Title: Potential spread of the invasive Ringed Crayfish and mechanisms of displacement on Coldwater Crayfish

Project Summary: This project will address uncertainties associated with conservation status designations as well as the decline of the imperiled Coldwater Crayfish. We will determine whether the invasive Ringed Crayfish is spreading within the Spring River drainage and mechanisms of displacement of Coldwater Crayfish by Ringed Crayfish. Crayfish are keystone species in most Ozark streams, so a replacement of one species by another may have impacts that are potentially far reaching throughout the ecosystem, and may include impacts on important fisheries (e.g., smallmouth bass).

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Project Partners:

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Total SWG Funding Requested: $76,852

Amount and Source of Matching Funds or In-kind Services: Total - $90,310
In-kind services, University of Arkansas – $60,810
- $13,495 graduate student tuition
- $33,815 indirect cost
- $13,500 equipment

In-kind services, Arkansas Game and Fish Commission – $8,500

In-kind services, Missouri Department of Conservation – $21,000

Total Project Cost: $76,852 SWG, $90,310 Match

Priority actions addressed

- Invasive Species Emerging Issue – "identify plant/animal native/exotic invasive species in Arkansas with special emphasis on impacts to Species of Greatest Conservation Need (SGCN) with recommendations for developing and prioritizing Conservation Actions to address impacts."

- *Orconectes eupunctus* – "Monitor their response to spread of the invading gapped ringed crayfish, *Orconectes neglectus chaenodactylus*, and possible displacement mechanisms related to early life history and drying disturbance."

Need

The Coldwater Crayfish (*Orconectes eupunctus*) is currently designated as a Species of Greatest Conservation Need by Arkansas Game and Fish Commission (AGFC), imperiled in Missouri and globally by Missouri Department of Conservation (MDC - S2/G2), and as threatened by the American Fisheries Society Endangered Species Committee. The Coldwater 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). Additionally, our research indicates that the Coldwater Crayfish has declined from significant portions of its range where the Ringed Crayfish (O*rconectes neglectus*) has invaded.

Crayfish are recognized as "keystone species" or "ecological dominants", integral to the fueling and functioning of many of our streams, 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.

Populations of Coldwater Crayfish are limited to the Eleven Point, Spring and Strawberry River drainages in Arkansas and Missouri. The Coldwater Crayfish has been documented from only about 20 Arkansas localities and 8 Missouri localities, but nearly all of those localities were in four streams. The full distribution of this species has never been assessed.

The Coldwater Crayfish has a very limited geographic distribution and is now being threatened by an invasive species that is spreading within the basin where it is found. The Ringed Crayfish (O*rconectes neglectus*) invaded the Spring River drainage after 1984 and has spread throughout the lower portion of the West Fork and into portions of the South Fork Spring River (Fig. 1), occupying similar habitats as those preferred by Coldwater Crayfish. Subsequently, Coldwater Crayfish abundances declined dramatically and it appears the species may have been extirpated from much of the West Fork where it formerly comprised 8–33% of the crayfish community. Furthermore, an ongoing study has confirmed additional introductions of the Ringed Crayfish in the range of the Coldwater Crayfish. Such loss of native biodiversity and crayfish species replacements are concerns because differing functional roles of these two species may lead to altered ecosystem structure and function. Another imperiled crayfish, the Mammoth Spring Crayfish (*Orconectes marchandi*) also occurs in the Spring River drainage, and is threatened by this invasion.

Our previous research suggests that differential susceptibility to seasonal drought and stream drying may be involved in the displacement of the Coldwater Crayfish by the Ringed Crayfish. However, we still know little about this potential mechanism and whether other factors, such as predation, are involved.

More importantly, we know little about how the Ringed Crayfish invasion will ultimately spread and affect the different populations of Coldwater Crayfish.

There has been little research on Coldwater Crayfish since it was first described. The threat of an advancing invasive species that may have displaced Coldwater Crayfish from a significant portion of its range increases the need for knowledge about this species. Major questions that need to be addressed include: How rapidly is the invasion of Ringed Crayfish spreading? Can we determine the causal mechanism by which the invasive crayfish might be displacing the native crayfish? How is the invasion of Ringed Crayfish affecting Coldwater Crayfish populations? What are the potential invasion impacts by Ringed Crayfish? For this proposal we will focus on the first two of these questions.

Based upon previous research, we hypothesize that 1) Ringed Crayfish are spreading slowly longitudinally along the Spring River, 2) Ringed Crayfish will successfully displace Coldwater Crayfish at the periphery of its range (e.g., in moderate sized, less thermally stable tributaries), 3) Ringed Crayfish will negatively affect Coldwater Crayfish.

Objectives

1. Monitor invasion "front" and potential spread of Ringed Crayfish in South Fork Spring River.
2. Determine displacement mechanisms of Coldwater Crayfish by Ringed Crayfish.

Approach

Objective 1

We will monitor the spread of the invasion by Ringed Crayfish in South Fork Spring River by sampling sites that were previously sampled and in newly selected sites. Sites that were previously sampled longitudinally along the South Fork Spring River (Magoulick and Distefano 2007, Larson et al. 2009) will be sampled again with similar methods. Additionally, new sites along the leading edge of the front will be sampled seasonally for 1.5 years. We will use a quantitative kicknet method to determine densities of individual crayfish species at each site. Crayfish will be dislodged from a 1-m^2 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 sample area. Replicate kicknet samples will be collected from riffle, run, and pool habitats in each of 11 previously sampled sites and 9 newly selected sites. New sites within streams will be selected randomly within river km 40-70, the previous range of leading edge of the invasion front. Site selection will also be based partly on accessibility (e.g., landowner permission), and sample locations within habitats will be randomly chosen. Habitat types will be delineated by qualitatively assessing depth and flow rate of the stream. At all sampling locations, physical characteristics of habitats will be collected. Substrate size composition within the habitat will be quantified by visually estimating percent area of silt (<0.02 cm diameter), sand (0.02-0.1 cm), gravel (0.1-3 cm), pebble (3-6 cm), cobble (6-25 cm), and boulder ($\geq$ 26 cm) within the 1m^2 sample area. Following collection of crayfish, stream depth and mean (0.6 depth) current velocity in front of the sample area will be determined using a meter stick and Marsh-McBirney® flow meter. Crayfish densities will be compared between time periods using ANOVA. We will also use crayfish presence data to estimate occupancy rates using program PRESENCE. Relationships between occupancy rates and environmental variables will be determined using covariates.

Objective 2

We will conduct a series of experiments to determine mechanisms of displacement of Coldwater Crayfish

by Ringed Crayfish. We will conduct field and mesocosm experiments to determine interactive effects of predation and stream drying on growth and survival of Ringed Crayfish and Coldwater Crayfish. Field experiments will be conducted as below during seasonal drying to examine affects of drying and predation on crayfish growth and survival. Additionally, we will examine whether drying (wet/dry water levels) and predation (smallmouth bass present/absent) interact to effect growth and survival of Ringed Crayfish and Coldwater Crayfish in replicated 190 liter mesocosms and in field enclosures.

We will examine field predation rates of coldwater and Ringed Crayfish with tethering experiments at an upstream location on the West Fork (Ringed Crayfish present, Coldwater Crayfish absent) and downstream location on South Fork Spring River (Coldwater Crayfish present, Ringed Crayfish absent). In each experiment, we will place 30 crayfish of each species in run habitats. We will use acrylamide gel to glue 6-lb-test monofilament fishing line to the dorsal portion of the carapace of each crayfish. We will anchor the free end of the tether to a rock and adjust the monofilament line so that each crayfish can move freely within an area of 30-cm radius around the rock. The tether will not impede crayfish from seeking shelter under available substrates within their range of mobility. Preliminary tests of the tethering technique will be conducted to examine frequency of crayfish escapes. The experimental area in each habitat will be systematic with grid points 1 m apart with a random starting point for crayfish species. The number and size of crayfish missing from their tethered location in each habitat will be recorded daily for 2 d during both experiments. This period of time has shown substantial predation in previous studies. The downstream experiment will use only Coldwater Crayfish so as not to introduce Ringed Crayfish at this site. Predation on Coldwater Crayfish from the downstream location will be used as a reference comparison to upstream. This experiment will determine whether predation may be a mechanism in the displacement but will also elucidate the relative importance of each species to predators.

Expected Results and Benefits

Results of objectives 1 and 2 should provide relevant information about the cause of observed Coldwater Crayfish declines, and whether Ringed Crayfish are spreading further in the system. This will facilitate management and policy decisions regarding Coldwater Crayfish and the invasive species. Specifically, potential documentation of the invasive Ringed Crayfish as a causal factor in the decline of Coldwater Crayfish will largely influence the direction and extent of management of Coldwater Crayfish. Additionally, this information will be used in evaluating the currently assigned conservation status ranks and determine whether those ranks should be modified. Such a decision would significantly affect future management of the species by AGFC and MDC, and possibly by federal agencies. For example, these data would be a primary tool used by AGFC, MDC and U.S. Fish & Wildlife Service in potential deliberations concerning federal listing of the Coldwater Crayfish as a federally “threatened” species.

Principal investigators and students will share information from this project via manuscripts, theses and dissertations, presentations at local to international meetings, and updates of the Arkansas Wildlife Action Plan. As part of this project we will add data to the National Monitoring Partnership database, and we will add the project to the Arkansas Portal in the Conservation Directory. Finally, we will provide annual progress reports and a final report after the completion of the project.

Location of Work

Studies related to objective 1 and 2 will be at sites in the West Fork and South Fork Spring River (Fig. 1) and potentially the Eleven Point River. Other experiments for objective 2 will be conducted at the University of Arkansas streams mesocosm facility.

Budget

State Wildlife Grant Input: $ 76,852
Total Project Match: $90,310
Total Project cost: $167,162

Requested SWG Funds	**2012**	**2013**	**UA**	**AGFC**	**MDC**
Salary/Benefits					
Post-doc	$15,819	$15,819			
Technician	$7,589	$7,589			
Work-study labor	$3,048	$3,048			
Operating Expenses					
Travel	$4,620	$4,620			
Supplies	$6,200				
Capital Expenses	$8,500				
Subtotals	$45,776	$31,076			
Match			$60,810	$8,500	$21,000
TOTAL	**$167,162**				

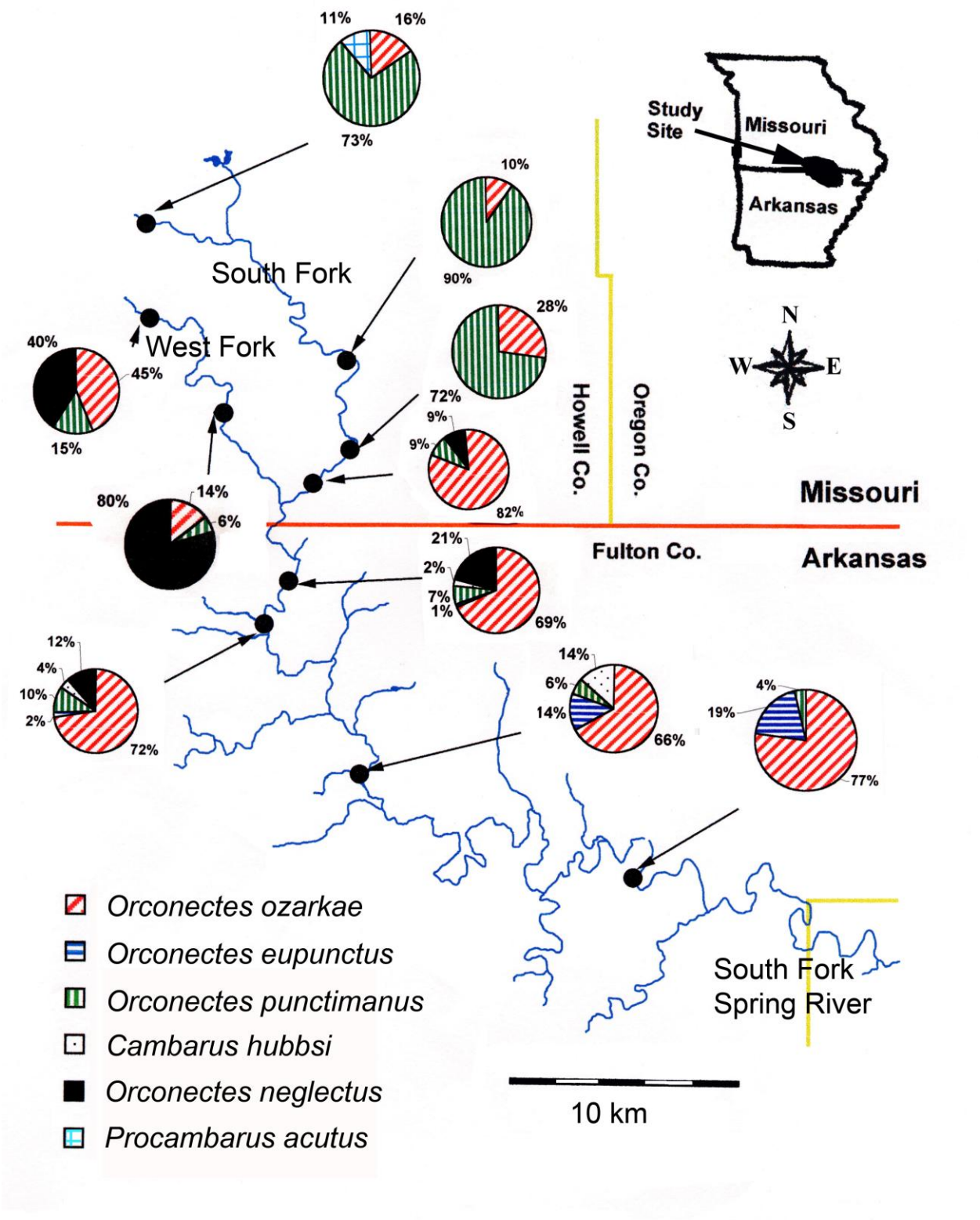

Figure 1. Map of Coldwater Crayfish (*O. eupunctus*) and Ringed Crayfish (*O. neglectus*) distribution in the Spring River drainage of Arkansas and Missouri (Magoulick and DiStefano 2007).

Project Leader

Dr. Dan 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
M.S. in Ecology from Eastern Michigan University – 1985
B.S. in Fisheries and Wildlife from Michigan State University – 1981
Active on Arkansas Wildlife Action Plan Fish and Crayfish Taxa Teams.
Author of 29 peer-reviewed scientific publications, many dealing with aquatic species conservation and biological invasions.

Previous SWG Grants

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.

Arkansas Game and Fish Commission. 2010-2012. Magoulick, D.D., S. Longing, J.W. Quinn, J. Jackson, J. Duzan and J. Petersen. Classification of Arkansas flow regimes, regional ecological-flow response relationships and environmental flows assessment for the Ozark region. $172,008.

Project Partners

Mr. Brian Wagner – Nongame Aquatics Biologist, Coordinator of Nongame Aquatics Program, Arkansas Game and Fish Commission 1997-present, research in aquatic conservation 1988-present
M.S. in Fisheries from Virginia Tech – 1991
B.S. in Systematics and Ecology from University of Kansas – 1986
Certified Fisheries Scientist through American Fisheries Society – 1993-present
Leads Arkansas Wildlife Action Plan Crayfish Taxa Team, and active on the Fish, Cave, and Invertebrate Taxa Teams.
Author of 9 peer-reviewed scientific publications on sport fish, nongame fish, crayfish, reptiles, and amphibians.

Mr. Robert DiStefano – Resource Scientist, Missouri Department of Conservation, 1986-present
M.S. in Fisheries Science from Virginia Tech – 1987
B.S. in Fisheries Management from Eastern Kentucky University – 1983
American Fisheries Society Certified Fisheries Scientist
Direct Missouri's Statewide Crayfish Conservation & Management Program
Author of 31 peer-reviewed scientific publications on aquatic species conservation and management, including invasive species issues.