Title: Population status and population genetics of the imperiled Mammoth Spring crayfish, *Orconectes marchandi*, in the Spring River drainage

Project Summary: The Mammoth Spring crayfish, *Orconectes marchandi*, is one of our most geographically restricted stream crayfish and is considered imperiled in Arkansas, Missouri and globally, and a candidate for listing by the USFWS. Crayfish are extremely important in most freshwater systems, typically acting as keystone or dominant species in these systems. The threat of an advancing invasive species, along with potential habitat loss and fragmentation, makes determining *O. marchandi* population status and population genetics extremely important. We propose to determine *O. marchandi* population status by comparing abundance and occupancy rates from 1998-1999 to those from a recent study in 2010-2011, and by comparing current and historical genetic diversity. We will also use genetic data to examine population structure, gene flow among sub-populations, and potential ESU's. Simulation models will be used to determine potential effects of an invasive crayfish on *O. marchandi* populations.

Project Leader: Dr. Daniel D. Magoulick, Assistant Unit Leader and Professor, USGS, Arkansas Cooperative Fish & Wildlife Research Unit, University of Arkansas

Mailing Address: Arkansas Cooperative Fish & Wildlife Research Unit, Department of Biological Sciences, SCEN 632, 1 University of Arkansas, Fayetteville, AR 72701
Contact Info: danmag@uark.edu; 479-575-5449; 479-575-3330Fax

Project Partners: Brian K. Wagner, Nongame Aquatics Biologist, Arkansas Game and Fish Commission, 915 E. Sevier Street, Benton, AR 72015, 877-847-2690, bkwagner@agfc.state.ar.us

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

Dr. James W. Fetzner, Jr., Assistant Curator, Section of Invertebrate Zoology, Carnegie Museum of Natural History, 4400 Forbes Ave., Pittsburgh, Pennsylvania 15213, FetznerJ@CarnegieMNH.org

Total SWG Funding Requested: $69,400

Total Project Cost: $167,836

Amount and Source of Matching Funds or In-kind Services: $98,436*

- $28,000 graduate student tuition, University of Arkansas
- $30,536 indirect cost, University of Arkansas
- $ 8,000 in-kind services, Arkansas Game and Fish Commission
- $20,000 in-kind services, Missouri Department of Conservation
- $11,900 indirect cost, Carnegie Museum of Natural History

Funding priorities addressed:

- Project that addresses the need to monitor populations of Mammoth Spring crayfish, *Orconectes marchandi*, a species of greatest conservation need
- Project that addresses need to examine genetic isolation of Mammoth Spring crayfish populations in advance of potential invasion of ringed crayfish, *Orconectes neglectus*
- Project that addresses the emerging issue of invasive species
- Research projects that lead to on-the-ground conservation

Ecobasin targeted: Spring River basin of Arkansas and Missouri

Problem and Justification

The Mammoth Spring crayfish, *Orconectes marchandi*, is considered imperiled in Arkansas and Missouri and globally (G2) and classified as threatened by the American Fisheries Society Endangered Species Committee (Taylor et al. 2007). The Mammoth Spring crayfish is one of our most geographically restricted stream crayfish and an introduced species is spreading within the basin where it is found (Flinders and Magoulick 2005, Magoulick and DiStefano 2007). Populations of *O. marchandi* are limited to the Spring River drainage of southern Missouri and northern Arkansas (Pflieger 1996, Flinders and Magoulick 2005). In Arkansas, *O. marchandi* are restricted to small streams in the eastern portion of the Spring River drainage (Flinders and Magoulick 2005). These populations appear to be isolated from each other with the cold water Spring River mainstem acting as a potential barrier (Flinders and Magoulick 2005).

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. Crayfish convert more of this organic matter into usable energy for other organisms than all other invertebrates combined, and they play an important role in nutrient cycling. Crayfish also create habitat for other organisms, including many fish, invertebrates, plants, fungi and microbes.

In addition to *O. marchandi* being rare and imperiled, an invasive species, *Orconectes neglectus*, now occurs in high numbers in the West Fork and extends into the South Fork Spring River (Flinders and Magoulick 2005). Our previous research has shown that; 1) *O. neglectus* was introduced into the West Fork of the Spring River between 1984 and 1998, 2) *O. neglectus* appear to be negatively impacting the native crayfish community (Larson et al. 2009), especially *Orconectes eupunctus* and *Cambarus hubbsi*, and 3) *O. neglectus* has the potential to expand its distribution in the Spring River drainage affecting other species including the imperiled *O. marchandi* (Flinders and Magoulick 2005, Taylor et al. 2007).

The threat of an advancing invasive species that has displaced two native species, along with potential habitat loss and fragmentation, makes determining *O. marchandi* population status, population genetics and potential isolation extremely important. Major questions that need to be addressed include "Are populations of *O. marchandi* stable since sampling last occurred in

1999?", "Is metapopulation structure evident, how much gene flow occurs among sub-populations of *O. marchandi*, and are subpopulations Evolutionarily Significant Units (ESU's)?", and "How will invasion of *O. neglectus* potentially affect *O. marchandi* populations?". Based on our previous research, we hypothesize that *O. marchandi* populations are currently stable, but are at risk due to small geographic range and are susceptible to invasion by *O. neglectus*. We also hypothesize that populations of *O. marchandi* in small streams are relatively isolated with little gene flow among them and the sub-populations are ESU's.

Objectives

1. Examine population status of *Orconectes marchandi* in the Spring River drainage
2. Determine the extent of gene flow (ESU's) and compare current and historical genetic diversity of *Orconectes marchandi*
3. Examine population structure of *Orconectes marchandi* and determine potential for invasion impacts by *Orconectes neglectus*

Methods

Objective 1

We will examine population trends in distribution and abundance of *Orconectes marchandi* in the Spring River drainage by comparing sites that were sampled in 1998-1999 (Flinders and Magoulick 2005) and again in 2010-2011 as part of another study (Magoulick et al. unpublished data). Sites were sampled using a quantitative kicknet method to determine densities of crayfish at each site. Replicate kicknet samples were collected from a minimum of three riffle, run, and pool habitats in each of 54 stream segments in 1998-1999 and 104 stream segments in 2010-2011. At all sampling locations, physical characteristics of habitats were also measured including substrate size, depth, current velocity, habitat area, canopy cover, temperature, pH, dissolved oxygen, and conductivity. GIS will be used to incorporate landscape anthropogenic and natural variables. These variables will include factors related to soils, geology, land cover, and hydrology, while anthropogenic variables will include factors related to agriculture, urbanization, mining, hydrology, and water quality. We will examine *O. marchandi* occupancy rates using program PRESENCE. We will compare changes in occupancy over time, and shifts in presence between time periods. Relationships between occupancy rates and environmental variables will be determined using covariates. At sites where *O. marchandi* were found in both time periods, we will compare densities between time periods using ANOVA. GIS will be used to link the *O. marchandi* distribution and density data to landscape and stream segment scale anthropogenic and natural variables.

Objective 2

Twenty sites will be selected from sites where *O. marchandi* are known to occur. At each site, 20 individuals of *O. marchandi* will be collected to investigate gene flow and phylogeographic patterns, including effective population sizes and current and historic genetic diversity. Comparisons of current and recent historical genetic diversity will indicate if populations are growing or have undergone bottlenecks. A tissue sample will be taken from each individual (a claw or leg which are regenerated) and crayfish will be returned unharmed to their collection

site. In the laboratory, genomic DNA will be extracted from each individual tissue sample. The COI mtDNA gene will be amplified with polymerase chain reaction (PCR) with universal primers previously developed (Folmer et al. 1994). The COI mtDNA gene is highly variable in freshwater crayfish (e.g., Fetzner and DiStefano 2008; Taylor and Knouft 2006), and therefore optimal for use in examining gene flow and phylogeographic patterns. Current and historical genetic diversity and migration rates will be estimated using programs DNASP and MIGRATE, and effective population sizes will be calculated. The program TCS 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., sites within streams, streams within watersheds) to determine levels of genetic differentiation within and among sites.

Objective 3

We will use data from the previous section to determine whether *O. marchandi* 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 program R to determine potential effects of *O. neglectus* invasion on populations of *O. marchandi* using the population structure previously determined.

Expected Results and Benefits

It is vital for managers to understand the status and threats to the Mammoth Spring crayfish, given that it is one of our most geographically restricted stream crayfish and an invasive species is spreading within the basin where it is found. Monitoring and population genetics of the Mammoth Spring crayfish 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 the Mammoth Spring crayfish 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 the Mammoth Spring crayfish, and will inform decisions regarding at least two other species that are potentially at risk from similar threats (*Orconectes eupunctus* and *Cambarus hubbsi*). 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 Natural Heritage Commission. A graduate student will be mentored and trained as part of this study. Finally, we expect reports, 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: $69,400

Total Project Match: $98,436*

Total Project cost: $167,836

Requested SWG Funds	U of A	CMNH
Salary/Benefits		$22,000
Graduate Student	$30,900	
Operating Expenses		
Travel	$5,000	
Supplies	$2,000	$7,500
Capital Expenses	$2,000	
Subtotals	$39,900	$29,500
TOTAL	**$69,400**	

Project Leader

Dr. Daniel D. Magoulick - Assistant 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 32 peer-reviewed scientific publications

Previous SWG Grants

Arkansas Game and Fish Commission. Magoulick, D.D., S. Longing, J.W. Quinn, J. Jackson, J. Duzan and J. Petersen. 2011-2014. Classification of Arkansas flow regimes, regional ecological-flow response relationships and environmental flows assessment for the Ozark region. $172,000.

Arkansas Game and Fish Commission. Magoulick, D.D. 2005-2007. Effect of the introduced crayfish, *Orconectes 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
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 37 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
Immediate Past-President and Board Member of the International Association of Astacology
Member of the IUCN Freshwater Crab and Crayfish Specialist Group
Author of 16 peer-reviewed scientific publications and 1 book chapter

Recent Related Publications

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.

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.

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.

Larson, E.R., R.J. DiStefano, D.D. Magoulick, and J. Westhoff. 2008. Efficiency of quadrat sampling for riffle-dwelling crayfish. North American Journal of Fisheries Management 28:1036-1043.

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.