

2018 ARKANSAS STATE WILDLIFE GRANT PRE-PROPOSAL

PROJECT TITLE: A population genomic framework for prioritizing fish species of greatest conservation need in the White River Drainage in Arkansas



PROJECT SUMMARY: Connectivity among populations affects genetic diversity, local adaptation, and population structure. These factors play an integral role in the persistence of populations and species as well as the foundation of new ones. Therefore, measuring connectivity (geneflow) among populations and drawing comparisons among species —both common and rare —will be illuminating for conservation efforts. We propose to collect fish assemblages at 50+ sampling sites across the White River Drainage to determine 1) effective intraspecific geneflow of common and SGCN species; 2) population structure of species across the drainage; and 3) phylogenetic relationships of SGCN species and species complexes, including the *Etheostoma spectabile* species complex. This information will aid in prioritizing populations and species of greatest conservation need. The proposed research will form the basis of at least one University of Arkansas PhD student dissertation, provide research experience for several undergraduates, and result in a dataset of fish occurrence and relative abundance across a large portion of the state that will be informative for projects related to spatial ecology, taxonomic diversity and drainage-wide conservation efforts.

PROJECT LEADER: Dr. Marlis R. Douglas, Bruker Professor in Life Sciences, Department of Biological Sciences, University of Arkansas, Science and Engineering, Room 601, Fayetteville, AR 72701, mrd1@uark.edu, 479-575-4176

PROJECT PARTNER: Dr. Michael E. Douglas, Professor & 21st Century Chair in Global Change Biology, Department of Biological Sciences, University of Arkansas, med1@uark.edu

PROJECT PARTNER: Zachery D. Zbinden, Distinguished Doctoral Fellow, Department of Biological Sciences, University of Arkansas, Fayetteville, AR, zdzbinde@uark.edu

PROJECT BUDGET (35% cost-share):

SWG Grant Funds Requested:	\$74,500
Match Provided (>35%):	\$42,136
Total Project:	\$116,636

PROJECT STATEMENT

NEED: Dispersal among populations increases the chances of population persistence by increasing genetic diversity and via rescue effects, which occur when migrants help revitalize a population in decline (Fagan, 2002; Thomaz, Christie and Knowles, 2016). To better estimate the conservation needs of a species, both the status of individuals within populations (e.g. genetic diversity) and how populations interact with one another (e.g. geneflow) (Avisé, 1992; Vrijenhoek and Vrijenhoek, 1998) must be considered. Fish species restricted to headwater habitats and those with patchy distributions within watersheds (e.g. SGCN), are expected to have low population connectivity due to greater distances among populations (Thomaz, Christie and Knowles, 2016). Such isolated populations are particularly susceptible to extirpation due to natural and anthropogenic environmental change (Fagan, 2002). By measuring genetic isolation among populations, the degree of dispersal that has historically occurred can be inferred for any given species (Hughes, Schmidt and Finn, 2009). Such information is essential to aid managers in prioritizing populations and species of conservation concern.

For most fish species dispersal is poorly understood because monitoring and tracking individual fish in a river network is difficult and expensive (Tonkin *et al.*, 2017). Existing knowledge is skewed towards larger bodied fishes that are not encumbered by bulky tags required for tracking. And although passive integrated transponder (PIT) tags aim to rectify this, a drainage wide study of even one species would require purchasing and maintaining many tag reading stations; this proves to be cost prohibitive. Conservation genomic approaches are a much more cost-effective means of estimating dispersal, and, in addition, provide insights into both spatial and temporal population connectivity that would otherwise require many decades of tracking to produce (Bohonak, 1999; Tonkin *et al.*, 2017). Furthermore, genomic data allow estimation of diversity parameters relevant to conservation and management, including levels of genetic diversity, effective population size, and can help clarify taxonomic relationships —none of which can be estimated by tracking alone (Avisé, 1992; Douglas and Douglas, 2010).

It is more critical than ever to study population connectivity in stream dwelling species because these are the most at risk of population decline due to climate change (Tonkin *et al.*, 2017). Species constrained to river and stream networks cannot as readily move to suitable habitat in the face of a shifting environment. Increasing aridity that is expected in decades to come will predictably lead to less aquatic habitat and lower hydrologic network connectivity. Collecting baseline measurements of population connectivity now will allow future studies to determine the impact of climate change on the connectivity and persistence of fish populations (Vrijenhoek and Vrijenhoek, 1998).

PURPOSE & OBJECTIVES: The purpose of this study is to quantify genetic diversity within and genetic divergence among populations of stream fishes across the White River Drainage (WRD). These data will allow inferring population connectivity and aid in developing a framework for further prioritizing populations and species of greatest conservation concern. In addition, these data are necessary to clarify taxonomic relationships of cryptic species emblematic for the Ozarks, such as the *Etheostoma spectabile* complex, with unprecedented resolution. In turn, this will position the Arkansas Game and Fish Commission to establish pro-active conservation policies and practices for SGCN species.

Objectives are as follows:

1. Sample collection: collect fish assemblages throughout the WRD to quantify genetic diversity and population structure for at least 20 species. We will specifically target the following five SGCN species that occur in the WRD: *Etheostoma fragi*, *Etheostoma teddroosevelt*, *Etheostoma uniporum*, *Notropis ozarcanus*, and *Percina uranidea*.
2. Data generation: genotype each individual across 20,000 loci distributed throughout the genome using high-throughput Next-Generation-Sequencing approaches.
3. Data analysis: analyze genomic data with customized bioinformatics approaches; estimate genetic diversity within and among populations and species; assay genetic structure to determine connectivity among isolated populations; and analyze data within a comparative framework to resolve taxonomic relationships within species complexes and to identify intraspecific conservation units, such as evolutionarily significant units (ESUs) or management units (MUs) within each subdrainage.
4. Translate data into management applications: rank species and populations within species according to population connectivity and genetic diversity; translate this matrix into prioritization categories to guide conservation efforts and enhance effectiveness of management actions.

LOCATION: The White River Drainage (WRD) will be the focus of the multi-species population genomic study proposed here. It is the state's largest drainage (17,143 sq. mi. and 34% of Arkansas' land) and contains the Spring, Eleven Point, Strawberry, Kings, Black, and Little Red rivers. Across the WRD, collections of 50 fish assemblages have already been completed in June/July 2017 (Figure 1). Tissues for genomic analyses were collected from up to 10 individuals per species per location. Additional sampling is planned for spring and summer of 2018 to collect additional species and populations from across the drainage. Sampling will specifically target SGCN and areas of low sampling coverage. Permits to collect these samples have been secured.

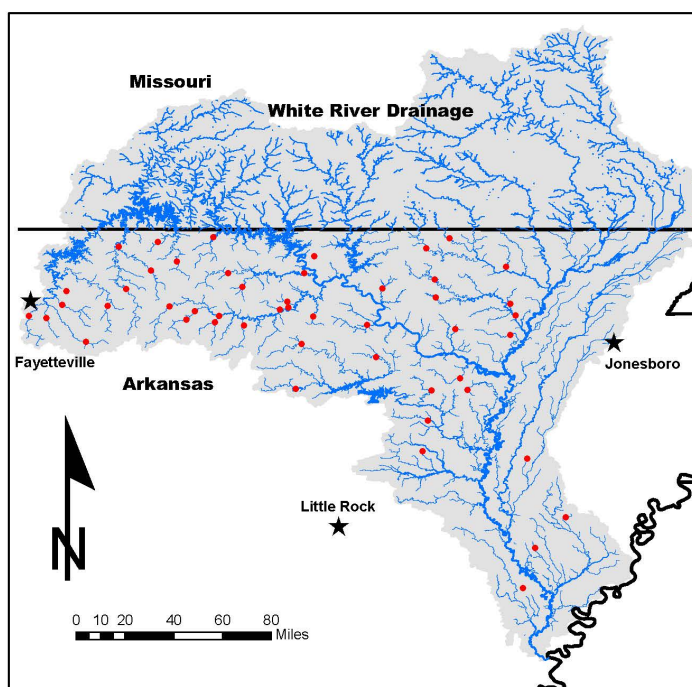


Figure 1. Distribution of sampling sites (red circles) across the White River Drainage collected during summer 2017.

APPROACH: Fish assemblages will be collected at more than 50 sites (i.e. individual stream reaches >200m in length) across the WRD. Up to 10 individuals per species per site will be preserved in ethanol for genomic analyses. Individuals will be identified to species and a tissue sample will be taken from each. DNA will be extracted from tissues using standard protocols and

prepared for Next-Generation-Sequencing using a reduced-representation library approach (ddRAD=double-digest Restriction-Associated-DNA) that consistently assays the same~20,000 nuclear DNA loci for each individual. Following preparation, the isolated loci will be sent for sequencing genomic sequencing will be conducted at the University of Oregon Genomics & Cell Characterization Core Facility. Genomic data will be analyzed to quantify genetic diversity, effective population size, geneflow, and isolation by distance. In addition, phylogenetic analyses will be conducted to identify distinct evolutionary lineages (ESUs) within each putative species and to clarify taxonomic relationships within and among species complexes.

EXPECTED RESULTS & BENEFITS:

1. A survey of species occurrence and assemblage structure across the WRD.
2. Estimates of genetic diversity and population connectivity for 20+ species across 50+ populations.
3. A list of species and populations ranked by level of genetic diversity and population connectivity.
4. A molecular taxonomic assessment of the darters in the Ozarks based on genomic data (>20,000 loci per individual).

This information will help managers to focus specifically on species and populations in the WRD that lack genetic diversity due to low population connectivity. It is possible that populations with low connectivity may be the result of habit degradation and/or seasonal stream drying. Having this information will help direct efforts for future habitat restoration projects.

BUDGET:

BUDGET CATGORY	YEAR 1	YEAR 2	TOTAL
Supplies for DNA work	\$10,000	\$10,000	\$20,000
Next-Generation-Sequencing	\$15,000	\$15,000	\$30,000
Total Direct Costs	\$25,000	\$25,000	\$50,000
Indirect Costs (49%)	\$12,250	\$12,250	\$24,500
Total SWG	\$37,250	\$37,250	\$74,500
UA Match (>35%)	\$20,735	\$21,401	\$42,136
Total Project	\$57,985	\$58,651	\$116,636

QUALIFICATIONS:

Dr. Marlis R. Douglas (Project Lead) is the Bruker Professor in Life Sciences in the Department of Biological Sciences at the University of Arkansas, Fayetteville. Dr. Douglas has over 20 years of experience in conservation genetics research involving rare, threatened or endangered, as well as invasive, aquatic species. Prior she held faculty positions at Colorado State University and the Illinois Natural History. She has a long history working closely with state and federal agencies to generate scientifically sound data that inform management and help refine conservation efforts. She serves on the Board of Governors for the American Society of Ichthyologists and Herpetologists (ASIH) and is the liaison between ASIH and the American Fisheries Society.

Dr. Michael E. Douglas (Project Partner) is the 21st Century Chair in Global Change Biology in the Department of Biological Sciences at the University of Arkansas, Fayetteville. Dr. Douglas held previous faculty positions at Oklahoma State (Biology), Arizona State University (Life Sciences), and Colorado State University (Fish, Wildlife and Conservation Biology), and was Director of the Division of Ecology and Conservation Science and Associate Director for Research at the Illinois Natural History Survey at the University of Illinois. He has over 30 years experience in conducting conservation and ecology oriented research in a wide variety of disciplines including taxonomy, phylogenetics, quantitative ecology, morphometrics, statistical analyses, primarily involving aquatic organisms, as well as wildlife species. He served as president of the American Society of Ichthyologists and Herpetologists and the Desert Fishes Council, and was for 15 years editor of the journal *Copeia*.

Mr. Zachery D. Zbinden is a Distinguished Doctoral Fellow in the Department of Biological Sciences at the University of Arkansas, Fayetteville. Mr. Zbinden completed a master's degree in biology at the University of Oklahoma under the guidance of Dr. William J. Matthews. There he led SWG-funded field efforts to survey drainages in southeastern Oklahoma for fish SGCN at 160 field sites across the Muddy Boggy, Kiamichi, and Little River drainages.

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