

STATE: KANSAS
DURATION: APRIL 1, 2008 – MARCH 31, 2010
TITLE: VIABILITY OF FRAGMENTED STREAMS IN KANSAS
SUBGRANT RECIPIENT: KANSAS STATE UNIVERSITY

GRANT #: T-20-R, AME #0
FAIMS #: T-20-R-1, AME #0

A. NEED

Habitat alteration and fragmentation are primary causes underlying the erosion of biodiversity in both terrestrial and aquatic habitats worldwide (Noss and Csuti 1997). Because of the linear nature of their habitat, stream organisms are particularly sensitive to habitat alterations and changes in connectivity that may isolate populations. Dams and their associated impoundments are common features in the contemporary landscape; particularly in Midwestern states, such as Kansas. As with most North American reservoirs, Kansas reservoirs, are young (≥ 50 years old), and the long-term ecological consequences of associated habitat alterations have been a subject of research focus over the past 20 years. Accordingly, there is a growing literature that documents the ecological effects of reservoir impoundment on fish assemblages.

In the Great Plains, reservoirs have been implicated in the extirpation or decline of native fishes in stream networks upstream of impoundments (Winston et al. 1991, Gido et al. 2002). At the assemblage level, species richness of macrohabitat generalists (Herbert and Gelwick, 2003) and centrarchids (Taylor et al., 2001) increases upstream of impoundments in this region. Falke and Gido (2006) also found differences in fish assemblage structure in small streams flowing into Tuttle Creek and Milford reservoirs when compared to those draining directly into the Kansas River. The construction of dams, therefore, can both isolate stream fishes that might ordinarily use rivers as corridors between tributary streams and provide a springboard for the spread of lake-adapted species. Although our understanding of the assemblage-level ecological effects of impoundments is gaining resolution, there is surprisingly little information about the genetic-level implications of these large-scale alterations of habitat.

The assessment of the effects of landscape features like impoundments on the genetic structure of populations is a central goal of the emerging field of landscape genetics (Manel et al. 2003, Holderegger and Wagner 2006, Storfer et al. 2007), and is an important undertaking for understanding the conservation of intraspecific genetic diversity in fishes in changing aquatic landscapes (Ryman et al. 1995). Genetic studies have addressed the influence of dams as barriers to movement in the primary habitat and migration routes of salmonids and other riverine fishes (e.g., Bessert and Orti 2007, Neraas and Spruell 2001, Meldgaard et al. 2003, Yamamoto et al. 2004, Alò and Turner 2005, Wofford et al. 2005, Cegelski et al. 2006, Crispo et al. 2006, Neville et al. 2006, Whiteley et al. 2006b). Nevertheless, the consequences of the transformation of an intervening water body, and putative migration corridor, from a lotic state to a lentic state for headwater stream populations are poorly understood. In particular, whether reservoirs alter population connectivity by removing main channel habitat thus altering among-tributary patterns of migration and genetic diversity is not well known.

Understanding patterns of *genetic diversity*¹, however, is of central importance to forming conservation strategies. Genetic diversity is one of the most important attributes of any population because sufficient genetic variation is necessary for populations to respond to shifting environmental conditions (Hartl and Clark 1997). Hence, changes in genetic diversity associated with habitat alteration and fragmentation can help to reveal the potential for negative long-term effects that might otherwise remain cryptic.

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For example, in the impounded Eno River of North Carolina, Skalski et al (in press) found that genetic diversity of creek chubs (*Semotilus atromaculatus*), measured using *gene diversity*¹, *allelic richness*¹, patterns of *isolation-by-distance*¹, and estimates of recent *gene flow*¹ from *microsatellite*¹ markers suggested an association between the type of movement corridor – whether a connecting waterbody was riverine or reservoir in type – and population genetic structure. Preliminary analyses of creek chub population structure in the Kansas River basin (Hudman and Skalski, unpublished) revealed contrasting patterns of isolation-by-distance and genetic differentiation associated with tributary position with respect to the location of the Bowersock Dam in Lawrence, KS. Combined results from these studies of creek chubs suggest that waterway impoundment results in significant habitat alteration and subsequent population sub-division in a widely distributed minnow species. Nevertheless, these results are correlative and should be replicated across reservoirs. Understanding the ecological effects of impoundment is particularly relevant to the conservation of fishes in Kansas because large reservoirs (> 800 ha surface area) occupy all major drainage basins (KDHE 1996, Figure 1), and small stream fishes occupying may be particularly vulnerable to fragmentation by impoundments or groundwater mining (Cross and Moss 1987).

The purpose of our study is to contribute to the Kansas Comprehensive Wildlife Conservation Plan by extending current understanding of assemblage-level effects of aquatic habitat alteration resulting from waterway impoundment in the Central Mixed-grass and Eastern Tall-grass Conservation Regions. Specifically, we will use microsatellite markers to assess the level of genetic isolation among stream fish populations inhabiting tributaries in the impounded Wakarusa, Elk Creek, and Republican River drainages (Figure 1). We will target creek chub for our genetic analyses because (1) they are common and can be sampled within the time frame of the proposal, (2) we have successfully developed and employed genetic markers for this species in Kansas, (3) they commonly co-occur with several species of conservation concern in Kansas (e.g., *Etheostoma spectabile*, *Loxilus cornutus*, *Lythrurus umbratilis*, *Nocomis biguttatus*, *Notropis topeka*, *Noturus exilis*, *Phoxinus erythrogaster*). The results from the study will directly contribute to the goals of the Kansas Comprehensive Wildlife Plan in two ways:

- (1) We will assess the potential for detrimental impacts of habitat alteration (fragmentation) on species needs (Statewide Issues #1 and #2, KCWCP page 18).
 - (a) Our use of a relatively common species (*S. atromaculatus*) of relatively low conservation priority will ensure large enough sample sizes to allow inferences about

Box 1. Definitions of genetic terms in the text; from Freeland (2005).

Allelic Richness: a measure of a population's genetic diversity calculated as the average number of alleles per locus.

F_{IS}: the inbreeding coefficient; the probability that an individual has two alleles at a particular locus that recently descended from a single common ancestor.

Gene Diversity: a measure of genetic diversity based on the probability that two alleles chosen randomly from the population will be different.

Gene Flow: the transfer of genetic material from one population to another following dispersal and subsequent reproduction of individuals, propagules, or gametes.

Genetic Diversity: the amount of genetic variation that is contained within a population.

Heterozygosity: the proportion of individuals in a population that have more than one type of allele at a particular genetic locus.

Isolation-by-Distance: a pattern of population differentiation in which the genetic dissimilarity of populations is correlated with the geographical distance that separates them.

Locus: the location of a particular gene or sequence of DNA on a chromosome.

Microsatellite: a stretch of DNA that consists of a short tandem sequence of up to five base pairs that is repeated multiple times.

Polymorphic: a locus that has multiple alleles in a population.

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the general effects of impoundment on genetic subdivision in the guild of fishes inhabiting small streams in Kansas (Strategy #12, KCWCP page 18).

- (b) Species-specific distribution data and genetic data collected during our fieldwork may be used for GIS-informed landscape-level analyses of associations between genetic diversity, population genetic structure and, for example, land-use or waterway management practices (Issue #2, Strategy #9, KCWCP page 19).
- (2) We will contribute to a general understanding of species populations and distributions (Statewide Issue #6, KCWCP page 21).
 - (a) We will estimate population-specific estimates of genetic diversity in altered and unaltered waterways of the Kansas River Basin (Strategy #11, KCWCP page 21)
 - (b) The genomic DNA samples collected for our population-level studies of genetic diversity may be used to develop baseline data for more research related to the ecology and systematics of fishes in Kansas (Strategy #13, KCWCP page 21).

B. OBJECTIVES

- (1) Estimate the effects of habitat fragmentation on population-level processes (genetic structure) of fishes inhabiting small streams in the Kansas River basin by using a relatively common species (*S. atromaculatus*) to allow inferences about the general effects of impoundment on genetic subdivision in members of the small stream guild.
- (2) Establishment of baseline data for more research related to the ecology and systematics of fishes in Kansas using the genomic DNA samples collected during our investigation of genetic diversity in impounded river systems.
- (3) Species-specific distribution data and genetic data collected during our fieldwork may be used for GIS-informed landscape-level analyses of associations between genetic diversity, population genetic structure and, for example, land-use or waterway management practices.

C. RESULTS

- (1) Increased understanding of the patterns of genetic diversity among populations of creek chub in impounded and un-impounded drainages in the Kansas River basin.
- (2) Increased potential for extrapolating effects observed in creek chubs to other fishes in the guild inhabiting small streams in Kansas; e.g., *Etheostoma* darters and *Noturus* catfishes.
- (3) Generation of genomic DNA suitable for the development of further investigations of the ecology and systematics of headwater stream fishes in Kansas.

D. APPROACH

Field Work. — We will collect fin tissue from creek chubs inhabiting tributaries surrounding Clinton, Perry, and Tuttle Creek Lakes (n = 12 tributaries around each lake) in the Kansas River basins and control tributaries (n = 12 control sites per lake) flowing into un-impounded sections of the Kansas River (N = 36 Lake sites, N = 36 control sites). Collections will be made by backpack electro-fishing during the Spring and Summer of 2008. Up to 32 fin clips (target n =

24-32) from each species in each tributary will be preserved in 70% ethanol and stored for processing in the lab. We will attempt to sample a complete set of neighboring control tributaries for each lake; we will target tributaries that are similar in terms of catchment area and geographic separation between sampling sites.

Lab Work. — We will isolate genomic DNA by tissue digestion in cell lysis buffer (10mM Tris, 168 mM EDTA, 2% SDS, pH=8.0) and proteinase K (Invitrogen, Inc.), treatment with RNase A (Qiagen, Inc.), protein precipitation with ammonium acetate, and a final DNA precipitation using isopropanol and ethanol washes. DNA will be re-hydrated for long-term storage in TLE buffer (10mM Tris, 0.1mM EDTA, pH=8.0). We will quantify isolated genomic DNA using a ND-1000 spectrophotometer (NanoDrop Technologies, Inc.) and prepare DNA templates (~20 ng/μl) for polymerase chain reaction (PCR) amplification.

For each species, our goal is to genotype 32 individuals at 20 microsatellite loci from each of our 24 sampling sites. Multiplexed PCR conditions will be optimized on a gradient thermalcycler (BioRad, Inc.). Once PCR conditions are optimized, fragments will be detected on an ABI 3130XL Genetic Analyzer, and sized by visual examination using GENEMAPPER 4.0 (Applied Biosystems, Inc.).

Genetic Data Analysis. — We will characterize population genetic diversity by assessing Hardy-Weinberg disequilibrium, linkage disequilibrium, and population differentiation across sites and microsatellite loci for each focal species. We will use standard approaches and software packages (e.g. GENEPOP 3.4; Raymond & Rousset 1995) to generate initial descriptive statistics. For each site within a species, we will calculate basic genetic summary statistics such as observed and expected *heterozygosity*¹, *F_{IS}*¹ (Weir and Cockerham 1984), allelic richness, and proportion of *polymorphic*¹ loci to characterize patterns of genetic diversity. To understand patterns of inbreeding, we will test the hypothesis that *F_{IS}* is different from zero by bootstrapping over loci. We will assess the effects of impoundment by, for example, comparing gene diversity and allelic richness between impounded and un-impounded sites. Further analyses will be based on the results of the preliminary analyses described here.

Evaluation. — This project will be evaluated at two levels. The first level is a description of the genetic diversity of fish populations in tributaries of impounded and un-impounded waterways. Because of the success of Hudman's preliminary work with creek chub in North Carolina and Kansas, we are confident that extending these methods to incorporate a larger spatial scale by replicating over reservoirs will be successful. At the second level, patterns of isolation will be evaluated statistically, and with the aid of statistical power estimates, we will evaluate the rigor of our sampling design. Given the descriptive nature of this work, it will be successful if it yields baseline information about genetic diversity in stream fishes of Kansas. Benefits of this work arise through a greater understanding of the association between habitat alteration and population-level in headwater stream fishes.

E. SCHEDULE

<u>Component</u>	<u>Target Start:</u>	<u>Target Completion:</u>
<i>Site Selection</i>	April 2008	May 2008
- Gido and Hudman will collaborate to coordinate selection of sampling sites.		
<i>Sample Collections</i>	May 2008	Sep 2008
- Hudman will coordinate collection of fin tissue from ~32 individuals of <i>E.</i>		

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spectabile, *N. exilis*, and *S. atromaculatus* at ≥ 12 sites around Milford Lake and ≥ 12 control sites.

DNA Extraction

Jun 2008

Oct 2008

- Hudman, using facilities at the University of Kansas or Kansas State University, will coordinate extraction of DNA from field-collected samples.

PCR Amplifications

July 2008

Nov 2008

- Hudman will coordinate PCR amplification of DNA samples at 20 loci for approximately 32 individuals from each of the 72 sample locations ($N = 2304$ individuals).

Data Analysis & Report Preparation

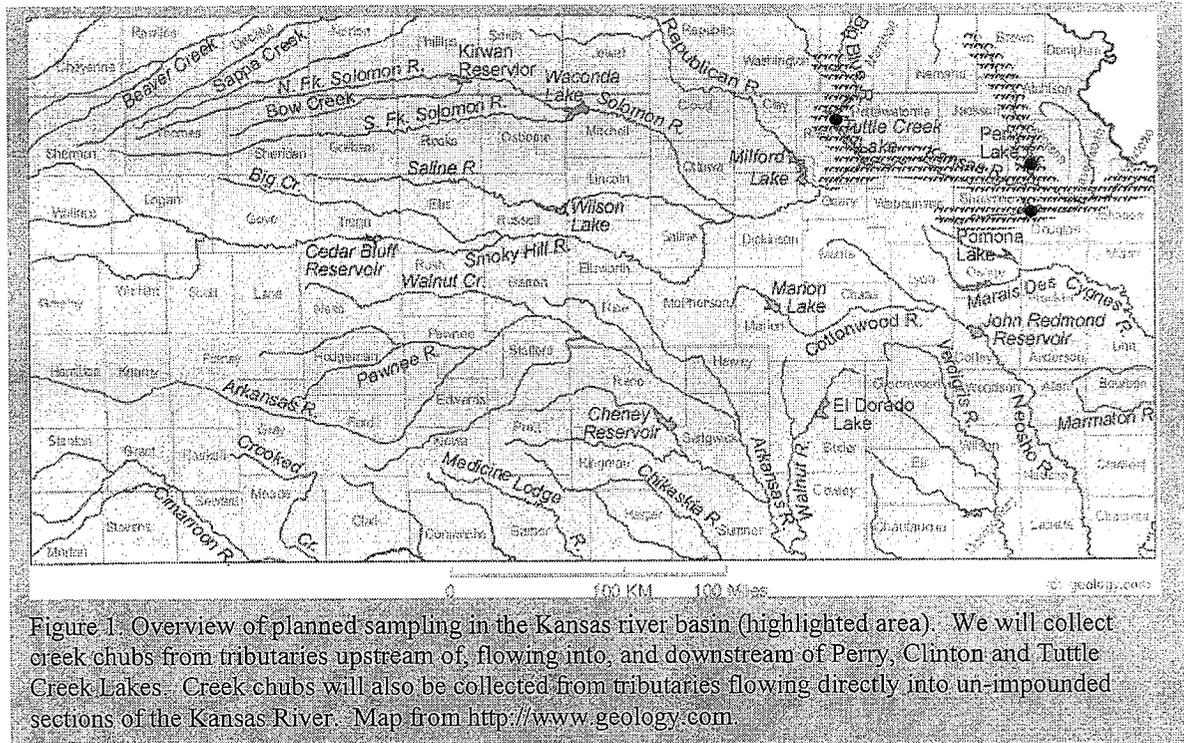
Nov 2008

Mar 2009

- Hudman will analyze the genetic data and summarize the primary findings from the genetic data.
- Hudman and Gido will collaborate to produce a progress report.

F. LOCATION

Field Work. — Gido and Hudman will collect 24-32 creek chubs from tributaries upstream of ($n = 3$), flowing into ($n = 6$), and downstream ($n = 3$) of Clinton Lake, Perry Lake, and Tuttle Creek Lake (Figure 1). Reservoir samples will be matched with an equal number of control samples collected from tributaries flowing directly into un-impounded sections of the Kansas River. Our sample sites will span two eco-regions in Kansas; the Eastern Tall-grass Prairie and the Central Mixed-grass Prairie (Fig. 2 in KCWCP; page 9).



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Counties in Kansas that will potentially be sampled

Marshal, Riley, Pottawatomie, Wabaunsee, Jackson, Shawnee, Jefferson, Atchison, Brown, Nemaha, Douglas, Leavenworth, Johnson, Wyandotte

Lab Work. — Hudman will supervise the lab work at Kansas State University including extraction of genomic DNA, curatorship and management of tissue sample and genomic DNA collections and databases, execution of multiplexed PCR reactions, curatorship, interpretation, and management of population genetic data set, analysis of population genetic data, and production of progress reports and peer-reviewed publications.

F. DELIVERABLE PRODUCTS

- (1) An evaluation of the level of genetic isolation between fish populations above three Kansas reservoirs in contrast to populations separated by similar distance but flowing into the Kansas River.

Assessment of the level of genetic isolation between fish populations above three Kansas Reservoirs to evaluate the potential for migration of stream fishes among tributary streams directly connected to a reservoir.

These assessments will be provided in progress reports and peer-reviewed publications

- (2) Detailed account of the approach used to evaluate population genetics of populations isolated by reservoirs so this information can be extended to other species or systems.
- (3) Whole specimens and tissues from fishes from collection sites will be submitted to the University of Kansas Natural History Museum for long term storage.

G. BUDGET

	MATCH	FEDERAL	TOTAL
SALARIES	\$16,706.00	\$29,193.00	\$47,101.00
SUPPLIES	\$ 0.00	\$16,500.00	\$16,500.00
TRAVEL	\$ 0.00	\$ 500.00	\$ 500.00
INDIRECT COSTS	<u>\$29,487.00</u>	<u>\$ 0.00</u>	<u>\$29,487.00</u>
Total	\$46,193.00	\$46,193.00	\$92,386.00

← 45,899.00

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The federal funds dedicated to "Salaries" will provide a portion of the salary for a postdoctoral research associate. The line item for supplies includes general field and lab supplies as well as those needed for genetic analysis. The subgrant recipient, Kansas State University, will provide the state share for the grant in part as a cash match through waived salary and indirect costs and as a direct cash contribution to the salary of the research associate.

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This proposal is completely covered by categorical exclusion 1.4B1 in 516 DM 2, Appendix 1; and/or 516 DM 6, Appendix 1.

This proposal does not have significant adverse effects on public health or safety.

This proposal does not have significant adverse effects on such natural resources and unique geographic characteristics as historic or cultural resources; park, recreation or refuge lands; wilderness areas; wild or scenic rivers; national natural landmarks; sole or principal drinking water aquifers; prime farmlands; wetlands (Executive Order 11990); floodplains (Executive Order 11988); national monuments; migratory birds (Executive Order 13186); and other ecologically significant or critical areas under Federal ownership or jurisdiction.

This proposal does not have highly controversial environmental effects or involve unresolved conflicts concerning alternative uses of available resources [NEPA Section 102(2)(E)].

This proposal does not have highly uncertain and potentially significant environmental effects or involve unique or unknown environmental risks.

This proposal does not have a precedent for future action or represent a decision in principle about future actions with potentially significant environmental effects.

This proposal does not have a direct relationship to other actions with individually insignificant but cumulatively significant environmental effects.

This proposal does not have significant adverse effects on properties listed or eligible for listing on the National Register of Historic Places as determined by either the bureau or office, the State Historic Preservation Officer, the Tribal Historic Preservation Officer, the Advisory Council on Historic Preservation, or a consulting party under 36 CFR 800.

This proposal does not have significant adverse effects on species listed, or proposed to be listed, on the List of Endangered or Threatened Species, or have significant adverse effects on designated Critical Habitat for these species.

This proposal does not have the possibility of violating a Federal law, or a State, local, or tribal law or requirement imposed for the protection of the environment.

This proposal does not have the possibility for a disproportionately high and adverse effect on low income or minority populations (Executive Order 12898).

This proposal does not have the possibility to limit access to and ceremonial use of Indian sacred sites on Federal lands by Indian religious practitioners or significantly adversely affect the physical integrity of such sacred sites (Executive Order 13007).

This proposal does not have the possibility to significantly contribute to the introduction, continued existence, or spread of noxious weeds or non-native invasive species known to occur in the area or actions that may promote the introduction, growth, or expansion of the range of such species (Federal Noxious Weed Control Act and Executive Order 13112).

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