Title: *Cambarus hubbsi* Distribution, Population Genetics and Potential Invasive Species Effects in the Ozark Highlands of Arkansas and Missouri

Project Summary: Crayfish are extremely important in most freshwater systems, typically acting as keystone species. Hubbs' crayfish, *Cambarus hubbsi*, is a narrow-range endemic occurring in north-central Arkansas and south-central Missouri. The threat of an advancing invasive species, along with potential habitat loss and fragmentation, makes determining potential invasive species effects, current and predicted distribution, and population genetics of this species a high priority. We propose to determine distribution and abundance of *C. hubbsi* by modeling species distribution then probabilistically sampling stream sites from its known range. We will also examine the distribution of genetic variation (=phylogeography) for *C. hubbsi* both within and among drainages where the species occurs. We will use the genetic data to examine population structure and gene flow among sub-populations, as well as identify any potential *C. hubbsi* Evolutionary Significant Units. Simulation models will be used to determine potential effects of an invasive crayfish on *C. hubbsi* populations. It is important for managers to understand the status and threats to the crayfish species of greatest conservation need (SGCN), especially *C. hubbsi*, given that they are geographically restricted stream crayfish and an invasive species known to displace *C. hubbsi* is spreading within drainage basins where they occur. Information gained here will ultimately be used to make decisions regarding the conservation of *C. hubbsi*, and will inform decisions regarding other species that are potentially at risk from similar threats (e.g., *Faxonius longidigitus, Faxonius meeki, Faxonius williamsi, Faxonius luteus, Faxonius punctimanus, Faxonius ozarkae, Faxonius neglectus chaeondactylus*).

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Robert J. DiStefano, Resource Scientist, Missouri Department of Conservation, Resource Science Center, 3500 East Gans Road, Columbia, Missouri 65201, 573-815-7901, Bob.DiStefano@mdc.mo.gov

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Total SWG Funding Requested: $89,702

Total Project Cost: $184,206

Amount and Source of Matching Funds or In-kind Services: $94,504 (51%)
$18,522 graduate student tuition, University of Arkansas
$27,310 unrecovered indirect costs, University of Arkansas
$ 6,000 in-kind services, Arkansas Game and Fish Commission
$23,832 in-kind services, Missouri Department of Conservation
$18,840 unrecovered indirect costs, Carnegie Museum of Natural History

Funding priorities addressed:

- Project that addresses the need to monitor populations of Hubbs' crayfish, *Cambarus hubbsi*, a species of greatest conservation need
- Project that addresses need to examine genetic isolation of Hubbs' crayfish populations and potential ESU's in advance of invasion of ringed crayfish, *Faxonius neglectus*
- Project that addresses physical, biological and water quality in three of the four priority drainage basins (Spring, Eleven Point and Strawberry River Basins)
- Research projects that lead to on-the-ground conservation

Ecobasins targeted: Spring, Eleven Point, Strawberry, St. Francis and portions of White River drainages of Arkansas and Missouri.

Problem and Justification

Crayfish are recognized as keystone species or ecological dominants, integral to the fueling and functioning of many freshwater systems, as well as serving an important role for terrestrial and avian species. They are prey for >208 species of invertebrates, fish, amphibians, reptiles, birds and mammals in North America, and are the most important prey item for several important sport fishes (e.g., smallmouth bass, Ozark bass). In the Ozarks, particulate organic matter is the primary energy source for most stream food webs and crayfish convert more of this organic matter into usable energy for other organisms than all other invertebrates combined. Given this, crayfish play an important role in nutrient cycling. Crayfish also create habitat for other organisms, including many fish, invertebrates, amphibians, plants, fungi and microbes. Therefore, crayfish are extremely important in most freshwater systems in North America.

Within the Ozark Highlands of Arkansas and Missouri, multiple narrow-ranged endemic crayfish are species of greatest conservation need (SGCN). One of these species, Hubbs' crayfish *Cambarus hubbsi*, has received much less attention than other SGCN. Previously, we examined current distribution and modeled predicted distribution of *C. hubbsi* in the upper Black River drainage (Fig. 1; Nolen et al. 2014) as part of a larger project examining distribution and population genetics of *Faxonius eupunctus*. We found that *C. hubbsi* were associated with larger rivers and deeper habitats in the Eleven Point, Spring and Strawberry River drainages. However, *C. hubbsi* also occurs in the St. Francis River drainage and portions of the White River drainage. We have also found that *C. hubbsi* occurs primarily in riffles and runs, and typically occurs at lower density than other crayfish species (Flinders and Magoulick 2005, 2007). Another of our previous studies examined *C. hubbsi* life history and determined that this species is comparatively slow growing, long-lived, with less reproductive output compared to many *Faxonius* species, suggesting it may be a K-selected strategist (Larson and Magoulick 2011). The relatively high habitat specificity, low densities and K-selected life history strategy suggest that *C. hubbsi* requires further study and greater conservation attention.

In addition to *C. hubbsi* being an imperiled endemic species, the invasive *Faxonius neglectus* now occurs in high numbers in portions of the Spring River drainage. Our previous research has shown that; 1) *F. neglectus* was introduced into the Spring River drainage between 1984 and 1998, 2) *F. neglectus* appears to be negatively impacting the native crayfish community,

including *C. hubbsi*, and 3) *F. neglectus* has the potential to expand its distribution in the Spring River drainage and elsewhere, potentially further affecting *C. hubbsi*.

The threat of an advancing invasive species that has displaced *C. hubbsi* and other native species, along with potential habitat loss and fragmentation, makes determining the distribution, population genetics and potential invasive species effects a high priority. Major questions that need to be addressed include "Is population structure evident, how much gene flow occurs among sub-populations of *C. hubbsi*, and are any subpopulations different enough to be considered Evolutionarily Significant Units (ESUs)?", and "How will the invasion of *F. neglectus* potentially affect *C. hubbsi* populations?". Based on our previous research, we hypothesize that *C. hubbsi* populations are currently stable, but are at risk due to a small geographic range and are susceptible to invasion by *F. neglectus*. We also hypothesize that populations of *C. hubbsi* in major drainages are relatively isolated from one another with little gene flow among them and that these sub-populations are ESUs.

Objectives

1. Examine distribution and abundance of *C. hubbsi* and develop species distribution models to predict its potential distribution
2. Determine the extent of gene flow and potential ESU's and compare current and historical genetic diversity of *C. hubbsi*
3. Examine population structure of *C. hubbsi*, and determine potential for invasion impacts by *F. neglectus*

Methods

Objective 1

We will determine distribution and densities of *C. hubbsi* populations in the Spring River, Eleven Point River, Strawberry River, St. Francis River and portions of the White River drainages in the known range of this species using occupancy estimation (and detection probability) methods by sampling stream segments (*as defined by* Westhoff et al. 2006, DiStefano et al. 2008). We will use data from our previous work (Nolen et al. 2014, Magoulick et al. 2017) to expand predictive models of *C. hubbsi* distribution and abundance throughout its known range. We will then randomly select 30-40 of the modeled stream segments with predicted *C. hubbsi* occupancy greater than 50% for sampling. Sampling reaches (locations within stream segments to be sampled) will be selected randomly, but will also be based on accessibility (e.g., landowner permission). Each stream segment will be considered a "site" (*sensu* MacKenzie et al. 2006). A minimum of two riffle habitats or "surveys" (*sensu* MacKenzie et al. 2006) and two run surveys will be sampled within each site. These sample sizes are known to have high power based on previous research (Magoulick et al. 2017). Riffles and runs will be delineated by qualitatively assessing depth and flow rate of the stream. We will use a quantitative kicknet method to determine densities of crayfish in each stream segment (Magoulick et al. 2017). Crayfish will be dislodged from a randomly chosen 1-m^2 quadrat "sub-sample" (*sensu* MacKenzie et al. 2006) area by thoroughly kicking and disturbing the substrate directly upstream of a 1.5 x 1.0-m seine net (3-mm mesh). Crayfish dislodged from the substrate will be washed into the seine net with the aid of the current and by pulling the seine through the sub-sample area. Crayfish will be

identified to species and sex. Replicate kicknet sub-samples will be collected from each riffle or run survey, leaving a 1-m "buffer" around any previous sub-sample locations to minimize the potential effect of previous sub-samples on subsequent ones. Sampling will occur only in water depths of $\leq$1.5 m because we are unable to use the seine effectively in deeper water. At all sampling reaches, physical characteristics of riffle and run surveys will be collected. Dominant substrate coarseness composition will be measured in each 1-m^2 sub-sample. Following collection of crayfish, stream depth and mean current velocity in front of each 1-m^2 sub-sample area will be determined. Landscape scale variables such as those used previously to model and examine crayfish occupancy and abundance will be collected via GIS. We will use crayfish presence data to estimate occupancy rates using package Unmarked in R. Relationships between occupancy rates and local and landscape scale environmental variables will be determined using covariates. Random forest models will be used to develop validated predictive distribution models based on landscape variables and probability of presence maps will be produced in ARC-GIS.

Objective 2

We will collect *C. hubbsi* for genetic analysis from 30 of the sites selected under Objective 1. At each site, 15 individuals of *C. hubbsi* will be collected in order to investigate phylogeographic patterns and levels of gene flow, and we will generate estimates of effective population size and various genetic diversity indices. A muscle tissue sample will be taken from each adult individual (claw or leg) and each sampled crayfish will be retained as a voucher and will be deposited and curated into the crustacean collection at the Carnegie Museum of Natural History (CMNH). In the CMNH laboratory, genomic DNA will be extracted from each individual tissue sample. The mitochondrial cytochrome oxidase subunit I (COI) gene will be amplified via the polymerase chain reaction (PCR) using universal primers previously developed and known to amplify in crayfish. The COI gene is highly variable in freshwater crayfish, and therefore is optimal for use in examining genetic variation and phylogeographic patterns at the geographic scale covered by this project. Current and historical genetic diversity and migration rates will be estimated using the programs DNASP and MIGRATE, and effective population sizes will also be calculated. Comparisons of current and recent historical genetic diversity will indicate if populations are growing or have undergone bottlenecks. The program PopART will be used to construct haplotype networks with 95% parsimoniously plausible branch connections. Isolation by distance will be determined with Mantel tests. Analysis of molecular variance (AMOVA) and fixation indices will be calculated at various hierarchical levels (e.g., within sampled sites, among sites within rivers, among rivers within watersheds, and among watersheds) to determine levels of genetic differentiation at varying geographic scales.

Objective 3

We will use data from the previous section to determine whether *C. hubbsi* distribution, population genetics and potential invasive species effects in the Ozark Highlands of Arkansas and Missouri consists of a single large population or smaller sub-populations, and if the latter, the structure of the sub-populations. Simulation models will be constructed in the program RAMAS-Metapop to determine potential effects of *F. neglectus* invasion on populations of *C. hubbsi* using the population structure previously determined.

Expected Results and Benefits

It is vital for managers to understand the status and threats to the SGCN crayfish species, especially *Cambarus hubbsi*, given that they are geographically restricted stream crayfish and an invasive species that known to displace *C. hubbsi* and other SGCN crayfish species is spreading in multiple drainage basins where *C. hubbsi* currently occur. Examining distribution and population genetics of *C. hubbsi* will help determine the status of this imperiled crayfish and potential for ESU's among sub-populations. Additionally, this study will allow us to determine population structure of *C. hubbsi* and potential threats to this population, including population bottlenecks, an invasive crayfish, and habitat loss. Information gained here will ultimately be used to make decisions regarding the conservation of *C. hubbsi*, and will inform decisions regarding other species that are potentially at risk from similar threats (e.g., *Faxonius longidigitus, Faxonius luteus, Faxonius punctimanus, Faxonius ozarkae, Faxonius neglectus chaeondactylus*). Data collected will also significantly enhance our understanding of crayfish distributions in Arkansas and will be incorporated into databases maintained by the Arkansas Game and Fish Commission and the Arkansas Wildlife Action Plan. A graduate student will be mentored and trained as part of this study. We will also provide annual progress reports and a final report that will be available for AGFC use only until published in a peer-reviewed journal. Finally, we expect multiple publications and presentations to result from this study.

Existing Resources Used

Facilities at the University of Arkansas are more than adequate to carry out the proposed project (e.g., computers, lab space, vehicles, etc.) and will be provided as in-kind support. Brian Wagner (AGFC) and Robert DiStefano (MDC) will provide in-kind support with technicians and field sampling assistance. Additionally, the laboratory of Dr. James Fetzner (Carnegie Museum) is well equipped to conduct the genetic analyses and this will be provided as in-kind support.

Budget

State Wildlife Grant Input: $89,702

Total Project Match: $94,504 (51%)

Total Project cost: $184,206

Requested SWG Funds	**U of A**	**CMNH**
Salary/Benefits		$28,424
Graduate Student	$36,832	
Research Technician	$10,353	
Operating Expenses		
Travel	$6,000	
Supplies	$3,000	$5,093
Capital Expenses	$0	
Subtotals	$56,185	$33,517
TOTAL REQUEST	**$89,702**	

Project Leader

Dr. Daniel D. Magoulick - Acting Unit Leader/ Professor, USGS, Arkansas Cooperative Fish and Wildlife Research Unit, Department of Biological Sciences, University of Arkansas 2000-present
Ph.D. in Ecology from University of Pittsburgh – 1994
Author of 47 peer-reviewed scientific publications

Previous SWG Grants

Magoulick, D.D. and J.W. Quinn. 2016-2018. Arkansas Game and Fish Commission. Flow-ecology relationships and environmental flows assessment for the Ozark-Ouachita Interior Highlands and the West Gulf Coastal Plains. $96,882.

Magoulick, D.D., R.J. DiStefano, B.K. Wagner and J.W. Fetzner, Jr. 2014-2016. Arkansas Game and Fish Commission. Invasive species effects, population status and population genetics of crayfish species of greatest conservation need (*Orconectes marchandi*, *Orconectes eupunctus*, and *Cambarus hubbsi*) in the Ozark Highlands of Arkansas and Missouri. $80,208.

Magoulick, D.D. and D.R. Leasure. 2014-2016. Arkansas Game and Fish Commission. Quantification of hydrologic alteration and relationships to biota in Arkansas streams: Development of tools and approaches for un-gaged streams. $53,000.

Magoulick, D.D., S. Longing, J.W. Quinn, J. Jackson, J. Duzan and J. Petersen. 2011-2013. Arkansas Game and Fish Commission. Classification of Arkansas flow regimes, regional ecological-flow response relationships and environmental flows assessment for the Ozark region. $172,000.

Magoulick, D.D. 2005-2007. Arkansas Game and Fish Commission. Effect of the Introduced Crayfish, *Faxonius neglectus*, on Native Crayfish in the Spring River Drainage. $68,390.

Project Partners

Brian K. Wagner, Non-Game Aquatics Biologist, Arkansas Game and Fish Commission
M.S. in Fisheries Science from Virginia Tech - 1991
Leads the Arkansas Wildlife Action Plan Crayfish Taxa Team
Certified Fisheries Scientist

Robert J. DiStefano, Resource Scientist, Missouri Department of Conservation, Director of Missouri Crayfish Conservation & Management Program
M.S. in Fisheries Science from Virginia Tech – 1987
Author of 47 peer-reviewed scientific publications

Dr. James W. Fetzner, Jr., Assistant Curator of Crustacea, Carnegie Museum of Natural History
Ph.D. in Zoology from Brigham Young University – 2001
Past-President and Managing Editor of the International Association of Astacology
Member of the IUCN Freshwater Crab and Crayfish Specialist Group
Author of 26 peer-reviewed scientific publications and 3 book chapters

Recent Related Publications

Yarra, A.N. and D.D. Magoulick. 2018. Stream permanence is related to crayfish occupancy and abundance in the Ozark Highlands, USA. Freshwater Science (In Press)

Magoulick, D.D., R.J. DiStefano, E.M. Imhoff, M.S. Nolen and B.K. Wagner. 2017. Landscape- and local-scale habitat influences on occupancy and detection probability of stream-dwelling crayfish: implications for conservation. Hydrobiologia 799:217-231.

DiStefano, R.J., C.A. Flinders, E.M. Imhoff and D.D. Magoulick. 2017. Conservation of imperiled crayfish – Faxonius (Crockerinus) marchandi Hobbs, 1948 (Decapoda: Cambaridae). Journal of Crustacean Biology 38:1-6.

Magoulick, D.D. and G.L. Piercey. 2016. Trophic overlap between native and invasive stream crayfish. Hydrobiologia 766:237-246.

Ludlam, J.P., B.T. Banks and D.D. Magoulick. 2015. Density-dependent effects of omnivorous stream crayfish on benthic trophic dynamics. Freshwater Crayfish 21:165-170.

Nolen, M.S., D.D. Magoulick, R.J. DiStefano, E.M. Imhoff and B.K. Wagner. 2014. Predicting probability of occurrence and factors affecting distribution and abundance of three Ozark endemic crayfish species at multiple spatial scales. Freshwater Biology 59:2374-2389.

Magoulick, D.D. 2014. Impacts of drought and crayfish invasion on stream ecosystem structure and function. River Research and Applications 30:1309-1317.

Dekar, M.P. and D.D. Magoulick. 2013. Effects of predators on fish and crayfish survival in intermittent streams. Southeastern Naturalist 12:197-208.

Westhoff, J.T., R.J. DiStefano and D.D. Magoulick. 2012. Do environmental changes or juvenile competition act as mechanisms of species displacement in crayfishes? Hydrobiologia 683:43-51.

Larson, E.R. and D.D. Magoulick. 2011. Life history notes on *Cambarus hubbsi* (Hubbs Crayfish) from the South Fork Spring River, Arkansas. Southeastern Naturalist 10:121-132.

Dekar, M.P., D.D. Magoulick and J. Beringer. 2010. Bioenergetics assessment of fish and crayfish consumption by otter (*Lontra canadensis*): integrating prey availability, diet, and field metabolic rate. Canadian Journal of Fisheries and Aquatic Sciences 67:1439-1448.

DiStefano, R.J., D.D. Magoulick, E.M. Imhoff, and E.R. Larson. 2009. Imperiled crayfishes use hyporheic zone during seasonal drying of an intermittent stream. Journal of the North American Benthological Society 28:142-152.

Larson, E.R., D.D. Magoulick, C. Turner and K.H. Laycock. 2009. Disturbance and species displacement: different tolerances to stream drying and dessication between a native and invasive crayfish. Freshwater Biology 54:1899-1908.

Fetzner Jr., J.W. and R.J. DiStefano. 2008. Population genetics of an imperiled crayfish from the White River drainage of Missouri, USA. Freshwater Crayfish 16:131-146.

Larson, E.R. and D.D. Magoulick. 2008. Comparative life history of native (*Orconectes eupunctus*) and introduced (*Orconectes neglectus*) crayfishes in the Spring River drainage of Arkansas and Missouri. American Midland Naturalist 160:323-341.

Flinders, C.A. and D.D. Magoulick. 2007. Effects of depth and crayfish size on predation risk and foraging profitability of a lotic crayfish. Journal of the North American Benthological Society 26:767-778.

Flinders, C.A. and D.D. Magoulick. 2007. Habitat use and selection within Ozark lotic crayfish assemblages: spatial and temporal variation. Journal of Crustacean Biology 27:242-254.

Magoulick, D.D. and R.J. DiStefano. 2007. Invasive crayfish *Orconectes neglectus* threatens native crayfishes in the Spring River drainage of Arkansas and Missouri. Southeastern Naturalist 6:141-150.

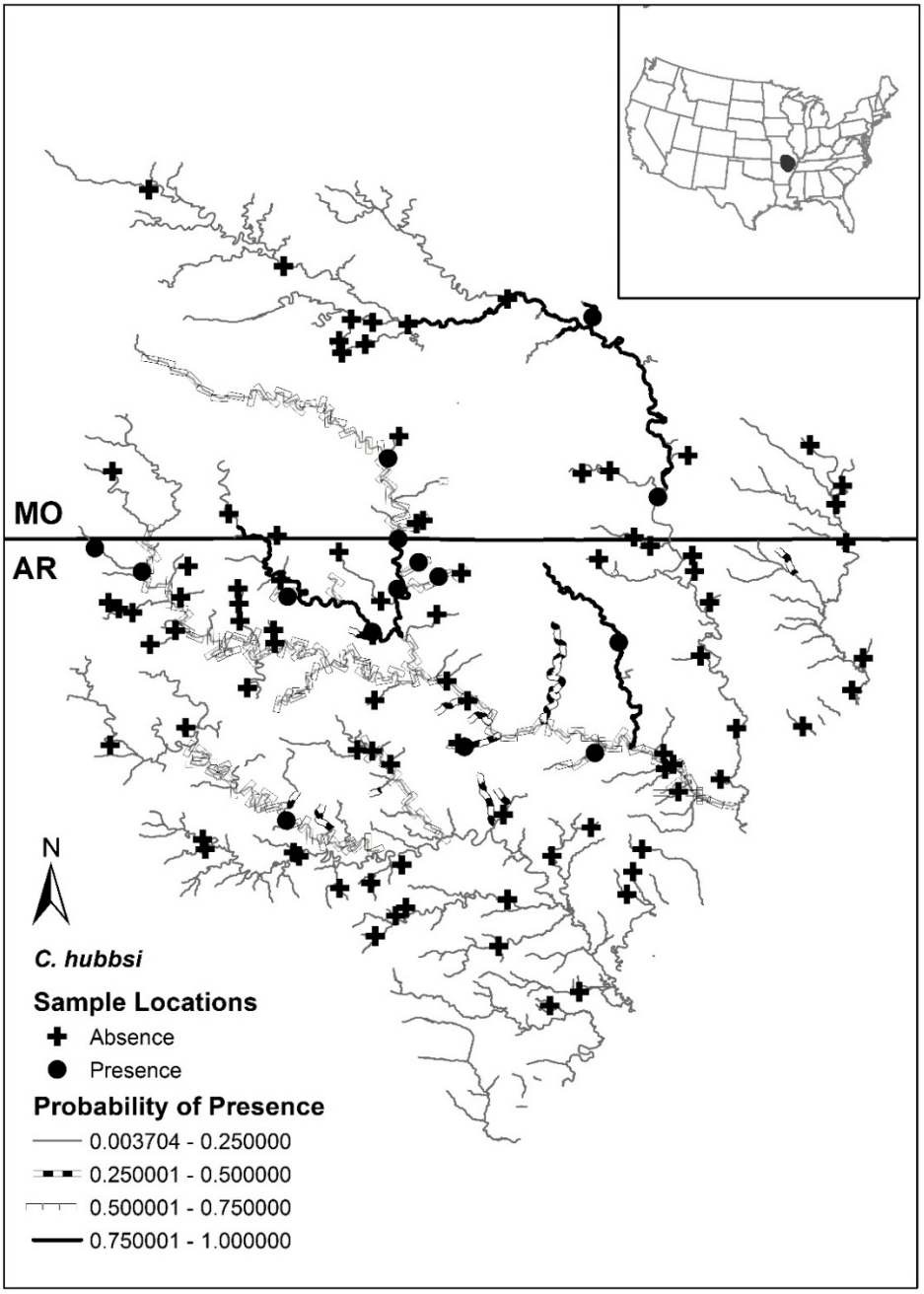

Figure 1. Sample locations and probability of presence of *C. hubbsi* in the Eleven Point, Spring and Strawberry River drainages of Arkansas and Missouri. Probability of presence is based on classification tree models related to landscape variables. From Nolen et al. (2014).