in knowledge gaps from our study and determine the cause of the mismatch between our molecular and morphological results. In the second component of this study, we utilized species distribution modeling (SDM) using the program MaxEnt and fine scale habitat modeling to analyze the distribution and habitat preferences of *C. causeyi*. Our SDM found average annual precipitation was by far the most important predictor of *C. causeyi* relative abundance. We collected habitat data from across *C. causeyi*'s known range, and we used our fine scale-data to ground-truth our SDM. We detected *C. causeyi* at only nine of 51 sites, potentially due to sampling outside of the peak of the reproductive season. We ran our fine-scale analysis by modeling zero-inflated Poisson generalized linear models and selecting with AICc. Our best model included proportion of sand in the soil and the presence of a competing burrower as explanatory variables. The interpolated MaxEnt output was found to be a poor predictor of finding *C. causeyi* in our fine-scale analysis potentially because it did not account for biotic interactions and lacked accurate soil data. Additionally, we found *C. causeyi* to still be vulnerable to a variety of threats such as climate change, interspecific competition, low local abundances, and relatively small range continue to pose a threat to conservation of this narrow endemic.

ACKNOWLEDGMENTS

I would like to thank my advisors Dr. Chris Taylor and Dr. Eric Larson for their guidance over the last two years. Thank you to Dr. Milton Tan for being on my committee and providing advice. Also, I want to thank my labmates Amanda Curtis, Caitlin Bloomer, Danny Szydlowski, Jordan Hartman, and Zack Rozansky for their constant friendship and support, even in the midst of a pandemic. I would like to thank everyone who assisted me with field work during this project including Brian Wagner, Caroline Caton, Dustin Lynch, Justin Stroman, Matthew Anderson, Maxwell Hartman, and Katie Morris, without whom this project would have been impossible. Additionally, I would like to thank my family and friends for supporting me as I pursued my Master's Degree.

TABLE OF CONTENTS

CHAPTER 1: GENERAL INTRODUCTION

Freshwater ecosystems cover less than one percent of the world’s surface but contain approximately ten percent of the world’s identified species (Poff et al. 2012; Strayer & Dudgeon 2010). This biodiversity has come under threat due to human activity such as damming, habitat destruction, pollution, and non-native species introductions (Poff et al. 2012; Richter et al.1997; Strayer 2010; Strayer & Dudgeon 2010, Taylor et al. 2007). The extinction rate and number of imperiled freshwater species is disproportionately high in comparison to that of terrestrial and marine ecosystems (Reid et al. 2019; Richter et al. 1997; Strayer & Dudgeon 2010). This relatively high extinction and imperilment rate among freshwater species is exacerbated by the high rates of endemism and narrow ranges of many freshwater species (Dudgeon et al. 2006; Taylor et al. 1996). Despite freshwater species being imperiled at a high rate, they receive far less study than terrestrial species and represent a situation in need of remedy (Dudgeon 2019; Strayer & Dudgeon 2010).

One group in need of further conservation attention is crayfish (Richman et al. 2015). Crayfish play important roles in freshwater ecosystems around the world and often make up a major portion of the benthic biomass in freshwater communities (Momot 1995). They can influence the abundance and diversity of aquatic animals such as fish, birds, and amphibians by being an important food source (Reynolds et al. 2013). Crayfish have also been shown to affect the abundance and biodiversity of the species that make up their diet, such as macroinvertebrates (Kreps et al. 2012), fish (Taylor & Soucek 2010; Thomas & Taylor 2013), and filamentous algae (Creed 1994). In addition, crayfish can modify their habitat in lotic environments by changing sediment transport (Statzner et al. 2003), and burrowing crayfish can also act as ecosystem engineers by mixing and disturbing the soil in which they burrow and by creating habitat for herpetofauna (Welch et al. 2008) and arthropods (Pintor and Soluk 2006).

Despite the substantial impact crayfish have on the world's ecosystems, they remain highly understudied (Moore et al. 2013) and highly endangered (Taylor et al. 2007, Richman et al. 2015). There are approximately 582 species of crayfishes in the world according to the International Union for Conservation of Nature and Natural Resources (IUCN), and over seventy percent of those species are native to North America (Richman et al. 2015). Of North American species, over two-thirds are endemic to the southeastern United States (Taylor et al. 2007). As conservation efforts continue to be made to protect and increase knowledge about crayfish, focusing on the diverse array of species in the southeastern United States will be crucial to protecting crayfish biodiversity.

Accurate taxonomic information for crayfishes of the United States is needed for effective conservation (Taylor et al. 2019). Ensuring the taxonomy of crayfishes remains current is important because species and evolutionarily significant units (ESUs) are often used as the primary unit in conservation policy (Beheregaray & Caccone 2007; Funk et al. 2012; Theodoridis et al. 2019). Evolutionary significant units are defined as populations within a species with high genetic distinctiveness that deserve separate management or priority for conservation (Barbosa et al. 2018; Funk et al. 2012). Cryptic diversity, usually represented by unique lineages that appear morphologically similar or identical, can impede process of identifying unique conservation units (Beheregaray & Caccone 2007; Voelker et al. 2013). These seemingly similar cryptic lineages may react differently to changes in habitat and carry a unique evolutionary history; these ecologically and evolutionarily distinct lineages can be lost if not identified and managed accordingly (Theodoridis et al. 2019). Because crayfishes have been understudied, many instances of cryptic diversity have only recently been identified and signals the need for further investigation of cryptic diversity within the North American crayfishes (Fetzner & Taylor 2018; Glon et al. 2019a; Glon et al. 2019b; Larson et al. 2012).

Species distribution models (SDMs) are important tools for conserving rare and threatened species (Fois et al. 2018; Peterman et al. 2013). To effectively conserve a species, resource managers must understand potential threats, and discerning a species distribution and habitat requirements is integral to knowing those threats (Richter et al. 1997; Taylor et al. 2007). Species distribution models use species locality data and environmental data to make a correlative model of the conditions that predict the relative suitability of habitat for a given species (Warren & Seifert 2011). A common and effective program for creating a SDM is the maximum entropy modeling software MaxEnt (Elith et al. 2011; Phillips et al. 2006). Additionally, MaxEnt is a suitable tool for rare and understudied species because it performs well with small sample sizes (Hernandez et al. 2006; Rhoden et al. 2017).

While SDMs can be an effective tool for analyzing the drivers of a species distribution, model validation should be included as an important part of the process (Peterman et al. 2013; Rhoden et al. 2017; Stirling et al. 2016). Traditional sampling can incorporate variables driving a species' distribution that are not easily accounted for by exclusively using large scale spatial data and possibly unreliable historical data (Hirzel et al. 2006). Species distribution models can fail to account for the influence of biotic variables, lack fine-scale enough data for a question, or be subject to sampling bias (Fourcade et al. 2014; Guisan & Thuiller 2005; Peterman et al. 2013). All of these potential problems illustrate the need for ground-truthing and utilizing traditional habitat sampling when studying a species' habitat needs and distribution.

The goal of this study is to deepen our understanding of *Cambarus hubbsi*, Hubbs' Crayfish, and *Cambarus causeyi*, the Boston Mountain Crayfish, so they can be more effectively conserved and managed. *Cambarus hubbsi* and *C. causeyi* are endemic to the Ozarks, and are classified as Species of Greatest Conservation Need in Arkansas. The relatively small ranges and rarity of these species makes them vulnerable to extinction, and both have knowledge gaps that need to be addressed to facilitate conservation. Utilizing the techniques outlined above (taxonomic delineation of cryptic diversity and species distribution modeling and ground-truthing) will be key to addressing these knowledge gaps.

Previous work has hinted that *C. hubbsi* may harbor unique lineages; in portions of their range *C. hubbsi* have been anecdotally noted to have a difference in coloration and habitat usage (Fowler 2015; Pflieger 1996). Additionally, *C. hubbsi* is currently believed to be in decline in portions of their range (Flinders & Magoulick 2005; Fowler 2015; Magoulick & DiStefano 2007). The discovery of cryptic diversity within *C. hubbsi* would allow managers to address the conservation of those unique lineages. To address these knowledge gaps within *C. hubbsi*, we will analyze the morphological and molecular characteristics of *C. hubbsi* across its range using well-established methods.

The most recent survey for *C. causeyi* by Robison et al. (2009) suggested that the range and abundance of *C. causeyi* had both appeared to have decreased. In this study we set out to determine the distribution of *C. causeyi* and create models to ascertain its preferred habitat. Both efforts will aid in determining if there has been a decline in their range and identify habitat needed to protect the species. The SDM will assist in our understanding of suitable habitat for *C. causeyi*. The fine-scale habitat analysis will ground-truth the SDM and provide information to managers for future *C. causeyi* distributional studies using traditional sampling methods.

There are a wide variety of tools and techniques being used to manage and conserve freshwater taxa. This project aims to utilize a multi-dimensional approach of using molecular and morphological taxonomic assessments, SDMs, and fine-scale habitat modeling to address knowledge gaps in freshwater conservation. In particular, we hope to expand our knowledge about rare and understudied species of crayfishes from one of the most biodiverse areas for crayfishes as a whole.

LITERATURE CITED

Barbosa, S., Mestre, F., White, T. A., Paupério, J., Alves, P. C., & Searle, J. B. (2018). Integrative approaches to guide conservation decisions: Using genomics to define conservation units and functional corridors. *Molecular Ecology*, *27*(17), 3452–3465. https://doi.org/https://doi.org/10.1111/mec.14806

Beheregaray, L. B., & Caccone, A. (2007). Cryptic biodiversity in a changing world. *Journal of Biology*, *6*(4), 9. doi:10.1186/jbiol60

Crandall, K. A., & De Grave, S. (2017). An updated classification of the freshwater crayfishes (Decapoda: Astacidea) of the world, with a complete species list. *Journal of Crustacean Biology*, *37*(5), 615–653. https://doi.org/10.1093/jcbiol/rux070

Creed Jr., R. P. (1994). Direct and Indirect Effects of Crayfish Grazing in a Stream Community. *Ecology*, *75*(7), 2091–2103. https://doi.org/10.2307/1941613

Dudgeon, D. (2019). Multiple threats imperil freshwater biodiversity in the Anthropocene. *Current Biology*, *29*(19), R960–R967. https://doi.org/https://doi.org/10.1016/j.cub.2019.08.002

Dudgeon, D., Arthington, A., Gessner, M., Kawabata, Z., Knowler, D., Lévêque, C., Naiman, R. J., Prieur-Richard, A.-H., Soto, D., Stiassny, M. L. J., Sullivan, C. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews, 81*(2), 163-182. doi:10.1017/S1464793105006950

Elith, J., Phillips, S. J., Hastie, T., Dudík, M., Chee, Y. E., & Yates, C. J. (2011). A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions*, *17*(1), 43–57. https://doi.org/10.1111/j.1472-4642.2010.00725.x

Fetzner, J. W., Jr., & Taylor, C. A. (2018). Two new species of freshwater crayfish of the genus *Faxonius* (Decapoda: Cambaridae) from the Ozark Highlands of Arkansas and Missouri. Zootaxa, 4399(4), 491–520. doi:http://dx.doi.org/10.11646/zootaxa.4399.4.2

Flinders, C. A., & Magoulick, D. D. (2005). Distribution, Habitat Use and life History of Stream-dwelling Crayfish in the Spring River Drainage of Arkansas and Missouri with a Focus on the Imperiled Mammoth Spring Crayfish (*Orconectes marchandi*). *American Midland Naturalist*, *154*(2), 358–374. http://10.0.6.138/0003-0031(2005)154[0358:DHUALH]2.0.CO

Fois, M., Cuena-Lombraña, A., Fenu, G., & Bacchetta, G. (2018). Using species distribution models at local scale to guide the search of poorly known species: Review, methodological issues and future directions. *Ecological Modelling*, *385*, 124–132. https://doi.org/10.1016/j.ecolmodel.2018.07.018

Fourcade, Y., Engler, J. O., Rödder, D., & Secondi, J. (2014). Mapping species distributions with MAXENT using a geographically biased sample of presence data: A performance assessment of methods for correcting sampling bias. *PLoS ONE*, 9(5). https://doi.org/10.1371/journal.pone.0097122

Fowler, Allison (Ed) (2015). *Arkansas Wildlife Action Plan*. Arkansas Game and Fish Commission, Little Rock, Arkansas. 1678 pp.

Funk, W. C., McKay, J. K., Hohenlohe, P. A., & Allendorf, F. W. (2012). Harnessing genomics for delineating conservation units. *Trends in Ecology and Evolution*, *27*, 489–496. https://doi.org/10.1016/j.tree.2012.05.012

Glon, M. G., Thoma, R. F., Daly, M., & Freudenstein, J. V. (2019a). *Lacunicambarus chimera*: a new species of burrowing crayfish (Decapoda: Cambaridae) from Illinois, Indiana, Kentucky, and Tennessee. Zootaxa, 4544(4), 451–478. doi:http://dx.doi.org/10.11646/zootaxa.4544.4.1

Glon, M. G., Williams, B. W., & Loughman, Z. J. (2019b). *Lacunicambarus dalyae*: a new species of burrowing crayfish (Decapoda: Cambaridae) from the southeastern United States. Zootaxa, 4683(3), 361–380. doi:http://dx.doi.org/10.11646/zootaxa.4683.3.3

Guisan, A., & Thuiller, W. (2005). Predicting species distribution: offering more than simple habitat models. *Ecology Letters*, *8*(9), 993–1009. https://doi.org/https://doi.org/10.1111/j.1461-0248.2005.00792.x

Hernandez, P. A., Graham, C. H., Master, L. L., & Albert, D. L. (2006). The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography*, *29*(5), 773–785. https://doi.org/10.1111/j.0906-7590.2006.04700.x

Hirzel, A. H., Le Lay, G., Helfer, V., Randin, C., & Guisan, A. (2006). Evaluating the ability of habitat suitability models to predict species presences. *Ecological Modelling*, *199*(2), 142–152. https://doi.org/https://doi.org/10.1016/j.ecolmodel.2006.05.017

Kreps, T. A., Baldridge, A. K., & Lodge, D. M. (2012). The impact of an invasive predator (*Orconectes rusticus*) on freshwater snail communities: Insights on habitat-specific effects from a multilake long-term study. *Canadian Journal of Fisheries and Aquatic Sciences*, *69*, 1164–1173. https://doi.org/10.1139/f2012-052

Magoulick, D. D., & DiStefano, R. J. (2007). Invasive Crayfish *Orconectes neglectus* threatens native crayfishes in the Spring River drainage of Arkansas and Missouri. *Southeastern Naturalist*, *6*(1), 141–150. http://www.jstor.org/stable/4540986

Momot, W. T. (1995). Redefining the role of crayfish in aquatic ecosystems. *Reviews in Fisheries Science*, *3*(1), 33–63. https://doi.org/10.1080/10641269509388566

Moore, M. J., DiStefano, R. J., & Larson, E. R. (2013). An assessment of life-history studies for USA and Canadian crayfishes: Identifying biases and knowledge gaps to improve conservation and management. *Freshwater Science*, *32*(4), 1276–1287. http://10.0.7.107/12-158.1

Peterman, W. E., Crawford, J. A., & Kuhns, A. R. (2013). Using species distribution and occupancy modeling to guide survey efforts and assess species status. *Journal for Nature Conservation*, *21*(2), 114–121. https://doi.org/https://doi.org/10.1016/j.jnc.2012.11.005

Pflieger, W. L. (1996). *The Crayfishes of Missouri*. Missouri Department of Conservation, Jefferson City.

Phillips, S. J., Anderson, R. P., & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, *190*(3–4), 231–259. https://doi.org/10.1016/J.ECOLMODEL.2005.03.026

Phillips, S. J., Dudík, M., & Schapire, R. E. [Internet] MaxEnt software for modeling species niches and distributions (Version 3.4.1). Available from url: http://biodiversityinformatics.amnh.org/open_source/maxent/. Accessed on 2019-12-13.

Pintor, L. M., & Soluk, D. A. (2006). Evaluating the non-consumptive, positive effects of a predator in the persistence of an endangered species. *Biological Conservation*, *130*(4), 584–591. https://doi.org/10.1016/j.biocon.2006.01.021

Poff, N. L., Olden, J. D., & Strayer, D. L. (2012). Climate change and freshwater fauna extinction risk. In L. Hannah (Ed.), *Saving a Million Species: Extinction Risk from Climate Change* (309–336). https://doi.org/10.5822/978-1-61091-182-5_17

Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J., Kidd, K.A., MacCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., Taylor, W.W., Tockner, K., Vermaire, J.C., Dudgeon, D. and Cooke, S.J. (2019), Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, 94: 849-873. https://doi.org/10.1111/brv.12480

Reynolds, J., Souty-Grosset, C., & Richardson, A. (2013). Ecological roles of crayfish in freshwater and terrestrial habitats. *Freshwater Crayfish*, *19*(2), 197–218. https://doi.org/10.5869/fc.2013.v19-2.197

Rhoden C.M., Peterman W.E., Taylor C.A. (2017) MaxEnt-directed field surveys identify new populations of narrowly endemic habitat specialists. *PeerJ* 5:e3632 https://doi.org/10.7717/peerj.3632

Richman, N. I., Böhm, M., Adams, S. B., Alvarez, F., Bergey, E. A., Bunn, J. J. S., Burnham, Q., Cordeiro, J., Coughran, J., Crandall, K. A., Dawkins, K. L., DiStefano, R. J., Doran, N. E., Edsman, L., Eversole, A. G., Füreder, L., Furse, J. M., Gherardi, F., Hamr, P., Holdich, D. M., Horwitz, P., Johnston, K., Jones, C. M., Jones, J. P. G., Jones, R. L., Jones, T. G., Kawai, T., Lawler, S., López-Mejía, M., Miller, R. M., Pedraza-Lara, C., Reynolds, J. D., Richardson, A. M. M., Schultz, M. B., Schuster, G. A., Sibley, P. J., Souty-Grosset, C., Taylor, C. A., Thoma, R. F., Walls, J., Walsh, T. S., & Collen, B. (2015). Multiple drivers of decline in the global status of freshwater crayfish (Decapoda: Astacidea). *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *370*(1662), 20140060. https://doi.org/10.1098/rstb.2014.0060

Richter, B. D., Braun, D. P., Mendelson, M. A., & Master, L. L. (1997). Threats to Imperiled Freshwater Fauna. *Conservation Biology*, *11*(5), 1081–1093. https://doi.org/https://doi.org/10.1046/j.1523-1739.1997.96236.x

Robison, H. W., Crandall, K. A., & Wagner, B. (2009). *Distribution, Genetics, and Conservation Status of Three Arkansas Crayfishes*(Project No. T26R-11). https://www.wildlifearkansas.com/ProjectReports.html

Statzner, B., Peltret, O., & Tomanova, S. (2003). Crayfish as geomorphic agents and ecosystem engineers: effect of a biomass gradient on baseflow and flood-induced transport of gravel and sand in experimental streams. *Freshwater Biology*, *48*(1), 147–163. http://10.0.4.22/j.1365-2427.2003.00984.x

Stirling, D. A., Boulcott, P., Scott, B. E., & Wright, P. J. (2016). Using verified species distribution models to inform the conservation of a rare marine species. *Diversity and Distributions*, *22*(7), 808–822. https://doi.org/https://doi.org/10.1111/ddi.12447

Strayer, D. L. (2010). Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology*, *55*(1), 152–174. https://doi.org/https://doi.org/10.1111/j.1365-2427.2009.02380.x

Strayer, D. L., & Dudgeon, D. (2010). Freshwater biodiversity conservation: recent progress and future challenges. *Journal of the North American Benthological Society*, *29*(1), 344–358. https://doi.org/10.1899/08-171.1

Taylor, C. A., DiStefano, R. J., Larson, E. R., & Stoeckel, J. (2019). Towards a cohesive strategy for the conservation of the United States' diverse and highly endemic crayfish fauna. *Hydrobiologia*, *846*(1), 39–58. https://doi.org/10.1007/s10750-019-04066-3

Taylor, C. A., Schuster, G. A., Cooper, J. E., DiStefano, R. J., Eversole, A. G., Hamr, P., Hobbs, H. H., Robison, H. W., Skelton, C. E., Thoma, R. F. (2007). A reassessment of the conservation status of crayfishes of the United States and Canada after 10+ years of increased awareness. *Fisheries*, *32*(8), 372–389. https://doi.org/10.1577/1548-8446(2007)32[372:arotcs]2.0.co;2

Taylor, C. A., & Soucek, D. J. (2010). Re-examining the importance of fish in the diets of stream-dwelling crayfishes: Implications for food web analyses and conservation. *The American Midland Naturalist*, *163*(2), 280–293. https://doi.org/10.1674/0003-0031-163.2.280

Taylor, C.A., Warren Jr., M.L., Fitzpatrick Jr., J.F, Hobbs III, H.H., Jezerinac, R.F., Pflieger, W.L., & Robison, H.W. (1996). Conservation status of crayfishes of the United States and Canada. *Fisheries*, 21, 25-38. https://doi.org/10.1577/1548-8446(2007)32[372:AROTCS]2.0.CO;2

Theodoridis, S., Nogués-Bravo, D., & Conti, E. (2019). The role of cryptic diversity and its environmental correlates in global conservation status assessments: Insights from the threatened bird's-eye primrose (Primula farinosa L.). *Diversity and Distributions*, *25*(9), 1457–1471. https://doi.org/https://doi.org/10.1111/ddi.12953

Thomas, C., & Taylor, C. A. (2013). Scavenger or predator? Examining a potential predator–prey relationship between crayfish and benthic fish in stream food webs. *Freshwater Science*, *32*, 1309–1317. https://doi.org/10.1899/12-169.1

Voelker, G., Marks, B. D., Kahindo, C., A'genonga, U., Bapeamoni, F., Duffie, L. E., Huntley, J. W., Mulotwa, E., Rosenbaum, S. A., Light, J. E. (2013). River barriers and cryptic biodiversity in an evolutionary museum. *Ecology and Evolution*, *3*(3), 536–545. https://doi.org/10.1002/ece3.482

Warren, D. L., & Seifert, S. N. (2011). Ecological niche modeling in MaxEnt: the importance of model complexity and the performance of model selection criteria. *Ecological Applications*, *21*(2), 335–342. https://doi.org/10.1890/10-1171.1

Welch, S. M., Waldron, J. L., Eversole, A. G., & Simoes, J. C. (2008). Seasonal Variation and Ecological Effects of Camp Shelby Burrowing Crayfish (*Fallicambarus gordoni*) Burrows. *The American Midland Naturalist*, *159*(2), 378–384. https://doi.org/10.1674/0003-0031(2008)159[378:SVAEEO]2

CHAPTER 2: A TAXONOMIC ASSESSMENT OF HUBBS' CRAYFISH (*CAMBARUS HUBBSI*) IN ARKANSAS AND MISSOURI

INTRODUCTION

Crayfish play important roles in freshwater ecosystems around the world and often compose a significant portion of the benthic biomass in those systems (Momot 1995). Crayfish can influence the abundance and diversity of aquatic, terrestrial, and amphibious animals by being an important food source (Reynolds et al. 2013) and affect biodiversity by predating on macroinvertebrates (Kreps et al. 2012; Reynolds et al. 2013), fish (Taylor & Soucek 2010; Thomas & Taylor 2013), aquatic vegetation (Reynolds et al. 2013), and filamentous algae (Creed 1994). Despite the significant impact crayfish have on the world's ecosystems, they remain highly understudied (Moore et al. 2013) and highly endangered (Taylor et al. 2007, Richman et al. 2015). Due to being understudied in comparison to other taxa, many crayfishes have only recently been described, and from 1972 to 2007 an average of 3.4 new species were described a year (Moore et al. 2013). More attention needs to be paid to crayfish conservation and taxonomy by addressing relevant issues, such as cryptic diversity (Helms et al. 2015; Larson et al. 2012).

Cryptic diversity, usually represented by unique lineages that appear morphologically very similar or identical, can slow the process of identifying unique conservation units (Beheregaray & Caccone 2007; Voelker et al. 2013). These seemingly similar, cryptic lineages may react differently to changes in habitat and carry a unique evolutionary history; it is important to identify ecologically and evolutionarily unique lineages so they can be managed accordingly (Theodoridis et al. 2019). Because crayfish have been so understudied, many instances of cryptic diversity have only recently been identified and signals the need for further investigation of cryptic diversity within the taxa (Fetzner & Taylor 2018; Glon et al. 2019a; Glon et al. 2019b; Larson et al. 2012). This study focuses on the issue of cryptic diversity within *Cambarus hubbsi* (Hubbs' Crayfish).

Cambarus hubbsi is a stream dwelling crayfish endemic to the Ozark Highlands and Boston Mountains ecoregions of Missouri and Arkansas in the United States of America (USA), and is considered a Species of Greatest Conservation Need by the Arkansas Game and Fish Commission (AGFC) (Fowler 2015). *Cambarus hubbsi* is associated with riffles and runs of larger, more permanent streams (Flinders & Magoulick 2005; Magoulick et al. 2017; Nolen et al. 2014), and appears to be intolerant of environmental stressors, such as drought (Yarra & Magoulick 2018). Additionally, *C. hubbsi* is noted to prefer streams with large cobble or boulders, which provide shelter, and it is rarely seen in open habitats (Pflieger 1996; Flinders & Magoulick 2005). *Cambarus hubbsi* has a *K*-life history strategy relative to sympatric crayfish species in the genus *Faxonius* due to its slow growth rate, late age of reaching maturity, and overall low reproductive potential (Larson & Magoulick 2011). *Cambarus hubbsi*'s intolerance of drought conditions combined with its *K*-life history strategy may make it vulnerable to disturbance, such as the introduction of an invasive species (Yarra & Magoulick 2018). A recent decrease in the abundance of *C. hubbsi* in the Spring River watershed has been hypothesized to have been caused by the introduction of *Faxonius neglectus*, the Ringed Crayfish (Flinders & Magoulick 2005, Magoulick & DiStefano 2007).

Cambarus hubbsi's range is comprised of the White River drainage in Arkansas and Missouri (Fowler 2015; Pflieger 1996). *Cambarus hubbsi* appears to be more common in the Black River drainage than the remainder of the White River basin, and there have been color and habitat differences noted between these two portions of *C. hubbsi*'s range according to anecdotal evidence from members of AGFC (Fowler 2015). Pflieger (1996) noted that *C. hubbsi* in the upper White River drainage of Missouri have a “bright orange-red” coloration instead of an “olive-tan or a reddish-tan” coloration. These differences in coloration of *C. hubbsi* within its range indicates possible cryptic diversity. This study seeks to understand the taxonomic status of *C. hubbsi* by analyzing morphological and genetic diversity within the White River drainage. We hope to improve the conservation of *C. hubbsi* by identifying unique lineages, that might differ in conservation needs, through analyzing two mitochondrial DNA gene regions and specimens from across their historic range.

METHODS

Materials Analyzed

This study used a combination of historic collections and newly sampled specimens from across *C. hubbsi*’s known range (Fig. 2.1). The drainages that make up *C. hubbsi*’s range include the Strawberry River, Eleven Point River, Spring River, Black River, Upper White River, Lower White River, Little North Fork of the White River, Buffalo River, Current River, and Saint Francis River. The upper and lower White River drainages are defined respectively as anything upstream and downstream of Bull Shoals Reservoir. Not all of these drainages were represented equally in the dataset due to a lack of available data. Only two individuals from the Current River drainage and two individuals from the Saint Francis River drainage were included in the molecular analysis, and no individuals from the Black River drainage were included in the molecular analysis. New specimens were collected by kick seining using a 3.18 m wide x 1.52 m high, 3.2 mm mesh seine or by hand picking in rivers within the White River drainage. Tissue samples were taken from the abdomen of *C. hubbsi* for genetic analyses. All tissue samples were stored in 99% ethanol, and specimens were stored in 70% ethanol. We analyzed the genetic data of 34 individuals for the molecular analysis (Table 2.1). The genetic data were gathered from the National Center for Biotechnology Information's website GenBank or were amplified from isolated DNA. For the morphological analysis, we measured 166 specimens from 10 drainages within the White River basin that were newly collected or were in the Illinois Natural History Survey's (INHS) Crustacean Collection (Table 2.2).

Molecular Analyses

For the molecular analysis of *C. hubbsi*, we examined two mitochondrial gene regions: the cytochrome oxidase subunit one (COI) DNA bar coding region and the 16S rDNA region. We did not use any nuclear gene regions for our molecular analyses because available nuclear markers for crayfish have been uninformative for the genus *Cambarus* (Breinholt et al. 2012). DNA from tissue samples were extracted by using the DNeasy® Tissue and Blood Kit, samples were amplified using PCR with Folmer one and two COI primers (Folmer et al. 1994) and 1472 and 16S17sub 16S primers (Taylor et al. 2014), and samples were purified with the QIAquick PCR Purification Kit. To amplify our sequences we used a combination of MasterMix or PuReTaq Ready-To-Go (RTG) PCR Beads. We used the following conditions during PCR: 95° C for 5 minutes; then 40 cycles of 95° C for 30 seconds, 52° C for one minute, and 72° C for one minute; then 72° C for 7 minutes; hold on 4° C. The purified double-stranded DNA samples were Sanger sequenced at the University of Illinois at Urbana-Champaign Core Sequencing Facility. The sequencing results were trimmed and cross-checked by visually analyzing chromatograms and aligned using Sequencher 5.4 (Gene Codes Corporation, Ann Arbor, Michigan). The COI region was trimmed to 621 base pairs, and the 16S gene region was trimmed to about 400 base pairs. Additional sequence data were taken from GenBank. We used *Cambarus maculatus, C. tenebrosus, C. striatus, C. gentryi*, *C. hubrichti*, *C. pyronotus*, and *Procambarus clarkii* as outgroups in our analyses. *Procambarus clarkii* was used to root the tree, and we chose the other species used as outgroups because they were in the same genus as *C. hubbsi* and there was complete sequence data available for the COI and 16S gene regions.

We conducted both Bayesian and maximum parsimony phylogenetic analyses on our molecular data. For our Bayesian analysis, models of DNA sequence evolution were tested for their fit to the COI and 16S data by using jModelTest 2.1.10 (Darriba et al. 2012; Guindon & Gascuel 2003) with the best model selected by Bayesian information criteria (BIC) in mrBayes v3.2.7 (Ronquist et al. 2012). HKY + G was selected for both gene regions, and the molecular data were partitioned by gene region in the Bayesian analysis. We conducted two runs with two million generations and a sampling frequency of 200 in mrBayes and created a 50% majority rule consensus tree.

We inferred phylogenetic relationships and established nodal support using maximum parsimony with bootstrapping in PAUP* version 4.0b10 (Swofford 2002). For the maximum parsimony analysis we used the heuristic search, characters were treated as unordered, gaps were treated as a “fifth base,” and all character transformations were given equal weight. Also, we used the tree-bisection-reconnection (TBR) algorithm for branch swapping, and we used 1000 bootstrap replicates. Major lineages from our recovered phylogenetic trees were mapped onto the morphological results and used to assist in the identification of unique lineages.

Morphological Analyses

Morphological traits were recorded from specimens collected during the study and from the INHS's Crustacean Collection. Counts and measurements taken on the chelae, antennal scales, carpus, gonopods, and mesial ramus were taken on the right side of the crayfish unless the right side appeared damaged or regenerated. Measurements were taken to the nearest 0.1 millimeter using digital calipers. Individuals were classified as either a form I male, form II male, or female. Form I males are mature males in a reproductively active state, and form II males are males in a reproductively inactive state. A form I male can be identified based on its sclerotized gonopod. Juveniles were considered to be any individuals possessing a carapace that is less than 15 mm long (Larson & Magoulick 2011) and were not included in this study. Form I males were also omitted from this study to help control for the effect form has on morphometrics, although our results were insensitive to inclusion of form I males (Appendix A). Additionally, individuals with a post-orbital carapace length longer than 30 mm were excluded from this study, so the variation in size of individuals between drainages was more even.

We recorded the following standard morphological traits for crayfish: chela total length, chela palm length, chela palm width at its widest point, the number of tubercles in the first row on the mesial margin of the palm of the chela, the number of rows of tubercles on mesial surface of the palm of the chela, the number of tubercles on the opposable margin of the dactyl, the number of tubercles on the opposable margin of the propodus, number of spines on the mesial margin of the merus, carapace length, post-orbital carapace length, areola length, areola width at its narrowest point, rostrum width at the base of rostrum, rostrum width at the base of the acumen, rostrum length, presence or absence of a terminal spine or a tubercle on the rostral margin, antennal scale length, antennal scale width at the widest point, and the number of lateral terminal spines on the mesial ramus of the uropod (Fetzner & Taylor 2018). Chelae measurements were only taken from non-regenerated chelae. Spines were differentiated from tubercles based on sclerotization; spines are sclerotized and usually have an acute tip, and tubercles are not.

After measurements were taken, we created the following ratios: areola length/areola width, rostrum length/rostrum width at the base of the acumen, rostrum width at the base/rostrum width at the base of the acumen, rostrum length/rostrum width at the base, post-orbital carapace total length/rostrum length, chela length/palm width, palm width/palm length, antennal scale length/antennal scale width, post-orbital carapace length/chela length, post-orbital carapace length/palm width, and post-orbital carapace length/palm length (Fetzner & Taylor 2018). Ratios were used in analyses, rather than direct measurements, to control for differences in size among individuals. We included post-orbital carapace length in our analyses instead of total carapace length to account for variation that may have been caused by some individuals having a regenerated or damaged acumen. We omitted the number of lateral terminal spines on the mesial ramus of the uropod, the presence of a spine or a tubercle at the end of the lateral margins of the rostrum, and the number of rows of tubercles on mesial surface of the palm from the analysis due to a lack of variation in the dataset. The number of tubercles on the propodus and dactyl were omitted from the analysis because they were positively correlated with the post-orbital carapace length.

We ran a principal coordinates analysis (PCoA) and a permutational multivariate analysis of variance (PERMANOVA) in R version 3.5.2 on the ratios described above, the number of spines on the mesial margin of the merus, and the number of tubercles in the first row of tubercles on the mesial margin of the chela. The PCoA was run with the vegan and ape packages and used Gower's distance due to its ability to accommodate mixed variable types (Oksanen et al. 2019; Paradis & Schliep 2018). The PERMANOVA was used to test for the effect of drainage on our morphological variables, and it was conducted by using the adonis function in the vegan package (Oksanen et al. 2019). We ran 1000 permutations and used Gower's distance for the PERMANOVA. Additionally, we ran a pairwise post-hoc test comparing drainages using the package pairwiseAdonis (Martinez 2020).

RESULTS

Molecular Results

The standard deviation of the split frequencies during our Bayesian analysis went below 0.01 indicating convergence in the analysis. In the Bayesian analysis *C. hubbsi* was found to be monophyletic, and we recovered three major, reciprocally monophyletic clades within *C. hubbsi* (Fig. 2.2). Clade A consists of the Strawberry River drainage and the White River drainage upstream of its confluence with the Black River (Fig. 2.1). Clade B consists of the Eleven Point River and Spring River drainages. Clade C consists of individuals from the Saint Francis River and Current River Drainage. The average uncorrected p-distance between clade A and B was 0.039. Between clade A and C, average uncorrected p-distance was 0.038. The average uncorrected p-distance was lower between clades B and C at 0.03. Clades B and C were recovered as sister clades in a larger clade, but this was poorly supported with 62% posterior probability (Fig. 2.2). Therefore, we believe clade C should be treated as an independent clade based on the currently available data.

In the parsimony analysis, the ten shortest trees of 499 steps were retained during the heuristic search, and 4,839,133 rearrangements were tried. One-hundred-fifty of 1,013 total characters were parsimony informative. The 50% majority-rule consensus of those ten trees contained the same major clades recovered in the Bayesian analysis. The only exception was that clade B and C were found to be sister clades, further supporting that clade B and C should be treated as separate.

Morphological Results

We analyzed the first two principal coordinates in our PCoA, based on the broken stick method and scree plot (Jackson 1993). The proportion of variance explained by the first principal coordinate was 0.412, while the second principal coordinate explained 0.181. In our PCoA the Spring and Eleven Point individuals clustered together, matching the results of our pairwise test and clade B of our molecular analysis (Fig. 2.3). However, individuals that we would expect to be separated into clade A and C, based on our molecular analysis, overlapped in our PCoA.

All morphological measurements contributed a significant amount to the results (Table 2.3). Most of the separation between clade B and the other clades can be seen on the first principal coordinate axis (Fig. 2.3). Traits that loaded negatively on the first principal coordinate were associated with clades A and C, and traits that loaded positively on the first principal coordinate were associated with clade B (Fig. 2.4). Larger values for areola length/width loaded negatively on to the first principal coordinate, meaning individuals from the Spring and Eleven Point drainages had wider areolas relative to their length. Larger values for the rostrum base width/ rostrum tip width and for the post-orbital carapace total length/rostrum length loaded positively on the first principal coordinate, which means the Spring and Eleven Point individuals had shorter, more angled rostrums. In comparison, larger values for the rostrum length/rostrum width at the base loaded negatively on the first principal coordinate, meaning crayfish from clades A and C had longer rostrums. Larger values for the post-orbital carapace total length / palm width loaded negatively on the first principal coordinate, meaning that the Spring and Eleven Point crayfish had wider chelae. Our PERMANOVA analysis, when controlling for sex as an explanatory variable, found that drainage has a significant effect on our suite of morphological variables. The p value was 0.001 and the R^2 was 0.428. The post-hoc pairwise comparisons on all individuals found that 12 of 45 comparisons were significantly different between drainages (Fig. 2.5).

DISCUSSION

Our molecular analyses recovered three unique lineages within *C. hubbsi*; these lineages are clade A (Strawberry River drainage and the White River drainage upstream of its confluence with the Black River), clade B (Eleven Point and Spring river drainages), and clade C (Saint Francis and Current river drainages) (Fig. 2.2). The average p-distances between these clades of ~3% is below what is typically seen between different species of crayfishes in the genus *Cambarus* (usually 5% or higher), but biodiversity below the species level is still worthy of recognition (Breinholt et al. 2012; Diéguez-Uribeondo et al. 2008; Foltz et al. 2019). Cryptic biodiversity within *C. hubbsi* needs to be recognized when managers are making decisions on how to conserve the species (Beacham et al. 2004; Reiss et al. 2009). The unique lineages may represent populations in the process of speciation and that have unique habitat needs and threats (Theodoridis et al. 2019). A method of more formally recognizing and protecting this cryptic biodiversity would be to designate clades A, B, and C as separate evolutionarily significant units (ESUs) (Funk et al. 2012).

Evolutionarily significant units are defined broadly as populations within a species with high genetic distinctiveness that deserve separate management or priority for conservation (Barbosa et al. 2018; Funk et al. 2012). Evolutionarily significant units were originally defined by Ryder (1986) as a method of identifying diversity below the species level. There have been a variety of ESU concepts that emphasize various types supporting evidences such as reciprocally monophyletic clades, morphological differences, and geographical separation (Barbosa et al. 2018; Dizon et al. 1992; Funk et al. 2012; Moritz 1994). Based on the strongly supported, monophyletic clades in both of our molecular analyses, the average p-distances of approximately 3% between these major clades, and the defined geographic separation between clades, we believe clades A, B, and C should be treated as separate ESUs.

A potential partial explanation for the distribution of the three ESUs is that the range of *C. hubbsi* is on the edge of the Coastal Plain and more upland areas. The abruptness of the physiographic change may be impeding gene flow and dispersal between the ESUs, as it has in some fishes in the area (Cashner & Suttkus 1977; Page & Cordes 1983). The distribution of the ESUs could be furthered explained by the fact that until the Pleistocene, the Black River was a direct tributary of the Mississippi River (Fisk 1944; Page & Cordes 1983). However, the Mississippi River was diverted from the western edge of the Mississippi Valley, which caused the Black River to shift westward and merge with the White. *Cambarus hubbsi* may have then spread downstream to other White River tributaries and other parts of the upper White drainage. Other aquatic organisms endemic to the Ozarks, such as the “saddleback darters” in the family Percidae (Page & Cordes 1983), show a similar biogeographic pattern.

Defining these three clades as separate ESUs informs managers, so they may address threats unique to each lineage. Some potential threats throughout *C. hubbsi*’s range include gravel mining, introduction of invasive species, and seasonal and supraseasonal drought (Brown et al. 1998; Flinders & Magoulick 2005; Larson & Magoulick 2011; Yarra & Magoulick 2018). In particular, *C. hubbsi* has anecdotally been found to be less common in the drainages found in clade A (Fowler 2015) and should be given additional conservation attention. Also, there are several large dams forming reservoirs in the portion of the range where clade A resides, which could be blocking gene flow within the ESU (Barnett et al. 2020). Additionally, a potential threat to clade B is the introduction and spread of the *F. neglectus* in the Spring River and Eleven Point River drainages (Flinders & Magoulick 2005, Imhoff et al. 2012; Magoulick & DiStefano 2007).

A limitation of our study is that we currently have gaps in our *C. hubbsi* genetic data, such as missing nuclear genome data and portions of their range being under-represented, that should be a focus of future research. In particular, we currently have limited genetic data from the populations that make up clade C. These populations include the upper Black River (which currently has no genetic data), Saint Francis River, and Current River drainages (Fig. 2.1). Additional collection of genetic data from the population in the Black, Saint Francis and Current River drainages would be beneficial to further defining the range and uniqueness of clade C. Based on the limited genetic data available for clade C, it appears it may be more closely related to clade B than A, but the apparent distinctiveness of clade C could be further validated with more data. Additionally, next generation sequencing or sequencing of additional gene regions could provide additional support for the separation of these clades into separate ESUs or change our understanding of the genetic relationship between the *C. hubbsi* populations (Barbosa et al. 2018; Funk et al. 2012).

Supplementary data might also be useful to resolve the conflict between our morphological and molecular results. In both analyses, clade B (Spring and Eleven Point drainages) was shown to be distinctive. However, the drainages that are home to clades A and C were not shown to be distinctive in our morphological analysis. We hypothesize this may be connected to the higher population density in the Spring and Eleven Point drainages that we noticed while conducting this study (Appendix B) and was noted anecdotally by Fowler (2015). Crayfish have been shown to exhibit different morphology when under a higher amount of competition, notably longer and wider chelae (Haddaway et al. 2012). Our PCoA results showing that the Spring and Eleven Point had generally larger chelae than the other drainages support this finding. Also, crayfish have been found to have phenotypic plasticity than can be affected by other habitat qualities, such as stream flow rate (Haddaway et al. 2012). Crayfish with shorter chelae and a fusiform body have been shown to tolerate high water velocities better (Messager & Olden 2019). Therefore, other habitat differences between drainages could be causing morphological differences that are incongruent with the molecular data, but further research would be needed to support this. The flow regime across the range of *C. hubbsi* has been shown to vary greatly and could be another potential cause of the morphological results (Leasure et al. 2014). It is also possible that the morphology has just not yet evolved to reflect the genetic divergence seen between clades A and C (De Quieroz 2007).

Cambarus hubbsi is vulnerable to disturbance and environmental stressors due to its life history strategy and habitat preferences (Flinders & Magoulick 2005; Larson & Magoulick 2011; Yarra & Magoulick 2018). This vulnerability combined with the newly found cryptic diversity means that a large disturbance, such as an invasive species introduction (James et al. 2015), in one portion of its range has the potential to not only reduce the overall range of the species, but also, potentially lose a lineage unique to that drainage. We hope to see the three ESUs acknowledged by managers as further work is done to conserve *C. hubbsi* within its entire range.

Additionally, we hope that the new knowledge of cryptic biodiversity within the species will aid in conservation efforts as *C. hubbsi* continues to face anthropogenic and natural threats.

TABLES AND FIGURES

Table 2.1 *Cambarus hubbsi* individuals and outgroups in our molecular analysis with the Illinois Natural History Survey (INHS) Crustacean Collection museum number, GenBank accession numbers, and drainage of origin. N/A means not applicable.

Sample	INHS Museum #	GenBank- COI	GenBank- 16S	Drainage
CT641	16739	N/A	N/A	Current
CT646	16739	N/A	N/A	Current
JF16129	N/A	MG872959	N/A	Eleven Point
JF16071	N/A	MG872958	N/A	Eleven Point
KC124	N/A	JX514446	JX514518	Eleven Point
CT406	15419	N/A	N/A	Eleven Point
CT644	15430	N/A	N/A	Eleven Point
CT643	13732	N/A	N/A	Little N. Fork
CT638	16811	N/A	N/A	Lower White
CT639	16811	N/A	N/A	Lower White
CT628	16811	N/A	N/A	Lower White
CT640	16811	N/A	N/A	Lower White
CT647	16738	N/A	N/A	Saint Francis
CT648	16738	N/A	N/A	Saint Francis
CT634	16818	N/A	N/A	Spring
CT627	16818	N/A	N/A	Spring
CT635	16818	N/A	N/A	Spring
CT637	16818	N/A	N/A	Spring
CT622	16823	N/A	N/A	Spring
CT623	16823	N/A	N/A	Spring
CT631	16823	N/A	N/A	Spring
CT624	16823	N/A	N/A	Spring
CT618	16895	N/A	N/A	Spring
JF16104	N/A	MG872957	N/A	Spring
CT625	16814	N/A	N/A	Strawberry
CT619	16814	N/A	N/A	Strawberry
CT626	16814	N/A	N/A	Strawberry
CT629	16817	N/A	N/A	Strawberry
CT620	16817	N/A	N/A	Strawberry
CT621	16817	N/A	N/A	Strawberry
CT630	16817	N/A	N/A	Strawberry
CT632	16914	N/A	N/A	Upper White
CT636	16914	N/A	N/A	Upper White
CT633	16914	N/A	N/A	Upper White
P. clarkii	N/A	AY701195	AF235990	
C. maculatus	N/A	JF737746	AF235988	
C. striatus	N/A	JX514441	JX514514	
C. gentryi	N/A	DQ411785	AY853664	
C. hubrichti	N/A	JX514484	JX514519	
C. pyronotus	N/A	JX514439	JX514511	
C. tenebrosus	N/A	JX514444	JX514521	

Table 2.2 *Cambarus hubbsi* individuals included in our morphological analysis denoted by their drainage and Illinois Natural History Survey museum collection number. Museum numbers refer to lots that may contain multiple individuals. Total is the total number of *C. hubbsi* measured, and Females and Males refers to the number of females and form II males within that total.

Drainage	**INHS Catalog Numbers**	**Total**	**Females**	**Males**
Black	13198, 12918, 12689	6	6	0
Buffalo	10527, 16586	5	1	4
Current	15658, 13397	2	1	1
Eleven Point	10501, 10592, 15430, 16067, 16575	18	7	11
Little N. Fork White	13742	2	0	2
Lower White	10598, 16580	6	3	3
Spring	10705, 12821, 16576, 16592, 16632	19	7	12
Saint Francis	4917, 12449, 12865, 13046, 13056, 13111, 13113, 13116, 13181, 13873, 13899	29	14	15
Strawberry	10783, 16584, 16583, 15928	7	3	4
Upper White	16578, 9549, 16579	4	0	4

Table 2.3 The linear correlation and significance of morphological variables on the first and second principal coordinates. "TL" means total length of the preceding anatomical structure, or it means the post-orbital carapace length if written by itself. "MM" means mesial margin.

Morphological Count or Ratio	**PC 1**	**PC 2**	**Pr(>r)**
# Spines Merus MM	0.757	0.653	0.020
# Tubercles Chela MM	0.191	0.982	0.001
Areola Length/Width	-0.956	0.293	0.001
Rostrum TL/Tip Width	0.745	-0.667	0.001
Rostrum Base W/Tip Width	0.951	-0.308	0.001
Rostrum TL/ Base Width	-0.996	0.086	0.001
TL/Rostrum TL	0.999	0.036	0.001
Chela TL/Palm Width	-0.765	0.644	0.001
Palm Width/Palm Length	0.548	-0.837	0.001
Scale Length/Width	-0.058	-0.998	0.001
TL/Chela TL	-0.434	-0.901	0.001
TL/Palm Width	-0.925	-0.381	0.001
TL/Palm Length	-0.431	-0.902	0.001

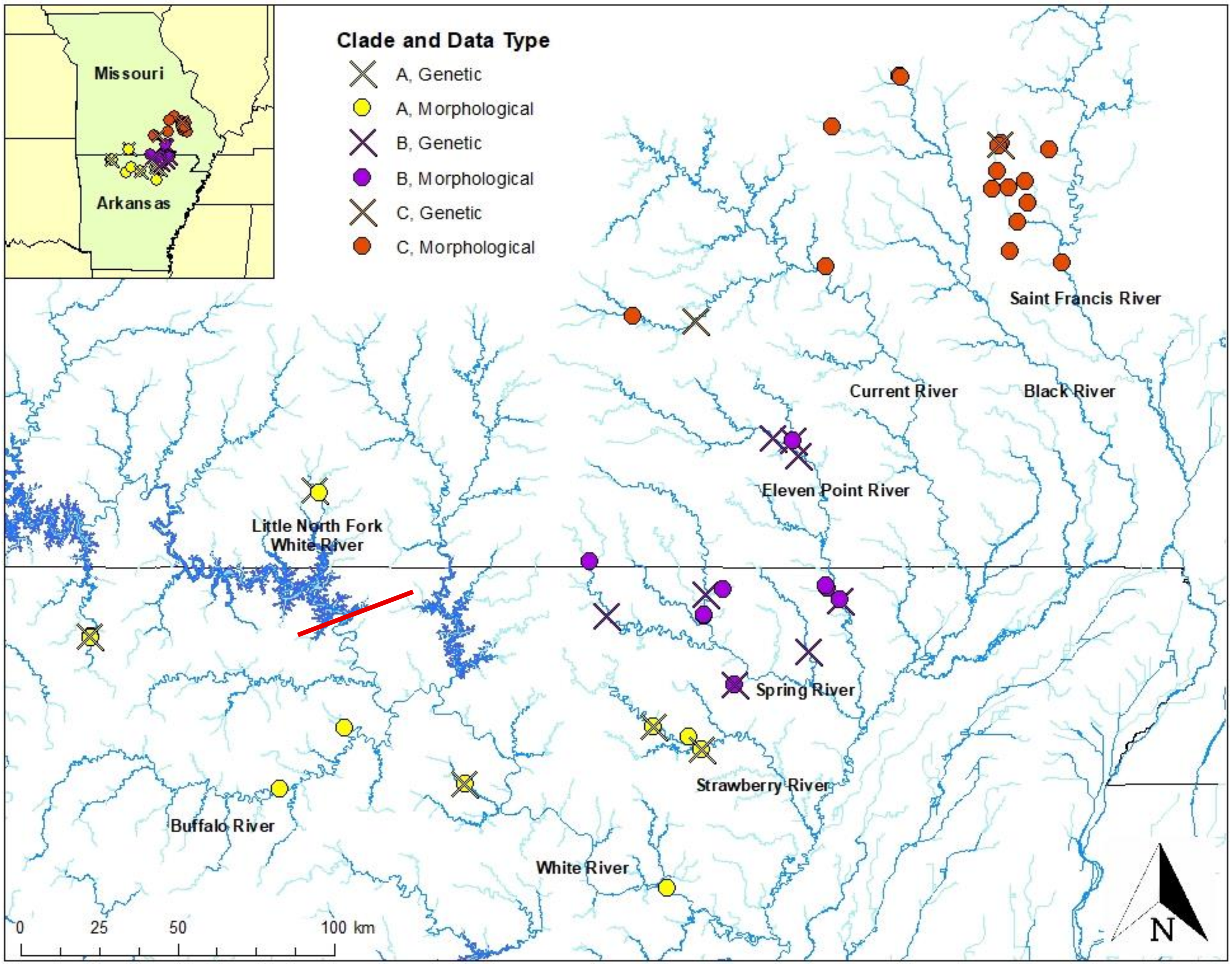


Figure 2.1 A map of specimen collection sites used in the study of molecular and morphological variation of *Cambarus hubbsi* (Hubbs' Crayfish) within Missouri and Arkansas, USA. Molecular genetic data sites are depicted with Xs, and morphological data is depicted with circles. Clades A, B, and C are yellow, purple, red respectively, and were assigned based on the results of the molecular analyses. The upper and lower White River drainages are defined respectively as anything upstream and downstream of Bull Shoals Reservoir; this border is indicated by the red line.

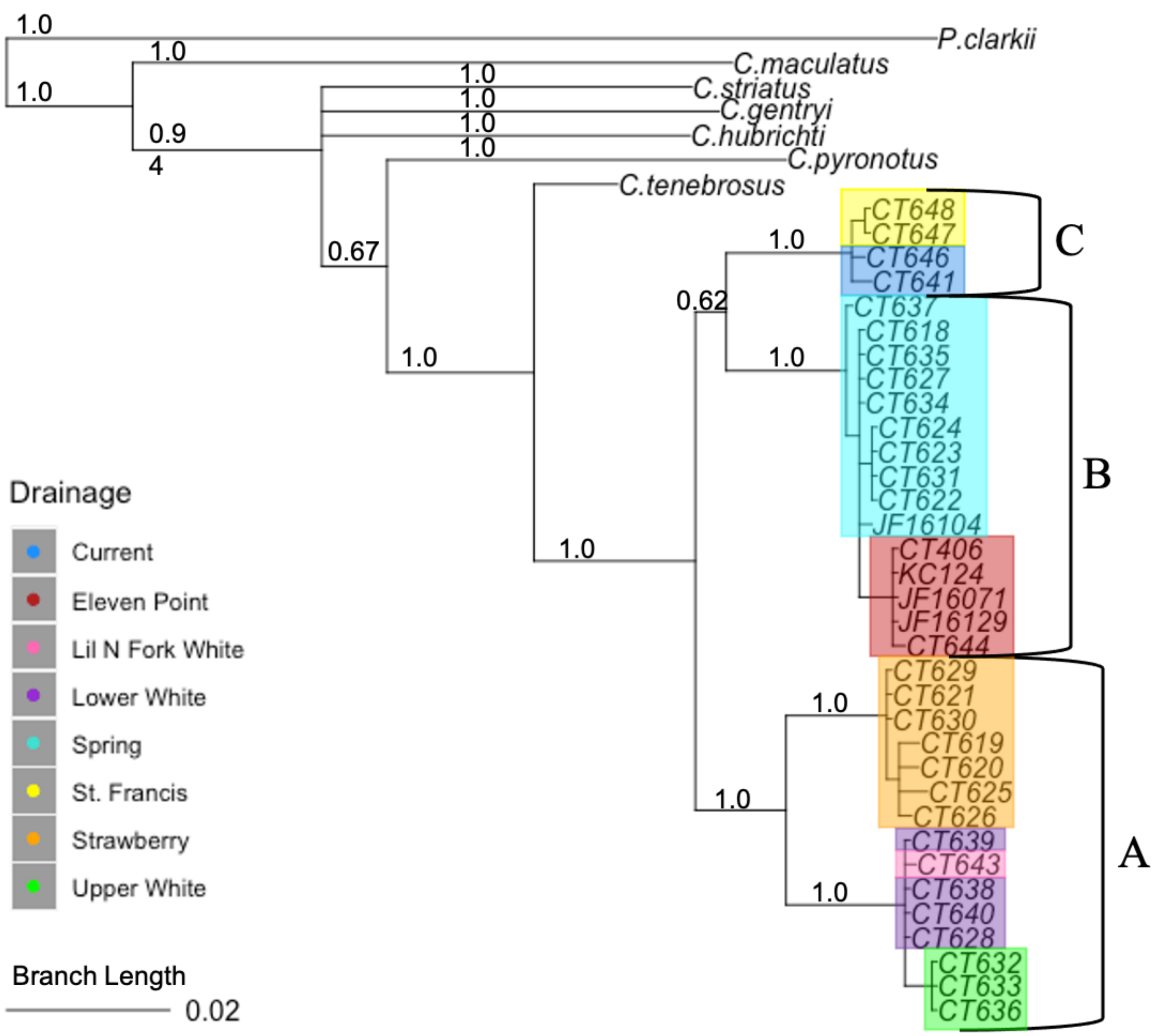


Figure 2.2 Phylogram showing the 50% majority rule consensus tree from our Bayesian analyses of *Cambarus hubbsi* (Hubbs' Crayfish) from Arkansas and Missouri. Posterior probabilities of nodes are given on branches. The three major clades of *C. hubbsi* are labeled A, B, and C, and drainages are color coded.

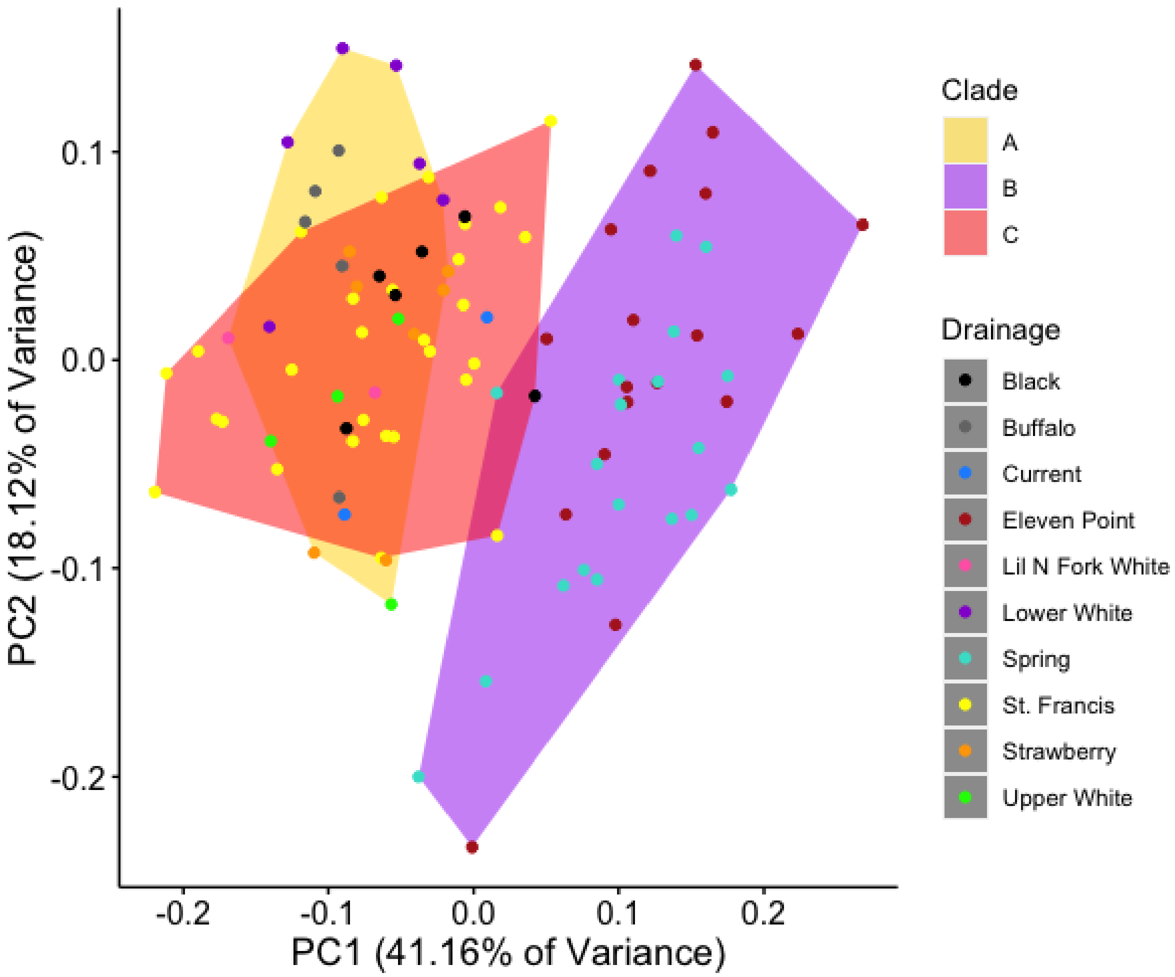


Figure 2.3 The first and second principal coordinates of the PCoA analysis on *Cambarus hubbsi*'s (Hubbs' Crayfish) morphology, color coded by drainage of origin. The polygons depict the associated clade based on the outcome of the molecular analysis.

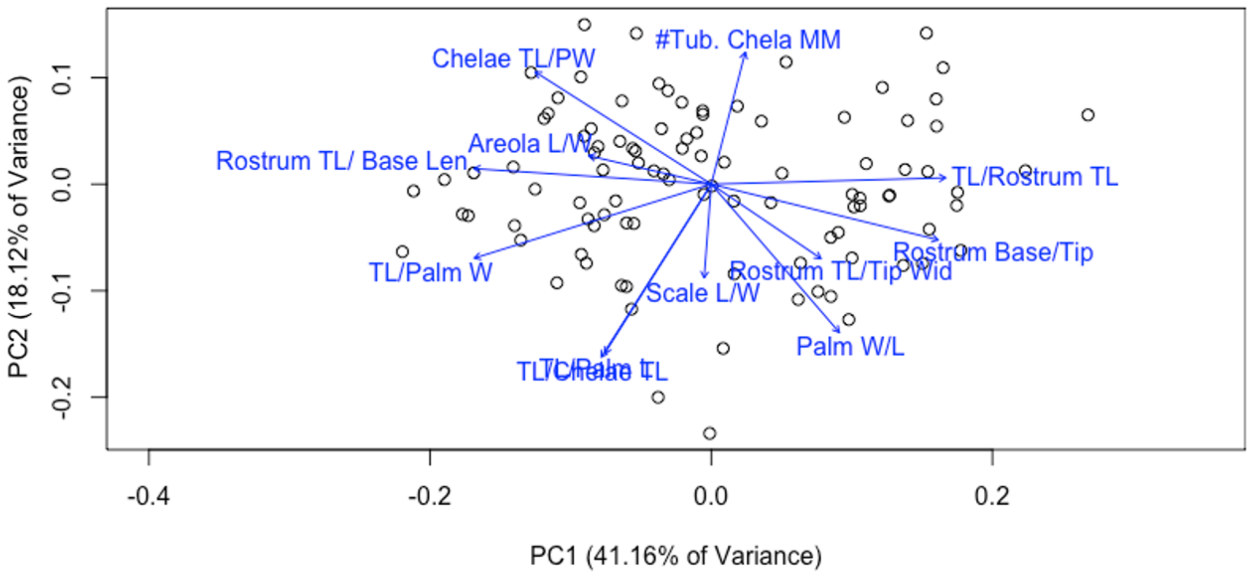


Figure 2.4 A vector plot of the first and second principal coordinates of the PCoA of *Cambarus hubbsi* depicting the influence of each morphological measurement ratio on the coordinates. The abbreviations used in the figure are the following; # Spines Merus MM = number of spines on the merus mesial margin, #Tub. Chela MM = number of tubercles on the chela mesial margin, Areola L/W = areola length divided by areola width, Rostrum TL/Tip Wid = rostrum length divided by rostrum width at the tip, Rostrum Base/Tip = rostrum width at the base divided by rostrum width at the tip, Rostrum TL/ Base Len = rostrum total length divided by the width of the rostrum at the base, TL/Rostrum TL = post-orbital carapace length divided by rostrum length, Chelae TL/PW = chela total length divided by palm width, Palm W/L = palm width divided by palm length, Scale L/W = antennal scale length divided by antennal scale width, TL/Chelae TL = post-orbital carapace length divided by chela length, TL/Palm W = post-orbital carapace length divided by palm width, TL/Palm L = post-orbital carapace length divided by palm length.

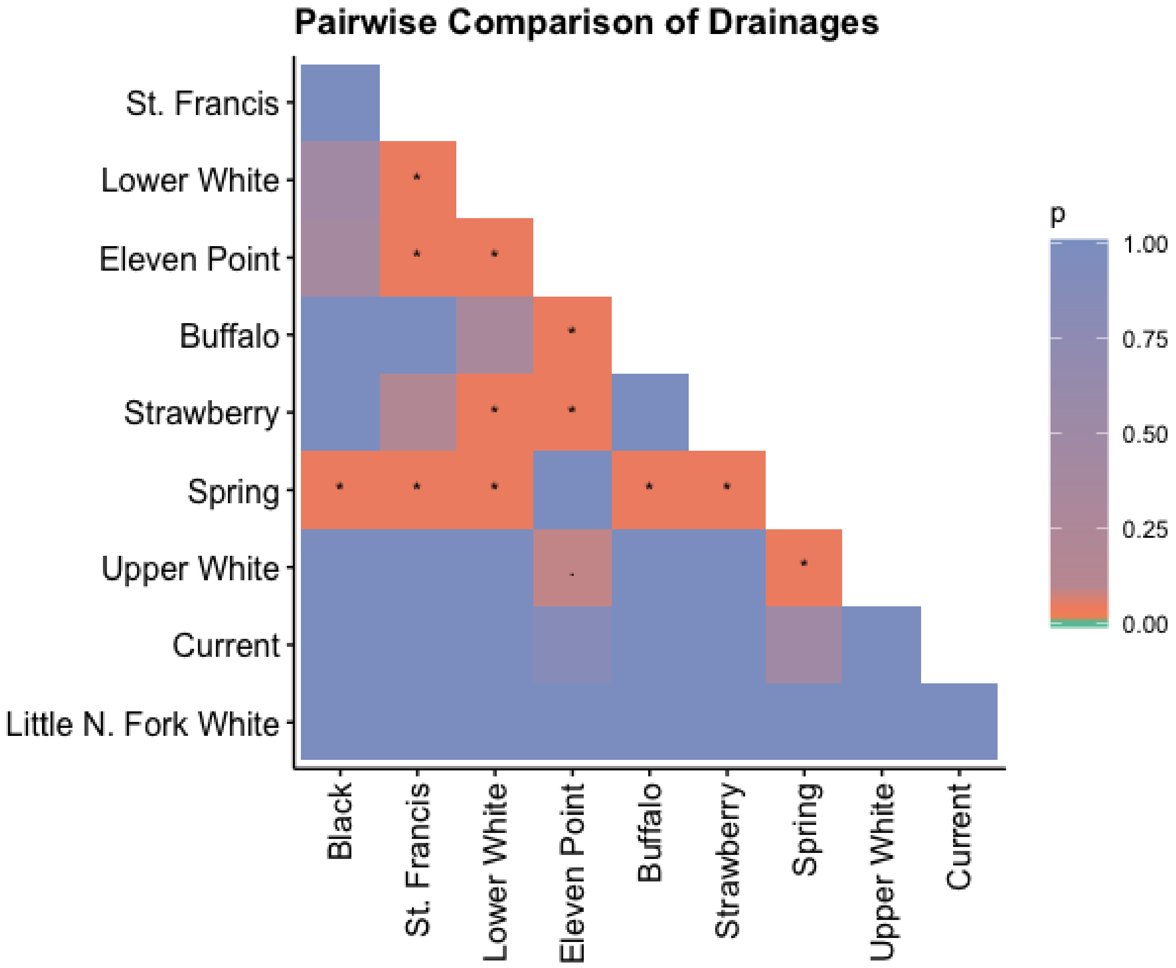


Figure 2.5 Comparisons between drainages of *Cambarus hubbsi* (Hubbs' Crayfish) from a pairwise, post-hoc test on the PERMANOVA conducted by using the R package pairwiseAdonis (Martinez 2020). Red blocks are statistically significant p values; '*' indicates $p \leq 0.05$ and '.' indicates $p \leq 0.10$.

LITERATURE CITED

Barbosa, S., Mestre, F., White, T. A., Paupério, J., Alves, P. C., & Searle, J. B. (2018). Integrative approaches to guide conservation decisions: Using genomics to define conservation units and functional corridors. *Molecular Ecology*, *27*(17), 3452–3465. https://doi.org/https://doi.org/10.1111/mec.14806

Barnett, Z.C., Adams, S.B., Ochs, C.A. and Garrick, R.C., 2020. Crayfish populations genetically fragmented in streams impounded for 36–104 years. *Freshwater Biology*, *65*(4), 768-785. https://doi.org/10.1111/fwb.13466

Beacham, T. D., Lapointe, M., Candy, J. R., Miller, K. M., & Withler, R. E. (2004). DNA in action: Rapid application of DNA variation to sockeye salmon fisheries management. *Conservation Genetics*, *5*(3), 411–416. https://doi.org/10.1023/B:COGE.0000031140.41379.73

Beheregaray, L. B., & Caccone, A. (2007). Cryptic biodiversity in a changing world. *Journal of biology*, *6*(4), 9. doi:10.1186/jbiol60

Breinholt, J. W., Porter, M. L., & Crandall, K. A. (2012). Testing phylogenetic hypotheses of the subgenera of the freshwater crayfish genus *Cambarus* (Decapoda: Cambaridae). *PLoS ONE*, *7*(9). https://doi.org/10.1371/journal.pone.0046105

Brown, A. V, Lyttle, M. M., & Brown, K. B. (1998). Impacts of gravel mining on gravel bed streams. *Transactions of the American Fisheries Society*, *127*(6), 979–994. https://doi.org/10.1577/1548-8659(1998)127<0979:IOGMOG>2.0.CO;2

Cashner, R., & Suttkus, R. (1977). *Ambloplites constellatus*, a new species of Rock Bass from the Ozark Upland of Arkansas and Missouri with a review of western Rock Bass populations. *The American Midland Naturalist, 98*(1), 147-161. doi:10.2307/2424721

Creed Jr., R. P. (1994). Direct and indirect effects of crayfish grazing in a stream community. *Ecology*, *75*(7), 2091–2103. https://doi.org/10.2307/1941613

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, *9*(8), 772. https://doi.org/10.1038/nmeth.2109

De Queiroz, K. (2007). Species concepts and species delimitation. *Systematic Biology*, *56*(6), 879–886. https://doi.org/10.1080/10635150701701083

Diéguez-Uribeondo, J., Royo, F., Souty-Grosset, C., Ropiquet, A., & Grandjean, F. (2008). Low genetic variability of the White-Clawed Crayfish in the Iberian Peninsula: Its origin and management implications. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *18*(1), 19–31. https://doi.org/https://doi.org/10.1002/aqc.811

Dizon, A., Lockyer, C., Perrin, W., Demaster, D., & Sisson, J. (1992). Rethinking the stock concept: A phylogeographic approach. *Conservation Biology*, *6*(1), 24-36. http://www.jstor.org/stable/2385848

Fetzner, J. W., & Taylor, C. A. (2018). Two new species of freshwater crayfish of the genus *Faxonius* (Decapoda: Cambaridae) from the Ozark Highlands of Arkansas and Missouri. *Zootaxa*, *4399*(4), 491–520. https://doi.org/10.11646/zootaxa.4399.4.2

Fisk, H. N. (1944). *Geological Investigation of the Alluvial Valley of the Lower Mississippi River*. Mississippi River Commission

Flinders, C. A., & Magoulick, D. D. (2005). Distribution, habitat use and life history of stream-dwelling crayfish in the Spring River drainage of Arkansas and Missouri with a focus on the imperiled Mammoth Spring Crayfish (*Orconectes marchandi*). *American Midland Naturalist*, *154*(2), 358–374. http://10.0.6.138/0003-0031(2005)154[0358:DHUALH]2.0.CO

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, *3*(5), 294–299. https://doi.org/10.1071/ZO9660275

Foltz, D. A., II, Sadecky, N. M., Myers, G. A., Fetzner, J. W., Welsh, S. A., Stocker, G. W., Glon, M. G., & Thoma, R. F. (2018). *Cambarus loughmani*, a new species of crayfish (Decapoda: Cambaridae) endemic to the pre-glacial Teays River Valley in West Virginia, USA. *Journal of Natural History*, *52*(45–46), 2875–2897. https://doi.org/10.1080/00222933.2018.1557271

Fowler, Allison (Ed) (2015). *Arkansas Wildlife Action Plan*. Arkansas Game and Fish Commission, Little Rock, Arkansas.

Funk, W. C., McKay, J. K., Hohenlohe, P. A., & Allendorf, F. W. (2012). Harnessing genomics for delineating conservation units. *Trends in Ecology and Evolution*, *27*, 489–496. https://doi.org/10.1016/j.tree.2012.05.012

Guindon, S., & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, *52*(5), 696–704. https://doi.org/10.1080/10635150390235520

Haddaway, N. R., Mortimer, R. J. G., Christmas, M., Grahame, J. W., & Dunn, A. M. (2012). Morphological diversity and phenotypic plasticity in the threatened British white-clawed crayfish (*Austropotamobius pallipes*). *Aquatic Conservation: Marine and Freshwater Ecosystems*, *22*(2), 220–231. https://doi.org/https://doi.org/10.1002/aqc.2225

Helms, B. S., Vaught, R. C., Suciu, S. K., & Santos, S. R. (2015). Cryptic diversity within two endemic crayfish species of the Southeastern US revealed by molecular genetics and geometric morphometrics. *Hydrobiologia*, *755*(1), 283–298. https://doi.org/10.1007/s10750-015-2311-4

Imhoff, E., Moore, M., & DiStefano, R. (2012). Introduced alien ringed crayfish (*Orconectes neglectus neglectus* [Faxon, 1885]) threaten imperiled coldwater crayfish (*Orconectes eupunctus* Williams, 1952) in the Eleven Point River drainage, Missouri, USA. *Aquatic Invasions*, *7*(1), 129–134. https://doi.org/10.3391/ai.2012.7.1.014

Jackson, D. A. (1993). Stopping rules in principal components analysis: A comparison of heuristical and statistical approaches. *Ecology*, *74*(8), 2204–2214. https://doi.org/https://doi.org/10.2307/1939574

James, J., Slater, F. M., Vaughan, I. P., Young, K. A., & Cable, J. (2015). Comparing the ecological impacts of native and invasive crayfish: could native species' translocation do more harm than good? *Oecologia*, *178*(1), 309–316. https://doi.org/10.1007/s00442-014-3195-0

Kreps, T., Elgin, A., & Lodge, D. (2012). The impact of an invasive predator (*Orconectes rusticus*) on freshwater snail communities: Insights on habitat-specific effects from a multilake long-term study. *Canadian Journal of Fisheries and Aquatic Sciences*, *69*, 1164–1173. https://doi.org/10.1139/f2012-052

Larson, E. R., Abbott, C. L., Usio, N., Azuma, N., Wood, K. A., Herborg, L.-M., & Olden, J. D. (2012). The signal crayfish is not a single species: cryptic diversity and invasions in the Pacific Northwest range of *Pacifastacus leniusculus*. *Freshwater Biology*, *57*(9), 1823–1838. https://doi.org/https://doi.org/10.1111/j.1365-2427.2012.02841.x

Larson, E. R., & Magoulick, D. D. (2011). Life-history notes on *Cambarus hubbsi* Creaser (Hubbs Crayfish) from the South Fork Spring River, Arkansas. *Southeastern Naturalist*, *10*(1), 121. https://doi.org/10.1656/058.010.0110

Leasure, D. R., Magoulick, D. D., & Longing, S. D. (2016). Natural flow regimes of the Ozark–Ouachita Interior Highlands Region. *River Research and Applications*, *32*(1), 18–35. https://doi.org/https://doi.org/10.1002/rra.2838

Loughman, Z. J., Welsh, S. A., & Thoma, R. F. (2017). *Cambarus (C.) appalachiensis*, a new species of crayfish (Decapoda: Cambaridae) from the New River Basin of Virginia and West Virginia, USA. *Zootaxa*, *4243*(3), 432–454. https://doi.org/10.11646/zootaxa.4243.3.2

Martinez Arbizu, P. (2020). pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version 0.4

Magoulick, D. D., & DiStefano, R. J. (2007). Invasive crayfish *Orconectes neglectus* threatens native crayfishes in the Spring River Drainage of Arkansas and Missouri. *Southeastern Naturalist*, *6*(1), 141–150. http://www.jstor.org/stable/4540986

Magoulick, D., DiStefano, R., Imhoff, E., Nolen, M., & Wagner, B. (2017). Landscape- and local-scale habitat influences on occupancy and detection probability of stream-dwelling crayfish: Implications for conservation. *Hydrobiologia*, *799*, 217-231. https://doi.org/10.1007/s10750-017-3215-2

Messager, M. L., & Olden, J. D. (2019). Phenotypic variability of Rusty Crayfish (*Faxonius rusticus*) at the leading edge of its riverine invasion. *Freshwater Biology*, *64*(6), 1196–1209. https://doi.org/https://doi.org/10.1111/fwb.13295

Momot, W. T. (1995). Redefining the role of crayfish in aquatic ecosystems. *Reviews in Fisheries Science*, *3*(1), 33–63. https://doi.org/10.1080/10641269509388566

Moore, M. J., DiStefano, R. J., & Larson, E. R. (2013). An assessment of life-history studies for USA and Canadian crayfishes: identifying biases and knowledge gaps to improve conservation and management. *Freshwater Science*, *32*(4), 1276–1287. http://10.0.7.107/12-158.1

Moritz, C. (1994). Defining ‘Evolutionarily Significant Units’ for conservation. *Trends in Ecology & Evolution*, *9*(10), 373–375. https://doi.org/https://doi.org/10.1016/0169-5347(94)90057-4

Nguyen, L.-T., Schmidt, H. A., von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, *32*(1), 268–274. https://doi.org/10.1093/molbev/msu300

Nolen, M. S., Magoulick, D. D., DiStefano, R. J., Imhoff, E. M., & Wagner, B. K. (2014). Predicting probability of occurrence and factors affecting distribution and abundance of three Ozark endemic crayfish species at multiple spatial scales. *Freshwater Biology*, *59*(11), 2374–2389. https://doi.org/10.1111/fwb.12442

Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., and Wagner, H. (2019). vegan: Community Ecology Package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan

Page, L., & Cordes, L. (1983). Variation and systematics of *Etheostoma euzonum*, the Arkansas Saddled Darter (Pisces: Percidae). *Copeia, 1983*(4), 1042-1050. doi:10.2307/1445107

Paradis, E. & Schliep, K. (2018). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35: 526-528.

Pflieger, W. L. (1996). *The Crayfishes of Missouri*. Missouri Department of Conservation, Jefferson City.

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reiss, H., Hoarau, G., Dickey-Collas, M., & Wolff, W. J. (2009). Genetic population structure of marine fish: Mismatch between biological and fisheries management units. *Fish and Fisheries*, *10*(4), 361–395. https://doi.org/https://doi.org/10.1111/j.1467-2979.2008.00324.x

Reynolds, J., Souty-Grosset, C., & Richardson, A. (2013). Ecological roles of crayfish in freshwater and terrestrial habitats. *Freshwater Crayfish*, *19*(2), 197–218. https://doi.org/10.5869/fc.2013.v19-2.197

Richman, N. I., Böhm, M., Adams, S. B., Alvarez, F., Bergey, E. A., Bunn, J. J. S., Burnham, Q., Cordeiro, J., Coughran, J., Crandall, K. A., Dawkins, K. L., DiStefano, R. J., Doran, N. E., Edsman, L., Eversole, A. G., Füreder, L., Furse, J. M., Gherardi, F., Hamr, P., Holdich, D. M., Horwitz, P., Johnston, K., Jones, C. M., Jones, J. P. G., Jones, R. L., Jones, T. G., Kawai, T., Lawler, S., López-Mejía, M., Miller, R. M., Pedraza-Lara, C., Reynolds, J. D., Richardson, A. M. M., Schultz, M. B., Schuster, G. A., Sibley, P. J., Souty-Grosset, C., Taylor, C. A., Thoma, R. F., Walls, J., Walsh, T. S., & Collen, B. (2015). Multiple drivers of decline in the global status of freshwater crayfish (Decapoda: Astacidea). *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *370*(1662), 20140060. https://doi.org/10.1098/rstb.2014.0060

Ronquist, F., M. Teslenko, P. van der Mark, D. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology 61*:539–542. DOI: 10.1093/sysbio/sys029Sequencher® version 5.4.6 DNA sequence analysis software, Gene Codes Corporation, Ann Arbor, MI USA http://www.genecodes.com

Ryder, O. A. (1986). Species conservation and systematics: the dilemma of subspecies. *Trends in Ecology & Evolution*, *1*(1), 9–10. https://doi.org/10.1016/0169-5347(86)90059-5

Swofford, D.L. (2002) PAUP: Phylogenetic analysis using parsimony (and other methods), Version 4.0 Beta 10. Sinauer Associates, Sunderland.

Taylor, C. A., Adams, S. B., & Schuster, G. A. (2014). Systematics and biogeography of *Orconectes*, Subgenus *Trisellescens*, in the Southeastern United States, a test of morphology-based classification. *Journal of Crustacean Biology*, *34*(1), 1–14. https://doi.org/10.1163/1937240X-00002196

Taylor, C. A., Schuster, G. A., Cooper, J. E., DiStefano, R. J., Eversole, A. G., Hamr, P., Hobbs, H. H., Robison, H. W., Skelton, C. E., Thoma, R. F. (2007). A reassessment of the conservation status of crayfishes of the United States and Canada after 10+ years of increased awareness. *Fisheries*, *32*(8), 372–389. https://doi.org/10.1577/1548-8446(2007)32[372:arotcs]2.0.co;2

Taylor, C. A., & Soucek, D. J. (2010). Re-examining the importance of fish in the diets of stream-dwelling crayfishes: Implications for food web analyses and conservation. *The*

American Midland Naturalist, *163*(2), 280–293. https://doi.org/10.1674/0003-0031-163.2.280

Thomas, C., & Taylor, C. A. (2013). Scavenger or predator? Examining a potential predator–prey relationship between crayfish and benthic fish in stream food webs. *Freshwater Science*, *32*, 1309–1317. https://doi.org/10.1899/12-169.1

Voelker, G., Marks, B. D., Kahindo, C., A'genonga, U., Bapeamoni, F., Duffie, L. E., Huntley, J. W., Mulotwa, E., Rosenbaum, S. A., Light, J. E. (2013). River barriers and cryptic biodiversity in an evolutionary museum. *Ecology and Evolution*, *3*(3), 536–545. https://doi.org/10.1002/ece3.482

Yarra, A. N., & Magoulick, D. D. (2019). Modelling effects of invasive species and drought on crayfish extinction risk and population dynamics. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *29*(1), 1–11. https://doi.org/10.1002/aqc.2982

CHAPTER 3: MULTI-SCALE MODELING OF THE DISTRIBUTION OF A RARE, ENDEMIC CRAYFISH (*CAMBARUS CAUSEYI*)

INTRODUCTION

Primary burrowing crayfishes are crayfish that spend the majority of their lives in burrows and only leave occasionally to mate and find food (Hobbs 1942). Burrowing crayfish play an important role as ecosystem engineers by disturbing the soil in which they burrow and by creating habitat for herpetofauna (Welch et al. 2008) and arthropods (Pintor and Soluk 2006). Crayfish are understudied relative to other aquatic taxa (Reid et al. 2019), and even among crayfishes, primary burrowers are particularly understudied (Bloomer et al. 2021; Moore et al. 2013). About 22% of North American crayfish species of conservation concern are primary burrowers (Taylor et al. 2007). Additionally, primary burrowers are known to inhabit a wide diversity of habitats such as open-canopied grasslands (Rhoden et al. 2016), pitcher plant bogs (Welch et al. 2008), and upland forested seeps (Loughman 2010), and can be habitat specialists or generalists (Loughman et al. 2012). To effectively conserve a species, resource managers must understand a species distribution and habitat requirements, so they can make informed decisions (Richter et al. 1997; Taylor et al. 2007).

A tool for analyzing the habitat variables driving the range of species, especially rare and endemic taxa, is species distribution modeling (SDMs) (Fois et al. 2018; Peterman et al. 2013; Rhoden et al. 2017). Species distribution models use species locality and environmental data to make a correlative model that depicts the relative suitability of habitat for a given species (Warren & Seifert 2011). A common and effective program for creating SDMs is the maximum entropy modeling software MaxEnt (Elith et al. 2011; Phillips et al. 2006). MaxEnt is a suitable tool for rare and understudied species because it performs well with small sample sizes and is capable of using presence-only data (Elith et al. 2011; Hernandez et al. 2006, Rhoden et al. 2017).

While SDMs can be used for analyzing the drivers of a species' distribution, ground-truthing should be included as an important part of the process (Egly & Larson 2018; Peterman et al. 2013; Rhoden et al. 2017; Stirling et al. 2016). Traditional field sampling can incorporate variables motivating a species' distribution that are not easily accounted for by using only large-scale spatial data and possibly unreliable historical data (Hirzel et al. 2006). This is particularly true for sessile organisms or organisms with a small home range, which benefit from finer grain analyses (Guisan et al. 2007). Species distribution models can poorly account for the influence of biotic variables, lack fine-scale habitat information, or have sampling bias (Fourcade et al. 2014; Guisan & Thuiller 2005; Peterman et al. 2013). These potentially illustrate the need for incorporating ground-truthing and utilizing traditional habitat sampling when studying a species' habitat needs and distribution. This study seeks to use SDMs and fine-scale habitat modeling to understand the habitat preferences and range of the narrowly endemic Boston Mountain Crayfish (*Cambarus causeyi*).

Cambarus causeyi is a rarely observed burrowing crayfish that is endemic to the Boston Mountains of Arkansas, United States of America (USA). It was initially described by Reimer (1966) using individuals collected from Pope County, Arkansas. However, the undescribed *C. causeyi* was first collected by Horton H. Hobbs in 1941 in Pope County (Robison et al. 2009). *Cambarus causeyi* is described as spending the majority of its life in generally shallow, yet complex, burrows, but it is believed to occasionally occur in "small springs and tiny creeks under rocks" for reproduction (Robison et al. 2009). Additionally, *C. causeyi* was noted to be associated with intermittent mountain seeps and streams, and burrows were often found underneath large stones (Robison & Leeds 1996). Reproductively active form I males were found during the months of February, March, April, June, July, August, September, and November (Robison et al. 2009). The only egg-bearing female was found in July, which suggests breeding occurs in the spring or early summer (Robison & Leeds 1996).

Cambarus causeyi is understudied and has only been observed a few number of times since being described. Between 1941 and 1986, only nine *C. causeyi* individuals were captured over six surveys. These specimens were collected from four localities in Pope County and one locality in Stone County (Robison & Leeds 1996). Robison and Leeds (1996) reported the capture of 87 individuals during 47 collections from 39 separate localities (erroneously reported as 40). The 1996 study expanded the known range of *C. causeyi* to include Madison, Johnson, Franklin, Newton, and Searcy counties and reported 67 of the individuals in this study were collected from Johnson County, making it the county with the highest abundance.

Robison et al. (2009) published a follow-up study on the distribution of *C. causeyi*. The authors visited 39 localities but found 14 *C. causeyi* in total at only four sites (only three described in Table 3.1). The new localities were in Madison, Newton, and Searcy counties. Robison et al. (2009) located *C. causeyi* at four localities, relative to the 40 localities where Robison and Leeds (1996) found *C. causeyi*, which signaled a potential decrease in range for the species. The extreme decrease in the number of individuals caught in the 2009 study caused *C. causeyi* to be updated to the status of Vulnerable on the 2009 IUCN assessment (Robison et al. 2009). Only two additionally records of *C. causeyi* have been made by Arkansas Game and Fish Commission (AGFC) since 2009; they are from Newton and Van Buren counties in 2017 and 2019, respectively.

We set out to determine the distribution of *C. causeyi* and create models to ascertain its preferred habitat. The SDM and fine-scale modeling will aid in determining if there has been a decline in the range of *C. causeyi* as suggested in Robison et al. (2009) and identify habitat needed to protect the species. The SDM will assist in our understanding of what is suitable habitat for *C. causeyi* and predict its distribution, and the fine-scale habitat analysis will ground-truth the SDM and provide information to managers for future *C. causeyi* sampling.

METHODS

Species Distribution Modeling

Using presence-only historical occurrences, we created a SDM of suitable habitat for *C. causeyi*. This model was created in the maximum entropy modeling software MaxEnt version 3.4.1. Due to the vagueness of many of the historical site descriptions from Robison and Leeds (1996) and Robison et al. (2009), we sorted the 44 historical sites by the precision of their location descriptions. Out of the 44 sites there are two exact locations, eight good locations, 27 fair locations, two poor locations, and five unusable locations (Fig. 3.1). Exact locations were sites where coordinates were provided by the original source. Good locations lacked coordinates but could be narrowed down to a specific location and no other locations matched the historical description. An example of a site that was classified as good was the “roadside seepage on St. Hwy. 16, 3.1 mi. S of jct. of St. Hwys. 16 and 23 and 0.4 mi. W. of Dutton” (Robison et al. 2009). Fair locations provided a township, range, and section, but an exact location was not identifiable. Fair locations were either placed on a road crossing at a stream that seemed to match the description, or in the center of the section if a specific stream crossing could not be selected. Poor locations did not provide a township, range, and section and had vague site descriptions.

Unusable sites either had a description that made it unfeasible to locate the site, or were in the same township, range, and section as another vaguely identified site. Only sites labelled as exact, good, or fair were used in the model; this included 37 historical sites in total. We had a small number of samples included in our SDM, but Maxent has been shown to produce reliable models, even with smaller sample sizes (Fois et al. 2018, Galante et al. 2018).

The extent of the model projection was all Hydrologic Unit Code 8's (HUC 8's) that significantly overlap with the counties of historical sites (Fig. 3.1). These HUC 8's were clipped to the boundaries of Arkansas. HUC8 Petit Jean was excluded because it minimally overlapped with *C. causeyi*'s range. The model's grain size was 30 x 30 meters because most of the available environmental layers were at this resolution and it is fine scale enough to be informative for crayfish (Rhoden et al. 2017). Any layers that were not available at a 30 x 30 meters grain size were converted to that scale using ArcMap.

Environmental variables included in the SDM were precipitation, Euclidean distance from a stream, elevation, slope, solar radiation, and average available water storage for the top 150 cm of soil. Average annual precipitation data (Fig. 3.2) was taken from the 30-year Parameter-elevation Regressions on Independent Slopes Model (PRISM) data for 1981-2010 (Daly et al. 1997), and it was included in the model because of C. *causeyi*'s association with ephemeral, precipitation dependent streams (Robison et al. 2009). The Euclidean distance from a stream was calculated with the National Hydrography Dataset (U.S. Geological Survey) and was selected because *C. causeyi* is associated with small streams. Elevation data were taken from the National Elevation Dataset (U.S. Geological Survey & EROS Data Center), and we hypothesized that *C. causeyi* may be associated with seepages in high elevation areas (Robison & Leeds 1996). Slope and solar radiation were calculated from the elevation dataset using ArcMap with the ESRI

Spatial Analysts Tools. Slope was included because we anecdotally noted that *C. causeyi* seemed to be found on steeper inclines and solar radiation was used because it was found to be positively associated with other burrowers (Rhoden et al. 2017). Soil data were taken from the gridded Soil Survey Geographic Database (gSSURGO); we included average available water storage for the top 150 cm of soil because burrowing crayfish are associated with hydric environments (Rhoden et al. 2016).

We tested for correlation between all predictor variables by using the Collection Band Statistics tool in ArcMap. A 45-replicate run was conducted in MaxEnt with bootstrapping for replication using a 20% random test percentage. Variable importance was measured with a jackknife analysis and response curves. We set a maximum of 5,000 iterations of the MaxEnt models, and used pseudoabsences, due to a lack of absence data for the species. The above settings and the defaults were used in the final MaxEnt model. Due to the narrow range of *C. causeyi* and the small number of presences, we did not use a bias file. Additionally, we calculated the test area under the curve (AUC), percent contribution, and permutation importance.. Area under the curve is a measure of how well a model can differentiate between presences and background points. Area under the curve is on a scale of 0 to 1, and above 0.5 the model is better than random. Percent contribution is how much training gain each variable provided to the model. Permutation importance is how much the training AUC is impacted by a variable being permuted randomly.

Fine-scale Habitat Modeling

Field Methods

In March of 2019 we conducted a preliminary survey with hand excavation at fourteen sites to ensure that *C. causeyi* was still present and detectable in its known range. Following this preliminary survey, we conducted habitat sampling over the summers of 2019 and 2020. We had intended to conduct habitat sampling during the spring, when *C. causeyi* activity is at its peak (Robison et al. 2009), but we were unable to sample due to the global pandemic. For *C. causeyi* fine-scale habitat analysis, historical sites (including known sites from March 2019 sampling) and new sites were sampled for *C. causeyi* and habitat. New sites were located where streams were present, and they were selected to cover the entire range of MaxEnt output values and predicted range of *C. causeyi* to confirm the validity of the SDM. Habitat data was collected from 51 sites in total. Habitat data and crayfish were collected from within quadrats, and there was a thirty-minute timed search for crayfish at each site. There were five one m^2 quadrats placed at a site. The quadrats were marked out by using a $1m^2$ polyvinyl chloride (PVC) pipe quadrat sampler and flags. The first quadrat was placed within the stream channel, or the dried stream channel, haphazardly. Then a quadrat was placed ten meters from the central quadrat upstream, downstream, on the left ascending bank, and on the right ascending bank. In situations where there was a barrier preventing a quadrat being placed exactly ten meters away from the central quadrat, the quadrat was placed as close to ten meters away as possible. The timed searched was used to aid in detection of *C. causeyi* due to its low abundance at most sites. The field crew searched anywhere in the nearby vicinity, excluding the areas within the five quadrats. All crayfish were collected with hand excavation or dip net, which was used if there was standing water present and the burrows were found empty.

At each site, the following variables were recorded: average canopy cover as a proportion, proportion of quadrats with large rock present, sand proportion in soil, the presence of another primary burrower (*Procambarus liberorum*) and the presence of a stream dwelling crayfishes (*Faxonius meeki* and *Faxonius williamsi*). Canopy cover was measured at each quadrat by using a Model-A spherical densiometer from Forestry Suppliers, Incorporated. A lack of tree canopy cover was shown to be an important positive driver for other burrowing crayfish's occupancy (Rhoden et al. 2016). We considered any rocks larger than 128mm x 128mm to be large. We expected large rock to have a positive effect on *C. causeyi*'s presence due to it being associated with large rocks in past surveys (Robison et al. 2009). Sand proportion was calculated from a soil core collected at the central quadrat at each site. The soil samples were analyzed with laser diffraction by the Illinois State Water Survey (Appendix C). Sandy soils has been shown to have a negative association with other burrowers because they are not suitable for constructing burrows, and we expected to find the same with *C. causeyi* (Dorn & Volin 2009; Grow 1982; Grow & Merchant 1980). We expected the presence of other crayfish species to have a negative effect on whether *C. causeyi* occupied a site due to interspecies competition (James et al. 2015; Reynolds et al. 2013).

Modeling

We modeled our fine-scale data with zero-inflated Poisson generalized linear models in the program R version 3.5.2 with the package glmmTMB version 1.0.2.1 (Brooks et al. 2017). Our response variable was the number of quadrats occupied by *C. causeyi*. We treated the timed search for *C. causeyi* as a sixth quadrat in this analysis. We ran a zero-inflated model because we had poor detection of *C. causeyi*. We used the habitat data collected from the quadrat sampling and the interpolated MaxEnt output to create a suite of potential models. We ground-truthed the MaxEnt model by using the interpolated MaxEnt output for the site as a predictor variable of the number of quadrats occupied by *C. causeyi*. Models included in model selection were the null model, sand only, interpolated MaxEnt output only, canopy cover only, proportion of quadrats containing a large rock, presence/absence of *P. liberorum*, presence/absence of stream dwelling crayfish species, sand and *P. liberorum*, interpolated MaxEnt output and sand and *P. liberorum*, and sand and large rock. We selected the most supported model with Akaike information criterion corrected for small sample size (AICc). Additionally, we analyzed the accuracy of our MaxEnt predictions by calculating the AUC of the receiver operating characteristic of occupied sites compared to unoccupied sites from our fine-scale habitat sampling (Fawcett 2006; Rhoden et al. 2017). We conducted this analysis with the pROC package in program R (Robin et al., 2011).

RESULTS

Species Distribution Modeling

Our MaxEnt model's test AUC was 0.911 (Fig. 3.3). Our jackknife analysis results show that average annual precipitation was by far the best predictor of *C. causeyi* in our model (Fig. 3.4). Precipitation also had the highest percent contribution and permutation importance in the model (Table 3.1). There was a unimodal relationship between the average annual precipitation and the likelihood *C. causeyi* was predicted to live in an area, which peaked at approximately 54 inches per year (Fig. 3.5). Above this limit of 54 inches per year the predicted suitability for *C. causeyi* decreased, plateauing around 60 inches per year. In the jackknife analysis, all other variables performed poorly; models without the other environmental variables performed about as well or better than the global model. Additionally, all variables, besides precipitation, had a percent contribution below 14 percent and a permutation importance below seven percent. Solar radiation was consistently shown to be the least important variable to the MaxEnt model.

Fine-scale Habitat Modeling

We only detected *C. causeyi* at nine of 51 sites during our habitat sampling (Fig. 3.6). Three sites lacking *C. causeyi* during our habitat sampling contained individuals during our preliminary March 2019 survey, and they were treated as absences in our statistical analysis (Appendix D). We found male and female pairs sharing burrows during the spring preliminary sampling and found only one female in berry and many young instars during the summer habitat sampling. Our most supported model from our fine-scale analysis included the proportion of sand and presence of *P. liberorum* as predictor variables (Table 3.2), and the proportion of sand and the presence of *P. liberorum* had a negative association with *C. causeyi* relative abundance (Fig. 3.7). Proportion of sand and presence of *P. liberorum* were also the only single variable models to perform better than the null model. The interpolated MaxEnt output generally performed poorly. In the only model better than the null model that included the MaxEnt output, the MaxEnt output had a negative impact on the number of quadrats occupied by *C. causeyi*. The AUC for the field validation of our MaxEnt model was 0.548.

DISCUSSION

Our MaxEnt model appeared to perform well with a high AUC and identified average annual precipitation as an important factor in defining the distribution of this species. However, upon ground-truthing the model, the MaxEnt model performed poorly, as it was not included in our most supported generalized linear models. We found site-specific measures of soil texture and presence of another burrower (*P. liberorum*) best explained *C. causeyi*'s relative abundance. GIS-based SDMs like MaxEnt may lack fine-resolution data that determines occupancy or abundance (Anderson et al. 2002; Guisan & Thuiller 2005; Peterman et al. 2013), although the low detectability of *C. causeyi* and coronavirus preventing field sampling during the spring of 2020 might also have affected our results. Both scales of analyses (range-wide SDM predictors and fine-scale predictors from ground-truthing) may inform future management and conservation of *C. causeyi.*

Precipitation may have a positive effect on habitat suitability for *C. causeyi* because the species inhabits ephemeral streams, which dry up in certain areas or parts of the year (Robison et al. 2009). Increased levels of precipitation would ensure that there is water available for longer periods of time. Additionally, *C. causeyi* is known for having shallow burrows, and increased precipitation would bring the water table closer to the surface (Novakowski & Gillham 1988; Robison et al. 2009). A shallow water table means individuals would not have to burrow as deep to reach water. The importance of high precipitation to defining *C. causeyi*'s distribution, may explain the low detection by Robison et al. (2009), which was preceded by and coincided with the 2006-2007 extreme drought in Arkansas, according to the U.S. Drought Monitor.

While precipitation may be good at broadly defining the range of *C. causeyi*, available GIS environmental data may miss factors important for this organism. For example, in our fine-scale modeling, the amount of sand in the soil was shown to be negatively associated with *C. causeyi* relative abundance, but the water storage capacity of the soil was not shown to be important in our MaxEnt model. Water storage capacity of the soil from gSSURGO had a poor relationship with our field-observed sand proportion at these sites, which should have a negative relationship to water holding capacity due to sands poor water holding capacity. We ran a linear regression on the proportion of sand as a function of the soil water storage capacity, and the multiple R-squared was 0.021, and the p value was 0.305. (Fig. 3.8). Soil conditions are important to burrowing crayfish, and our MaxEnt models lacked high enough resolution soil data to explain occupancy or relative abundance for this species. Similarly, biotic interactions appear to be important to *C. causeyi*'s distribution; *C. causeyi* and *P. liberorum* were never found at the same site. Biotic interactions can be incorporated in species distribution models (Anderson et al. 2002; Guisan & Thuiller 2005; Peterman et al. 2013), but we lacked a priori field data to know that *P. liberorum* may have been such an effective competitor excluding *C. causeyi* from otherwise suitable sites.

We encountered several limitations during the course of our study. Our most supported generalized linear model performed better than the null model but has a mediocre fit overall (Fig. 3.7). We initially designed our study to be analyzed with occupancy and detection probability models (Durso et al. 2011; MacKenzie et al. 2003) using replicated quadrats or timed searches as units for estimating detection problems, but we did not detect *C. causeyi* at enough sites for our models to converge. We then shifted to zero-inflated Poisson generalized linear models. We had planned to sample during the spring of 2020 because that is when water levels and crayfish breeding activity are highest (Robison et al. 2009), aiding in detection, but unfortunately COVID-19 restrictions made this impossible. We suggest that future sampling for *C. causeyi* be done during the spring months, given the species higher activity rate during that season.

Spring sampling may have improved model performance and detectability of *C. causeyi*, as evidenced by the three sites where we could only find individuals during March and not the summer. This point is further supported by the fact that Rhoden et al. (2017) had better success field validating their SDMs of similar narrowly endemic burrowing crayfish after sampling in the spring months. Additionally, the fact that we found only one female in berry and many young instars during the summer sampling and that we did not find any male and female pairs sharing a burrow during the summer, suggests we missed the peak of the reproductive, wet season, when the crayfish may have been easier to detect.

Multi-scale studies can be useful for understanding rare, endemic species because they allow you to gain the benefits of working in different scales, while also balancing out the potential drawbacks of each scale. We mapped the range limit of *C. causeyi* with MaxEnt and identified a potentially strong effect of precipitation on this species. Conversely, when ground-truthed, the MaxEnt model did not predict *C. causeyi* relative abundance very well. This is perhaps due to limited field sampling in the non-optimal time of year, but also potentially because MaxEnt missed fine resolution habitat data like biotic interactions, soil texture, and soil composition, which is especially important for burrowing crayfishes. It has been shown in other studies, such as Egly & Larson (2018) and Anderson et al. (2002) that not accounting for biotic interactions in your SDM can impair its accuracy; both studies represent cases where a species was consistently not found in an area predicted to be good habitat, but instead, a competitor seemed to have excluded it from the area. Higher resolution, more accurate soil GIS data and knowledge of biotic interactions would benefit future SDMs made for burrowing crayfishes.

Future work might investigate biotic, competitive interactions with other burrowers like *P. liberorum*, which could be a displacement risk for *C. causeyi* if climate or other factors facilitate its spread into *C. causeyi*'s range. Additionally, *C. causeyi* may be at risk from climate change because it appears to be dependent on precipitation keeping their ephemeral streams high, and climate change is known to cause extreme weather patterns, such as droughts (Mukherjee et al. 2018). Despite finding *C. causeyi* at more sites than during the last survey for the species, it continues to deserve its vulnerable status and extra protection due to the threats described above, its narrow range, and the low local abundances found during the course of this study (Appendix D).

TABLES AND FIGURES

Table 3.1 The percent contribution and premutation importance of each variable to our MaxEnt model of *Cambarus causeyi*'s distribution. Percent contribution: how much training gain each variable provided. Permutation importance: how much the training AUC is impacted by a variable being permuted randomly.

Variable	**Percent Contribution**	**Permutation Importance**
Precipitation	65.0	78.9
Euclidean Distance from a Stream	13.4	6.7
Elevation	12.0	6.1
Slope	5.0	3.7
Available Water Storage	3.1	2.8
Solar Radiation	1.5	1.7

Table 3.2 Our candidate models for explaining *Cambarus causeyi*'s presence in our quadrats and timed search with their predictor variables, K, AICc, delta AICc, and weighted AICc.

Models	**K**	**AICc**	**Delta_AICc**	**AICcWt**
Sand + *P. liberorum*	4	63.084	0.000	0.207
P. liberorum + Large Rock	3	63.969	0.885	0.133
Sand + Large Rock	4	64.034	0.949	0.129
Sand	4	64.102	1.017	0.124
Interpolated MaxEnt Output + Sand + *P. liberorum*	3	64.799	1.714	0.088
P. liberorum	5	65.209	2.124	0.072
Null Model	2	65.486	2.401	0.062
Interpolated MaxEnt Output + Sand	3	65.912	2.827	0.050
Large Rock	4	66.176	3.092	0.044
Interpolated MaxEnt Output + *P. liberorum*	4	66.990	3.905	0.029
Stream Dwelling Crayfish	3	67.659	4.574	0.021
Interpolated MaxEnt Output	3	67.714	4.630	0.020
Canopy Cover	3	67.734	4.650	0.020

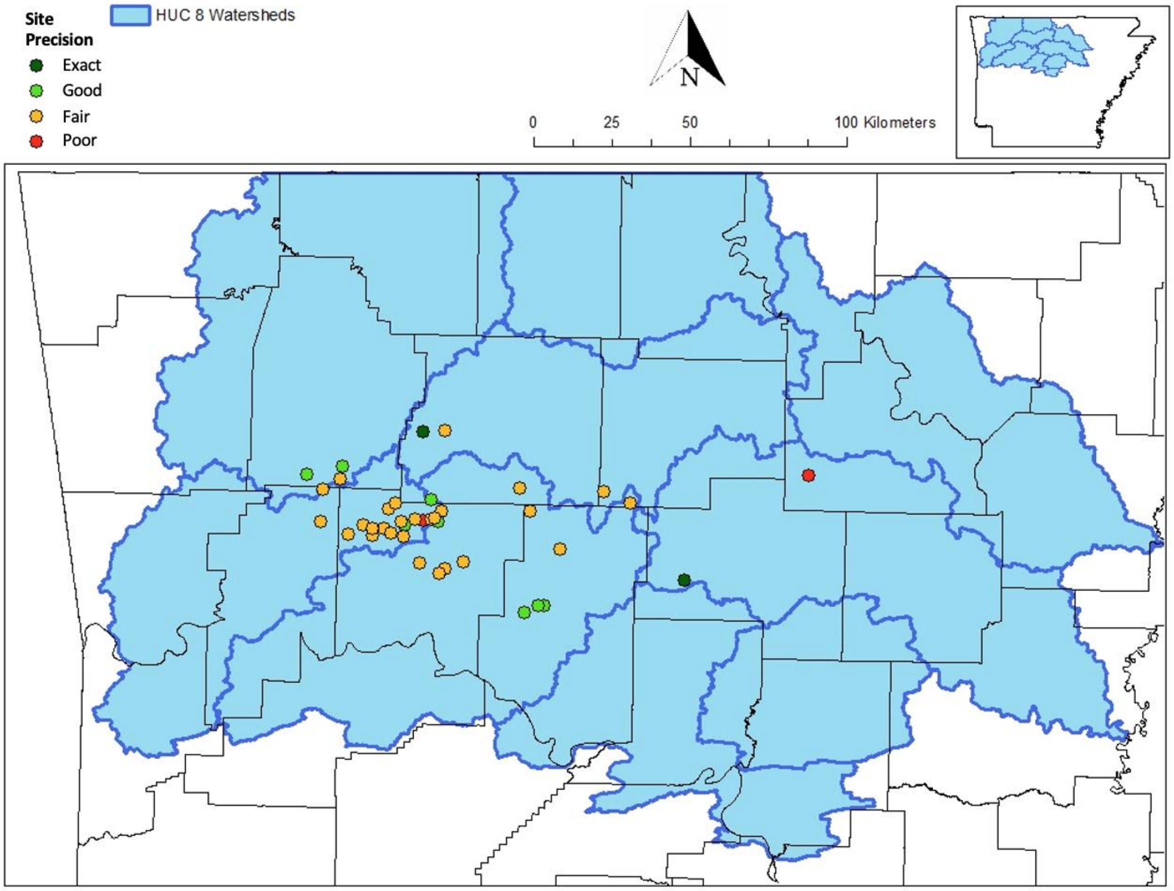


Figure 3.1 A map of Arkansas, USA showing the extent of the MaxEnt model for *C. causeyi*, all historical sites, the confidence in the sites' accuracies, and the HUC8's that make up the extent. "Poor" sites were not used in MaxEnt model, and "unusable" sites are absent from this map because they could not be georeferenced.

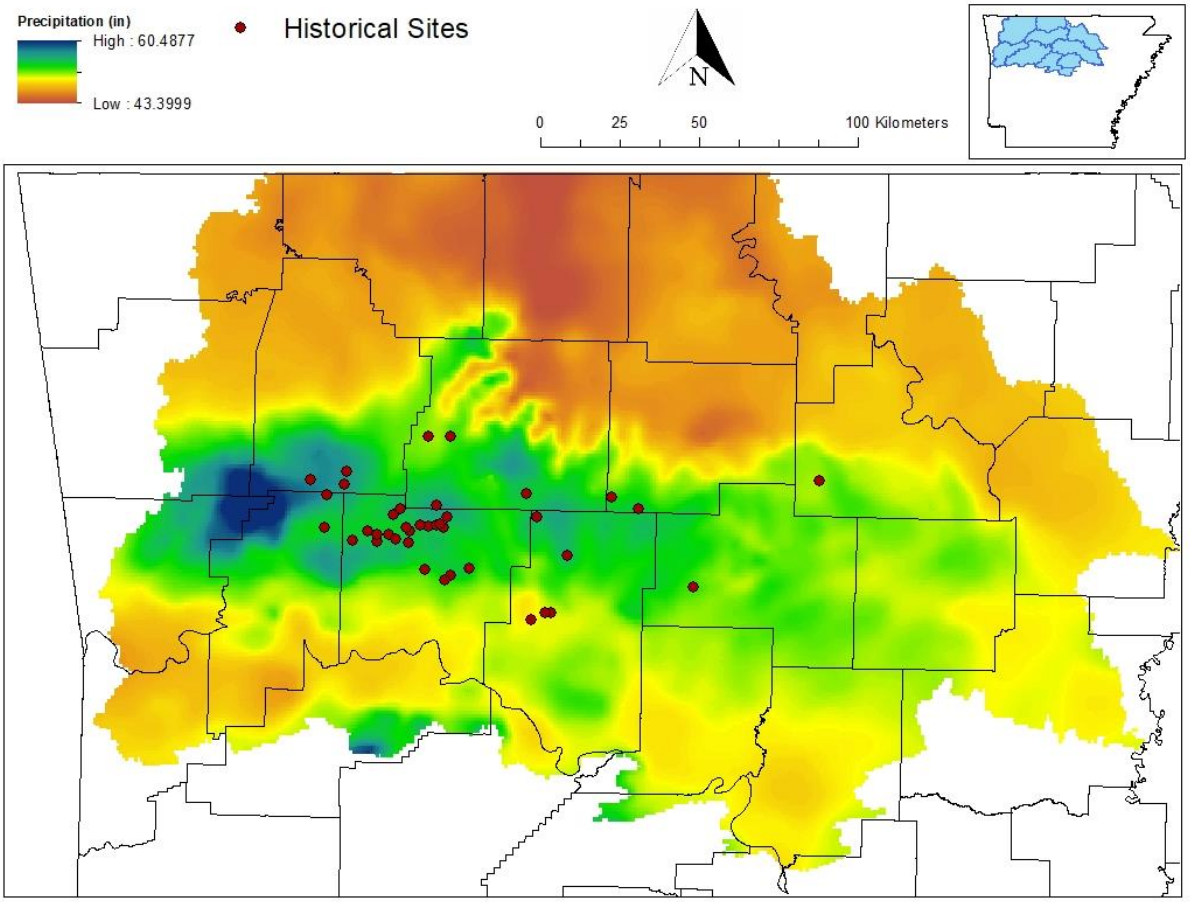


Figure 3.2 A map of Arkansas, USA showing the average annual precipitation in inches from 1981-2010, all historical *Cambarus causeyi* sites, and the HUC8's that make up the extent of our MaxEnt model.

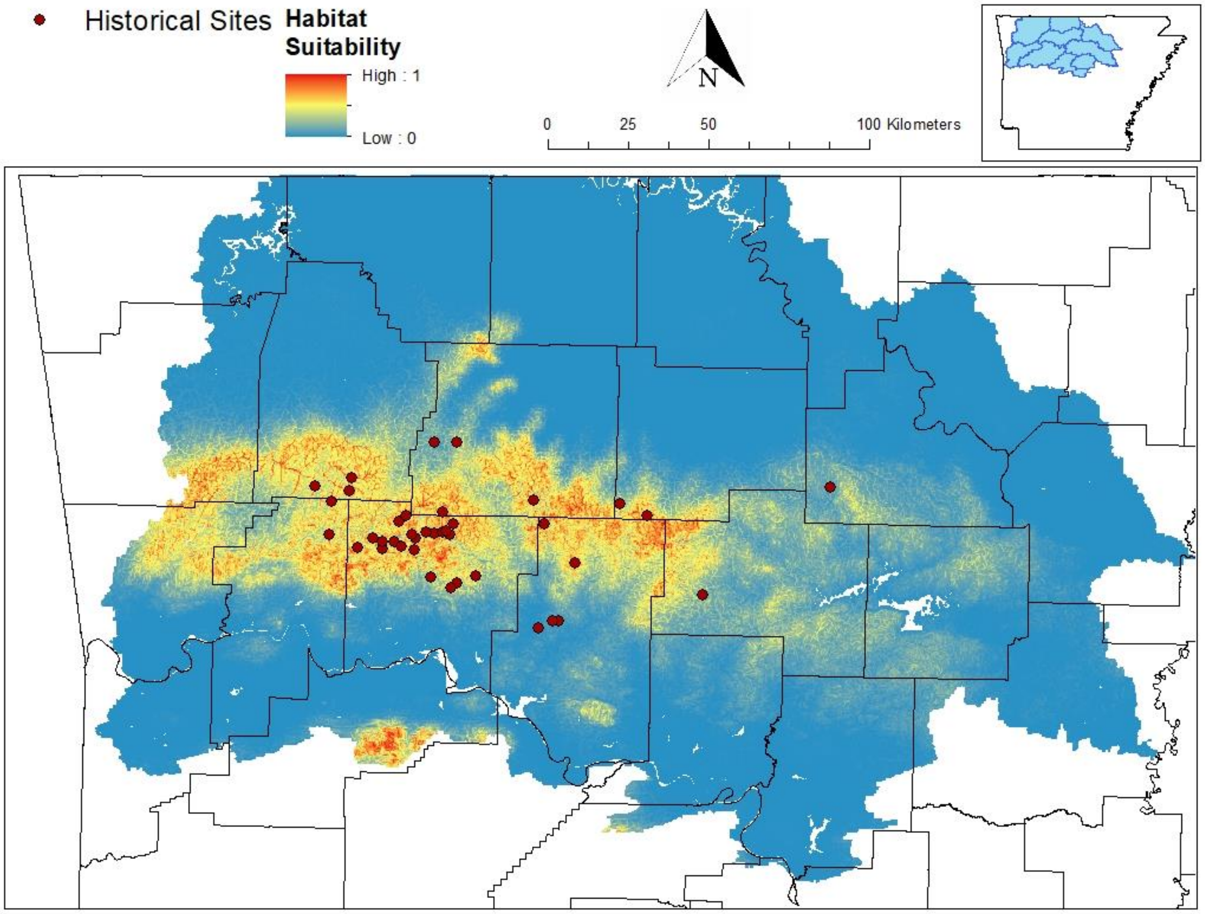


Figure 3.3 A map of Arkansas, USA showing the predicted distribution of *Cambarus causeyi* from MaxEnt, all historical *Cambarus causeyi* sites, and the HUC8's that make up the extent of our MaxEnt model.

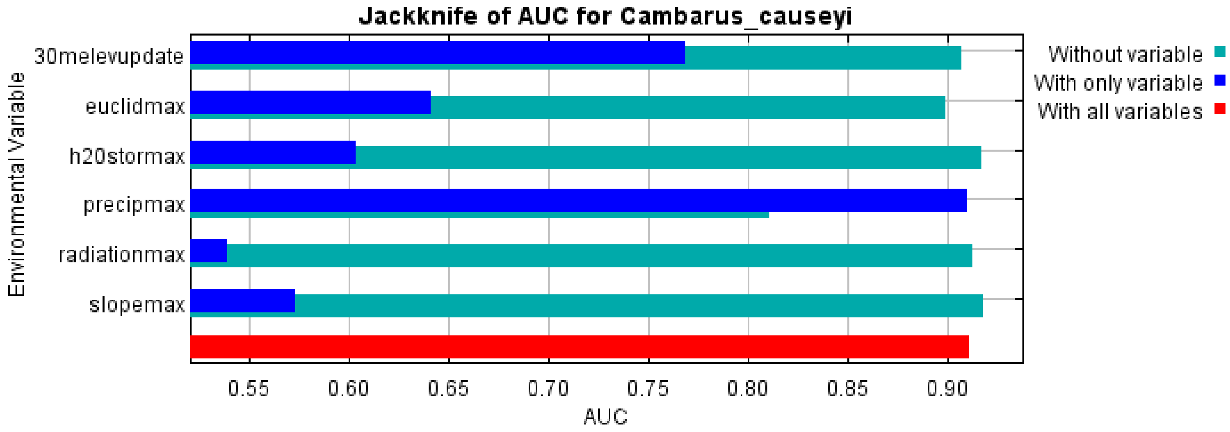


Figure 3.4 The results of our MaxEnt jackknife analysis on the area under the curve (AUC) for our *Cambarus causeyi* model. "30melevupdate" = elevation, "euclidmax" = Euclidean distance from a stream, "h2Ostormax" = average available water storage for the top 150 cm of soil, "precipmax" = 1981-2010 annual average precipitation, "radiationmax" = solar radiation, and "slopemax" = slope. Precipitation is the only variable that, when it was the only variable included in a model, had a higher AUC than in a model without that variable. Additionally, the AUC of the model with all variables was approximately equal to the AUC of the model with just precipitation.

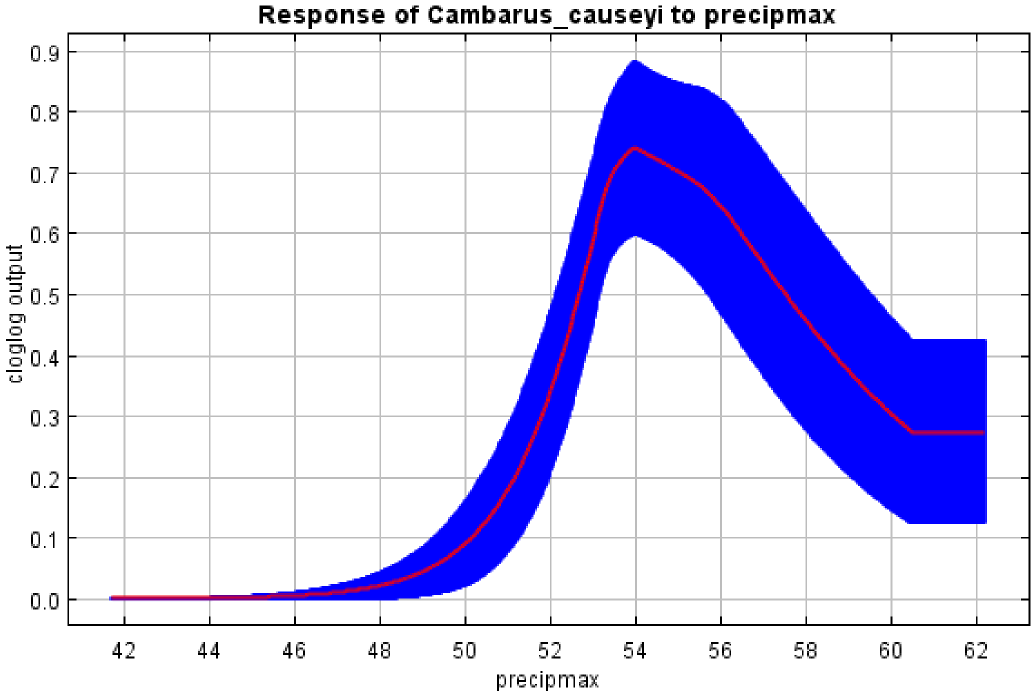
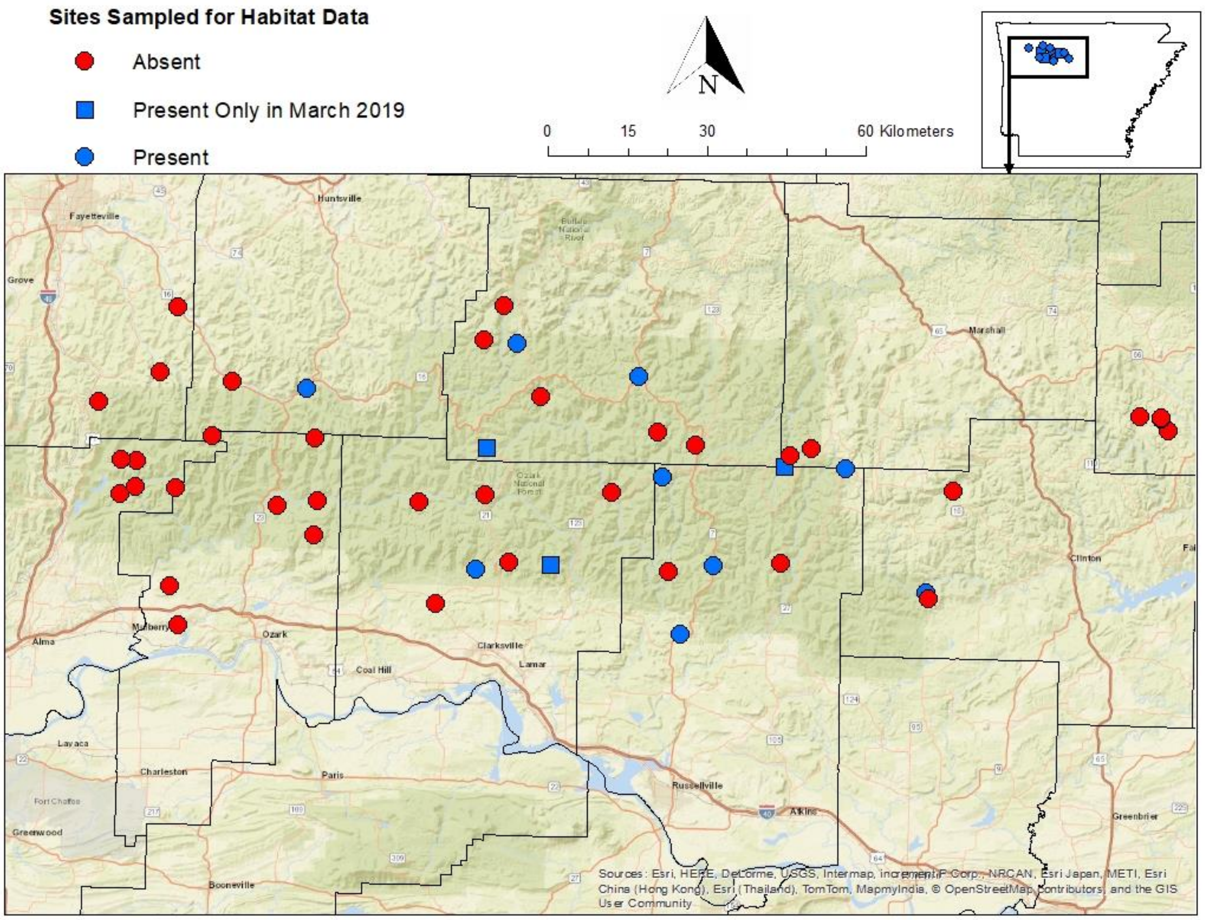


Figure 3.5 The response of *Cambarus causeyi*'s estimated suitability in cloglog to the annual average precipitation in inches from 1981-2010 from the PRISM climate data (Daly et al. 1997) generated in our MaxEnt analysis.



Sources: Esri, HERE, DeLorme, USGS, Intermap, increment P Corp., NRCAN, Esri Japan, METI, Esri China (Hong Kong), Esri (Thailand), TomTom, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

Figure 3.6 Map of Arkansas, USA depicting all sites included our fine-scale habitat modeling. Red sites were absences and blue sites were present during the course of our study. Squares indicate sites where we detected *Cambarus causeyi* in March 2019, but not during the course of our habitat sampling during the following summers. The squares were treated as absences in our fine-scale habitat statistical analysis.

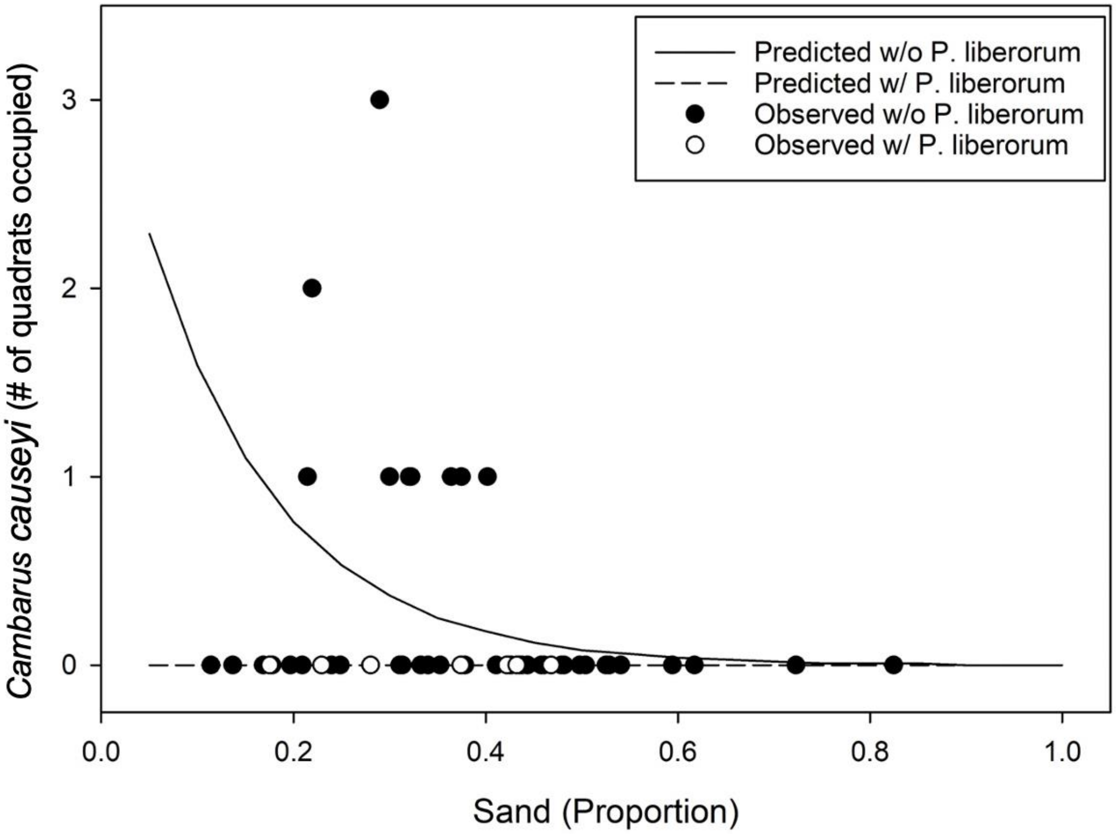


Figure 3.7 The observed and predicted results from our most supported model for explaining *Cambarus causeyi*'s habitat. The points represent observed values, and the model predicted values are lines. Our most supported model's predictor variables were the proportion of sand in the soil and the presence or absence of *Procambarus liberorum*.

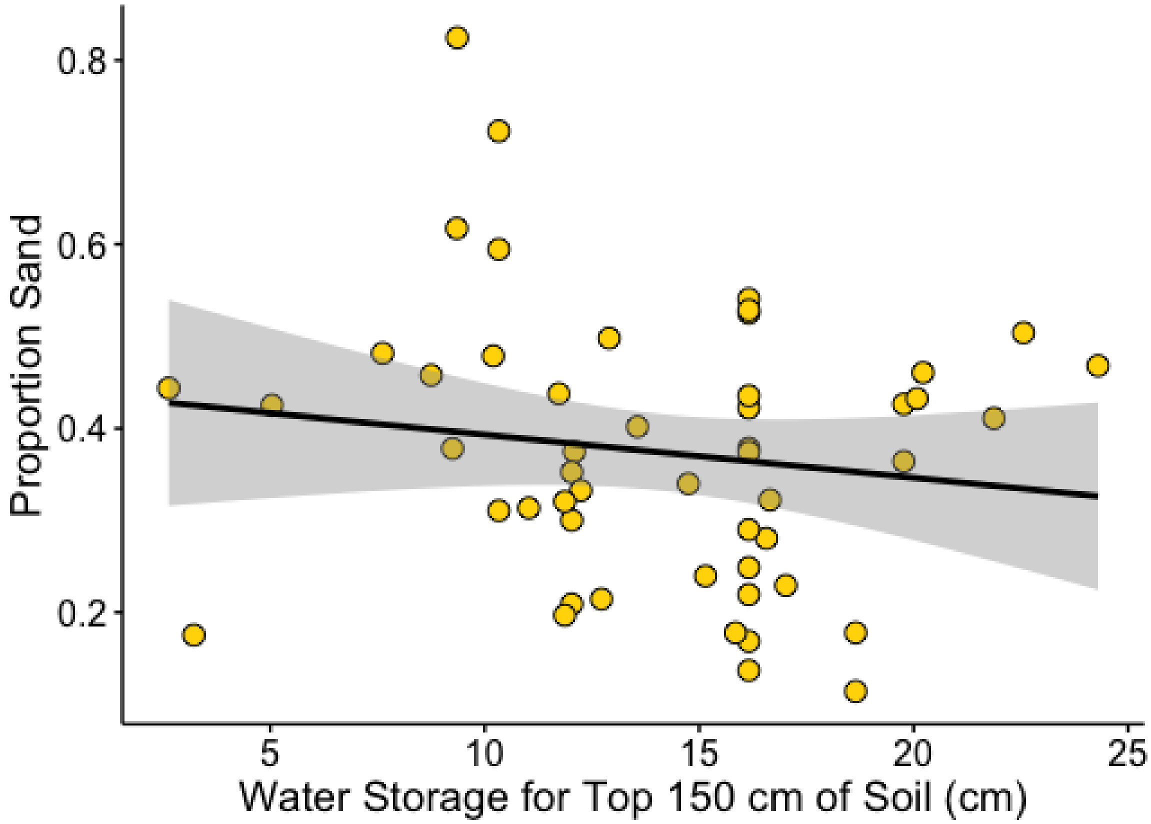


Figure 3.8 A linear regression with 95% confidence intervals showing the lack of relationship between the proportion of sand in our soil samples and the water storage for the top 150 cm of soil from gSSURGO. The multiple R-squared is 0.021, and the p value is 0.305.

LITERATURE CITED

Anderson, R. P., Peterson, A. T., & Gómez-Laverde, M. (2002). Using niche-based GIS modeling to test geographic predictions of competitive exclusion and competitive release in South American pocket mice. *Oikos*, *98*(1), 3–16. https://doi.org/https://doi.org/10.1034/j.1600-0706.2002.t01-1-980116.x

Bloomer, C. C., DiStefano, R. J., & Taylor, C. A. (2021). A global review of life history studies on burrowing crayfish. *Crustaceana*, *94*(3), 357–379. https://doi.org/https://doi.org/10.1163/15685403-bja10098

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Maechler, M., &. Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, 9(2), 378-400.

Daly, C., G. Taylor, and W. Gibson. 1997. The PRISM approach to mapping precipitation and temperature. 10th AMS Conf. on Applied Climatology, Reno, NV, 10-12.

Dorn, N.J., & Volin, J.C., 2009. Resistance of crayfish (*Procambarus* spp.) populations to wetland drying depends on species and substrate. *Journal of the North American Benthological Society*, *28*(4), 766-777.

Durso, A. M., Willson, J. D., & Winne, C. T. (2011). Needles in haystacks: Estimating detection probability and occupancy of rare and cryptic snakes. *Biological Conservation*, *144*(5), 1508–1515. https://doi.org/10.1016/J.BIOCON.2011.01.020

Egly, R. M., & Larson, E. R. (2018). Distribution, habitat associations, and conservation status updates for the pilose crayfish *Pacifastacus gambelii* (Girard, 1852) and Snake River Pilose Crayfish *Pacifastacus connectens* (Faxon, 1914) of the western United States. *PeerJ*, *6*, e5668. https://doi.org/10.7717/peerj.5668

Elith, J., Phillips, S. J., Hastie, T., Dudík, M., Chee, Y. E., & Yates, C. J. (2011). A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions*, *17*(1), 43–57. https://doi.org/10.1111/j.1472-4642.2010.00725.x

Evans, J.S., Oakleaf, J., Cushman, S.A., & Theobald, D. (2014). *An ArcGIS Toolbox for Surface Gradient and Geomorphometric Modeling*, version 2.0-0. Available at: https://evansmurphy.wixsite.com/evansspatial/arcgis-gradient-metrics-toolbox

Fawcett, T. (2006). An introduction to ROC analysis. *Pattern Recognition Letters*, *27*(8), 861 874. https://doi.org/10.1016/j.patrec.2005.10.010

Fois, M., Cuena-Lombraña, A., Fenu, G., & Bacchetta, G. (2018). Using species distribution models at local scale to guide the search of poorly known species: Review, methodological issues and future directions. *Ecological Modelling*, *385,* 124–132. https://doi.org/10.1016/j.ecolmodel.2018.07.018

Fourcade, Y., Engler, J. O., Rödder, D., & Secondi, J. (2014). Mapping species distributions with MAXENT using a geographically biased sample of presence data: A performance assessment of methods for correcting sampling bias. *PLoS ONE*, 9(5). https://doi.org/10.1371/journal.pone.0097122

Galante, P. J., Alade, B., Muscarella, R., Jansa, S. A., Goodman, S. M., & Anderson, R. P. (2018). The challenge of modeling niches and distributions for data-poor species: a comprehensive approach to model complexity. *Ecography*, *41*(5), 726–736. https://doi.org/https://doi.org/10.1111/ecog.02909

Grow, L. 1982. Burrowing/soil-texture relationships in the crayfish, *Cambarus diogenes diogenes* Girard (Decapoda, Astacidea). *Crustaceana*, 42(2):150-157.

Grow, L. and H. Merchant. 1980. The burrow habitat of the crayfish *Cambarus diogenes diogenes* (Girard). *The American Midland Naturalist*, 103(2):231-237.

Guisan, A., Graham, C. H., Elith, J., Huettmann, F., & the NCEAS Species Distribution Modelling Group. (2007). Sensitivity of predictive species distribution models to change in grain size. *Diversity and Distributions*, *13*(3), 332–340. https://doi.org/https://doi.org/10.1111/j.1472-4642.2007.00342.x

Guisan, A., & Thuiller, W. (2005). Predicting species distribution: offering more than simple habitat models. *Ecology Letters*, *8*(9), 993–1009. https://doi.org/https://doi.org/10.1111/j.1461-0248.2005.00792.x

Hernandez, P. A., Graham, C. H., Master, L. L., & Albert, D. L. (2006). The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography*, *29*(5), 773–785. https://doi.org/10.1111/j.0906-7590.2006.04700.x

Hirzel, A. H., Le Lay, G., Helfer, V., Randin, C., & Guisan, A. (2006). Evaluating the ability of habitat suitability models to predict species presences. *Ecological Modelling*, *199*(2), 142–152. https://doi.org/https://doi.org/10.1016/j.ecolmodel.2006.05.017

Hobbs, H. H., Jr., 1942. The crayfishes of Florida. (University of Florida Press, Gainesville, Florida, U.S.A.).

James, J., Slater, F. M., Vaughan, I. P., Young, K. A., & Cable, J. (2015). Comparing the ecological impacts of native and invasive crayfish: could native species' translocation do more harm than good? *Oecologia*, *178*(1), 309–316. https://doi.org/10.1007/s00442-014-3195-0

Loughman, Z. J. (2010). Ecology of *Cambarus dubius* (Upland Burrowing Crayfish) in north-central West Virginia. *Southeastern Naturalist*, *9*(3), 217–230. https://doi.org/10.1656/058.009.s311

Loughman, Z. J., Welsh, S. A., & Simon, T. P. (2012). Occupancy rates of primary burrowing crayfish in natural and disturbed large river bottomlands. *Journal of Crustacean Biology*, *32*(4), 557–564. https://doi.org/10.1163/193724012X637339

MacKenzie, D. I., Nichols, J. D., Hines, J. E., Knutson, M. G., & Franklin, A. B. (2003). Estimating site occupancy, colonization, and local extinction when a species is detected imperfectly. *Ecology*, *84*(8), 2200–2207. https://doi.org/10.1890/02-3090

Mukherjee, S., Mishra, A., & Trenberth, K. E. (2018). Climate change and drought: A perspective on drought indices. *Current Climate Change Reports*, *4*(2), 145–163. https://doi.org/10.1007/s40641-018-0098-x

Moore, M. J., DiStefano, R. J., & Larson, E. R. (2013). An assessment of life-history studies for USA and Canadian crayfishes: identifying biases and knowledge gaps to improve conservation and management. *Freshwater Science*, *32*(4), 1276–1287. http://10.0.7.107/12-158.1

Novakowski, K. S., & Gillham, R. W. (1988). Field investigations of the nature of water-table response to precipitation in shallow water-table environments. *Journal of Hydrology*, *97*(1–2), 23–32. https://doi.org/10.1016/0022-1694(88)90063-7

Peterman, W. E., Crawford, J. A., & Kuhns, A. R. (2013). Using species distribution and occupancy modeling to guide survey efforts and assess species status. *Journal for Nature Conservation*, *21*(2), 114–121. https://doi.org/https://doi.org/10.1016/j.jnc.2012.11.005

Phillips, S. J., Anderson, R. P., & Schapire, R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, *190*(3–4), 231–259. https://doi.org/10.1016/J.ECOLMODEL.2005.03.026

Pintor, L. M., & Soluk, D. A. (2006). Evaluating the non-consumptive, positive effects of a predator in the persistence of an endangered species. *Biological Conservation*, *130*(4), 584–591. https://doi.org/10.1016/j.biocon.2006.01.021

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reid, A.J., Carlson, A.K., Creed, I.F., Eliason, E.J., Gell, P.A., Johnson, P.T.J., Kidd, K.A., MacCormack, T.J., Olden, J.D., Ormerod, S.J., Smol, J.P., Taylor, W.W., Tockner, K., Vermaire, J.C., Dudgeon, D. and Cooke, S.J. (2019), Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, 94: 849-873. https://doi.org/10.1111/brv.12480

Reimer, R. D. (1966). Two new species of the genus *Cambarus* from Arkansas (Decapoda, Astacidae). *Tulane Studies in Zoology.*, *13*, 9–15. https://doi.org/10.5962/bhl.part.19672

Reynolds, J., Souty-Grosset, C., & Richardson, A. (2013). Ecological roles of crayfish in freshwater and terrestrial habitats. *Freshwater Crayfish*, *19*(2), 197–218. https://doi.org/10.5869/fc.2013.v19-2.197

Rhoden C.M., Peterman W.E., Taylor C.A. (2017) MaxEnt-directed field surveys identify new populations of narrowly endemic habitat specialists. *PeerJ* 5:e3632 https://doi.org/10.7717/peerj.3632

Rhoden, C. M., Taylor, C. A., & Peterman, W. E. (2016). Highway to heaven? Roadsides as preferred habitat for two narrowly endemic crayfish. *Freshwater Science*, *35*(3), 974–983. https://doi.org/10.1086/686919

Richter, B. D., Braun, D. P., Mendelson, M. A., & Master, L. L. (1997). Threats to Imperiled Freshwater Fauna. *Conservation Biology*, *11*(5), 1081–1093. https://doi.org/https://doi.org/10.1046/j.1523-1739.1997.96236.x

Robin, X., Turck, N., Hainard, A., Tiberti, N., Lisacek, F., Sanchez, J.-C., & Müller, M. (2011). pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics, 12, 77. DOI: 10.1186/1471-2105-12-77 http://www.biomedcentral.com/1471-2105/12/77/

Robison, H. W., Crandall, K. A., & Wagner, B. (2009). *Distribution, Genetics, and Conservation Status of Three Arkansas Crayfishes*(Project No. T26R-11). https://www.wildlifearkansas.com/ProjectReports.html

Robison, H. W., & Leeds, G. (1996). Distribution and natural history aspects of the Arkansas endemic crayfish, *Cambarus causeyi* Reimer. *Journal of the Arkansas Academy of Science, 50*(21), 105–109.

Stirling, D. A., Boulcott, P., Scott, B. E., & Wright, P. J. (2016). Using verified species distribution models to inform the conservation of a rare marine species. *Diversity and Distributions*, *22*(7), 808–822. https://doi.org/https://doi.org/10.1111/ddi.12447

Taylor, C. A., Schuster, G. A., Cooper, J. E., DiStefano, R. J., Eversole, A. G., Hamr, P., Hobbs, H. H., Robison, H. W., Skelton, C. E., Thoma, R. F. (2007). A reassessment of the conservation status of crayfishes of the United States and Canada after 10+ Years of increased awareness. *Fisheries*, *32*(8), 372–389. https://doi.org/10.1577/1548-8446(2007)32[372:arotcs]2.0.co;2

U.S. Geological Survey, National Hydrography Dataset (1:24,000 resolution) (NHD), accessed November 6, 2019 at URL https://datagateway.nrcs.usda.gov/

U.S. Geological Survey & EROS Data Center, National Elevation Dataset (30 meter resolution) , accessed November 18, 2019 at URL https://datagateway.nrcs.usda.gov/

Warren, D. L., & Seifert, S. N. (2011). Ecological niche modeling in MaxEnt: the importance of model complexity and the performance of model selection criteria. *Ecological Applications*, *21*(2), 335–342. https://doi.org/10.1890/10-1171.1

Welch, S. M., Waldron, J. L., Eversole, A. G., & Simoes, J. C. (2008). Seasonal variation and ecological effects of Camp Shelby Burrowing Crayfish (*Fallicambarus gordoni*) burrows. *The American Midland Naturalist*, *159*(2), 378–384. https://doi.org/10.1674/0003-0031(2008)159[378:SVAEEO]2.0.CO

CHAPTER 4: SUMMARY

In Chapter 2, we identified three unique evolutionarily significant units (ESUs) in need of separate conservation attention within *Cambarus hubbsi*. *Cambarus hubbsi* is vulnerable to disturbance and environmental stressors due to its life history strategy and habitat preferences. This vulnerability, combined with the newly found cryptic diversity, means that a large disturbance in one portion of its range has the potential to not only reduce the overall range of the species, but also, potentially lose a lineage unique to that drainage. We hope to see the three ESUs acknowledged by managers as further work is done to conserve *C. hubbsi* within its entire range. Additionally, we hope that the new knowledge of cryptic biodiversity within the species will aid in conservation efforts as *C. hubbsi* continues to face anthropogenic and natural threats. We also suggest that future studies analyze additional genetic data, particularly from clade C, to fill in knowledge gaps. This would include sequencing individuals from parts of *C. hubbsi*'s range that are underrepresented, sequencing more genes, and potentially using next generation sequencing techniques.

In Chapter 3 we utilized species distribution modeling (SDM) and fine scale habitat modeling to analyze the distribution and habitat preferences of *C. causeyi*. Our SDM found average annual precipitation was by far the most important predictor of *C. causeyi* relative abundance. We ran our fine-scale analysis by modeling zero-inflated Poisson generalized linear models and selecting with AICc. Our most supported model included proportion of sand in the soil and the presence of a competing burrower as explanatory variables. The interpolated MaxEnt output was found to be a poor predictor of *C. causeyi* relative abundance in our fine-scale analysis due to issues with accounting for site specific soil data and biotic interactions. Our results suggest that for accurate field assessments, future sampling for *C. causeyi* should be conducted during the spring, when *C. causeyi* is more active. Finally, issues such as climate change, interspecific competition, low local abundances, and relatively small range continue to pose a threat to the conservation of this narrow endemic, and its status should continue to be monitored.

APPENDIX A: *CAMBARUS HUBBSI* MORPHOLOGICAL DATA

Appendix A is in the associated .csv file. It contains the raw morphological data for the *Cambarus hubbsi* individuals used in the study, and the excluded form I males. L = length, W= width, TL = Post-orbital total length. Measurements are in millimeters.

APPENDIX B: *CAMBARUS HUBBSI* CATCH DATA

Appendix B is in the associated .csv file. It contains the data on all *Cambarus hubbsi* individuals collected during the course of the study. It also contains the available catch per unit effort data. NA means not applicable.

APPENDIX C: SOIL TEXTURE DATA

Table C.1 The soil texture data collected during *Cambarus causeyi* field sampling and analyzed using laser diffraction at the Illinois State Water Survey.

Field #	Date	Latitude	Longitude	County	% Sand	% Silt	% Clay <2µm	USDA Class
KBQ 19-16	2019-05-21	35.62994	-93.74456	Franklin	22.93	63.65	13.42	ZL
KBQ 19-17	2019-05-21	35.67702	-93.73857	Franklin	28.03	59.21	12.76	ZL
KBQ 19-18	2019-05-21	35.83255	-93.75476	Madison	36.41	54.91	8.68	ZL
KBQ 19-20	2019-05-21	35.76342	-93.74209	Franklin	17.78	69.80	12.42	ZL
KBQ 19-21	2019-05-22	35.67571	-93.56564	Johnson	37.84	55.48	6.68	ZL
KBQ 19-22	2019-05-22	35.67506	-93.56461	Johnson	52.53	42.10	5.37	SL
KBQ 19-23	2019-05-22	35.68594	-93.453	Johnson	41.08	54.73	4.19	ZL
KBQ 19-24	2019-05-22	35.75005	-93.45097	Newton	49.79	45.13	5.08	SL
KBQ 19-25	2019-05-22	35.59256	-93.41238	Johnson	24.88	59.33	15.79	ZL
KBQ 19-26	2019-05-23	35.58891	-93.34202	Johnson	16.85	74.57	8.58	ZL
KBQ 19-27	2019-05-23	35.70903	-93.1517	Pope	21.94	61.72	16.35	ZL
KBQ 19-30	2019-05-23	35.58723	-93.06491	Pope	21.45	62.57	15.98	ZL
KBQ 19-32	2019-05-23	35.49232	-93.12066	Pope	32.22	61.65	6.13	ZL
KBQ 19-34	2019-05-24	35.58287	-93.46875	Johnson	37.47	58.21	4.32	ZL
KBQ 19-36	2019-06-04	35.68678	-94.07263	Crawford	37.42	58.01	4.57	ZL
KBQ 19-37	2019-06-04	35.69694	-94.04698	Crawford	61.72	34.16	4.12	SL
KBQ 19-38	2019-06-04	35.73243	-94.04498	Crawford	54.07	43.79	2.14	SL
KBQ 19-39	2019-06-04	35.73484	-94.07069	Crawford	42.21	49.71	8.07	L

Table C.1 (cont.)

KBQ 19-40	2019-06-04	35.76623	-93.91526	Madison	47.86	46.49	5.65	SL
KBQ 19-41	2019-06-05	35.89798	-93.45442	Newton	35.25	59.97	4.78	ZL
KBQ 19-42	2019-06-05	35.94535	-93.42053	Newton	20.90	62.58	16.52	ZL
KBQ 19-43	2019-06-05	35.89432	-93.39811	Newton	28.98	53.75	17.27	ZL
KBQ 19-45	2019-06-05	35.72516	-92.94542	Searcy	48.15	46.71	5.14	SL
KBQ 19-46	2019-06-05	35.73959	-92.93436	Searcy	33.24	58.73	8.03	ZL
KBQ 19-47	2019-06-06	35.55	-92.70353	Van Buren	40.18	52.60	7.22	ZL
KBQ 19-51	2019-06-06	35.54151	-92.69925	Van Buren	17.78	71.94	10.28	ZL
KBQ 19-52	2019-06-06	35.79359	-92.3409	Stone	82.45	15.94	1.60	LS
KBQ 20-74	2020-06-02	35.81398	-94.10864	Washington	42.62	49.27	8.11	L
KBQ 20-75	2020-06-03	35.67097	-93.80508	Franklin	43.75	48.92	7.33	L
KBQ 20-76	2020-06-10	35.74936	-92.89838	Searcy	31.33	57.05	11.62	ZL
KBQ 20-77	2020-06-09	35.82126	-93.35729	Newton	43.53	49.54	6.93	SL
KBQ 20-78	2020-06-09	35.84814	-93.19154	Newton	30	59.18	10.82	ZL
KBQ 20-79	2020-06-10	35.72161	-92.84055	Pope	32.04	63.12	4.84	ZL
KBQ 20-80	2020-06-02	35.94375122	-93.974275	Washington	46.79	51	2.2	ZL
KBQ 20-81	2020-06-02	35.84113	-93.8818	Madison	23.96	67	9.04	ZL
KBQ 20-82	2020-06-02	35.854	-94.00474	Washington	33.99	56.16	9.85	ZL
KBQ 20-83	2020-06-03	35.69476	-93.97803	Franklin	37.81	53.51	8.67	ZL
KBQ 20-84	2020-06-03	35.55908	-93.98834	Franklin	17.53	72.19	10.28	ZL
KBQ 20-85	2020-06-03	35.53491	-93.53657	Johnson	43.22	51.89	4.9	ZL
KBQ 20-86	2020-06-04	35.50522	-93.9748	Franklin	42.51	52.01	5.48	ZL

Table C.1 (cont.)

KBQ 20-87	2020-06-04	35.688404	-93.238246	Johnson	44.35	48.56	7.09	L
KBQ 20-88	2020-06-04	35.57977	-93.14053	Pope	19.69	74.21	6.1	ZL
KBQ 20-89	2020-06-04	35.59046	-92.95174	Pope	46.05	47.67	6.28	SL
KBQ 20-90	2020-06-09	35.82127	-93.35725	Newton	52.86	44.88	2.26	SL
KBQ 20-91	2020-06-09	35.82127	-93.35725	Newton	13.68	60.7	25.62	ZL
KBQ 20-92	2020-06-09	35.77106	-93.159	Newton	50.38	45.27	4.35	SL
KBQ 20-93	2020-06-10	35.7537	-93.0954	Newton	11.41	70.69	17.9	ZL
KBQ 20-94	2020-06-11	35.68989	-92.6584	Van Buren	45.76	50.33	3.9	ZL
KBQ 20-95	2020-06-11	35.7737	-92.29342	Stone	72.29	24.28	3.43	SL
KBQ 20-96	2020-06-11	35.78939	-92.30533	Stone	59.45	38.52	2.03	SL
KBQ 20-97	2020-06-11	35.79129	-92.30531	Stone	31.05	59.71	9.24	ZL

APPENDIX D: *CAMBARUS CAUSEYI* COLLECTION SITES

Table D.1 The collection data for the summer field sampling seasons for *Cambarus causeyi*.

Field Number	Number Collected	Date	Latitude	Longitude	County	Present in Spring 2019
KBQ19-16	0	5/21/19	35.62994	-93.74456	Franklin	No
KBQ19-17	0	5/21/19	35.67702	-93.73857	Franklin	No
KBQ19-18	1	5/21/19	35.83255	-93.75476	Madison	Yes
KBQ19-20	0	5/21/19	35.76342	-93.74209	Franklin	No
KBQ19-21	0	5/22/19	35.67571	-93.56564	Johnson	No
KBQ19-22	0	5/22/19	35.67506	-93.56461	Johnson	No
KBQ19-23	0	5/22/19	35.68594	-93.45300	Johnson	No
KBQ19-24	0	5/22/19	35.75005	-93.45097	Newton	Yes
KBQ19-25	0	5/22/19	35.59256	-93.41238	Johnson	No
KBQ19-26	0	5/23/19	35.58891	-93.34202	Johnson	Yes
KBQ19-27	3	5/23/19	35.70903	-93.15170	Pope	Yes
KBQ19-30	1	5/23/19	35.58723	-93.06491	Pope	Yes
KBQ19-32	1	5/23/19	35.49232	-93.12066	Pope	No
KBQ19-34	1	5/24/19	35.58287	-93.46875	Johnson	Yes
KBQ19-36	0	6/4/19	35.68678	-94.07263	Crawford	No
KBQ19-37	0	6/4/19	35.69694	-94.04698	Crawford	No
KBQ19-38	0	6/4/19	35.73243	-94.04498	Crawford	No
KBQ19-39	0	6/4/19	35.73484	-94.07069	Crawford	No
KBQ19-40	0	6/4/19	35.76623	-93.91526	Madison	No
KBQ19-41	0	6/5/19	35.89798	-93.45442	Newton	No
KBQ19-42	0	6/5/19	35.94535	-93.42053	Newton	No
KBQ19-43	4	6/5/19	35.89432	-93.39811	Newton	No
KBQ19-45	0	6/5/19	35.72516	-92.94542	Searcy	Yes
KBQ19-46	0	6/5/19	35.73959	-92.93436	Searcy	No
KBQ19-47	3	6/6/19	35.55000	-92.70353	Van Buren	No
KBQ19-51	0	6/6/19	35.54151	-92.69925	Van Buren	No
KBQ19-52	0	6/6/19	35.79359	-92.34090	Stone	No
KBQ20-74	0	6/2/20	35.81398	-94.10864	Washington	No
KBQ20-80	0	6/2/20	35.94375	-93.97428	Washington	No
KBQ20-81	0	6/2/20	35.84113	-93.88180	Madison	No
KBQ20-82	0	6/2/20	35.85400	-94.00474	Washington	No
KBQ20-75	0	6/3/20	35.67097	-93.80508	Franklin	No
KBQ20-83	0	6/3/20	35.69476	-93.97803	Franklin	No
KBQ20-84	0	6/3/20	35.55908	-93.98834	Franklin	No

Table D.1 (cont.)

KBQ20-85	0	6/3/20	35.53491	-93.53657	Johnson	No
KBQ20-86	0	6/4/20	35.50522	-93.97480	Franklin	No
KBQ20-87	0	6/4/20	35.68840	-93.23825	Johnson	No
KBQ20-88	0	6/4/20	35.57977	-93.14053	Pope	No
KBQ20-89	0	6/4/20	35.59046	-92.95174	Pope	No
KBQ20-77	0	6/9/20	35.82126	-93.35729	Newton	No
KBQ20-78	1	6/9/20	35.84814	-93.19154	Newton	No
KBQ20-90	0	6/9/20	35.82127	-93.35725	Newton	No
KBQ20-91	0	6/9/20	35.82127	-93.35725	Newton	No
KBQ20-92	0	6/9/20	35.77106	-93.15900	Newton	No
KBQ20-76	0	6/10/20	35.74936	-92.89838	Searcy	No
KBQ20-79	1	6/10/20	35.72161	-92.84055	Pope	No
KBQ20-93	0	6/10/20	35.75370	-93.09540	Newton	No
KBQ20-94	0	6/11/20	35.68989	-92.65840	Van Buren	No
KBQ20-95	0	6/11/20	35.77370	-92.29342	Stone	No
KBQ20-96	0	6/11/20	35.78939	-92.30533	Stone	No
KBQ20-97	0	6/11/20	35.79129	-92.30531	Stone	No