UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

MULTI-SCALE EFFECTS OF HABITAT ALTERATION ON STREAM FISHES: FROM GENOTYPES TO COMMUNITIES

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

Degree of

DOCTOR OF PHILOSOPHY

By

NATHAN R. FRANSSEN
Norman, Oklahoma
2011

Downloaded from SHAREOK. Persistent Handle URL: https://hdl.handle.net/11244/319409

MULTI-SCALE EFFECTS OF HABITAT ALTERATION ON STREAM FISHES: FROM GENOTYPES TO COMMUNITIES

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF ZOOLOGY

BY

_____
Dr. Edith Marsh-Matthews, Chair

_____
Dr. William Matthews

_____
Dr. Aondover Tarhule

_____
Dr. Caryn Vaughn

_____
Dr. Gary Wellborn

© Copyright by NATHAN R. FRANSSEN 2011
All rights reserved.

I dedicate this dissertation to my parents, Richard and Anita Franssen.

ACKNOWLEDGEMENTS

This dissertation would not have been completed if it were not for help and support from many people. I need to thank Dr. Edie Marsh-Matthews for taking me into her lab and providing logistical and financial support. My committee members, Dr. Edie Marsh-Matthews, Dr. William Matthews, Dr. Aondover Tarhule, Dr. Caryn Vaughn, and Dr. Gary Wellborn, were not only very instrumental in shaping the research presented here, but also other projects not included in this dissertation. I also thank Dr. Keith Gido and Dr. Michael Tobler for their contributions to my final chapter.

I would also like to thank my fellow graduate students at OU for their advice, support, and willingness to discuss science, most notably: Michi Tobler, Don Shepard, Matt Dugas, Rickey Cothran, and David Donoso. Several people also provided invaluable help in the field: Dan Allen, Judith Barkstedt, Rickey Cothran, Matt Dugas, Allison Ethridge, Courtney Franssen, Amber Makowicz, Edie Marsh-Matthews, Bill Matthews, Carrie Miller-Deboer, Emily Remmel, Michi Tobler, and Rich Zamor. Robbie Stinchcomb, Carol Baylor, George Davis, George Martin, Rosemary Knapp and Anne Harris graciously provided much needed logistical support.

I also thank my sources of funding during my tenure as a graduate student: Sam Noble Oklahoma Museum of Natural History, OU Department of Zoology, Adam's scholarships, OU College of Arts and Sciences, and the Oklahoma Biological Survey.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS....iv
TABLE OF CONTENTS....v
LIST OF TABLES....vii
LIST OF FIGURES....ix
ABSTRACT....xii

CHAPTER 1 – GENETIC STRUCTURE OF A NATIVE CYPRINID IN A RESERVOIR-ALTERED STREAM NETWORK

Abstract....1
Introduction....2
Materials and Methods....5
Study system and sampling....5
Extraction of microsatellite DNA....6
Analyses....7
Results....11
Variation in microsatellites....11
Population differentiation....11
Discussion....13
Conclusions....16
Acknowledgements....17
Literature Cited....18
Tables....25
Figures....29
Appendix I....36

CHAPTER 2 – ANTHROPOGENIC HABITAT ALTERATION INDUCES RAPID MORPHOLOGICAL DIVERGENCE IN A NATIVE STREAM FISH

Abstract....40
Introduction....41
Materials and Methods....44
Field collections....44
Morphological divergence and phenotypic plasticity....44
Geometric morphometric analyses....46
Data analysis....47
Field collections....47
Geographic distance, time, and shape divergence....48
Morphological divergence and phenotypic plasticity....49
Results....51
Field collections....51
Geographic distance, time, and shape divergence....52
Genetic divergence and phenotypic plasticity....52

Discussion.....53
Field collections.....53
Geographic distance, time, and shape divergence.....55
Genetic-level and phenotypic plasticity.....56
Conservation implications.....59
Conclusions.....60
Acknowledgements.....61
Literature Cited.....62
Tables.....69
Figures.....74
Appendix A.....80
Appendix B.....82

CHAPTER 3 – ANNUAL VARIATION OF COMMUNITY BIOMASS IS LOWER IN MORE DIVERSE STREAM FISH COMMUNITIES

Abstract.....86
Introduction.....87
Materials and Methods.....91
Stream hydrology.....91
Fish communities.....92
Species richness and community/population stability.....94
Community stabilizing mechanisms.....94
Community structures.....95
Results.....96
Stream hydrology.....96
Species richness and community/population stability.....97
Community stabilizing mechanisms.....97
Community structures.....98
Discussion.....98
Community stabilizing mechanisms.....99
Effects of environmental variability and community structure.....101
Community stability and conservation.....103
Acknowledgements.....105
Literature Cited.....106
Tables.....112
Figures.....113
Appendix A.....119
Appendix B.....120
Appendix C.....121
Appendix D.....122
Appendix E.....124

LIST OF TABLES

CHAPTER 1

Table 1. Site location, site ID, date of collection, type of habitat, latitude (dd) and longitude (dd) of 12 sites where *C. lutrensis* individuals were collected to assess genetic structure of *C. lutrensis* in and near Lake Texoma, OK/TX, USA, in 2008 and 2009.....25

Table 2. Locus, repeat type, primer sequences, multiplex reaction group, GenBank accession number, and source of microsatellite locus primer. Labeled primers are indicated with (1 = 6FAM, 2 = NED, 3 = TET).....26

Table 3. Summary statistics of 12 populations of *C. lutrensis* collected in or near Lake Texoma, OK/TX between 2008 and 2009. Population names are the same as in Table 1. Mean number of individuals genotyped per locus (N), mean number of alleles per locus (N_{all}), mean allelic richness per locus (A_r), mean gene diversity per locus (G_{div}), total number of private alleles (P_{all}), mean expected (H_e) and observed (H_o) heterozygosites per locus, and the inbreeding coefficient averaged over all loci (F_{IS}).....27

Table 4. Summary data for spatial population genetic structure of *C. lutrensis* in the Lake Texoma basin (coded as in Table 1). The entries below the diagonal are pairwise F_{ST} values (bold text and * indicate significant at $p < 0.05$). Entries above the diagonal are Reynolds coancestry coefficient (above) and the number of loci with significant pairwise genotypic differentiation over 5 loci (below).....28

CHAPTER 2

Table 1. Reservoir basin system (system ID in Appendix B) and specific site (site ID in Appendix B) data of *C. lutrensis* collected for geometric morphometric analysis to assess body shape divergence in reservoirs. Lot numbers of specimens obtained from the Sam Noble Oklahoma Museum of Natural History are indicated under Oklahoma Identification (OID)....67

Table 2. Mixed-model ANCOVA results testing for effects of habitat (stream or reservoir), centroid size (allometry), reservoir basin of capture (Basin), and population nested within the basin × habitat interaction on morphological index scores from individuals collected from stream and reservoir habitats.....68

Table 3. Pearson correlation coefficients between superimposed landmarks and morphological index scores. Coefficients >0.40 are in bold and the directionality of the landmark shifts is presented for stream populations

relative to reservoir populations (i.e., movement of landmarks reflect the shifts from stream populations to reservoir populations).....69

Table 4. Results from three mixed-model ANOVAs testing for population level differences, treatment (predator or non-predator) and parental effects on PC scores of size corrected body shape variables (PC I, II, and III).....70

Table 5. Results from repeated measure ANOVAs testing the effects of treatment (predator or non-predator) and population of origin (reservoir or stream) on PC scores of size corrected shape variables (PC I, II, and III).....71

CHAPTER 3

Table 1. Regression results of independent variables not included in the final model from stepwise regression predicting community stability, population stability, and community turnover using species richness and flow variability (PC I, II, and III) as independent variables. Each estimate is the standardized regression coefficient that would result if the variable were entered into the equation at the next step.....110

LIST OF FIGURES

CHAPTER 1

Figure 1. Location of sampling sites in the study area near Lake Texoma, OK/TX. Sites are coded by site numbers as stated in Table 1. Note the Red River flows west to east and the Washita River flows north-west to south-east and both debouch into Lake Texoma.....29

Figure 2. Neighbor-joining rooted (Whiskey Creek) tree from Cavalli-Sforza chord distances among *Cyprinella lutrensis* populations in the Lake Texoma basin. Numbers at the branches indicate the percent of times (>50 %) the populations of the branch occurred among the trees over 1,000 bootstrap replicates.....30

Figure 3. STRUCTURE results showing three (k = 3) genetic clusters. Each vertical bar is an individual (grouped by population). Different colors in each bar demonstrate the proportion of times each individual was grouped in that cluster.....31

Figure 4. Relationship between geographic distance (km) and genetic distance ($F_{ST}/1-F_{ST}$) among *C. lutrensis* in the Lake Texoma basin, OK/TX, USA. The solid line denotes the best-fit linear regression model.....32

Figure 5. Results of multiple random reductions for estimating mean allelic richness. Each ascending line is a population. The vertical dotted line at 18 genotypes indicates where allelic richness was compared among populations. The three direct-tributary populations are noted: 8 = Brier Creek 6, 12 = Little Glasses Creek, 9 = Brier Creek 5. Brier Creek 5 had significantly lower allelic richness than predicted by the null distribution (see Fig. 6).....33

Figure 6. The null distribution of allelic richness using a sample size of 18 individuals. Arrows indicate observed allelic richness in Brier Creek 5 (black arrow), Little Glasses Creek (white arrow), Brier Creek 6 (dashed arrow). P-value indicates Brier Creek 5 had allelic richness significantly lower than predicted by the null distribution.....34

Figure 7. A) Mean daily surface elevation (m) above sea level of Lake Texoma from 2007 – 2010. The inset graph in A) is the mean daily surface elevation (black line) and ± standard deviation (gray vertical bars) of Lake Texoma from 1995 through 2006. B) Number of *C. lutrensis* collected from two sites in Brier Creek, a direct tributary of Lake Texoma between 2007 and 2010. Filled circles and solid lines are Brier Creek 5, and open circles and dotted lines are Brier Creek 6. Samples where tissue was collected for microsatellite analysis are indicated with (*).....35

CHAPTER 2

Figure 1. The 10 landmark locations set on *C. lutrensis* photographs for geometric morphometric analyses: 1) tip of the snout, 2) corner of the mouth, 3) center of the eye, 4) back of the skull, 5) anterior insertion of the dorsal fin, 6) insertion of the last dorsal ray on the caudal fin, 7) insertion of the last ventral ray on the caudal fin, 8) anterior insertion of the anal fin, 9) anterior insertion of the pelvic fin, and 10) anterior insertion of the pectoral fin.....72

Figure 2. Morphological variation in *C. lutrensis* between stream and reservoir habitats. Grids are thin-plate spline transformations from specimen means along the morphological index at the observed scale. Vectors below transformations reflect the direction and magnitude (magnified 3 times to ease interpretation) of each landmark movement between habitats. Vectors point in the direction landmarks moved from stream habitats to reservoir habitats. Lines are drawn between landmarks to aid visualization.....73

Figure 3. Mean±SE morphological index scores of stream populations (closed circles) and reservoir populations (open circles) from each reservoir basin. Stream sites from Lake Thunderbird and Lake Texoma are numbered according to Table 1.....74

Figure 4. Relationship between the distance of stream populations from their respective reservoirs and the difference between reservoir and stream morphological index scores ($r^2 = 0.34$, $F_{1,6} = 6.22$, $p = 0.047$). Symbols indicate the different reservoir basins: Arcadia (●), Canton (○), Fort Cobb (▼), Grand (△), Oogalah (■), Texoma (□), and Thunderbird (◆).....75

Figure 5. Morphological variation of *C. lutrensis* between stream population and reservoir population offspring, and between offspring reared with and with out a predator. Grids are thin-plate spline transformations from specimen means (observed range) between populations (above) and predator treatments (below). Vectors below transformations reflect the direction and magnitude (magnified 3 times to ease interpretation) of each landmark movement between populations and between predator and non-predator reared offspring. Vectors point in the direction landmarks move from the stream population to the reservoir population and from the non-predator reared offspring to the predator reared offspring. Lines are drawn between landmarks to aid visualization.....76

Figure 6. Mean±SE PC I and II scores of mean offspring from each parent-treatment combination (i.e., each mesocosm) of offspring spawned from a

reservoir (n = 4 parents) and stream population (n = 8 parents) and reared in predator and non-predator treatments.....77

CHAPTER 3

Figure 1. (a), Relationship between $\log_{10}$ species richness and $\log_{10}$ community stability (n = 35, $F_{1,34} = 7.38$, $R^2 = 0.18$, P = 0.01). (b), Relationship between stream hydrology (PC I) and $\log_{10}$ mean population stability (n = 35, $F_{1,34} = 23.59$, $R^2 = 0.42$, P < 0.001). Positive PC I scores associated with mean annual flow, date of minimum flow, and flow rise and fall rates; negative PC I scores associated with coefficient of variation of mean annual flow, and median number of days with no flow.....111

Figure 2. (a), Relationship between all species' mean annual biomass and their temporal variance ($s^2 = 5.95m^{1.69}$; n = 979, $F_{1,977} = 39207.05$, $r^2 = 0.98$, P < 0.001). The fitted lines are the power functions where z = 1 and z = 2 using the constant 5.95. Axes are $\log_{10}$ scaled to allow maximum separation of points. (b), Relationship between the most abundant species' mean annual biomass (i.e., species that comprised at least 20% of yearly biomass collections at each site) and their temporal variance ($s^2 = 8.70m^{1.60}$; n = 218, $F_{1,216} = 1764.98$, $r^2 = 0.87$, P < 0.001). Fitted lines are power functions where z = 1 and z = 2 using the constant 8.70. Axes are $\log_{10}$ scaled to allow maximum separation of points. (c), Relationship between species richness and sum variance of population biomass (n = 35, $F_{1,34} = 6.53$, $r^2 = 0.17$, P = 0.015).....112

Figure 3. Relationship between $\log_{10}$ species richness and mean community evenness (J) (n = 35, $F_{1,34} = 6.36$, $r^2 = 0.16$, P = 0.017).....113

Figure 4. Relationship between $\log_{10}$ species richness and sum covariance (Square root(X + 60000)); (n = 35, $F_{1,34} = 12.23$, $r^2 = 0.27$, P < 0.001). The dashed line indicates zero covariance on untransformed data.....114

Figure 5. Relationship between stream hydrology (PC I) and mean species turnover in each community (n = 35, $F_{1,34} = 20.69$, $r^2 = 0.39$, P < 0.001). Positive PC I scores associated with mean annual flow, date of minimum flow, and flow rise and fall rates; negative PC I scores associated with coefficient of variation of mean annual flow, and median number of days with no flow.....115

Figure 6. (a), Mean number of species in each family and mean number of species at each site over the sampling period. (b), Relationship between mean species richness and mean numbers of families present at each site over the sampling period. Dashed line represents a 1:1 relationship.....11

ABSTRACT

Ever increasing anthropogenic habitat alteration affects organisms at several levels of biological organization over multiple spatial and temporal scales. Understanding how evolutionary and ecological processes interact with altered habitats at these various scales will be a major challenge to conservation biologists in the coming decades, and will be crucial for predicting and alleviating deleterious effects of habitat modification.

Although the immediate effects of habitat alteration on organisms are often easily recognized, consequences that may emerge over larger spatial and temporal scales may not be as evident. At the landscape scale, populations can exhibit metapopulation structures, where, in order to remain viable, sink populations are reliant on migrants from source populations. Habitat alteration may reduce the suitability of migration corridors among source and sink populations, disrupting natural metapopulation dynamics. However, the resultant effects on populations may only be evident after a significant lag-time, when the deleterious effects of population isolation are manifested.

The same habitat alterations that can reduce migration rates also have the potential to interact with populations over longer time scales. Native species persisting in locally altered habitats are subjected to novel selective pressures; yet, the evolutionary impacts of these novel selections on resident populations are often overlooked. Local selective pressures may drive local adaptation in modified environments, altering the evolutionary trajectories of populations and potentially making individuals maladapted to more natural habitats.

Regardless of the spatial and temporal scale used to examine the effects of human-induced habitat modification on organisms, the end point is often the extinction or local extirpation of species. Loss of species from communities may influence other biotic and abiotic components of ecosystems such as community dynamics, nutrient fluxes, and ecosystem function. Thus, habitat alteration not only has the potential to affect population-level dynamics over space and time, but also to alter larger components of ecological systems through extirpation of species.

In the first chapter, I assessed the potential for habitat alteration, specifically reservoirs, to alter gene flow among reservoir fragmented stream fish populations. Using microsatellite markers, I assessed the spatial genetic structure of populations of a common minnow (Cyprinidae), red shiner (*Cyprinella lutrensis*), in and around Lake Texoma, (OK/TX), USA, and tested for lower genetic diversity in two direct tributary populations that have historically experienced population declines and recently have increased in abundance. I found populations were genetically isolated by distance with little differentiation among most populations. However, in one direct tributary population, there was substantial genetic differentiation, and genetic diversity was significantly lower compared to other populations. Gene flow appeared to be lower in reservoir habitats compared to intact stream segments, suggesting reservoirs may be reducing migration among historically connected populations.

In the second chapter, I explored how habitat alteration may result in novel selective pressures that could drive morphological divergence in resident populations. I quantified body shape variation of *C. lutrensis* in streams and

reservoirs from seven reservoir basins in Oklahoma, USA. Body shape significantly and consistently diverged in reservoirs compared to stream habitats within reservoir basins; individuals from reservoir populations were deeper-bodied and had shorter heads compared to stream populations. Stream populations were also increasingly different from reservoir populations as distance from reservoirs increased. I also assessed the relative contribution of population-level and predator-induced phenotypic plasticity on observed body shape variation by rearing offspring from a reservoir and a stream population with or without a piscivorous fish. Significant population-level differences in body shape persisted in offspring, and both populations demonstrated similar predator-induced phenotypic plasticity. My results suggest that, although components of body shape are plastic, anthropogenic habitat modification can drive trait divergence in native fish populations.

In the third chapter, we (myself, Dr. Michael Tobler, and Dr. Keith B. Gido) investigated the potential effects of biodiversity losses on community-level dynamics. Using a long-term dataset of 35 stream fish communities matched with hydrologic data, we showed that community stability (annual variation of standing biomass of fishes) was less variable in more species-rich communities and was not associated with stream hydrology. Our findings suggest anthropogenically induced extirpation of vertebrate consumers may lower community biomass stability in complex ecosystems.

CHAPTER 1

GENETIC STRUCTURE OF A NATIVE CYPRINID IN A RESERVOIR-ALTERED STREAM NETWORK

Formatted for Journal of Evolutionary Ecology

Abstract

Reservoirs modify riverine ecosystems worldwide, and often with deleterious impacts on native biota. The immediate effects of reservoirs on native fishes below dams and in impounded reaches have received considerable attention, but it is unclear how reservoirs affect fishes at larger spatial and temporal scales. Documented declines of stream fish populations in direct tributaries of reservoirs suggest the reservoir pools may be reducing gene flow among historically connected populations. Here, using genetic microsatellite markers, I assessed the spatial genetic structure of populations of a common minnow (Cyprinidae), red shiner (*Cyprinella lutrensis*), in a reservoir-fragmented stream network. I also tested the prediction that populations in two direct tributaries that have historically experienced population declines would have low genetic diversity. Results suggest most populations were isolated by distance with little differentiation among populations. In one direct tributary population, however, there was substantial genetic differentiation, and genetic diversity was significantly lower than in other populations. Gene flow was also likely lower in reservoir habitats than in intact stream habitats, suggesting reservoir habitats may be reducing gene flow among the reservoir-separated populations. These results suggest reservoirs may functionally

reduce gene flow among reservoir-fragmented stream fish populations, contributing to declines of populations in direct tributaries of reservoirs.

Introduction

Predicting consequences of habitat fragmentation on ecological systems is a major challenge for conservation biologists (Tilman et al. 1994). Riverine impoundments fragment lotic ecosystems worldwide (Nilsson and Berggren 2000), frequently with deleterious impacts on aquatic systems at multiple spatial and temporal scales (Benke 1990, Ward 1998, Pringle et al. 2000, Poff et al. 2007). Numerous immediate and adverse effects of impoundments are relatively well documented, but it is not clear how reservoirs affect biota over larger temporal and spatial scales (Fullerton et al. 2010).

The degree to which impoundments alter natural stream habitats is typically implicated as the driving factor behind changes to native fish communities in reservoir-altered systems. More often than not, conversion of lotic riverine habitats to lentic reservoirs results in fish community shifts through extirpation of riverine species (Holden and Stalnaker 1975, Martinez et al. 1994), increased abundance of native habitat generalists (Ruhr 1973, Gido and Matthews 2000, Edds et al. 2002, Herbert and Gelwick 2003), and increased densities of native and introduced piscivores (Matthews 1985, Martinez et al.1994, Edds et al. 2002). Fish communities downstream of impoundments are similarly altered by extirpations or introductions caused by changes in temperature, flow regime, sediment loads, and turbidity levels (Vanicek et al. 1970, Holden and Stalnaker 1975, Edwards 1978, Berkman and Rabeni 1987). Although the effects of reservoirs on localized fish

communities up- and downstream of impoundments are well studied, the potential for inundated stream reaches to act as barriers to migration of stream fishes has received little attention.

Fishes inhabiting small streams in undisturbed riverine systems can exhibit natural source-sink population dynamics, with coalescing streams and mainstems serving as migration corridors among populations (Fagan et al. 2002, Fagan 2002). Conversely, studies which have documented declines or extirpation of small-bodied fishes in streams that flow directly into reservoirs (i.e., direct tributaries) posit that a lack of migration through reservoir habitats (and hence reduced rescue effects) contributes to population declines (Winston et al. 1991, Luttrell et al. 1999, Lienesch et al. 2000, Herbert and Gelwick 2003, Falke and Gido 2006, Matthews and Marsh-Matthews 2007). Reservoir habitats could functionally reduce gene flow among once-connected populations, subjecting reservoir-fragmented populations to deleterious effects associated with genetic isolation and small population sizes (i.e., inbreeding depression, genetic drift; Vrijenhoek 1998). Moreover, even if extirpated populations in direct tributaries can be re-colonized, subsequent reestablished populations could suffer from similar deleterious effects (e.g., deleterious founder effects; Mayr 1942, Lande 1988).

The extent of reservoir-based population fragmentation will likely be modulated by how disparate reservoir habitats are compared to natural streams and the species-specific ecologies of stream fishes. Stream fishes with strict habitat preferences (i.e., habitat specialists), may be most susceptible to reservoir-based habitat fragmentation because of the extant reservoir pools alter habitats (Schlosser

et al. 2000, Herbert and Gelwick 2003, Skalski et al. 2008). In addition, the increased density of piscivorous fishes in reservoirs may also lower the suitability reservoir habitats to act as migration corridors by increasing predation pressure on small-bodied stream fishes (Schlosser et al. 2000). Indeed, reservoir-based population fragmentation of a stream habitat specialist (creek chub, *Semotilis atromaculatus*) revealed population isolation and reduced genetic diversity in reservoir-fragmented populations (Skalski et al. 2008). Reservoir-isolating effects may not be limited to stream habitat specialists. Many small-bodied fishes that commonly inhabit streams can demonstrate lower densities in reservoirs, with their abundances decreasing further downstream in inundated reaches (e.g., Gido et al. 2002, Matthews et al. 2004), suggesting reservoirs may be poor migration corridors for even generalist, small-bodied species.

The small-bodied habitat generalist *Cyprinella lutrensis* (Cyprinidae) experienced near, if not complete, extirpation in six of seven direct tributaries of Lake Texoma, OK/TX, U.S.A., whereas populations in the un-fragmented riverine networks upstream of the reservoir remained intact (Matthews and Marsh-Matthews 2007). However, subsequent sampling (2008-2009) in one 'extirpated' direct tributary population (Brier Creek), revealed *C. lutrensis* had reappeared and then disappeared in two reaches of Brier Creek (Marsh-Matthews et al. 2011). The declines of direct tributary populations and the failure of *C. lutrensis* to become reestablished were particularly surprising given *C. lutrensis* is hardy (Matthews and Hill 1977), widespread (Matthews 1987), and can numerically dominate fish assemblages in its native range (Marsh-Matthews and Matthews 2000). Matthews

and Marsh-Matthews (2007) and Marsh-Matthews et al. (2011) have suggested increased predator densities and local habitat changes and reduced migration rates through reservoir habitats as possible mechanisms contributing to the decline in and reestablishment failure of *C. lutrensis* populations in direct-tributaries.

Here, I assessed the genetic structure of the habitat generalist *C. lutrensis* from intact riverine and reservoir-fragmented stream populations in the Lake Texoma basin, OK/TX, USA. I tested the prediction that the reservoir is acting as a barrier to gene flow among populations, assessed whether reduction of population sizes in direct-tributaries has lowered genetic diversity in reservoir-fragmented populations, and examined the potential population-of-origin of recently collected individuals in one previously 'extirpated' direct tributary population.

Materials and methods

Study system and sampling

Denison dam impounded the Red and Washita Rivers in 1944, and formed Lake Texoma on the border of Oklahoma and Texas, USA (Fig. 1). Lake Texoma is a large (36,000 ha) and shallow (maximum depth 24 m) reservoir (Matthews et al. 2004). Because the impoundment was constructed near the confluence of the Red and Washita Rivers, the reservoir has two distinct arms, the Red River and Washita River arms (Fig. 1).

Twelve sites were sampled in or near Lake Texoma (Table 1; Fig. 1). Specimens (n = 28-30) at each site were collected by seine. Tissue was preserved in 95% ethanol in the field as whole individuals or as caudal fin clips and stored in 95% ethanol until DNA extraction. Six sites were in the un-fragmented Red River

system upstream of Lake Texoma, three sites were in the reservoir proper (Red River arm n = 2, Washita arm n =1), and three sites were in two direct tributaries of Lake Texoma (n = 2 in Brier Creek in the Red River arm, and n = 1 in Little Glasses Creek in the Washita River arm; Fig. 1). One site was sampled in both 2008 and 2009 (Brier Creek Cove; Table 1). In 2008 and 2009, sampling in Brier Creek at six different sites yielded only enough *C. lutrensis* individuals for genetic analysis only at two sites, Brier Creek station 5 in 2008 and ~4 km downstream at Brier Creek station 6 in 2009. No other direct tributaries of Lake Texoma (on the Oklahoma side) were included in genetic analyses because of the scarcity or absence of *C. lutrensis* in those habitats (see Matthews and Marsh-Matthews 2007). Although Hickory Creek (site 6 in Fig. 1) appears to be a direct tributary of Lake Texoma, here it was considered a Red River tributary because this end of Lake Texoma has silted in and during high flow the Red River flows past the Hickory Creek-Red River confluence.

Extraction of microsatellite DNA

DNA was extracted from dorsal muscle tissue from whole individuals or fin clips using a modified simple Chelex extraction (Walsh et al. 1991): approximately 100 mg tissue and 300 μl of 10% Chelex solution was incubated at 99°C for 12 min. Genetic variation was analyzed at seven different microsatellite loci (Table 2). The forward primers were end-labeled with fluorescent dyes and the polymerase chain reaction (PCR) was performed on a DNA Engine Dyad (MJ Research) thermocycler using two different multiplexed 12 μl reactions. The first reaction mixture contained 6.25 μl of Type-it Microsatellite Master Mix (Qiagen, Chatsworth, CA,

USA), 2.5 μl of 50 μmol primers for three loci (Ca6, Nme 24B6.211, and Nme25C8.208), 0.5 μl template DNA, and 4.5 μl ddH_20. The second multiplexed PCR contained 6.25 μl of Type-it Microsatellite Master Mix, 1.25 μl of 50 μmol primers of four loci (Rhca20, Rhca24, Nme 18C2.178, and Nme 24B6.191), 0.5 μl template DNA, and 3.25 μl ddH_20. Thermocycler settings for both PCRs after denaturation at 95°C for 5 min were: 30 cycles of 95°C for 30 s, 57°C annealing temperature for 1.5 min followed by extension at 72°C for 30 s. The final polymerization step was extended to 30 min at 60°C. PCR products were electrophoresed using a 3130xl Genetic Analyzer (Applied Biosystems) with the GS600 size standard, and alleles were scored using Peak Scanner v1.0 software (Applied Biosystems).

Analyses

Preliminary scoring of alleles indicated one locus (Ca6) amplified products using PCR, but also amplified three alleles in some samples and was therefore not included in further analyses. Microsatellite allele frequencies at each locus and population were tested for departures from Hardy-Weinberg equilibrium (Fisher's exact tests) and linkage-disequilibrium between loci in GENEPOP ver. 4.0.10 (Raymond and Rousset 1995). Probability tests were based on the Markov chain method with 10,000 dememorizations, 20 batches, and 5,000 iterations per batch. Genotypic frequencies of pairwise single and multilocus differences among populations (G-based tests) were tested using the permutation procedures with 10,000 dememorizations, 100 batches, and 5,000 iterations per batch in GENEPOP.

General descriptive statistics (e.g., number of samples, observed and expected heterozygosities, and F_{IS}) were calculated in GenAlEx ver. 6.4 (Peakall and Smouse 2006) or FSTAT ver. 2.9.3.2 (Goudet 1995). Genetic differentiation among populations was estimated using pairwise F_{ST} (=θ Weir and Cockerham 1984) with 10,000 permutations in ARLEQUIN ver. 3.5.1 (Excoffier and Lischer 2010) and Reynolds coancestry coefficient (Reynolds et al. 1983) calculated with default settings in FSTAT. I used Analysis of Molecular Variance (AMOVA) with 1,000 permutations in ARLEQUIN to assess the relative contribution of genetic variation attributable to within individuals, individuals within sites, and among sitess. Where multiple tests were performed; all associated p-values were adjusted for multiple comparisons using a sequential Bonferroni correction (Holm 1979, Rice 1989).

I tested for patterns of isolation-by-distance and effects of the reservoir on genetic distance among populations using a partial Mantel test with 10,000 randomizations in FSTAT. The genetic distance matrix was pairwise F_{ST} between populations (with Brier Creek Cove 2008 and 2009 samples combined) and geographic distances between populations were based on stream segment and reservoir shoreline distances. The matrix used to assess the potential influence of reservoir habitat on genetic distances was coded as 1's and 0's where 1 denoted populations separated by reservoir habitat and 0 denoted populations separated by stream habitat.

Genetic relationships among populations were estimated by Cavalli-Sforza and Edwards (1967) chord distances calculated in PHYLIP ver. 3.69 software

packages (Felsenstein 1993). Allele frequencies in each population were bootstrapped 1,000 times in SEQBOOT, and chord distances (GENDIS) were used to build rooted neighbor-joining trees (NEIGHBOR). The consensus tree (CONSENSE) and associated bootstrap values were then visualized in TreeView (Page 1996).

I used STRUCTURE ver 2.1 (Pritchard et al. 2000) to identify genetically distinct clusters (*k*) following the method presented by Evanno et al. (2005). For each value of *k* (*k* = 1 through 13, i.e., the total number of collections), 10 iterations were run using the admixture model with a burn-in period of 100,000 iterations followed by 100,000 iterations in the collection phase. Each run was performed using an ancestry model incorporating admixture, a model of correlated allele frequencies, and the prior population information as suggested by Pritchard et al. (2000).

Genetic diversity of populations was quantified using allelic richness (mean number of alleles per locus corrected for sample size) and Nei's gene diversity (the probability that, chosen at random, two copies of a gene (here, microsatellite loci) will be different alleles; Nei 1987). Only individuals that amplified all loci were included in these analyses. Gene diversity was calculated in FSTAT using default settings. Allelic richness was estimated using multiple random reductions (MRR; Leberg 2002) in R with the package standArich (http://www.ccmar.ualg.pt/maree/software.php?soft=sarich). Multiple random reduction analysis is similar to rarefaction in that it estimates allelic richness accounting for sample size. However, MRR resamples a subset of the individuals in

a population, but sequentially samples (with several iterations) 1 through *n* individuals, where *n* is the total number of individuals in the population (Leberg 2002). Here, the mean number of alleles at each locus and sampling effort (i.e., number of genotypes sampled) were quantified from 100 iterations. Allelic richness of populations was calculated at a sampling effort of 18 individuals (i.e., the smallest number of individuals that amplified all loci in one population). Differences in allelic richness and gene diversity among loci and populations were tested using General Linear Models (GLM) with allelic richness or gene diversity as the dependent variable and population and locus as fixed factors using SPSS v. 18 (SPSS, Inc., Chicago, IL, USA). The interaction term (population × locus) was not included because its addition would cause over-parameterization of each model (i.e., each data point would be represented in the model once). Moreover, a significant interaction term would indicate allelic richness varied by loci among populations, which was not of particular interest here. P-values of pairwise comparisons in significant models were adjusted using the Bonferroni correction.

Because of the low statistical power with only five loci (see results below), allelic richness of each population was also compared to a null distribution of allelic richness using all genotyped individuals. To generate the null distribution, allelic richness was quantified from a random selection of 18 individuals (with replacement) from all genotyped individuals with 1,000 iterations using the standArich package in R. The probability that the observed allelic richness of the three direct-tributary populations were a random subset of the null distribution was calculated using the Gaussian error function.

To test for recent bottlenecks in population sizes, the program BOTTLENECK (Cornuet and Luikart 1997) was implemented using 1,000 iterations with the Two Phase Model (TPM) as suggested by Cornuet and Luikart (1997) for microsatellite data. Probabilities of recent bottlenecks were assessed using the Wilcoxon sum rank test.

Results

Variation in microsatellites

A total of 385 individuals was genotyped at six microsatellite loci. There was little evidence for departures from Hardy-Weinberg equilibrium at each locus in each population, except two loci deviated significantly from equilibrium in Bills Creek (Rhca24) and Walnut Bayou Creek (Rhca20), in both cases due to an excess of homozygotes. Two locus pairs showed significant linkage disequilibrium ($p < 0.05$) in Brier Creek 5 (Nme 24B6.191 and Nme 24B6.211) and Little Glasses Creek (Nme 24B6.211 and Rhc24). Therefore, the locus shared in both cases (Nme 24B6.211) was removed from further analyses. The remaining microsatellite loci ($n = 5$) were all polymorphic (mean = 19.80 ± 12.09 SD alleles per locus, range = 4 – 36) and demonstrated variation among populations (Table 3, Appendix I). Most genetic variation was found within individuals (92 %), followed by among individuals within populations (6.56 %) and among populations (1.42 %), suggesting weak population structuring, overall.

Population differentiation

G-based exact tests showed significant differentiation among populations for some of the five microsatellite loci independently (Table 4) and combined (all $p <$

0.05). Calculation of F_{ST} values indicated some differentiation among populations across loci (mean $F_{ST} = 0.015 \pm 0.018$ SD, range = -0.016 – 0.078; Table 4). Significant pairwise F_{ST} values were found primarily between population pairs that were separated by a great distance (e.g., Whiskey Creek, Red River and the two populations in the Washita arm of Lake Texoma) and 5 of the 11 pairwise comparisons with Brier Creek 5 (Table 4). Reynolds coancestry coefficients between population pairs also demonstrated a similar pattern (Table 4). Cavalli-Sforza chord distances indicated weak population structuring among most populations, however, Brier Creek 5 and the two populations from the Washita River arm of Lake Texoma (Glasses Cove and Little Glasses Creek) demonstrated the most differentiation supported by 70.1 % and 90.2 % of the simulations, respectively (Fig. 2).

The program STRUCTURE also supported this differentiation among populations and suggested three genetic clusters coinciding with Brier Creek 5 and the two populations in the Washita arm (Glasses Cove and Little Glasses Creek) and the rest of the populations (Fig. 3).

Sites were significantly isolated by distance (Partial Mantel test, partial $r = 0.474$, $p < 0.001$; Fig. 4), but genetic distance did not correlate with reservoir habitats (partial $r = 0.123$, $p = 0.323$).

Allelic richness was variable among populations (Fig. 5) and the GLM demonstrated significant differences in allelic richness among loci ($F_{1,4} = 121.5$, $p < 0.001$) and populations ($F_{1,12} = 2.5$, $p = 0.014$). Bonferroni corrected pairwise comparisons revealed Brier Creek 5 had significantly lower allelic richness

compared to Brier Creek 6 and Hickory Creek. Conversely, gene diversity significantly differed among loci ($F_{1,4}$ = 135.2, $p < 0.0001$) but not among populations ($F_{1,12}$ = 1.4, $p = 0.194$). In addition, comparing observed allelic richness of direct-tributary populations to the null distribution revealed Brier Creek 5 ($p < 0.001$) had significantly lower allelic richness than would be predicted by chance but not Brier Creek 6 ($p = 0.35$) or Little Glasses Creek ($p = 0.81$; Figure 6).

There was no evidence for recent bottlenecks in population size among all populations (all $p > 0.05$) based on the results from BOTTLENECK.

Discussion

Genetic structuring of populations was primarily a function of isolation-by-distance and most differentiation was found between the two arms of the reservoir. However, one population in one of the two direct tributaries showed increased genetic differentiation less explained by distance. Mean allelic richness was also lower (compared to a null distribution based on all populations) in one but not the other direct tributary.

Although many studies have assessed the effects of physical barriers (e.g., dams, waterfalls, weirs) on the genetic structure of fish populations (National Resource Council 1996), few have investigated the potential for reservoir habitats to act as barriers to gene flow (Skalski et al. 2008). Reservoirs can reduce gene flow of habitat specialists in reservoir-fragmented populations, where fragmented populations experience isolating effects (i.e., many fixed alleles, low genetic diversity) compared to intact riverine populations (Skalski et al. 2008). Here, *C. lutrensis* (a habitat generalist) occurred in reservoir habitats, albeit in lower densities

compared to nearby streams (Gido et al. 2002, Matthews et al. 2004). However, significant divergence among populations primarily occurred between the two arms of the reservoir and in only one (and at only one site: Brier Creek 5) of the two direct tributaries of Lake Texoma.

The significant isolation-by-distance correlation suggests a spatial structuring of *C. lutrensis* populations due to geographic distances among populations. However, the two populations from the Washita arm of Lake Texoma (i.e., Glasses Cove and the direct tributary, Little Glasses Creek) likely drove much of this relationship. Removal of these two populations did result in a non-significant isolation-by-distance relationship among the remaining populations (Mantel test, $r = 0.27$, $p = 0.118$). The clustering of the two Washita arm populations based on Cavalli-Sforza chord distances and the program STRUCTURE, also suggests there is likely less gene flow between the two arms of the reservoir compared to gene flow within the Red River arm. This is not a surprising result given the upstream reaches of reservoirs tend to resemble large rivers (Wetzel 1990), and densities of small-bodied stream fishes can decrease further downstream in reservoirs (Gido et al. 2002). Thus, low numbers of potential migrants in downstream reaches of the reservoir, coupled with reservoir habitats being potentially poor corridors for small-bodied fish migration (Schlosser et al. 2000), may explain the genetic disparity between the two arms of the reservoir.

There was little evidence that gene flow was restricted between the direct tributary population and the reservoir in the Washita arm of Lake Texoma, as both populations were genetically similar. However, populations in the direct tributary

(Brier Creek) in the Red River arm of the reservoir revealed genetic differentiation compared to the nearest reservoir populations, but only one of the two sites within Brier Creek demonstrated this differentiation. The furthest upstream site in Brier Creek (Brier Creek 5) was less similar compared to all other populations, including fish collected 4.5 km downstream at Brier Creek 6 and 10.0 km at Brier Creek Cove, suggesting some genetic isolation. This genetic disparity is unlikely to be a function of isolation-by-distance due to the close proximity of other populations, but the low *C. lutrensis* densities in Brier Creek (Matthews and Marsh-Matthews 2007) coupled with recent lake level fluctuations may explain this longitudinal genetic structure.

Fish collected from Brier Creek 5 in 2008 were potentially from a relict population that has not exchanged genes with the reservoir for some time, whereas fish from Brier Creek 6 in 2009 were likely recent immigrants from the reservoir proper. The low allelic richness observed in Brier Creek 5 suggests this population has potentially experienced genetic drift or founder effects, and the non-significant differences in Nei's gene diversity among populations was potentially due to our low statistical power with using only 5 loci. Conversely, Brier Creek 6 had the second highest observed allelic richness of all populations, suggesting these individuals have not suffered from genetic isolation. Rather, these individuals likely emigrated from the reservoir proper during a flood in May and June of 2009 (Fig. 7), and were recent arrivals to Brier Creek. Increases in reservoir pool elevation can inundate lower reaches of direct tributaries in Lake Texoma (Matthews and Marsh-Matthews 2007), potentially removing barriers to migration during periods of high

water (e.g., Franssen et al. 2006). The sudden appearance of individuals at Brier Creek 6 following a flood (Fig. 7), and their genetic similarity to the reservoir proper populations, suggests these individuals were recent emigrants from the reservoir.

Given that there is no obvious physical barrier between Brier Creek 5 and the reservoir proper; it is unclear why this population was genetically disparate or why *C. lutrensis* has appeared in small numbers and disappeared several times at both Brier Creek sites over the last few years (Marsh-Matthews et al. 2011). Matthews and Marsh-Matthews (2007) noted the increased incidence of deep pools with high predator densities (*Micropterus spp, Lepomis spp*.) in the lower reaches of direct tributaries of Lake Texoma. If lower reaches of direct tributaries are predator dense zones, this may impede the migration of individuals up or downstream, and this phenomenon could explain both the genetic isolation of Brier Creek 5 and the appearance of *C. lutrensis* individuals coinciding with a flood at Brier Creek 6. While much of this is speculative, future mapping of the longitudinal densities of piscivorous fishes in direct tributaries of Lake Texoma may shed light on the potential for piscivorous fishes to affect the longitudinal distribution of fishes in reservoir-fragmented streams.

Conclusions

The genetic structures of populations separated by large distances of reservoir habitat suggest reservoir habitats may functionally reduce gene flow among small-bodied fish populations in reservoir-fragmented stream systems. However, the isolating effect of the reservoir on populations inhabiting direct tributaries was

equivocal: only one of the two direct tributary populations demonstrated potential genetic isolation and reduced genetic diversity. Investigations of stream habitat specialists in reservoir-fragmented systems may prove to be better candidates to assess fragmentation by reservoirs; however, our results suggest reservoir habitats may fragment populations of even the most generalist and hardiest of species. Continued investigations of the effects of reservoirs on native biota at larger spatial and temporal scales will likely prove useful for conservation managers of reservoir-fragmented ecosystems.

Acknowledgements

Thanks to J. Barkstedt, W. Matthews, E. Marsh-Matthews, and R. Zamor for help with specimen collection, M. Schwemm, R. Broughton, and G. Wellborn for help with genetic analyses, M. Dugas and M. Tobler for statistical consultation. The ZEEB journal club and E. Marsh-Matthews made comments on earlier drafts of the manuscript. This manuscript is in partial fulfillment of a PhD degree from the Department of Zoology, University of Oklahoma (NRF). This study was approved by the University of Oklahoma IACUC (R08-004).

Literature cited

Benke, A.C. 1990. A perspective on America's vanishing streams. Journal of North American Benthological Society 9:77-88.

Berkman, H.E. and C.F. Rabeni. 1987. Effect of siltation on stream fish communities. Environmental Biology of Fishes 18:285-294.

Burridge, C.P. and J.R. Gold. 2003. Conservation genetic studies of the endangered Cape Fear Shiner, *Notropis mekistocholas* (Teleostei: Cyprinidae). Conservation Genetics 4:219-225.

Cavalli-Sforza, L.L. and A.W.F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. American Journal of Human Genetics 19:233-257.

Cornuet J.M. and G. Luikart. 1997. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001-2014.

Cross, F.B., and J.T. Collins. 1995. Fishes in Kansas. University of Kansas Museum of Natural History, Lawrence, p. 316.

Dimsoski, P., G.P. Toth GP and M.J. Bagley. 2000. Microsatellite characterization in central stoneroller *Campostoma anomalum* (Pisces: Cyprinidae). Molecular Ecology 9:2187-2189.

Edds, D.R., W.J. Matthews, and F.P. Gelwick. 2002. Resource use by large catfishes in a reservoir: is there evidence for interactive segregation and innate differences? Journal of Fish Biology 60:739-750.

Edwards, R.J. 1978. The effect of hypolimnion reservoir releases on fish

distribution and species diversity. Transactions of the American Fisheries Society 107:71-77.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611-2620.

Excoffier, L. and H.E. L. Lischer. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564-567.

Fagan, W.F., P.J. Unmack, C. Burgess, and W.L. Minckley. 2002. Rarity, fragmentation, and extinction risk in desert fishes. Ecology 83:3250-3256.

Fagan, W.F. 2002. Connectivity, fragmentation, and extinction risk in dendritic metapopulations. Ecology 83:3243-3249.

Falke, J.A. and K.B. Gido. 2006. Effects of reservoir connectivity on stream fish assemblages in the Great Plains. Canadian Journal of Fisheries and Aquatic Sciences 63:480-493.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author.

Franssen, N.R., K.B. Gido, T. R. Strakosh, K. N. Bertrand, C. M. Franssen, C. P. Paukert, K. L. Pitts, C. S. Guy, J. A. Tripe, and S. J. Shrank. 2006. Effects of floods on fish assemblages in an intermittent prairie stream. Freshwater Biology 51:2072-2086.

Fullerton, A.H., K.M. Burnett, E.A. Steel, R.L. Flitcroft, G.R. Pess, B.E. Fiest, C.E.

Torgersen, D.J. Miller, and B.L. Sanderson. 2010. Hydrological connectivity for riverine fish: measurement challenges and research opportunities. Freshwater Biology 55:2215-2237.

Gido, K.B. and W.J. Matthews. 2000. Dynamics of the offshore fish assemblage in a southwestern reservoir (Lake Texoma, Oklahoma–Texas). Copeia 2000: 917-930.

Gido, K.B., C.W. Hargrave, W.J. Matthews, G.D. Schnell, D.W. Pogue and G. Sewell. 2002. Structure of littoral-zone fish communities in relation to habitat, physical, and chemical gradients in a southern reservoir. Environmental Biology of Fishes 63:253-263.

Girard, P. and G. Angers. 2006. Characterization of microsatellite loci in longnose dace (*Rhinichthys cataractae*) and interspecific amplification in five other Leusciscinae species. Molecular Ecology Notes 6:69-71.

Goudet J. 1995. fstat version 1.2: a computer program to calculate *F*-statistics. Journal of Heredity 6:485-486.

Herbert, M.E. and F.P. Gelwick. 2003. Spatial variation of headwater fish assemblages explained by hydrologic variability and upstream effects of impoundment. Copeia 2003:273-284.

Holden, P.B. and C.B. Stalnaker. 1975. Distribution and abundance of mainstream fishes of the middle and upper Colorado River Basins, 1967–1973. Transactions of the American Fisheries Society 100: 217-231.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics 6:65-70.

Lande, R. 1988. Genetics and demography in biological conservation. Science 241:1455-1460.

Leberg, P.L. 2002. Estimating allelic richness: effects of sample size and bottlenecks. Molecular Ecology 11:2445-2449.

Lienesch, P.W., W.I. Lutterschmidt, and J.F. Schaefer. 2000. Seasonal and long-term changes in the fish assemblage of a small stream isolated by a reservoir. Southwestern Naturalist 45:274-288.

Luttrell, G.R., A.A. Echelle, W.L. Fisher, and D.J. Eisenhour. 1999. Declining status of two species of the *Macrhybopsis aestivalis* complex (Teleostei: Cyprinidae) in the Arkansas River Basin and related effects of reservoirs as barriers to dispersal. Copeia 1999:981-989.

Marsh-Matthews, E., W.J. Matthews, and N.R. Franssen. 2011. Can a highly invasive species re-invade its native community? The paradox of the red shiner. Biological Invasions DOI: 10.1007/s10530-011-9973-2.

Martinez, P.J., T.E. Chart, M.A. Trammell, J.G. Wullschlegef, and E.P. Bergersen. 1994. Fish species composition before and after construction of a main stem reservoir on the White River, Colorado. Environmental Biology of Fishes 40:227-239.

Marsh-Matthews, E.**,** and W.J. Matthews. 2000. Spatial variation in relative abundance of a widespread, numerically-dominant fish species and its effect on fish assemblage structure. Oecologia 125:283-292.

Matthews, W.J. 1985. Summer mortality of striped bass in reservoirs of the United States. Transactions of the American Fisheries Society 114:62-66.

Matthews, W.J., Hill L.G. 1977. Tolerance of the red shiner, *Notropis lutrensis* (Cyprinidae) to environmental parameters. Southwestern Naturalist 22:89-99.

Matthews, W.J. 1987. Geographic variation of *Cyprinella lutrensis* (Pisces: Cyprinidae) in the United States, with notes on *Cyprinella lepida*. Copeia 1987:616-637.

Matthews, W.J., K.B. Gido, and F.P. Gelwick. 2004. Fish assemblages of reservoirs, illustrated by Lake Texoma (Oklahoma-Texas, USA) as a representative system. Lake and Reservoir Management 20:219-239.

Matthews, W.J. and E. Marsh-Mathews. 2007. Extirpation of red shiner in direct tributaries of lake Texoma (Oklahoma-Texas): a cautionary case history from a fragmented river-reservoir system. Transactions of the American Fisheries Society 136:1041-1062.

Mayr, E. 1942. Systematics and the Origin of Species. Columbia University Press, New York.

National Resource Council. 1996. Upstream: salmon and society in the Pacific Northwest. Committee on Protection and Management of Pacific Northwest Anadromous Salmonids, Portland, OR and Seattle, WA.

Nei, M. 1987. Molecular evolutionary genetics. New York: Columbia University Press. 512 p.

Nilsson, C. and K. Berggren. 2000. Alterations of riparian ecosystems caused by river regulation. Bioscience 50:783-792.

Page, R.D.M. 1996. TreeView: an application to display phylogenetic trees on

personal computers. Computer Applications in the Biosciences 12:357-358.

Peakall, R., and P.E. Smouse. 2006. GENALEX 6: Genetic Analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288-295.

Poff, N.L., J.D. Olden, D.M. Merritt and D.M. Pepin. 2007. Homogenization of regional river dynamics by dams and global biodiversity implications. Proceedings of the National Academy of Science 104:5732-5737.

Pringle, C.M., M.C. Freeman and B.J. Freeman. 2000. Regional effects of hydrologic alterations on riverine macrobiota in the New World: Tropical-temperate comparisons. Bioscience 9:807-823.

Pritchard, J.K., M. Stephens, and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Raymond, M. and F. Rousset. 1995. GENEPOP (Version1.2): population genetics software for exact tests and eucumenicism. Journal of Heredity 86:248-249.

Reynolds, J., B.S. Weir, and C.C. Cockerham. 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. Genetics 105:767-779.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Ruhr, C.E. 1957. Effects of stream impoundment in Tennessee on the fish populations of tributary streams. Transactions of the American Fisheries Society 86:154-157.

Schlosser, I.J., J.D. Johnson, W.I. Knotek, and M. Lapinska. 2000. Climate variability and size-structured interactions among juvenile fish along a lake-stream gradient. Ecology 81:1046-1057.

Skalksi, G.T, J.B. Landis, M.J. Grose and S.P. Hudman. 2008. Genetic structure of creek chub, a headwater minnow, in an impounded river system. Transactions of the American Fisheries Society 137:962-975.

Tilman, D., May, R.M., Lehman, C.L. and Nowak, M.A. 1994. Habitat destruction and the extinction debt. Nature 371:65-66.

Vanicek, C.D., R.H. Kramer, and D.R. Franklin. 1970. Distribution of Green River fishes in Utah and Colorado following closure of Flaming Gorge Dam. The Southwestern Naturalist 15:297-315.

Vrijenhoek, R.C. 1998. Conservation genetics of freshwater fish. Journal of Fish Biology 53:394-412.

Walsh, P.S., D.A. Metzger, and R. Higuchi. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506-13.

Ward, J.V. 1998. Riverine landscapes: Biodiversity patterns, disturbance regimes, and aquatic conservation. Biological Conservation 3:269-278.

Weir, B.S. and C.C. Cockerham. 1984. Estimating *F*-Statistics for the analysis of population structure. Evolution 38:1358-1370.

Wetzel, R.G. 1990. Reservoir ecosystems: conclusions and speculations. Pages 227–238 in K. W. Thornton, B. L. Kimmel, and F. E. Payne, editors. Reservoir limnology: ecological perspectives. Wiley, New York.

Winston, M.R., C.M. Taylor, and J. Pigg. 1991. Upstream extirpation of four minnow species due to damming of a prairie stream. Transactions of the American Fisheries Society 120:98-105.

Table 1. Site location, site ID, date of collection, type of habitat, latitude (dd) and longitude (dd) of 12 sites where *C. lutrensis* individuals were collected to assess genetic structure of *C. lutrensis* in and near Lake Texoma, OK/TX, USA, in 2008 and 2009.

Site	ID	Date	Habitat	River Arm	Latitude	Longitude
Whiskey Creek	1	20-May-08	River tributary	Red	34.1529	-98.1565
Red River	2	20-May-08	River	Red	33.9377	-97.7320
Coffee Pot Creek	3	20-May-08	River tributary	Red	33.9403	-97.4518
Walnut Bayou Creek	4	10-Aug-09	River tributary	Red	33.9166	-97.2824
Bills Creek	5	10-Aug-09	River tributary	Red	33.8970	-97.1577
Hickory Creek	6	19-May-08	River tributary	Red	34.0377	-97.1434
Brier Creek Cove	7	23-Jun-08	Reservoir	Red	33.9248	-96.8654
	7	30-Jul-09				
Brier Creek Station 6	8	23-Jun-09	Reservoir tributary	Red	33.9539	-96.8422
Brier Creek Station 5	9	4-Jun-08	Reservoir tributary	Red	33.9990	-96.8286
Biostation Shore	10	12-Jun-08	Reservoir	Red	33.8794	-96.8021
Glasses Creek Cove	11	12-Aug-09	Reservoir	Washita	34.0281	-96.6611
Little Glasses Creek	12	2-Aug-09	Reservoir tributary	Washita	34.0256	-96.6983

Table 2. Locus, repeat type, primer sequences, multiplex reaction group, GenBank accession number, and source of microsatellite locus primer. Labeled primers are indicated with (1 = 6FAM, 2 = NED, 3 = TET).

Locus	Repeat type	Primer sequences (5'-3')	Multiplex reaction	Accession number	Source
Ca6	$(CA)_{14}CG(GA)_6$	F:CAGGTCTTGCCCACGTCTGAG[1]	1	AF277578	Dimsoski et al. 2000
		R:CACCTGTGGAACCGGCTTGA			
Rhca20	$(GA)_{17}$	F:CTACATCTGCAAGAAAGGC[1]	2	DQ106915	Girard and Anders 2006
		R:CAGTGAGGTATAAAGCAAGG			
Rhca24	$(GA)_{27}$	F:GTGGTGTTAGCAGAAACCCG[1]	2	DQ106917	Girard and Anders 2006
		R:CTGCTGTTTAATATGTCAC			
Nme 18C2.178	$(TG)_{15}$	F:TCAAACCCTACAGACAGCAAGACT[2]	2	AF532582	Burridge and Gold 2003
		R:TTTCTCAGGGGCTCCAACAAG			
Nme 24B6.191	$(AG)_6TGAC(AG)_6$	F:TTGCAGGGGAAACATACC[3]	2	AF532583	Burridge and Gold 2003
		R:GAATGGGCCGTTACTCTC			
Nme 24B6.211	$(CA)_{10}$	F:CGGACAGGTGTGATGGAATG[3]	1	AF532583	Burridge and Gold 2003
		R:ACCCTGTGGCTGTGAACGA			
Nme 25C8.208	$(TG)_9$	F:AAAAAGGCCTCCCAGTGC[2]	1	AF532584	Burridge and Gold 2003
		R:AATTATATGTCGGTGACCAGATTG			

Table 3. Summary statistics of 12 populations of *C. lutrensis* collected in or near Lake Texoma, OK/TX between 2008 and 2009. Population names are the same as Table 1. Mean number of individuals genotyped per locus (N), mean number of alleles per locus (N_{all}), mean allelic richness per locus (A_r), mean gene diversity per locus (G_{div}), total number of private alleles (P_{all}), mean expected (H_e) and observed (H_o) heterozygosites per locus, and the inbreeding coefficient averaged over all loci (F_{IS}).

Population	ID	N	N_{all}	A_r	G_{div}	P_{all}	H_e	H_o	F_{IS}
Whiskey Creek	1	28.8	10.60	8.84	0.77	1	0.77	0.75	0.02
Red River	2	27.6	11.20	9.69	0.79	3	0.79	0.74	0.06
Coffee Pot Creek	3	29.6	12.20	10.24	0.80	2	0.80	0.74	0.07
Walnut Bayou Creek	4	29.4	11.00	9.27	0.77	1	0.77	0.66	0.14
Bills Creek	5	27.8	9.80	8.58	0.77	0	0.76	0.60	0.22
Hickory Creek	6	28.8	13.20	10.94	0.77	5	0.77	0.73	0.06
Brier Cove 2008	7	29.0	11.20	9.75	0.75	1	0.75	0.66	0.12
Brier Cove 2009	7	25.6	10.80	9.59	0.76	0	0.76	0.59	0.22
Brier Creek 6	8	25.4	11.20	10.60	0.81	2	0.81	0.70	0.14
Brier Creek 5	9	28.4	8.00	6.85	0.73	0	0.73	0.63	0.14
Biostation Shore	10	27.4	11.00	9.51	0.80	1	0.80	0.67	0.16
Glasses Creek Cove	11	27.4	9.40	7.94	0.71	0	0.71	0.64	0.10
Little Glasses Creek	12	29.6	11.40	9.80	0.78	1	0.78	0.71	0.09

Table 4. Summary data for spatial population genetic structure of *C. lutrensis* in the Lake Texoma basin (coded as in Table 1). The entries below the diagonal are pairwise F_{ST} values (bold text and * indicate significant at sequential Bonferroni correction $p < 0.05$). Entries above the diagonal are Reynolds coancestry coefficient (above) and the number of loci with significant pairwise genotypic differentiation over 5 loci (below).

Population	1	2	3	4	5	6	7 (2008)	7 (2009)	8	9	10	11	12
1		0.0060	0.0120	0.0110	0.0090	0.0391	0.0250	0.0150	0.0080	0.0574	0.0060	0.0811	0.0701
2	0.006		0.0010	0.0010	0.0050	0.0210	0.0100	0.0170	0.0000	0.0410	0.0000	0.0431	0.0371
3	0.012	0.001		0.0030	0.0000	0.0060	0.0090	0.0030	0.0000	0.0352	0.0000	0.0160	0.0170
4	0.011	0.001	0.003		0.0000	0.0090	0.0000	0.0050	0.0000	0.0351	0.0000	0.0280	0.0320
5	0.009	0.005	-0.008	0.000		0.0050	0.0020	0.0010	0.0000	0.0321	0.0020	0.0260	0.0210
6	**0.038***	0.020	0.006	0.009	0.005		0.0000	0.0000	0.0000	0.0311	0.0050	0.0080	0.0110
7 (2008)	0.025	0.010	0.009	-0.001	0.002	-0.003		0.0010	0.0020	0.0401	0.0090	0.0210	0.0270
7 (2009)	0.015	0.017	0.003	0.005	0.001	-0.001	0.001		0.0040	0.0350	0.0060	0.0300	0.0290
8	0.008	0.000	-0.016	-0.002	-0.013	-0.006	0.002	0.004		0.0272	0.0000	0.0190	0.0020
9	**0.055***	**0.040***	**0.034***	0.034	0.032	0.030	**0.039***	0.034	0.026		0.0231	0.0501	0.0341
10	0.006	-0.006	-0.008	-0.001	0.002	0.005	0.009	0.006	-0.016	0.023		0.0350	0.0201
11	**0.078***	**0.042***	0.016	0.027	0.026	0.008	0.020	0.029	0.019	0.048*	0.035*		0.0000
12	**0.067***	**0.037***	0.016	**0.031***	0.021	0.011	0.027	0.028	0.002	0.033	0.020	-0.001	

Figure 1. Locations of sampling sites in the study area near Lake Texoma, OK/TX. Sites are coded by site numbers as stated in Table 1. Note the Red River flows west to east and the Washita River flows north-west to south-east and both debouch into Lake Texoma.

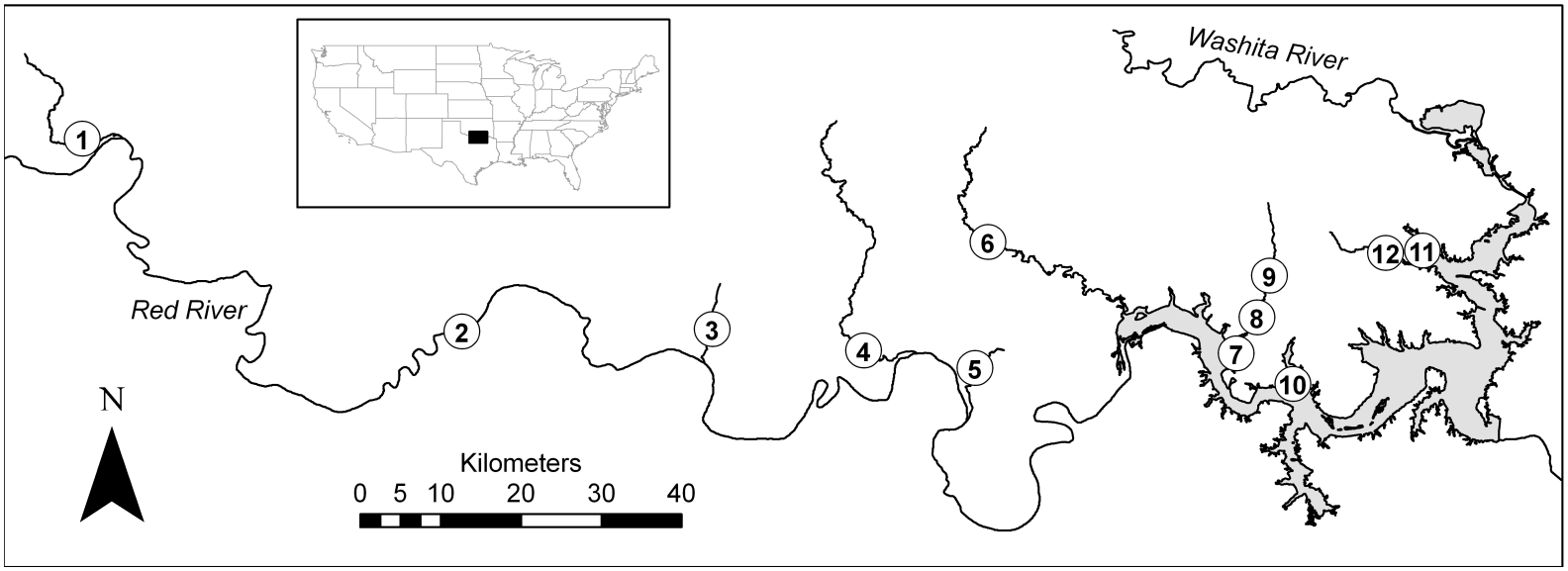


Figure 2. Neighbor-joining rooted (Whiskey Creek) tree from Cavalli-Sforza chord distances among *Cyprinella lutrensis* populations in the Lake Texoma basin. Numbers at the branches indicate the percent of times (>50 %) the populations of the branch occurred among the trees over 1,000 bootstrap replicates.

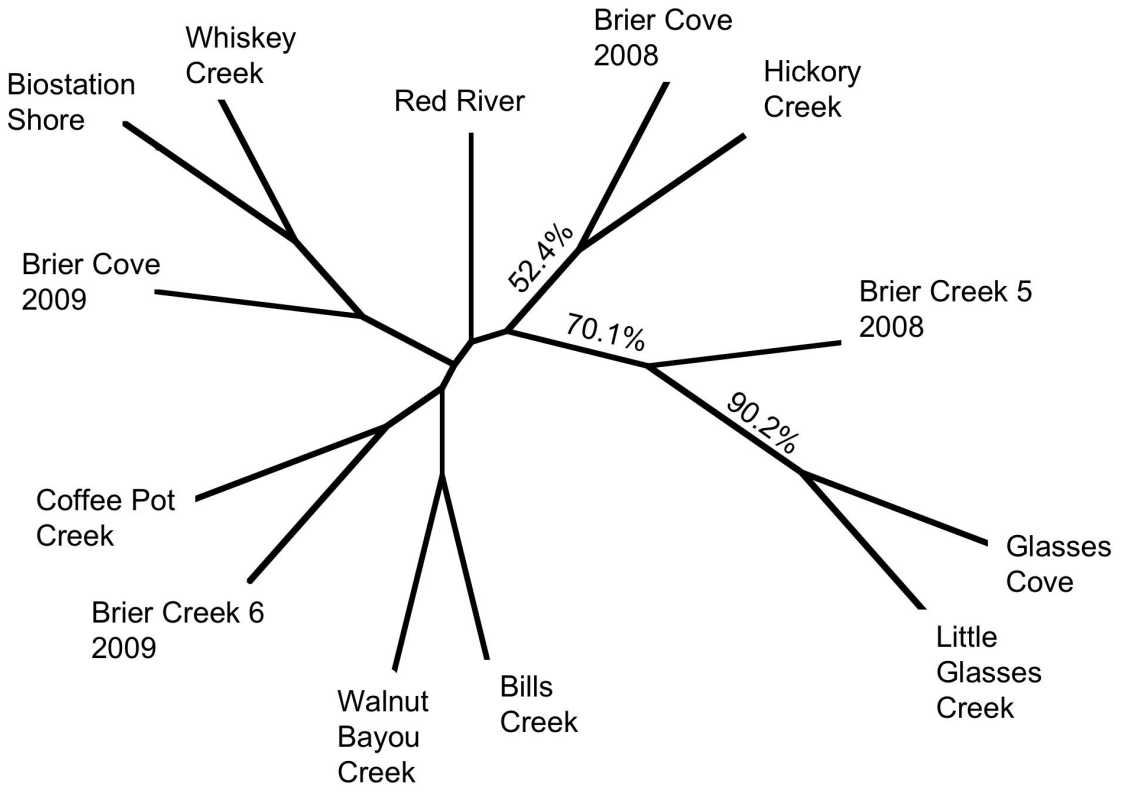


Figure 3. STRUCTURE results showing three (k = 3) genetic clusters. Each vertical bar is an individual (grouped by population). Different colors in each bar demonstrate the proportion of times each individual was grouped in that cluster.

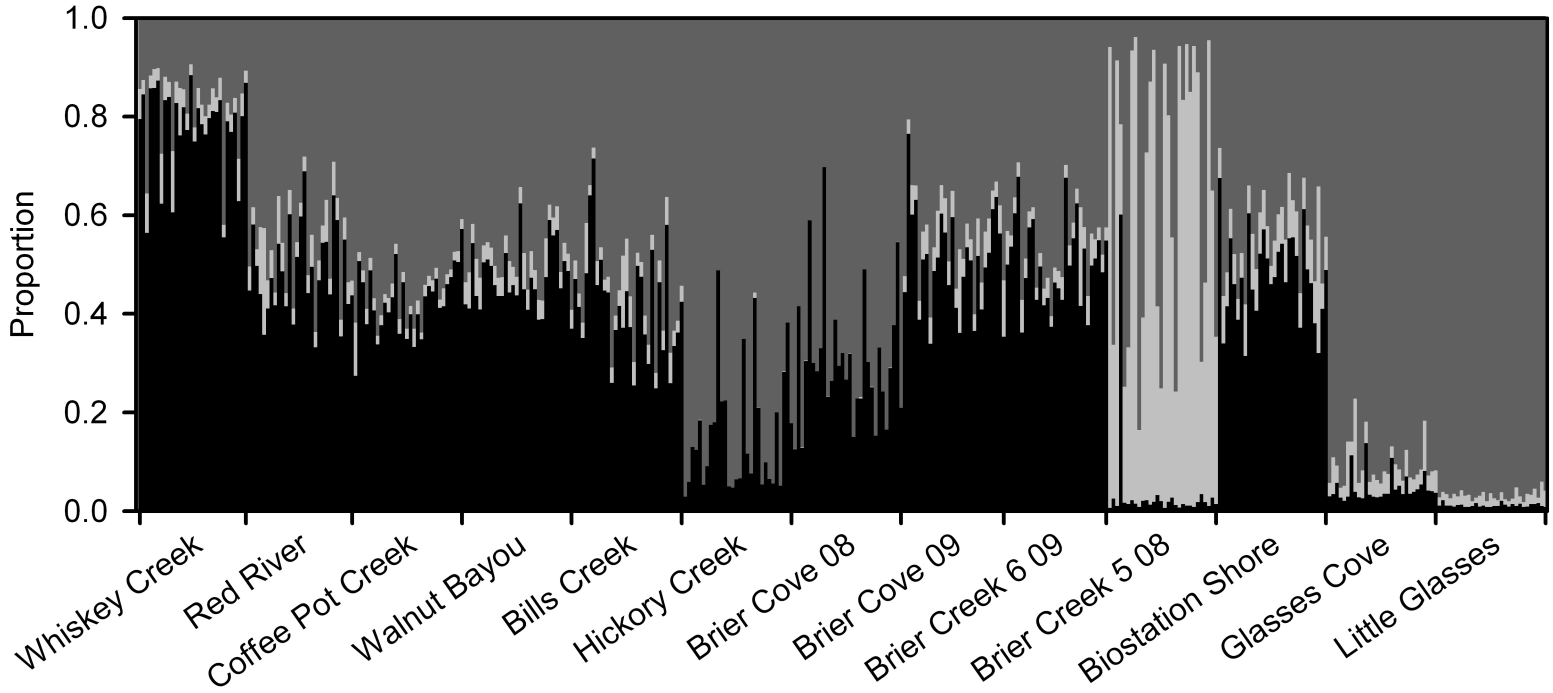


Figure 4. Relationship between pairwise geographic distance (km) and genetic distance ($F_{ST}/1-F_{ST}$) among *C. lutrensis* in the Lake Texoma basin, OK/TX, USA. The solid line denotes the best-fit linear regression model.

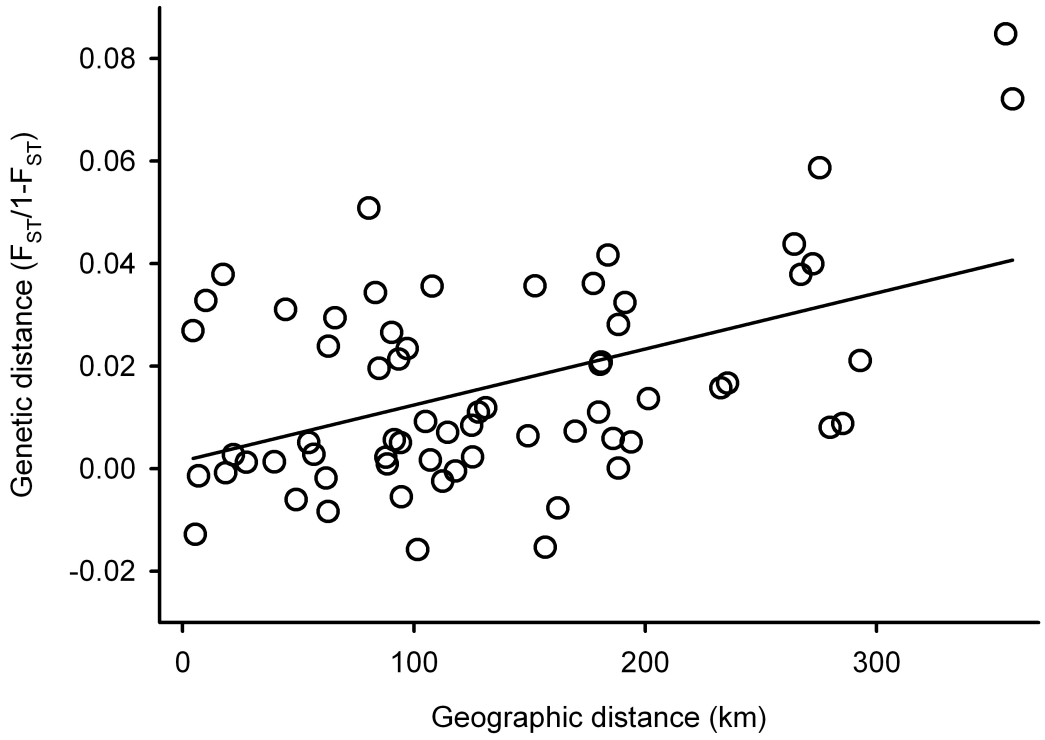


Figure 5. Results of multiple random reductions for estimating mean allelic richness. Each ascending line is a population. The vertical dotted line at 18 genotypes indicates where allelic richness was compared among populations. The three direct-tributary populations are noted: 8 = Brier Creek 6, 12 = Little Glasses Creek, 9 = Brier Creek 5. Brier Creek 5 had significantly lower allelic richness than predicted by the null distribution (see Fig. 6).

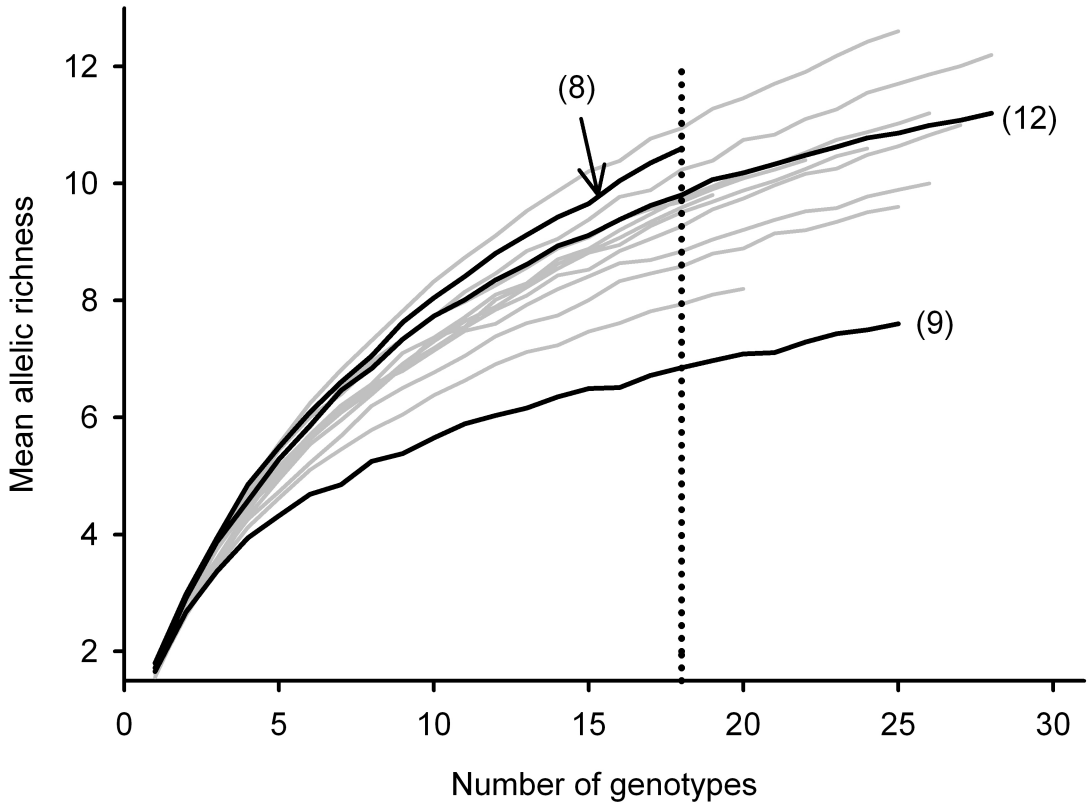


Figure 6. The null distribution of allelic richness using a sample size of 18 individuals. Arrows indicate observed allelic richness in Brier Creek 5 (black arrow), Little Glasses Creek (white arrow), Brier Creek 6 (dashed arrow). P-value indicates Brier Creek 5 had allelic richness significantly lower than predicted by the null distribution.

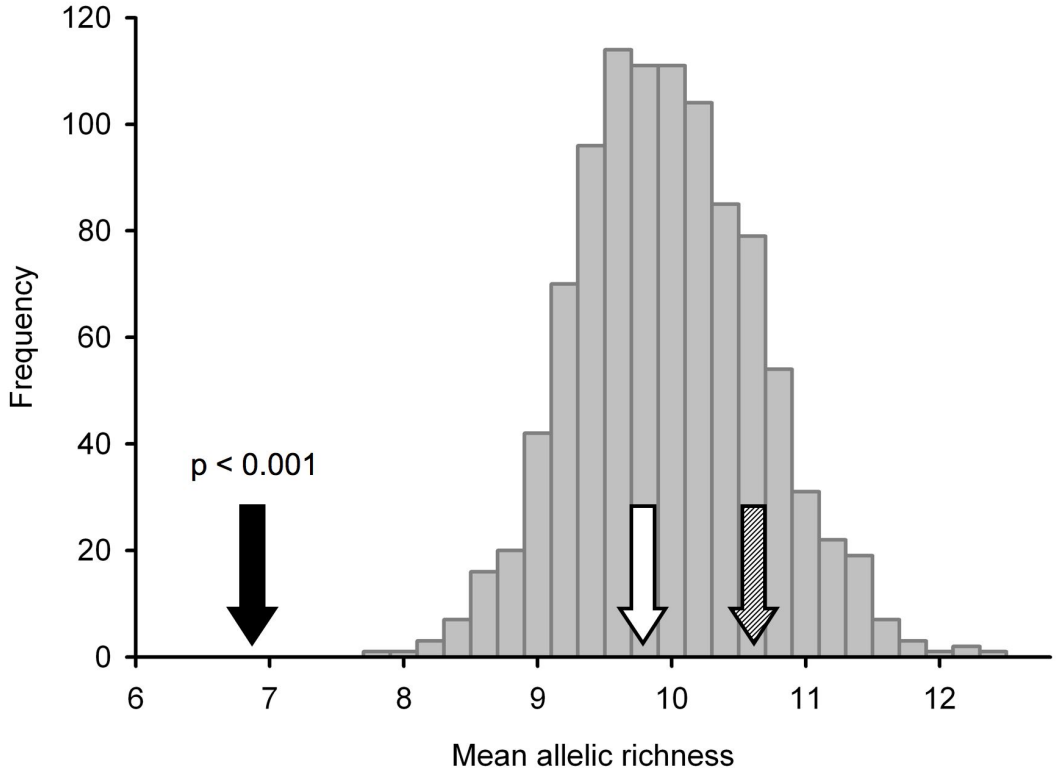
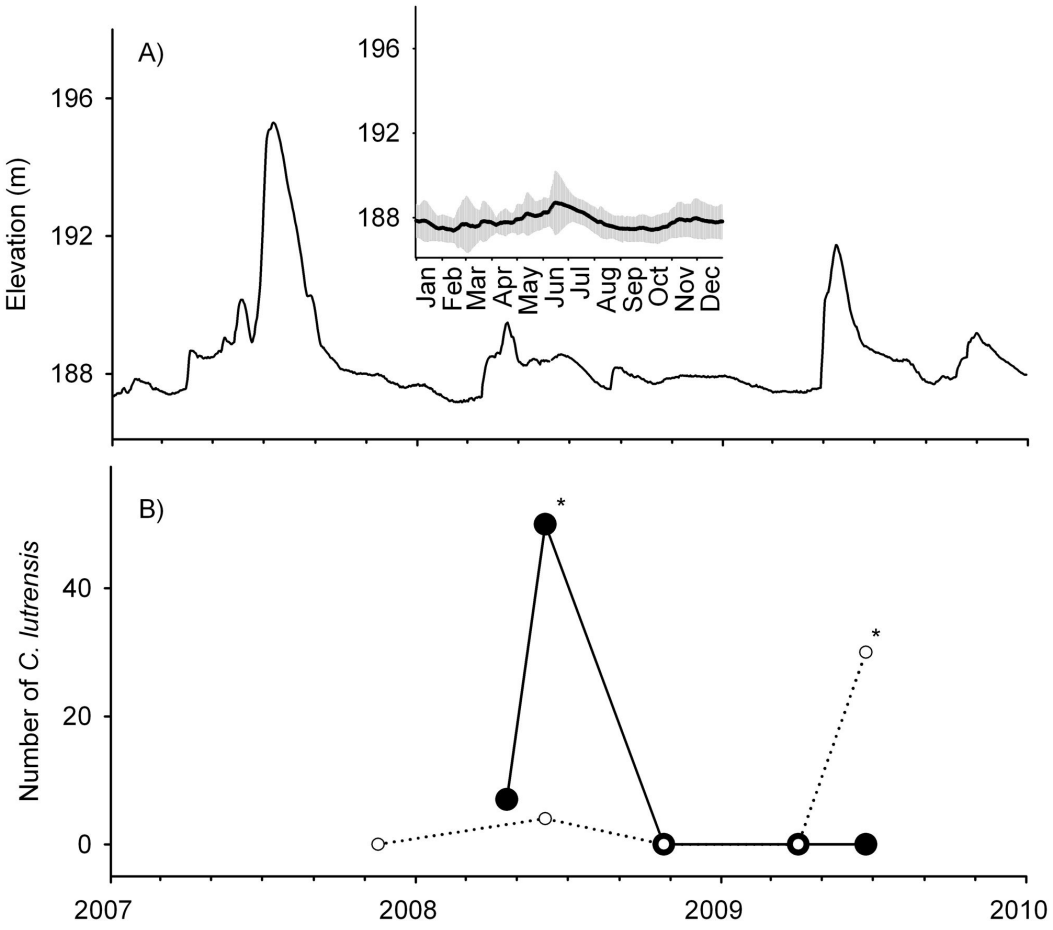


Figure 7. A) Mean daily surface elevation (m) above sea level of Lake Texoma from 2007 – 2010. The inset graph in A) is the mean daily surface elevation (black line) and ± standard deviation (gray vertical bars) of Lake Texoma from 1995 through 2006. B) Number of *C. lutrensis* collected from two sites in Brier Creek, a direct tributary of Lake Texoma between 2007 and 2010. Filled circles and solid lines are Brier Creek 5, and open circles and dotted lines are Brier Creek 6. Samples where tissue was collected for microsatellite analysis are indicated with (*).



Appendix I. Allele frequencies at each locus and population (coded as in Table 1). Alleles are coded as number of base pairs.

Locus	Allele	1	2	3	4	5	6	7 (2008)	7 (2009)	8	9	10	11	12
Rhca20	88	0.067	0.052	0.067	0.033	0.018	0.100	0.050	0.054	0.056	0.017	0.067	0.000	0.033
	90	0.700	0.638	0.583	0.750	0.696	0.667	0.767	0.696	0.556	0.617	0.617	0.603	0.483
	92	0.233	0.276	0.350	0.217	0.286	0.233	0.183	0.250	0.389	0.367	0.317	0.397	0.483
	96	0.000	0.034	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nme 18C2.178	167	0.103	0.241	0.317	0.300	0.357	0.533	0.433	0.462	0.296	0.383	0.327	0.707	0.517
	169	0.017	0.017	0.017	0.000	0.000	0.050	0.017	0.000	0.074	0.000	0.000	0.000	0.017
	171	0.397	0.397	0.200	0.200	0.232	0.067	0.167	0.173	0.167	0.100	0.231	0.034	0.000
	173	0.000	0.052	0.033	0.000	0.000	0.033	0.000	0.000	0.019	0.033	0.000	0.000	0.033
	175	0.000	0.034	0.067	0.067	0.018	0.017	0.050	0.038	0.019	0.017	0.000	0.052	0.083
	177	0.069	0.052	0.133	0.017	0.107	0.083	0.067	0.038	0.130	0.067	0.115	0.034	0.100
	179	0.000	0.000	0.000	0.017	0.036	0.017	0.017	0.000	0.019	0.000	0.000	0.000	0.000
	181	0.190	0.069	0.117	0.117	0.107	0.050	0.067	0.115	0.074	0.300	0.135	0.086	0.083
	183	0.103	0.069	0.050	0.167	0.107	0.083	0.117	0.096	0.093	0.083	0.000	0.034	0.083
	185	0.052	0.000	0.033	0.050	0.018	0.067	0.033	0.038	0.019	0.000	0.077	0.017	0.033
	187	0.017	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.019	0.000	0.038	0.017	0.000
	189	0.000	0.000	0.000	0.033	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.017
	191	0.000	0.000	0.000	0.017	0.000	0.000	0.017	0.000	0.019	0.000	0.019	0.000	0.017
	193	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	195	0.017	0.000	0.017	0.000	0.000	0.000	0.000	0.038	0.019	0.000	0.019	0.000	0.000
	197	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.038	0.000	0.000
	199	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017
	203	0.034	0.052	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.017	0.000	0.000	0.000
	210	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nme 24B6.191	189	0.000	0.017	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	191	0.138	0.310	0.233	0.233	0.143	0.268	0.214	0.125	0.185	0.321	0.288	0.271	0.259
	193	0.241	0.224	0.283	0.250	0.268	0.196	0.286	0.208	0.130	0.054	0.192	0.354	0.241
	195	0.121	0.052	0.033	0.117	0.000	0.054	0.107	0.104	0.148	0.000	0.077	0.000	0.000
	197	0.276	0.224	0.300	0.250	0.393	0.321	0.321	0.271	0.315	0.268	0.212	0.292	0.310
	199	0.034	0.034	0.067	0.017	0.054	0.018	0.000	0.063	0.074	0.000	0.019	0.000	0.000

Appendix 1. *Continued*

Locus	Allele	1	2	3	4	5	6	7 (2008)	7 (2009)	8	9	10	11	12
Nme 24B6.191	201	0.034	0.017	0.000	0.067	0.036	0.071	0.018	0.104	0.074	0.000	0.038	0.000	0.052
	203	0.069	0.052	0.033	0.033	0.089	0.018	0.036	0.063	0.056	0.304	0.077	0.083	0.052
	205	0.000	0.000	0.017	0.000	0.000	0.018	0.018	0.021	0.000	0.036	0.019	0.000	0.000
	207	0.034	0.000	0.017	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000
	209	0.000	0.034	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000
	211	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.069
	213	0.052	0.034	0.000	0.033	0.000	0.018	0.000	0.021	0.019	0.000	0.038	0.000	0.017
	217	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.018	0.000	0.000	0.000
Rhca24	249	0.000	0.000	0.034	0.000	0.000	0.000	0.018	0.019	0.019	0.018	0.000	0.000	0.000
	251	0.268	0.100	0.190	0.121	0.160	0.154	0.036	0.269	0.167	0.179	0.185	0.058	0.034
	253	0.018	0.000	0.034	0.017	0.100	0.058	0.054	0.019	0.000	0.000	0.019	0.019	0.000
	255	0.089	0.020	0.086	0.086	0.080	0.058	0.071	0.135	0.074	0.071	0.056	0.154	0.155
	257	0.089	0.040	0.017	0.034	0.020	0.019	0.018	0.019	0.019	0.000	0.019	0.096	0.069
	259	0.089	0.120	0.121	0.138	0.200	0.115	0.125	0.058	0.074	0.143	0.056	0.173	0.086
	261	0.143	0.140	0.052	0.103	0.040	0.038	0.125	0.096	0.093	0.000	0.111	0.077	0.052
	263	0.036	0.120	0.052	0.155	0.060	0.058	0.071	0.038	0.148	0.304	0.148	0.038	0.172
	265	0.071	0.080	0.086	0.172	0.040	0.058	0.089	0.000	0.111	0.054	0.037	0.077	0.052
	267	0.000	0.040	0.034	0.034	0.060	0.058	0.071	0.019	0.056	0.089	0.037	0.058	0.034
	269	0.036	0.020	0.000	0.000	0.060	0.019	0.018	0.058	0.000	0.018	0.037	0.000	0.000
	271	0.036	0.080	0.052	0.034	0.080	0.019	0.036	0.058	0.056	0.000	0.056	0.019	0.034
	273	0.018	0.080	0.034	0.000	0.000	0.077	0.054	0.038	0.056	0.000	0.037	0.019	0.017
	275	0.018	0.040	0.017	0.000	0.000	0.038	0.054	0.019	0.019	0.000	0.056	0.058	0.034
	277	0.000	0.020	0.000	0.000	0.000	0.058	0.036	0.058	0.019	0.000	0.019	0.000	0.052
	279	0.018	0.020	0.017	0.017	0.020	0.019	0.000	0.019	0.000	0.036	0.037	0.000	0.017
	281	0.000	0.040	0.034	0.000	0.000	0.019	0.054	0.000	0.019	0.000	0.000	0.077	0.034
	283	0.018	0.000	0.017	0.017	0.040	0.000	0.018	0.019	0.000	0.000	0.019	0.000	0.034
	285	0.000	0.000	0.017	0.000	0.000	0.019	0.000	0.019	0.019	0.000	0.019	0.019	0.017
	287	0.018	0.020	0.034	0.017	0.000	0.019	0.018	0.019	0.000	0.000	0.000	0.019	0.069
	289	0.000	0.000	0.017	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Appendix I. *Continued.*

Locus	Allele	1	2	3	4	5	6	7 (2008)	7 (2009)	8	9	10	11	12
Rhca24	291	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017
	293	0.000	0.000	0.017	0.000	0.000	0.000	0.018	0.000	0.000	0.018	0.019	0.019	0.017
	295	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.018	0.000	0.000	0.000
	297	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	299	0.000	0.000	0.000	0.017	0.020	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000
	301	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000
	303	0.000	0.000	0.017	0.000	0.000	0.019	0.000	0.000	0.019	0.018	0.000	0.019	0.000
	305	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000
	307	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.018	0.000	0.000	0.000
	309	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000
	311	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	313	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000
	315	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000
	319	0.000	0.020	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	323	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nme 25C8.208	199	0.000	0.000	0.017	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017
	201	0.000	0.019	0.000	0.000	0.033	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000
	203	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	205	0.000	0.000	0.017	0.018	0.000	0.050	0.017	0.042	0.000	0.077	0.036	0.034	0.033
	207	0.071	0.019	0.103	0.054	0.033	0.017	0.103	0.104	0.132	0.000	0.036	0.000	0.017
	209	0.179	0.231	0.259	0.268	0.150	0.100	0.121	0.208	0.211	0.135	0.214	0.190	0.083
	211	0.054	0.115	0.034	0.125	0.083	0.067	0.069	0.021	0.053	0.135	0.036	0.121	0.117
	213	0.268	0.192	0.121	0.161	0.183	0.217	0.276	0.354	0.184	0.385	0.196	0.121	0.117
	215	0.161	0.096	0.121	0.089	0.133	0.083	0.034	0.021	0.132	0.000	0.143	0.034	0.000
	217	0.107	0.019	0.069	0.018	0.100	0.067	0.017	0.021	0.053	0.000	0.089	0.034	0.117
	219	0.054	0.077	0.034	0.054	0.083	0.067	0.086	0.021	0.053	0.038	0.089	0.069	0.117
	221	0.000	0.019	0.052	0.018	0.067	0.017	0.017	0.042	0.053	0.000	0.000	0.086	0.050
	223	0.018	0.000	0.052	0.036	0.017	0.050	0.052	0.000	0.026	0.000	0.036	0.000	0.033
	225	0.018	0.019	0.034	0.036	0.050	0.017	0.017	0.063	0.026	0.019	0.018	0.103	0.033

Appendix 1. *Continued*.

Locus	Allele	1	2	3	4	5	6	7 (2008)	7 (2009)	8	9	10	11	12
Nme 25C8.208	227	0.000	0.038	0.034	0.018	0.017	0.017	0.052	0.000	0.026	0.000	0.000	0.069	0.050
	229	0.000	0.000	0.017	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033
	231	0.018	0.019	0.000	0.000	0.000	0.033	0.034	0.000	0.000	0.000	0.018	0.017	0.000
	233	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.033
	235	0.000	0.000	0.000	0.018	0.000	0.017	0.034	0.000	0.053	0.000	0.036	0.000	0.000
	237	0.018	0.019	0.017	0.000	0.000	0.017	0.000	0.000	0.000	0.135	0.000	0.034	0.000
	239	0.000	0.038	0.000	0.036	0.000	0.033	0.052	0.021	0.000	0.058	0.000	0.017	0.050
	241	0.018	0.058	0.000	0.000	0.017	0.017	0.017	0.042	0.000	0.019	0.018	0.000	0.033
	243	0.000	0.000	0.000	0.018	0.000	0.017	0.000	0.021	0.000	0.000	0.000	0.052	0.067
	245	0.000	0.000	0.017	0.018	0.017	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	247	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.018	0.017	0.000
	249	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

CHAPTER 2

ANTHROPOGENIC HABITAT ALTERATION INDUCES RAPID MORPHOLOGICAL DIVERGENCE IN A NATIVE STREAM FISH

Formatted for Evolutionary Applications

Abstract

Anthropogenic habitat alteration creates novel environments that can alter selection pressures. Construction of reservoirs worldwide has disturbed riverine ecosystems by altering biotic and abiotic environments of impounded streams. Changes to fish communities in impoundments are well documented, but effects of those changes on native species persisting in reservoirs, which are presumably subjected to novel selective pressures, are largely unexplored. I assessed body shape variation of a native stream fish in streams and reservoirs from seven reservoir basins in the Central Plains of the USA. Body shape significantly and consistently diverged in reservoirs compared to stream habitats within reservoir basins; individuals from reservoir populations were deeper-bodied and had shorter heads compared to stream populations. Stream populations were also increasingly different from reservoir populations as distance from reservoirs increased. I assessed the effects of genotypic divergence and predator induced phenotypic plasticity on observed body shape variation by rearing offspring from a reservoir and a stream population with or without a piscivorous fish. Significant population-level differences in body shape persisted in offspring, and both populations demonstrated similar predator-induced phenotypic plasticity. My results suggest that,

although components of body shape are plastic, anthropogenic habitat modification can drive trait divergence in native fish populations in reservoir-altered habitats.

Introduction

Species worldwide are subject to anthropogenic disturbances to ecosystems (Vitousek et al. 1997), and may consequently suffer extinction and contribute to the current unprecedented extinction rate (Pimm et al. 1995). The extent of environmental change and the subsequent responses of populations determine population viability in recently altered ecosystems. Stream impoundments are major contributors of habitat degradation and fragmentation in aquatic ecosystems (Baxter 1977, Dynesius and Nilsson 1994, Downing et al. 2006); threatening many imperiled freshwater organisms (Dudgeon et al. 2005). Generally, reservoirs have deleterious impacts on native biota, but for species that persist in these altered environments, they may serve as model systems to investigate population responses to rapid environmental disturbances because reservoirs are widespread, can be treated as replicated units, and potentially affect a wide-range of taxa.

When streams are impounded, they rapidly change from relatively shallow flowing habitats to deep standing bodies of water which most native stream fishes have likely not experienced during their evolutionary history (Baxter 1977). The presence and strength of novel biotic and abiotic selective pressures in reservoirs is evidenced by changes to historical structures of fish communities following impoundment: obligate stream fishes often suffer rapid extirpation or substantial declines in reservoirs of impounded streams (Taylor et al. 2001, Gido et al. 2009). Additionally, higher densities of native and nonnative piscivorous fishes are facilitated in reservoirs by newly formed

lentic habitats and game fish stockings (Gido and Brown 1999, Taylor et al. 2001, Paller 2005). Although many native stream fishes cannot persist in these novel ecosystems (*sensu* Hobbs et al. 2006), it is currently unclear how traits and evolutionary trajectories of resident populations may be impacted.

Intra- and interspecific phenotypic variation along natural environmental gradients of stream flow (Hubbs 1941, Brinsmead and Fox, 2002, Langerhans et al. 2003, McGuigan et al. 2003, Langerhans 2008, Pavey et al. 2010, Tobler and Carson 2010) and predator regimes (Endler 1980, Reznick et al. 1997, Langerhans et al. 2004, Hendry et al. 2006, Langerhans and Makowicz 2009, Pavey et al. 2010) can be used to generate *a priori* predictions of how fish morphologies may respond to reservoir habitats. Relationships between morphology and swimming performance likely constrain body shape variation along environmental gradients (Langerhans 2008, Tobler and Carson 2010). Specifically, selection on fishes in lotic habitats can result in fusiform body shapes that reduce drag and enable prolonged swimming, whereas increased body depth in lentic waters facilitates faster burst speeds and increased maneuverability (Gosline 1971, Alexander 1974, Langerhans 2008). The presence of piscivorous fishes can also select for increased caudal depths of small-bodied fishes, presumably increasing predator escape through high burst-swimming speed (Domenici and Blake 1997, Langerhans et al. 2004, Hendry et al. 2006, Langerhans 2009). Therefore, both loss of flow and increased predator densities in reservoirs have the potential to drive predictable morphological trait divergence between ancestral stream populations and populations in these newly altered habitats.

Most studies on fishes have offered observational evidence for adaptive trait divergence in response to varying predator and flow regimes. However, environmentally contingent phenotypes (i.e., phenotypic plasticity) are widespread (West-Eberhard 1989, Schlichting and Pigulucci 1998, Losos et al. 2000) and the contribution of phenotypic plasticity to observed morphological variation of fishes in the field has largely been overlooked (Langerhans 2008). I know of only two studies that have demonstrated predator- (Brönmark and Miner 1997) and flow-induced (Keeley et al. 2007) plastic morphological changes in fishes. Given that some fishes are plastic in response to the presence of predators or variable flow regimes, phenotypic plasticity could potentially be responsible for a portion of the morphological variation observed along environmental gradients. Haas et al. (2010) demonstrated morphological divergence of a stream fish in reservoirs using field-collected specimens. However, it is currently unclear if disparate morphologies are heritable, and how much body shape variation among populations could potentially be explained by phenotypic plasticity.

Here, I assessed whether newly formed lentic habitats drive morphological trait divergence of native stream-fish populations and predicted fish morphologies would demonstrate consistent divergence in replicated reservoir systems. I tested this prediction by quantifying body shape of a native small-bodied stream fish (*Cyprinella lutrensis* Baird and Girard) from field-collected individuals in streams near reservoirs and in reservoir habitats. Additionally, I assessed the relative contributions of genotypic variation and predator-induced phenotypic plasticity to morphological

divergence in reservoirs by rearing lab-spawned offspring of a reservoir and stream population in a common garden experiment with and without predators present.

Materials and methods

Field collections

Cyprinella lutrensis, a small-bodied Cyprinid (< 100 mm Total Length) native to and locally abundant in the Central Plains of the USA (Matthews 1987), were collected by seine in stream and reservoir habitats from seven reservoir basins in Oklahoma, USA (Table 1; Fig. A1). Specimens from five basins were collected between 1992 and 1999 and I obtained them from the Sam Noble Oklahoma Museum of Natural History (SNOMNH) in 2009 (Table 1). Specimens were fixed in 10% formalin in the field and transferred to 50% isopropyl alcohol for long-term preservation. I collected fish from the other two basins between 2007 and 2008 (Table 1) and preserved and stored them in 10% formalin before data acquisition (< 2 weeks). Museum collections consisted of one reservoir population and one stream population either upstream or downstream of each impoundment, and recent collections included one reservoir population and several stream populations near each impoundment (Table 1; Fig. A2). I only used males in breeding condition (determined by the presence of tubercles on the forehead; Koehn 1965) for analyses to reduce potential body shape variation due to sexual dimorphism, or in females, gravidity.

Morphological divergence and phenotypic plasticity

I assessed potential genotypic differences and predator-induced phenotypic plasticity in morphology between reservoir and stream populations, by spawning *C. lutrensis* adults from a reservoir and stream population in the lab and rearing their

offspring in a split-cohort common garden experiment with or without a predator present. I collected adult *C. lutrensis* from a reservoir (Lake Thunderbird) and a stream population down-stream of the reservoir (Pecan Creek; Table 1) in May 2009. I spawned individual breeding pairs from both populations (n = 4 pairs from stream population, n=8 pairs from reservoir population) in 40 l aquaria (i.e., one male and one female from the same population per aquarium) in a greenhouse at the Aquatic Research Facility (ARF) at the University of Oklahoma starting 1-July-2009. One round gravel-filled plastic tray (140 mm diameter, 35 mm deep) in each aquarium served as spawning substrate. Every third day, I replaced trays and hatched eggs in separate aerated plastic trays. Hatched juveniles from the same cohort were then haphazardly split into two outdoor 380 l mesocosms. I allowed each parental pair to spawn until I consistently observed at least 20 juvenile *C. lutrensis* in each paired mesocosm.

After each parental pair was finished spawning (i.e., ≥ 20 offspring in each mesocosm pair), I randomly assigned predator and non-predator treatments and introduced either a native piscivorous fish (*Micropterus salmoides* Lacepéde; Largemouth bass) or non-native non-piscivorous fish (*Cyprinus carpio* Linnaeus; Common carp) to each mesocosm. *Micropterus salmoides* (hereafter termed predator) is native to this region and have likely shared an evolutionary history with *C. lutrensis* whereas *C. carpio* (hereafter termed non-predator) is an exotic. I included the non-predator treatment in the paired mesocosms to control for the presence of a larger fish (i.e., the predator fish treatment) in the rearing environments. Parents did not successfully spawn offspring simultaneously; therefore, although split cohorts received

both predator and non-predator treatments the same day, I sequentially added predator/non-predator treatments through the summer. I placed hatched larval *C. lutrensis* in mesocosms between 5-July and 26-August, and stocked the first treatments 21-July and the last treatments 19-August. Predator treatment individuals were on average larger mean(range) = 122(90-180) mm Total Length (TL) than non-predator individuals 92(73-110) mm TL, therefore I added more than one non-predator to some mesocosms to approximate the length and biomass of the predator in the other paired mesocosm. The mean total length of predator and non-predators in paired mesocosms did not differ significantly (Paired t-test, $n = 12$, $t = -0.238$, $p = 0.815$). In addition, biomass estimated from published length-weight relationships of predator and non-predator fish (Carlander 1969, Schneider et al. 2000) did not differ significantly between treatments (Paired t-test, $n = 12$, $t = -1.073$, $p = 0.304$). I separated predator and non-predator fish from juvenile *C. lutrensis* with a screen barrier (window screen) held in place with silicone at ~1/3 end of each mesocosm. Juvenile *C. lutrensis* were on average 13 days old (range = 1 – 22) before I stocked treatment fish and juveniles were present with treatment fish on average 64 days (range = 45 – 77). I removed all juvenile *C. lutrensis* from mesocosms on 3-Oct-2009, euthanized, preserved, and stored them in 10% formalin solution until data acquisition (< 7 days), and only used individuals >10 mm Standard Length (SL) in analyses.

Geometric morphometric analysis

I quantified body shape of *C. lutrensis* specimens using geometric morphometric analyses (Zelditch et al. 2004) with tps software (http://life.bio.sunysb.edu/morph/). I photographed the left lateral side of each individual digitally with a reference scale,

randomized the photographs using tpsDig software (Rolhf 2004a), and set 10 homologous landmarks on each (Fig. 1). To account for bending of specimens, I unbent landmarks using the landmarks at the tip of the snout and middle of the eye and one temporary landmark set in the middle of the caudal peduncle (but removed in final analyses) using the "unbend specimens" function in tpsUtil (Rolhf 2004b). I resized landmark coordinates using the reference scale, and aligned landmark coordinates using least-squares superimposition to remove the effects of scale, translation, and rotation with the program tpsRelw (Rohlf 2004c). I calculated centroid size, partial warp scores and uniform components (i.e., weight matrix; hereafter referred to as shape variables) using tpsRelw and reserved them for analyses.

Data analysis

Field collections

To determine the consistency of shifts in morphological space between reservoir and stream habitats, I reduced the dimensionality of the shape variables by calculating morphological divergence scores for each individual along the stream-reservoir gradient based on a divergence vector (referred to as morphological index hereafter) as defined by Langerhans (2009). This morphological index does not distort morphological space and summarizes the linear combination of shape variables that contribute to the greatest difference in body shape between reservoir and stream habitats (Langerhans 2009). To quantify the morphological index, I created a score for each specimen on the stream-reservoir shape axis by multiplying the eigenvector of the effects (including centroid size) Sums of Squares and Cross Products (SSCP) matrix by the shape variables block to yield a column of morphological index scores for each individual controlling for

allometry (i.e., centroid size). I used the resulting scores as a dependant variable in subsequent analyses. I assessed individual landmark movement between habitat types by analyzing correlation coefficients between landmark positions and the morphological index scores of field collected specimens. Shape variation was then visualized using thin-plate spline transformation grids (Bookstein 1991) in tpsReg (Rohlf 2004d), along the morphological index of field caught specimens and the observed ranges of population and predator-induced plasticity of lab-reared individuals.

I tested for differences in body shape between stream and reservoir habitats using mixed model analysis of covariance (ANCOVA) following Langerhans et al. (2009). The ANCOVA model used the morphological index score as the dependant variable, centroid size as a covariate (to test for allometry), habitat type as a fixed factor (to test for effects of stream or reservoir habitats), basin as a random factor (to test for effects of basin), and population as a random factor nested within habitat by basin interaction (to test for unique population differentiation within habitat types). *F*-values were approximated using Wilks' lambda and effect strengths by use of partial eta squared (η_p^2). I also calculated the relative variance as the partial variance for a given term divided by the maximum partial variance value in the model. I removed non-significant interactions from the final model.

Geographic distance, time, and shape divergence

I assessed how distance from reservoirs and time since impoundment influenced shape variation among populations (i.e., stream versus reservoir) by quantifying the difference between mean morphological index scores from each stream and reservoir population in each reservoir basin. This analysis asked whether increased geographic

distance from reservoirs and time since impoundment would result in increased shape disparity between reservoir and stream populations. I used two separate linear regressions with $\log_{10}$ stream distance (km) from each reservoir or years since impoundment as independent variables, and the amount of shape divergence as the dependant variable (i.e., the difference between each stream population's mean morphological index score and the mean morphological index score of the reservoir population in each respective reservoir basin). Two reservoir systems had more than one stream population (Table 1), and inclusion of all populations would result in pseudo replication in these two reservoirs (i.e., differences in morphological index scores of all populations in these two reservoirs were calculated from the index scores of reservoir individuals). Therefore, I adjusted the denominator degrees of freedom from 13 to 6 to avoid the inflation of the degrees of freedom due to pseudo replication.

Because reservoirs may have more homogenous biotic and abiotic conditions compared to streams, and thus have more consistent and similar selection pressures among reservoir populations, I also quantified total variation in shape of all specimens in the two habitat types (i.e., stream and reservoir) using Coefficient of Variation (CV) of morphological index scores.

Morphologic divergence and phenotypic plasticity

I assessed genotypic differences in body shape between a reservoir and a stream population, and tested for predator-induced phenotypic plasticity in reared offspring using two approaches. However, prior to both analyses, I controlled for body size by performing a preparatory MANCOVA with shape variables as dependant variables and centroid size as a covariate and retained the unstandardized residuals. Because my focal

interests in this experiment were two-fold: genotypic differences and predator-induced phenotypic plasticity, I did not quantify a single morphological index as above with field-collected individuals, instead I used a Principal Component Analysis (PCA) to reduce the dimensionality of the shape variables. I performed the PCA, using a covariance matrix, of size-corrected shape variables (i.e., residuals) and only retained axes with eigenvalues >1 for analyses. Three PC axes explained 76.9% of the variation in sized corrected body shape (PC I = 49.9%, PC II = 17.4%, PC III = 9.6%) and I used these axes as dependant variables in the subsequent analyses.

For the first analytical approach, I conducted three separate mixed model ANOVAs. Each dependent variable was PC I, II, or III, and fixed factors were treatment (predator or non-predator; to test for predator-induced phenotypic plasticity), population-of-origin (to test for genotypic differences between populations), and the interaction between population and treatment. Parents nested within population (to control for non-independence of parents) was included as a random factor. Population of origin could arguably be a random factor; however, in this instance the two populations were chosen based on *a priori* knowledge of body shape differences between the populations (i.e., preliminary analysis of shape variation *C. lutrensis* were greatest between these populations). I removed non-significant interaction terms from each of the final models.

The second analytical approach used three separate repeated measures ANOVAs with mean parent-treatment combinations of PC scores (i.e., means of PC I, II, and III scores of juveniles from each mesocosm) as dependant variables. Population was a between-subjects factor and the within-subject was treatment (predator or non-

predator). Although repeated measures ANOVA is often used to test for differences over time, it was used here because variation introduced by parents was not specifically of interest, and it allowed me to test for population and treatment level effects while controlling for variation introduced by parents. I completed all analyses in SPSS v. 17.0 for Macintosh.

Results

Field collections

In the mixed model ANCOVA, habitat type (stream or reservoir), allometry (centroid size), and population nested within the basin-habitat interaction had significant effects on morphological index scores (Table 2). Conversely, basin and the interaction between basin and habitat were not significant. Habitat had the strongest effect on morphological index scores ($\eta^2_p = 0.48$), followed by centroid size ($\eta^2_p = 0.33$) and population nested within the basin-habitat interaction ($\eta^2_p = 0.20$; Table 2). Generally, *C. lutrensis* found in reservoir habitats had shorter heads with deeper body depths compared to individuals from stream habitats (Fig. 2). Specifically, body shape divergence in reservoir habitats was due to posterior movement of the tip of the snout, dorsal movement of the corner of the mouth, dorsal movement of the insertion of the dorsal fin, ventral movement of the insertion of the pelvic fin, and anterior movement pectoral fin (Table 3). Body shape diverged consistently in reservoir habitats in the replicated reservoir basins; however, there was substantial variation in the replicated stream populations in the one reservoir basin (Thunderbird) where several stream populations were collected (Fig. 3). Moreover, based on the CV of morphological

index scores, shape variation in reservoir habitats (CV = 19.8%) was half that compared to stream habitats (CV = 39.7%).

Geographic distance, time, and shape divergence

Stream populations became increasingly different from reservoir populations as distance from reservoirs increased ($n = 14$, $r^2 = 0.34$, $F_{1,6} = 6.22$, $p = 0.047$; Fig. 4). However, the relationship between morphological index scores among populations and year since impoundment was non-significant ($n = 14$, $r^2 = 0.125$, $F_{1,6} = 1.72$, $p = 0.238$).

Genetic divergence and phenotypic plasticity

Due to low spawning success and high juvenile mortality, only 4 parental pairs from the reservoir population and 8 parental pairs from the stream population were successfully spawned with offspring surviving in both predator and non-predator treatments. Overall, 257 individuals were analyzed for shape variation and the mean from each mesocosm was 10.7 (range = 1-25).

When testing for genotypic and phenotypic plasticity effects on body shape of *C. lutrensis* offspring using the three mixed model ANOVAs, population of origin ($\eta^2_p = 0.34$), parents nested within population ($\eta^2_p = 0.15$) and treatment ($\eta^2_p = 0.02$), had significant effects on PC I (Table 4). Parents nested within population ($\eta^2_p = 0.38$), and treatment ($\eta^2_p = 0.05$) had significant effects on PC II. When testing PC III, parents nested within population ($\eta^2_p = 0.31$), and the interaction between treatment and population ($\eta^2_p = 0.03$) were significant (Table 4).

Qualitatively similar results were obtained when testing for population-level differences in body shape and predator induced phenotypic plasticity using repeated measures ANOVA. With PC I as the dependant variable, only population had a

significant effect on body shape with no significant effects of predator treatment or their interaction (Table 5). Conversely, when testing differences on PC II, only treatment had a significant effect on body shape (Table 5). There were no significant main effects or their interaction when testing PC III (Table 5). Thus, PC I largely reflected variation due to population-level genetic differences and PC II reflected predator induced phenotypic plasticity. Offspring from the stream population had larger caudal areas and smaller head regions compared to offspring from the reservoir population and resembled similar body shapes to adult male *C. lutrensis* collected from reservoir habitats (Fig. 5). Juvenile *C. lutrensis* reared with predators also had smaller heads and larger caudal areas compared to individuals reared with non-predators (Fig. 5). Because there was not a significant interaction between population and predator treatment, both populations demonstrated similar predator-induced phenotypic plasticity (Fig. 6).

Discussion

My results suggest consistent morphological divergence of a native small-bodied fish in anthropogenically altered riverine systems. Experimental results from rearing offspring of a reservoir and stream population with and without predators verified that 1) shape variation between the two studied populations had a genetic basis, and 2) both populations exhibited similar predator-induced phenotypic plasticity in body shape.

Field collections

Consistent morphological divergence between stream and reservoir populations within reservoir basins suggests habitat changes by impoundments are driving predictable phenotypic variation in *C. lutrensis*. Moreover, shape variation was overall

lower in the more homogenous reservoir environments. Body shape of *C. lutrensis* in reservoirs was less streamlined with deeper caudal areas and smaller heads. This morphological divergence was also qualitatively similar to morphological shifts found in reservoir-residing *C. venusta* (Haas et al. 2010), a small-bodied species ecologically similar to *C. lutrensis*. Such intra- and inter-specific trait divergence implies different reservoirs create similar selective pressures on small-bodied fishes. In response, phenotypes are potentially adapting to maximize fitness in these habitats. It is unlikely only one environmental factor is driving morphological divergence; a suite of novel selective pressures could potentially contribute to phenotypic differences between stream and reservoir-resident populations.

Because conversion of riverine systems to reservoir habitats is associated with multiple biotic and abiotic environmental changes (e.g., turbidity, flow, temperature, biotic communities), it may be difficult to isolate one factor independently without experimental manipulation. However, phenotypic variation of *C. lutrensis* did match predicted morphologies thought to be adaptive in both low flow conditions (Gosline 1971, Alexander 1974, Langerhans 2008) and habitats with high predator densities (Domenici and Blake 1997, Langerhans et al. 2004, Hendry et al. 2006, Langerhans 2009). These two factors in concert could be driving observed morphological shifts of small-bodied fishes. The increased body depth and caudal area could increase predator escape performance (through increased burst-speed; Langerhans 2008) and maneuverability for feeding on prey suspended in the water column (versus drifting prey in streams; Rincón et al. 2007) or through steady/unsteady-swimming performance tradeoffs (Langerhans 2008; 2009).

Assuming morphological divergence in reservoirs confers greater fitness to reservoir-resident individuals, divergent natural selection could lead to local adaptation in these habitats. Investigations of the morphologies of other fishes between lake-stream pairs suggest local habitats can drive phenotypic variation in spite of close proximities of populations (Brinsmead and Fox 2002, Hendry et al. 2002, Berner et al. 2009, Haas et al. 2010). Currently, the extent of gene flow among stream and reservoir populations is unknown, but high immigration rates among populations could limit the extent of local adaptation in reservoir habitats.

Habitat type explained the most variation in morphological divergence of *C. lutrensis*, followed by reservoir basin, although not significant. Given the geographic distances among reservoir basins (Fig. A1), a significant basin effect would likely be expected assuming fish from different basins have unique evolutionary histories, however, the use of museum and more recently collected specimens likely confounded this result. Because museum specimens were in preservative for at least 10 years, significant preservation effects on body shape could have masked a basin effect. Indeed, both time and the type of long-term preservative solution (i.e., formalin or 50% isopropyl alcohol) have significant effects on body shape of preserved *C. lutrensis* individuals (Appendix B). Therefore, it was not possible to isolate basin effects versus preservation effects with this data set.

Geographic distance, time, and shape divergence

The significant positive relationship between distance from reservoirs and difference in morphologic index scores between stream and reservoir populations suggests there is a spatial component to morphological divergence. In one reservoir

basin (Lake Thunderbird), two of the three stream populations that were most similar morphologically to reservoir individuals were collected from streams that flow directly into the reservoir. Thus, the close proximity of direct tributary populations could allow for increased gene exchange with reservoir populations, or streams closer to the reservoir could have environmental conditions more similar to reservoirs (e.g., fish communities; Falke and Gido 2006).

Although time since impoundment was not related to morphological differences between reservoir and stream populations, this could be potentially confounded by population distances from reservoirs or if morphological divergence occurred relatively early following the stream impoundments.

Genetic-level and phenotypic plasticity

Results from rearing offspring from a reservoir and stream population with and without a predator present suggest both genotypic and phenotypic plasticity contributed to observed phenotypic differentiation between these two populations. However, population level differences likely contributed more to phenotypic variation than plasticity. Both the mixed model and repeated measures ANOVAs demonstrated population of origin had significant effects on PC I (which explained 42.9% of the variance in size corrected body shape) and predator treatment had significant effects on PC II (explained 17.4% of the variance). Additionally, the mixed model found effects of predator treatment on PC I. Collectively, these results indicate body shape variation among offspring was most strongly influenced by their population of origin, followed by predator and non-predator treatments. Although I was unable to assess heritability directly by comparing parental morphologies to offspring morphologies (parents were

in very poor condition following spawning), or compare spawned offspring to field specimens (the size distributions between them were too large), results did support a heritable basis to body shape variation between the reservoir and stream populations.

When populations become isolated and divergent natural selection is strong, evolution of traits can occur over relatively short time scales (e.g., Reznick et al. 1997, Stockwell and Weeks 1999; Hendry et al. 2000). Because the reservoir and stream populations used here were separated by the physical stream impoundment, migration of individuals through the dam structure is improbable. Therefore, these two populations likely have had little or no low gene flow since construction of the reservoir in 1965. Additionally, *C. lutrensis* can spawn during its first year of life (Marsh-Matthews et al. 2002) potentially allowing for over 80 generations since these two populations became isolated, far more than needed to observe evolution under experimental conditions (Reznick et al. 1997). This suggests anthropogenic habitat alteration has likely facilitated adaptive trait divergence. Nonetheless, the effects of phenotypic plasticity as demonstrated by offspring reared with predators could also contribute to observed phenotypic divergence in reservoirs.

When reared with predators, the offspring of both populations demonstrated similar predator-induced phenotypic plasticity (i.e., the interaction was non-significant on PC I and PC II). However, based on the direction of the plastic shift in morphological space of both populations (Fig. 6), it is unlikely that the morphological divergence found in reservoirs is due to predator-induced phenotypic plasticity. Assuming reservoir-phenotypes are adaptive and predator-induced plasticity was contributing to the observed phenotypic variation in reservoirs, the plastic shift in lab-

reared individuals should have shifted in the direction of the reservoir population. However, when exposed to predators during development, offspring tended to resemble the stream population and not the reservoir population. Nonetheless, phenotypic plasticity for other environmental factors (e.g., flow) could contribute to observed phenotypic variation in the field.

The lab-reared offspring of the stream and reservoir populations exhibited disparate shape variation compared to their field-collected counterparts. However, this result needs to be interpreted with caution for several reasons. First, because *C. lutrensis* offspring were much smaller (mean SL (mm) = 22.3 ± 4.88 SD) than field collected individuals (mean SL (mm) = 46.6 ± 6.62 SD) and allometric shape variation may confound comparisons between such large size differences (Zelditch et al. 2004), therefore direct comparisons between field and lab-reared individuals may not be appropriate. Second, sex and breeding condition of individuals could also confound comparisons between the two groups; shape analyses of field individuals were restricted to only males in breeding condition (i.e., individuals in breeding color with head tubercles) while lab-reared individuals were not sexed and none exhibited breeding condition. *Cyprinella lutrensis* can reach sexual maturity as small as 29 mm SL (Marsh-Matthews et al. 2002), therefore most lab-reared individuals were not of reproductive age. Whereas population level differences were apparent in the lab-reared individuals, in light of these confounding effects, it is unclear if the same shape differences observed in the field would be observed in the lab-reared individuals had they be reared to a similar size as field individuals.

While interpretation of morphological comparisons between field-collected and lab-reared individuals were likely confounded, it is also unclear if the population-level morphological differences in the lab were driven by divergent selection in the two habitats or was merely a function of genetic differences due to distance between populations. Moreover, the results of the plasticity experiment were limited by having only one reservoir replicate. Further experiments assessing population-level morphological divergence with other reservoir and stream populations may elucidate the consistency of genetic divergence in replicated reservoir systems.

Conservation implications

The implications of rapid evolutionary change on conservation efforts have gained interest in recent years (Stockwell et al. 2003, Carroll et al. 2007). While reservoirs create novel environmental conditions, they are also relatively young. Yet evidence suggests stream fishes that can persist in these habitats have undergone divergent evolution in under 100 years (Haas et al. 2010, this paper). Assuming contemporary evolution of reservoir resident fishes has adapted them to impounded habitats, these reservoir-adapted traits may not be adaptive in other environments. For example, reservoir-adapted phenotypes would likely have lower fitness in flowing water habitats compared to resident stream fishes. Therefore, reservoir-adapted individuals would potentially be poor candidates to re-colonize extirpated populations in streams that flow into a reservoir proper (i.e., direct tributaries of reservoirs). Matthews and Marsh-Matthews (2007) documented the near or complete extirpation of *C. lutrensis* from several direct tributaries of Lake Texoma, Oklahoma-Texas, USA, whereas stream populations upstream of the reservoir remained intact, in spite of the fact *C. lutrensis*

still inhabits the reservoir proper. In addition, recent re-colonization of at least one direct tributary did not result in reestablishment of the species (Matthews and Marsh-Matthews pers. comm.). Because these streams flow directly into the reservoir, new colonists are likely to be derived from reservoir populations. Although other factors could have influenced the extirpation of *C. lutrensis* in these direct tributaries (e.g., habitat changes, increased predation pressure; Matthews and Marsh-Matthews 2007), reservoir-adapted individuals colonizing extirpated stream habitats are potentially ill-adapted to successfully reestablish a viable population. Moreover, introgression of reservoir-adapted genotypes into resident stream populations may also decrease the mean fitness of stream populations, increasing the chances of extirpations. However, experimental manipulation such as environmental transplanting or swimming performance estimates will be needed in order to assess if reservoir individuals are ill-adapted to stream habitats.

Conclusions

This study documented consistent morphological divergence in body shape of a native stream fish in reservoirs of impounded riverine systems. A common garden experiment revealed body shape differences between a reservoir and stream population had a genetic basis and the rearing of offspring with and without predators induced phenotypic plasticity in body shape. However, based on the direction of the plastic shift in morphological space, increased predator densities in reservoirs are likely not driving the observed divergence (due to predator-induced phenotypic plasticity). Although this study provided evidence of genetic-based morphological divergence in reservoirs, assessment of several other lines of investigation are needed. First, migration levels

among stream and reservoir populations will be needed to assess the extent to which gene flow may limit local adaptation to reservoir habitats. Second, although *C. lutrensis* demonstrated predator-induced plasticity, the potential for plasticity in regard to flow variation has not been examined. The relative contribution of plasticity versus genetic components in observed phenotypic variation will also elucidate the extent of local adaptation in these systems. Finally, relationships between body morphology and fitness in stream and reservoir habitats will need to be assessed to determine if body shape influences fitness in various habitats.

Acknowledgements

M. Tobler, C. Tobler, E. Marsh-Matthews, W. Matthews, E. Remmel, R. Zamor, M. Dugas, R. Cothran helped with specimen collection. The Sam Noble Oklahoma Museum of Natural History and E. Marsh-Matthews provided funding. A. Tarhule, C. Vaughn, G. Wellborn, E. Marsh-Matthews, W. Matthews, M. Tobler, A. Stiff, and OU ZEEB journal club made comments on the manuscript. M. Dugas and M. Tobler helped with data analyses and the preservation study. This manuscript is in partial fulfillment of a Ph.D. degree from the Department of Zoology, University of Oklahoma. This project was approved by IACUC # R08-004 from the University of Oklahoma.

Literature cited

Alexander, R.M. 1967. Functional design in fishes. Hutchinson, London.

Baxter, R.M. 1977. Environmental effects of dams and impoundments. Annual Review of Ecology, Evolution and Systematics 8:255-283.

Berner, D,A. Grandchamp, and A.P. Hendry. 2009. Variable progress toward ecological speciation in parapatry: stickleback across eight lake-stream transitions. Evolution 7:1740-1753.

Bookstein, F.L. 1991. Morphometric tools for landmark data. Cambridge University Press, Cambridge, U.K.

Brinsmead, J., and M.G. Fox. 2002. Morphological variation between lake- and stream-dwelling rock bass and pumpkinseed populations. Journal of Fish Biology 61:1619-1638.

Brönmark, C., and J.G. Miner. 1992. Predator-induced phenotypic change in body morphology in Crucian carp. Science 258:1348-1350.

Carlander, K.D. 1969. Handbook of freshwater fishery biology. Volume One. The Iowa State University Press, Ames.

Carroll, S.P, A.P. Hendry, D.N. Reznick, and C.W. Fox. 2007. Evolution on ecological time scales. Functional Ecology 21:387-393.

Domenici, P., and R.W. Blake. 1997. Fish fast-start kinematics and performance. Journal of Experimental Biology 200:1165-1178.

Downing, J.A., Y.T. Prairie, J.J. Cole, C.M. Duarte, L.J. Tranvik, R.G. Striegl, W.H. McDowell, P. Kortelainen, N.F. Caraco, J.M. Melack, and J.J. Middelburg.

2006. The global abundance and size distribution of lakes, ponds, and impoundments. Limnology and Oceanography 51:2388-2397.

Dudgeon, D., A.H. Arthington, M.O. Gessner, Z. Kawabata, D.J. Knowler, C. Lévêque, R.J. Naiman, A. Prieur-Richard, D. Soto, M.L.J. Stiassny, and C.A. Sulliven. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological. Reviews 81:163-182.

Dynesius, M., and C. Nilsson. 1994. Fragmentation and flow regulation of river systems in the northern third of the world. Science 226:753-761.

Endler, J.A. 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution 34:76-91.

Falke, J.A., and K.B. Gido. 2006. Effects of reservoir connectivity on stream fish assemblages in the Great Plains. Canadian Journal of Fisheries and Aquatic Sciences 63:480-493.

Gido K.B., and J.H. Brown 1999. Invasion of North American drainages by alien fish species. Freshwater Biology 42:387-399.

Gido K.B., J.F., Schaefer, and J.A. Falke. 2009. Convergence of fish communities from the littoral zone of reservoirs. Freshwater Biology 54:1163-1177.

Gosline, W.A. 1971. Functional morphology and classification of teleostean fishes. University of Hawaii Press, Honolulu.

Haas, T.C., M.J. Blum, and D.C. Heins. 2010. Morphological responses of a stream fish to water impoundment. Biology Letters 6:803-806.

Hendry, A.P., J.K. Wenberg, P. Bentzen, E.C. Volk, and T.P. Quinn. 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced

salmon. Science 290:516-518.

Hendry, A.P., E.B. Taylor, and J.D. McPhail. 2002. Adaptive divergence and the balance between selection and gene flow: Lake and stream stickleback in the Misty system. Evolution 56:1199-1216.

Hendry, A.P., M.L. Kelly, M.T. Kinnison, and D.N. Reznick. 2006. Parallel evolution of the sexes? Effects of predation and habitat features on the size and shape of wild guppies. Journal of Evolutionary Biology 19:741-754.

Hobbs et al. 2006. Novel ecosystems: theoretical and management aspects of the new ecological world order. Global Ecology and Biogeography 15:1-7.

Hubbs C.L. 1941. The relation of hydrological conditions to speciation in fishes, In: Needham J.G., Sears P.B., Leopold A., editors. A symposium on hydrobiology. University of Wisconsin Press, Madison, pp 182–95.

Keeley, E.R., E.A. Parkinson, and E.B. Taylor. 2007. The origins of ecotypic variation of rainbow trout: a test of environmental vs. genetically based differences in morphology. Journal of Evolutionary Biology 20:725-736.

Koehn, R.K. 1965. Development and ecological significance of nuptial tubercles of the red shiner: *Notropis lutrensis*. Copeia 1965:462-467.

Langerhans, R.B., C.A. Layman, A.K. Langerhans, and T.J. DeWitt. 2003. Habitat-associated morphological divergence in two Neotropical fish species. Biological Journal of the Linnaean Society 80:689-698.

Langerhans, R.B., C.A. Layman, A.M. Shokrollahi, and T.J. DeWitt. 2004. Predator-driven phenotypic diversification in *Gambusia affinis*. Evolution 58:2305-2318.

Langerhans, R.B. 2008. Predictability of phenotypic differentiation across flow regimes in fishes. Integrative and Comparative Biology 48:750-768.

Langerhans, R.B. 2009. Trade-off between steady and unsteady swimming underlies predator driven divergence in *Gambusia affinis*. Journal of Evolutionary Biology 22:1057-1075.

Langerhans, R.B., C.A. Layman, A.M. Shokrollahi, and T. J. DeWitt. 2009. Predator-driven phenotypic diversification in *Gambusia affinis*. Evolution 58:2305-2318.

Langerhans, R.B. and A.M. Makowicz. 2009. Shared and unique features of morphological differentiation between predator regimes in *Gambusia caymanensis*. Journal of Evolutionary Biology 22:2231-2242.

Losos, J.B., D.A. Creer, D. Glossip, R. Goellner, A. Hampton, G. Roberts, N. Haskell, P. Taylor, and J. Ettling. 2000. Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard *Anolis sagrei*. Evolution 54:301-305.

Marsh-Matthews, E., Matthews, W.J., K.B. Gido, and R.L. Marsh. 2002. Reproduction by young-of-year red shiner (*Cyprinella lutrensis*) and its implications for invasion success. Southwestern Association of Naturalists 47:605-610.

Matthews, W.J. 1987. Geographic variation of *Cyprinella lutrensis* (Pisces: Cyprinidae) in the United States, with notes on *Cyprinella lepida*. Copeia 1987:616-637.

Matthews, W.J. and E. Marsh-Mathews. 2007. Extirpation of red shiner in direct tributaries of lake Texoma (Oklahoma-Texas): a cautionary case history from a fragmented river-reservoir system. Transactions of the American Fisheries Society 136:1041-1062.

McGuigan, K.A., C.E. Franklin, C. Moritz, and M.W. Blows. 2003. Adaptation of rainbow fish to lake and stream habitats. Evolution 57:104-118.

Paller, M.H. 2005. The influence of biomanipulation on fish community development in a southeastern United States cooling reservoir. Hydrobiologia 539:69-81.

Pavey, S.A., J.L. Nielsen, R.H. Mackas, T.R. Hamon, and F. Breden. 2010. Contrasting ecology shapes juvenile lake-type and riverine Sockeye Salmon. Transactions of the American Fisheries Society 139:1584-1594.

Pimm, S.L., G.J. Russel, J.L. Gittleman, and T.M. Brooks. 1995. The future of biodiversity. Science 269:347-350.

Reznick, D.N., F.H. Shaw, F.H. Rodd, and R.G. Shaw. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticula*). Science 275:1934-1937.

Rincón, P.A., M. Bastir, and G.D. Grossman. 2007. Form and performance: body shape and prey-capture success in four drift-feeding minnows. Oecologia 152:345-355.

Rohlf, F. 2004a. tpsDig. version 2.1 [computer program], Department of Ecology and Evolution, State University of New York at Stony Brook. Available at: http://life.bio.sunysb.edu/morph/.

Rohlf, F. 2004b. tpsUtil. version 1.45 [computer program], Department of Ecology and Evolution, State University of New York at Stony Brook. Available at: http://life.bio.sunysb.edu/morph/.

Rohlf, F. 2004c. tpsRelw. version 1.46 [computer program], Department of Ecology and Evolution, State University of New York at Stony Brook. Available at:

http://life.bio.sunysb.edu/morph/.

Rohlf, F. 2004d. tpsRegr. version 1.36 [computer program], Department of Ecology and Evolution, State University of New York at Stony Brook. Available at: http://life.bio.sunysb.edu/morph/.

Schlichting, C.D., and M. Pigliucci. 1998. Phenotypic evolution: A reaction norm perspective. Sinauer Associates, Sunderland, Maryland.

Schneider, J.C., P.W. Laarman, and H. Gowing. 2000. Length-weight relationships. Chapter 17 *in* Schneider, James C. (ed.) 2000. Manual of fisheries survey methods II:with periodic updates. Michigan Department of Natural Resources, Fisheries Special Report 25, Ann Arbor.

Stockwell, C.A. and S.C. Weeks. 1999. Translocations and rapid evolutionary responses in recently established populations of western mosquitofish (*Gambusia affinis*). Animal Conservation 2:103-110.

Stockwell, C.A., A.P. Hendry, and M.T. Kinnison. 2003. Contemporary evolution meets conservation biology. Trends in Ecology and Evolution 18:94-101.

Taylor, C.A., J.H. Knouft, and T.M. Hiland. 2001. Consequences of stream impoundment on fish communities in a small North American drainage. Regulated Rivers: Research and Management 17:687-698.

Tobler, M., and E.W. Carson. 2010. Environmental variation, hybridization, and phenotypic diversification in Cuatro Ciénegas pupfishes. Journal of Evolutionary Biology 23:1475-1489.

Vitousek, P.M, H.A. Mooney, J. Lubchenco, and J.M. Melillo. 1997. Human domination of Earth's ecosystems. Science 277:492-499.

West-Eberhard, M.J. 1989. Phenotypic plasticity and the origins of diversity. Annual Review of Ecology and Systematics 20:249-278.

Zelditch, M.L., D.L. Swiderski, D.H. Sheets, and W.L. Fink. 2004. Geometric morphometrics for biologists. Academic press. San Diego, CA.

Table 1. Reservoir basin system (system ID in Appendix B) and specific site (site ID in Appendix B) data of *C. lutrensis* collected for geometric morphometric analysis to assess body shape divergence in reservoirs. Lot numbers of specimens obtained from the Sam Noble Oklahoma Museum of Natural History are indicated under Oklahoma Identification (OID).

Basin system	Year Impounded	Name of site	Year of Collection	N	Lattitude	Longitude	Distance (km)	OID
Canton (a)	1986	Canton Lake (1)	1992	18	36.0813	-98.6037		51521
		Horse Creek (2)	1993	13	35.6800	-98.3810	66	67178
Lake Arcadia (b)	1948	Lake Arcadia (3)	1993	25	35.6102	-97.4129		49306
		Deep Fork River (4)	1993	31	35.6720	-97.1947	30	47771
Grand Lake (c)	1959	Grand Lake (5)	1994	15	36.6278	-94.8642		48542
		Neosho River (6)	1993	9	36.8589	-94.8757	26	49865
Oogalah (d)	1940	Lake Oogalah (7)	1993	14	36.6615	-95.5989		48093
		Verdigre River (8)	1999	14	36.8401	-95.5910	28	61628
Fort Cobb (e)	1963	Fort Cobb (9)	1992	11	35.2319	-98.5179		53711
		Cobb Creek (10)	1998	10	35.2902	-98.5942	8	63626
Lake Texoma (f)	1944	Lake Texoma (11)	2007-2008	39	33.8794	-96.8021		
		Caddo Creek (12)	2008	9	34.2637	-97.1643	80	
		Walnut Bayou (13)	2008	16	33.9166	-97.2823	85	
Lake Thunderbird (g)	1965	Lake Thunderbird (14)	2007-2008	68	35.2318	-97.2133		
		Bourbanais Creek (15)	2008	19	35.1779	-97.1421	10	
		Clear Creek (16)	2007	10	35.1788	-97.2651	2	
		Council Creek (17)	2007	27	35.1569	-97.0895	19	
		Dave Blue Creek (18)	2007-2008	29	35.1895	-97.3470	4	
		Elm Creek (19)	2007	15	35.2908	-97.3488	7	
		Hog Creek (20)	2007	18	35.3193	-97.2496	2	
		Pecan Creek (21)	2007-2008	145	35.2031	-97.1179	12	

Table 2. Mixed model ANCOVA results testing for effects of habitat (stream or reservoir), Centroid size (allometry), reservoir basin of capture (Basin), and population nested within the basin × habitat interaction on morphological index scores from individuals collected from stream and reservoir habitats.

Test for	Partial variance (η^2_p)	Relative variance	Significance		
			F	df	p
Habitat	0.48	1.00	12.63	1, 13.99	0.001
Basin	0.46	0.96	2.03	6, 14.03	0.13
Centroid size	0.33	0.69	261.89	1, 529	<0.001
Pop (Basin × Habitat)	0.20	0.42	9.92	13, 529	<0.001

Table 3. Pearson correlation coefficients between superimposed landmarks and morphological index scores. Coefficients >0.40 are in bold and the directionality of the landmark shifts is presented for stream populations relative to reservoir populations (i.e., movement of landmarks reflect the shifts from stream populations to reservoir populations).

Landmark	Coefficient	Direction
X1	**+0.84**	Posterior
Y1	+0.20	—
X2	+0.33	—
Y2	**+0.65**	Dorsal
X3	-0.02	—
Y3	-0.11	—
X4	-0.13	—
Y4	-0.25	—
X5	+0.15	—
Y5	**+0.42**	Dorsal
X6	-0.11	—
Y6	+0.37	—
X7	-0.05	—
Y7	+0.32	—
X8	+0.25	—
Y8	-0.35	—
X9	-0.31	—
Y9	**-0.62**	Ventral
X10	**-0.76**	Anterior
Y10	-0.27	—

Table 4. Results from three mixed model ANOVAs testing for population level differences, treatment (predator or non-predator) and parental effects on PC scores of size corrected body shape variables (PC I, II, and III).

		Partial	Relative	Significance		
Dependant	Test for	variance (η^2_p)	variance	F	df	p
PC I (49.9 %)	Population	0.342	1.00	5.473	1, 10.52	0.040
	Parents (Population)	0.152	0.44	4.385	10, 244	<0.001
	Treatment	0.022	0.06	5.435	1, 244	0.021
PC II (17.4 %)	Parents (Population)	0.384	1.00	15.224	10, 244	<0.001
	Treatment	0.047	0.12	12.003	1, 244	0.001
	Population	0.240	0.63	3.205	1, 10.20	0.103
PC III (9.6 %)	Parents (Population)	0.305	1.00	10.685	10, 243	<0.001
	Treatment × Population	0.033	0.11	8.36	1, 243	0.004
	Treatment	0.006	0.02	1.505	1, 243	0.221
	Population	0.044	0.14	0.47	1, 10.20	0.508

Table 5. Results from repeated measures ANOVAs testing the effects of treatment (predator or non-predator) and population of origin (reservoir or stream) on PC scores of size corrected shape variables (PC I, II, and III).

Response	Source	Hypothesis df	Error df	p
PC I (49.9 %)	Treatment	1	10	0.146
	Population	1	10	0.013
	Treatment × Population	1	10	0.514
PC II (17.4 %)	Treatment	1	10	0.017
	Population	1	10	0.108
	Treatment × Population	1	10	0.987
PC III (9.6 %)	Treatment	1	10	0.507
	Population	1	10	0.351
	Treatment × Population	1	10	0.252

Figure 1. The 10 landmark locations set on *C. lutrensis* photographs for geometric morphometric analyses: 1) tip of the snout, 2) corner of the mouth, 3) center of the eye, 4) back of the skull, 5) anterior insertion of the dorsal fin, 6) insertion of the last dorsal ray on the caudal fin, 7) insertion of the last ventral ray on the caudal fin, 8) anterior insertion of the anal fin, 9) anterior insertion of the pelvic fin, and 10) anterior insertion of the pectoral fin.

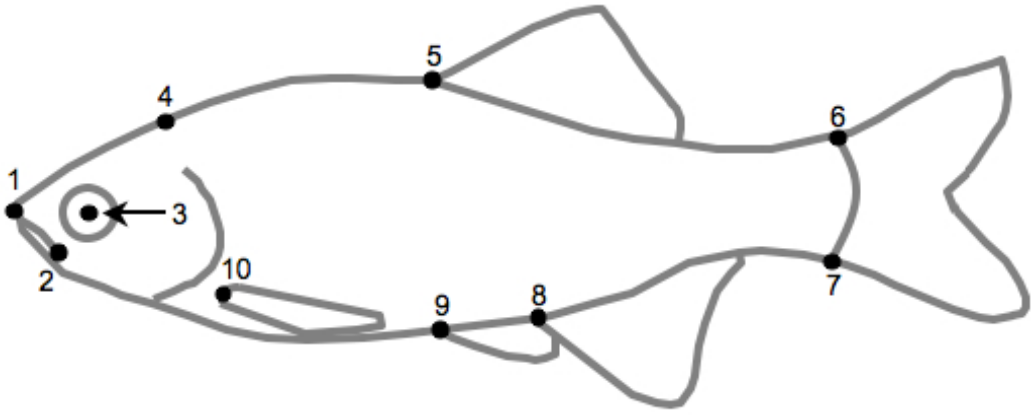


Figure 2. Morphological variation in *C. lutrensis* between stream and reservoir habitats. Grids are thin-plate spline transformations from specimen means along the morphological index at the observed scale. Vectors below transformations reflect the direction and magnitude (magnified 3 times to ease interpretation) of each landmark movement between habitats. Vectors point in the direction landmarks moved from stream habitats to reservoir habitats. Lines are drawn between landmarks to aid visualization.

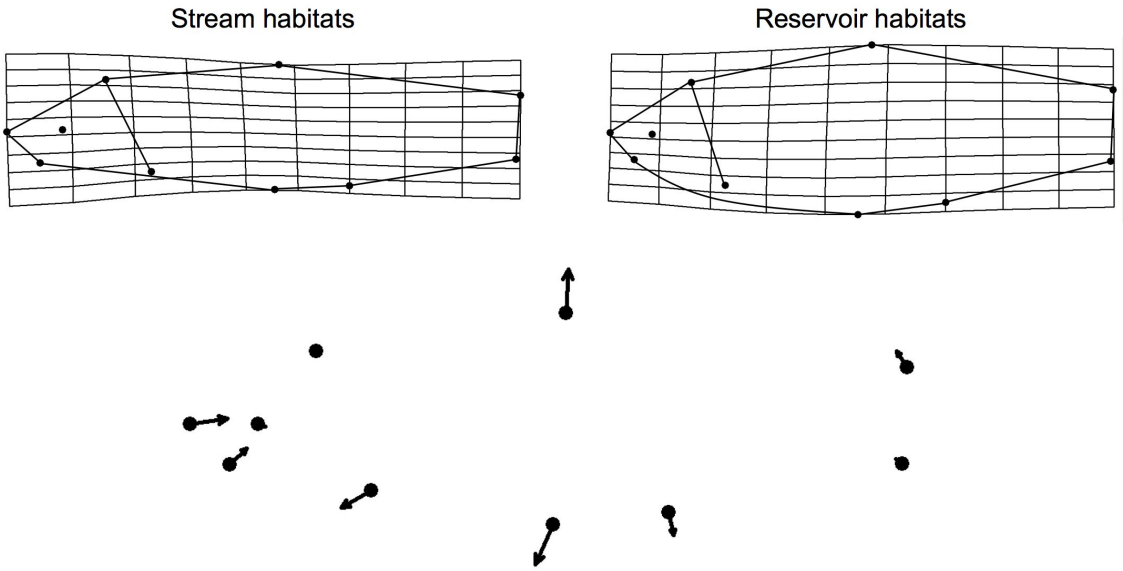


Figure 3. Mean±SE morphological index scores of stream populations (closed circles) and reservoir populations (open circles) from each reservoir basin. Stream sites from Lake Thunderbird and Lake Texoma are numbered according to Table 1.

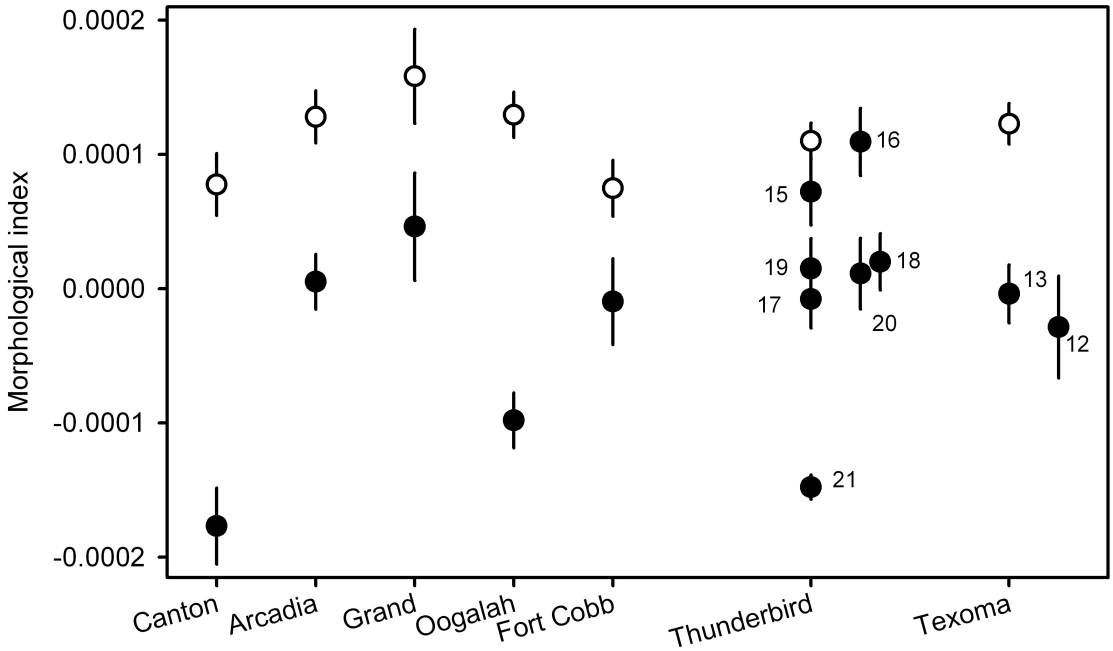


Figure 4. Relationship between the distance of stream populations from their respective reservoirs and the difference between reservoir and stream morphological index scores ($r^2 = 0.34$, $F_{1,6} = 6.22$, $p = 0.047$). Symbols indicate the different reservoir basins: Arcadia (●), Canton (○), Fort Cobb (▼), Grand (△), Oogalah (■), Texoma (□), and Thunderbird (◆).

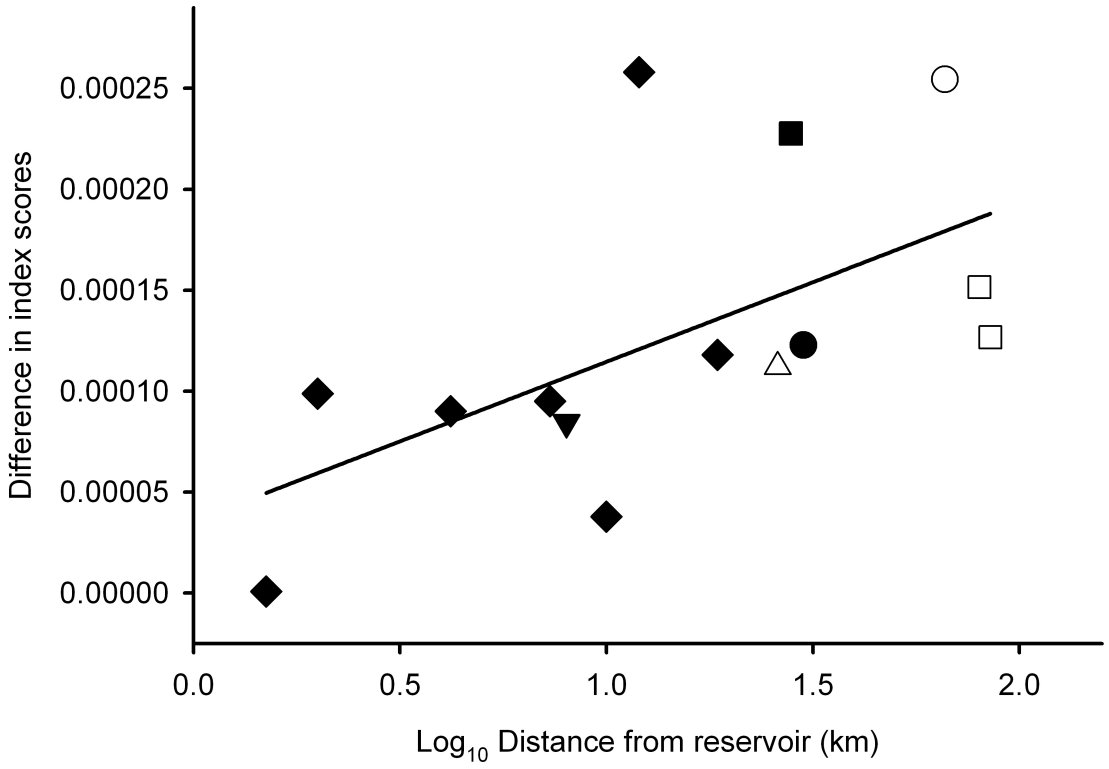


Figure 5. Morphological variation of *C. lutrensis* between stream population and reservoir population offspring, and between offspring reared with and with out a predator. Grids are thin-plate spline transformations from specimen means (observed range) between populations (above) and predator treatments (below). Vectors below transformations reflect the direction and magnitude (magnified 3 times to ease interpretation) of each landmark movement between populations and between predator and non-predator reared offspring. Vectors point in the direction landmarks move from the stream population to the reservoir population and from the non-predator reared offspring to the predator reared offspring. Lines are drawn between landmarks to aid visualization.

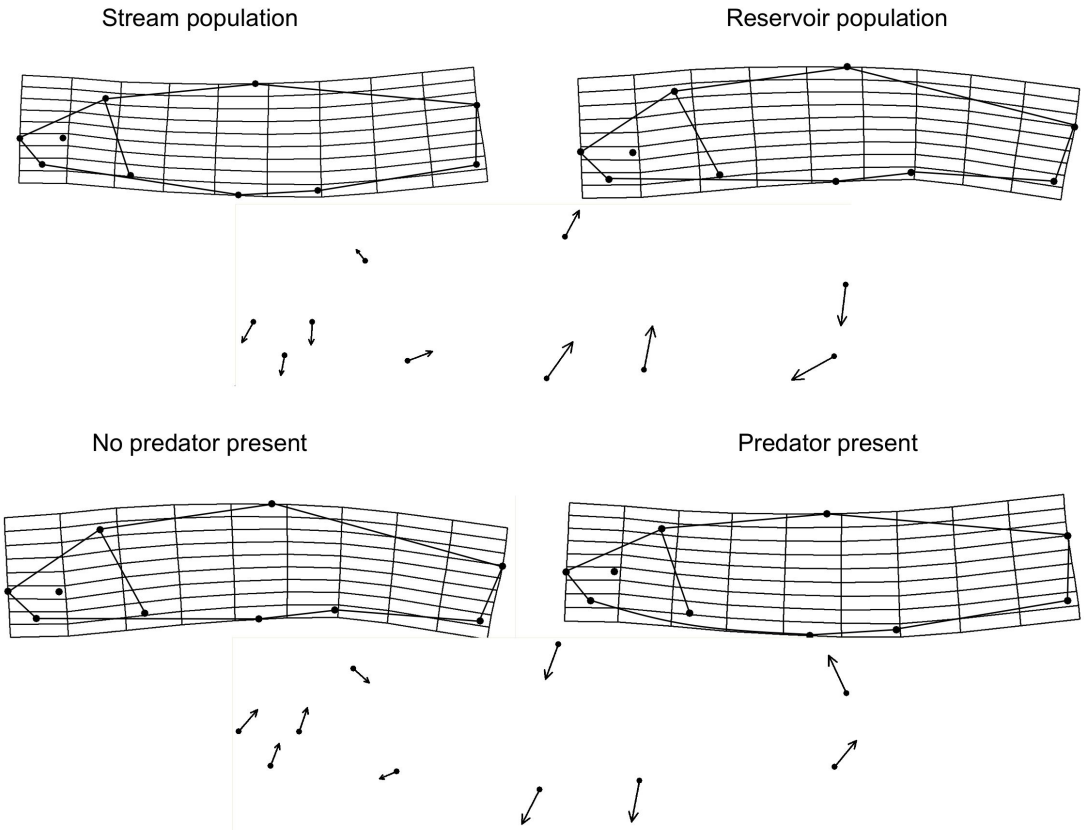
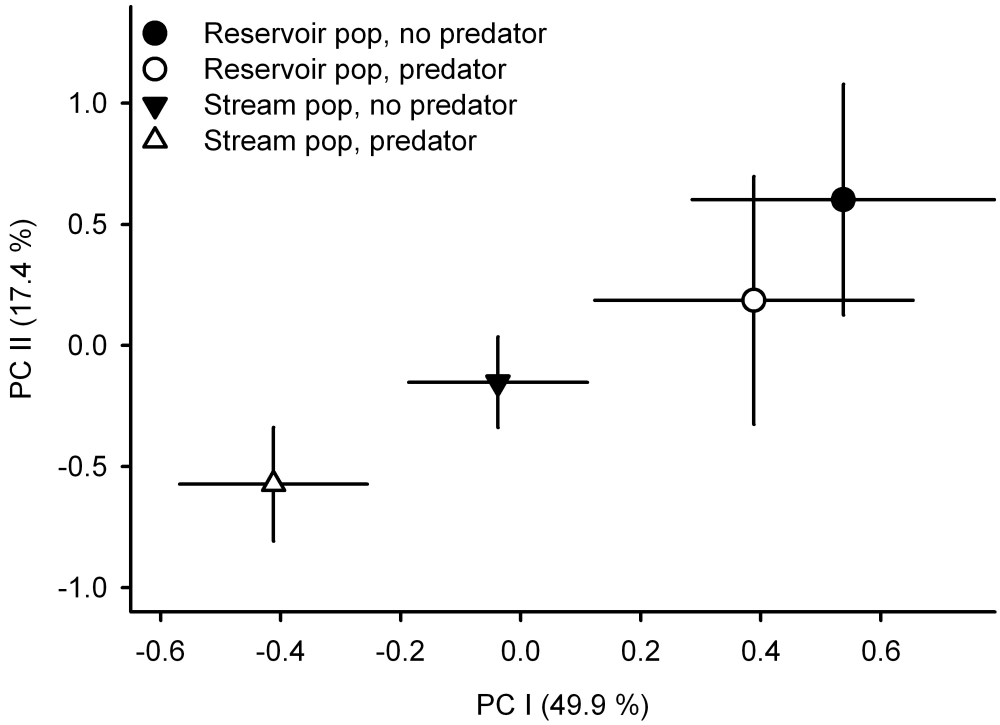


Figure 6. Mean±SE PC I and II scores of mean offspring from each parent-treatment combination (i.e., each mesocosm) of offspring spawned from a reservoir (n = 4 parents) and stream population (n = 8 parents) and reared in predator and non-predator treatments.



Appendix A: Spatial distribution of field collections.

Figure A1. Reservoir basins where body shape variation of *C. lutrensis* was assessed using geometric morphometrics. Letters next to reservoir basins correspond to letter IDs in Table 1.

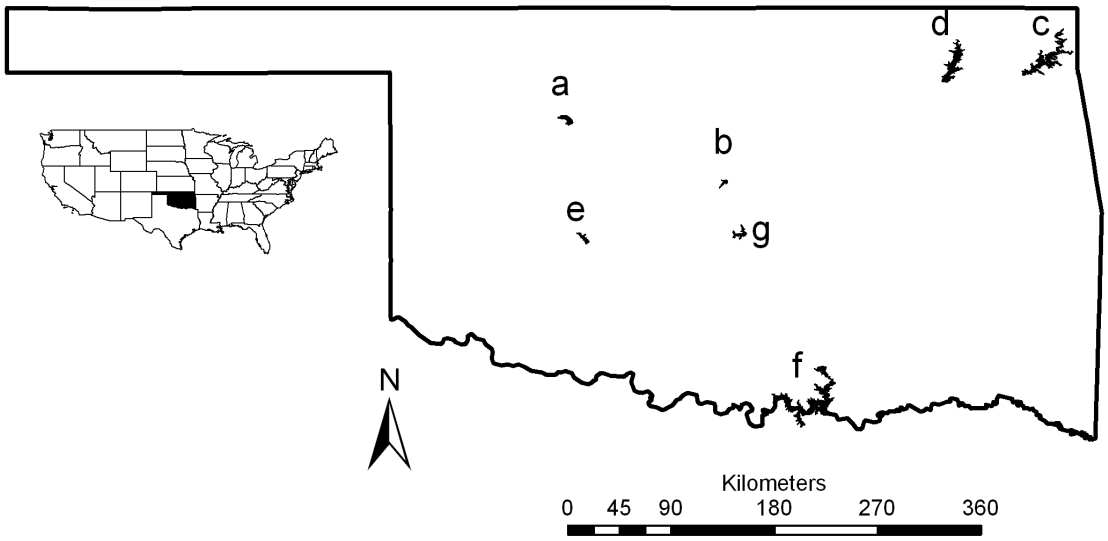
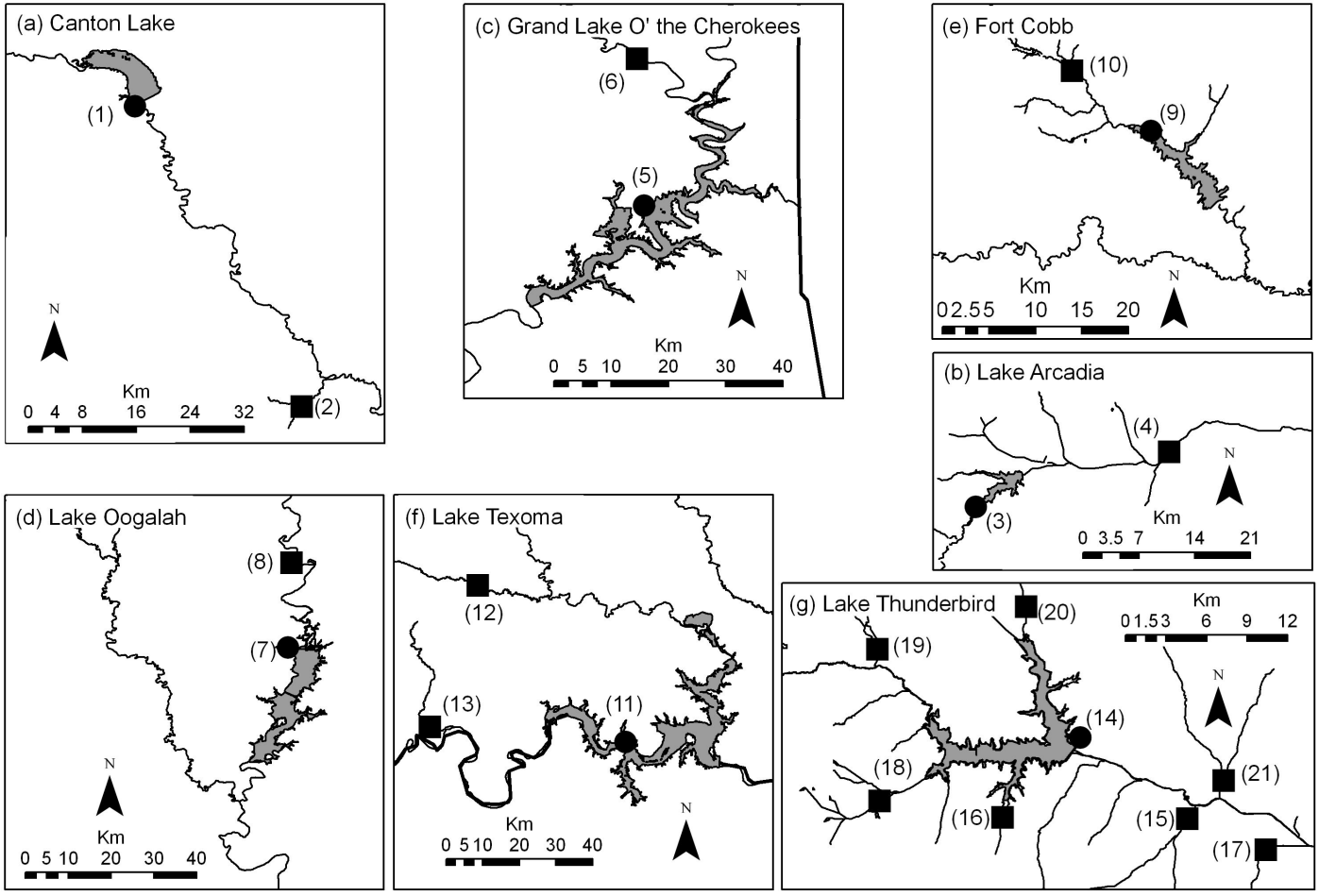


Figure A2. Sample locations of reservoir (filled circles) and stream populations (filled squares) within each basin. Reservoir basins are labeled with letters corresponding to letter IDs in Table 1 and Fig. A1. Sample sites are labeled with number IDs from Table 1.



Appendix B: Preservation effects on body shape variation

To assess the effects of type of preservative and time of preservation on shape variation of fish, 107 male *C. lutrensis* in breeding condition were collected from Pecan Creek near Lake Thunderbird, Oklahoma, USA on August 6, 2008 (Table 1). Fish were euthanized using MS222 and immediately preserved in 10% formalin solution and placed in individually numbered 177 ml glass jars. After 7 days in formalin, all individuals were photographed for geometric morphometric analyses. Formalin was then rinsed from 53 haphazardly selected individuals and replaced with tap water. The other 54 individuals were kept in 10% formalin. Three days later, the tap water was replaced with 50% isopropyl alcohol (a common museum preservative). All individuals were then photographed on September 22, 2008 (45 days after initial preservation) and November 18, 2009 (421 days after initial preservation).

Geometric morphometric and data analysis

All individuals (each photographed 3 times) were subjected to geometric morphometric analysis as described above (*Geometric morphometrics* section). To remove the effect of allometry on shape variation, unstandardized residuals were saved from a preparatory MANCOVA model with shape variables as dependant variables and centroid size as a covariate. A Principal Component Analysis (PCA) using a covariance matrix was then used to reduce the dimensionality of the shape variables. Only axes with eigenvalues > 1 were saved for analyses. To test the effects of preservation (50% isopropyl alcohol versus individuals kept in 10% formalin solution) and time on shape variation, separate repeated measures ANOVA

with each PC axis as the dependant variable were performed. The repeated measure was time (Day 7, 45, and 421) and the between subjects effect was type of preservation (i.e., formalin or isopropyl alcohol). When the assumption of sphericity was rejected (Mauchly's Test), degrees of freedom were adjusted using the Greenhouse-Geisser adjustment.

Results

Five PC axes explained 76% of the variation in shape. Repeated measures ANOVA indicated significant effects of time and preservation on shape (Table B1). Time had significant effects on all PC axes, type of preservative only had significant effects on the first PC axis, and the time-preservation interaction had significant effects on three PC axes (Table B1). Individuals placed in 50% isopropyl alcohol showed the greater divergence over time on the first PC axis compared to individuals retained in 10% formalin (Fig. B3).

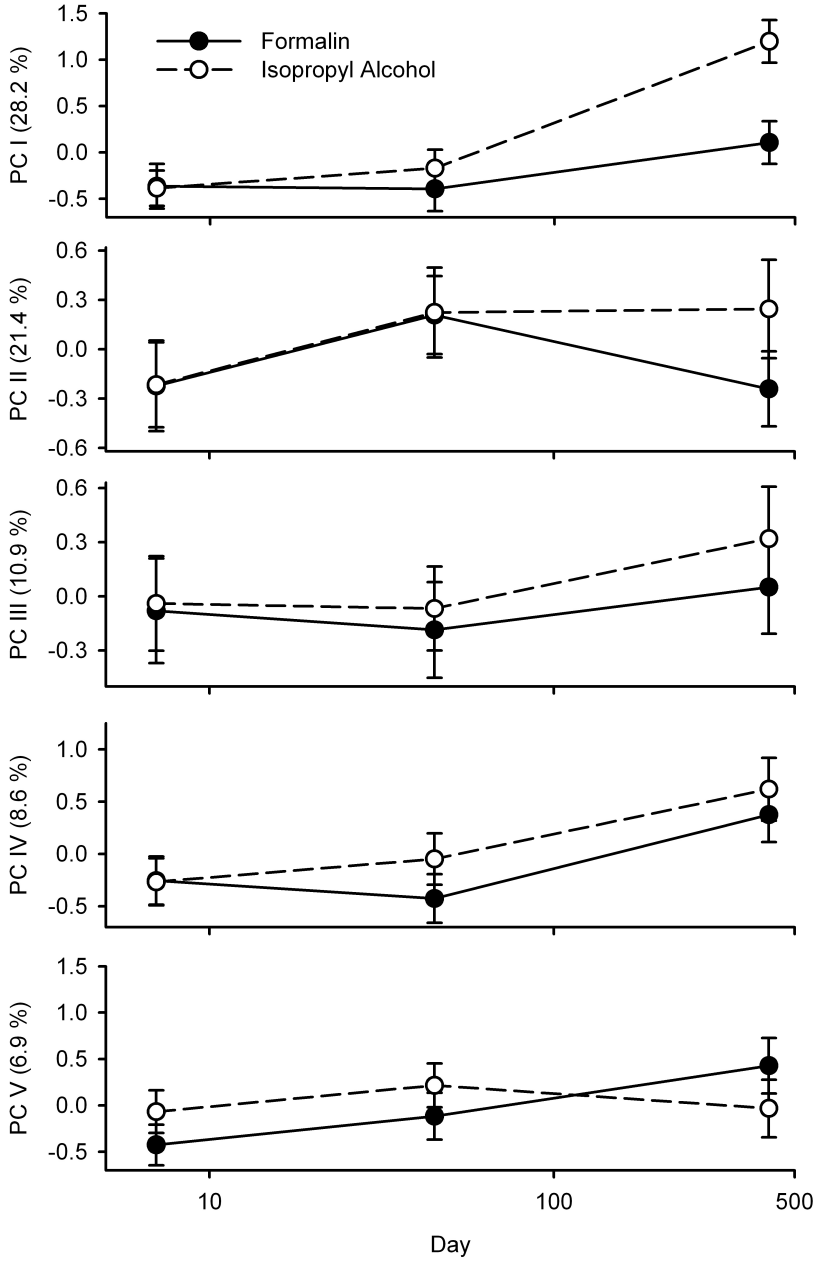
Conclusion

These results suggest time and type of preservation have significant effects on shape variation of *C. lutrensis*. Because type of preservation only had significant effects on the first PC axis and time had significant effects on all 5 PC axes, time since preservation likely has stronger effects on shape than type of preservation. Although museum specimens will undoubtedly be valuable assets for assessing shape variation using geometric morphometrics, one should be aware of potential variation in shape introduced into analyses attributable to effects of preservation and time.

Table B1. Results from 5 separate repeated measures ANOVA on each PC axis describing shape variation. Non-significant interactions are not presented. Error degrees of freedom with Greenhouse-Geiser adjustments are indicated with †.

Response	Source	Hypothesis df	Error df	P
PC I (28.2%)	time	1.65	172.72†	<0.001
	treatment	1	105	0.004
	time × treatment	1.65	172.72†	<0.001
PC II (21.4%)	time	1.68	176.20†	<0.001
	treatment	1	105	0.32
	time × treatment	1.68	176.20†	0.003
PC III (10.9%)	time	1.62	169.80†	0.001
	treatment	1	105	0.4
PC IV (8.6%)	time	2	210	<0.001
	treatment	1	105	0.136
PC V (6.9%)	time	1.66	173.98†	<0.001
	treatment	1	105	0.546
	time × treatment	1.66	173.98†	<0.001

Figure B3. Mean PC axes scores of size corrected shape variables of *C. lutrensis* preserved in 50 % isopropyl alcohol and individuals kept in 10 % formalin photographed 7, 45, and 421 days after initial preservation. Error bars are 95 % Confidence Intervals.



CHAPTER 3

ANNUAL VARIATION OF COMMUNITY BIOMASS IS LOWER IN MORE DIVERSE SREAM FISH COMMUNTIES

Nathan R. Franssen, Department of Zoology and Sam Noble Oklahoma Museum of Natural History, University of Oklahoma, Norman, OK

Michael Tobler, Department of Zoology, Oklahoma State University, Stillwater, OK

Keith B. Gido, Department of Biology, Kansas State University, Manhattan, KS

Formatted for Oikos

Abstract

Anthropogenic influences have disproportionally affected freshwater ecosystems, and a loss of biodiversity is forecasted to greatly reduce ecosystem function and services. Loss of species may destabilize communities by limiting the stabilizing forces of compensatory dynamics and/or statistical averaging, both of which are effects that can buffer variation in aggregate community properties. Currently, support for positive diversity-stability relationships stems from experiments with simple communities at small spatial and temporal scales, and application to natural communities is limited. Using a long-term dataset of 35 stream fish communities matched with hydrologic data, we show that community stability (annual variation of standing biomass of fishes) was less variable in more species-rich communities and was not associated with stream hydrology. Only the statistical averaging model of community stability was consistent with observed patterns of lower biomass variation in more species-rich communities. Our findings

suggest anthropogenically induced extirpation of vertebrate consumers may lower community biomass stability in complex ecosystems.

Introduction

Rapid loss of global biodiversity has prompted concern for the effects of reduced species richness on ecosystem stability and functioning (Naeem et al. 1994; Naeem and Li 1997; Loreau et al. 2001). The diversity-stability hypothesis predicts that higher species richness can reduce variation in community aggregate properties – such as biomass – through compensatory dynamics or statistical averaging; i.e., total community biomass remains relatively stable, whereas population biomass fluctuates over time (McNaughton 1977; Pimm 1984; Tilman and Downing 1994; Doak et al. 1998; Lehman and Tilman 2000). Experimental evidence from grassland and microbial communities suggests that increased species richness can indeed stabilize community aggregate properties and subsequently affect ecosystem-level processes (Cottingham et al. 2001; Balvanera et al. 2006; Cardinale et al. 2006; Hector et al. 2010). However, the effect of species richness on the stability of consumer communities (e.g., primary and secondary consumers) has received less attention, despite the fact that consumer biomass at least partially regulates energy availability to both higher trophic levels and decompositional components of food webs.

Although a positive relationship between species richness and community stability is supported by evidence from simple communities in field and laboratory experiments conducted at small spatial and temporal scales (Cardinale et al. 2002; Seabloom 2007; Jaing and Pu 2009), the influence of natural environmental

gradients on both species richness and stability has been largely ignored (Hooper et al. 2005; Hughes et al. 2007; Ives and Carpenter 2007). Because of potential nonlinear relationships between species richness and ecosystem function, extrapolation from small-scale empirical studies of simple communities to more complex community dynamics at larger spatial scales may be difficult (Emmerson et al. 2001; Hooper et al. 2005; Srivastava and Vellend 2005), and logistics often preclude manipulation of natural communities. Thus, observational studies have assessed relationships between species richness and community-level dynamics using natural ecological gradients or environmental variation (e.g., Frank and McNaughton 1991; Troumbis and Memtsas 2000; Valdivia and Molis 2009). Whereas directly testing mechanistic linkages between diversity and stability in observational studies may be limited, such studies can be used to evaluate relationships predicted from small-scale experiments at scales relevant to conservation (Hooper et al. 2005). Such studies are particularly needed in freshwater ecosystems (Dudgeon et al. 2006) and for species in higher trophic level communities (Petchey et al. 2004) that are disproportionately threatened by anthropogenic influences.

To assess the relationships between species richness and community stability in consumers, we investigated patterns between temporal fish community biomass stability (hereafter, community stability) and fish species richness using long-term community surveys at 35 sites in the central plains of North America (Appendix A). Because our fish communities were distributed over a large spatial scale (i.e., hundreds of kilometers), they naturally varied in species richness and composition,

and were exposed to gradients of stream size and flow conditions. Streams in this region are subjected to extreme flow conditions that can alter numerical abundances of fishes (Ross et al. 1985) and generally make these streams harsh environments (Dodds et al. 2004). Therefore, we tested for associations between species richness, hydrologic fluctuations, and temporal community stability.

We observed patterns to evaluate three potential, non-mutually exclusive mechanisms that could contribute to more species-rich communities being more stable: statistical averaging, overyielding, and covariance effects (Doak et al. 1998; Lehman and Tilman 2000; Cottingham et al. 2001). Statistical averaging (sensu portfolio effect; Tilman 1999) draws analogy with financial investments as relative fluctuations in a diversified portfolio are lower compared to a single or few investments. Statistical averaging effects would be supported if: 1) the temporal variance of species (s^2) scales with their mean biomass (m) with a constant (c) ($s^2 = cm^z$), such that $1 < z < 2$, and increases in strength as z approaches 2 (Doak 1998; Tilman et al. 1998) and, 2) sum variances decrease in more species-rich communities. In addition, increased biomass evenness among species would strengthen this effect by reducing the relative 'investment' in each species and increased synchrony of assets (i.e., species abundances) over time will weaken the strength of statistical averaging (Schindler et al. 2010). Accordingly, the lowered relative fluctuation in biomass of species-rich communities has been referred to as the 'insurance value' of species richness (Naeem and Li 1997). *Overyielding* occurs when total community biomass is increased while variation in total community biomass remains relatively constant. This may happen if higher species richness

increases overall niche occupancy, allowing more resources to be converted to biomass. For example, consider two communities over time: community A has 5 species with a mean annual biomass of 100 g per unit area and community B has 10 species with 200 g per unit area. If both communities demonstrate the same annual variation in biomass (e.g., standard deviation = 10 g), community B is relatively more stable than community A. The *overyielding effect* would be supported if mean community biomass increases as communities become more species rich while variability of biomass stays constant. Finally, the *covariance effect* stabilizes community biomass by reducing the covariance in biomass over time among species. Over time, the abundances of species can covary positively (species increase and decrease synchronously), neutrally (species increase and decrease randomly relative to each other), or negatively (species increase and decrease asynchronously, i.e., as species A increases, species B decreases proportionally). Higher species richness can increase niche overlap among community members, hence increasing competition and asynchronous species abundances over time. To support the *covariance effect,* total covariance (summed covariance between all species pairs) should be negatively associated with species richness. If increased species richness results in more asynchronous species fluctuations overtime, community stability may increase, however average species stability (population stability) may actually decrease in more species-rich communities.

In the present study, we tested for associations between species richness, environmental variability, and community and population stability. Overall, we show that the annual standing community biomass of more species-rich

communities was more stable over time and demonstrate that statistical averaging could explain this positive association.

Materials and Methods

Stream hydrology

To assess possible effects of variation in environmental conditions on community and population stability, we quantified stream hydrology using discharge data obtained from United States Geological Survey (USGS) gauging stations located at each sampling location (http://waterdata.usgs.gov/ok/nwis/rt; Appendix B). Specifically, we enumerated the magnitude, duration, and timing of extreme flow conditions to characterize stream hydrology using Indicators of Hydrologic Alteration (IHA) software, version 7, 2007 (Richter et al. 1996; Appendix C). Each site had sufficient data to quantify flow parameters for a minimum of 20 years as suggested by Richter et al. (1996; Appendix B). Parameters incorporated flow characteristics that potentially affect fishes over a wide range of temporal scales (i.e., days to years) and were chosen based on their potential influence on annual habitat availability (mean annual flow, number of zero flow days), variation and predictability of flow (annual Coefficient of Variation (CV) in flow, flow predictability, constancy/predictability), predictability of floods (percent of floods in 60 day period), flow constancy (base flow index), annual difference in extreme flows (date of minimum and maximum flow), and the rate of flow change during high flow events (rise rate, fall rate, number of reversals; Appendix C). The parameters chosen could affect fishes directly, e.g., through physical loss of habitat or disruption of reproductive efforts, and indirectly by

affecting resource availability (i.e., algae growth or abundance of aquatic invertebrates). Each parameter was appropriately transformed to approximate normality (Appendix C) and a Principal Components Analysis (PCA) based on a correlation matrix was used to summarize variation in flow conditions among sites. Only axes with eigenvalues above 1 were retained for analyses.

Fish communities

Fish communities were monitored at 35 sites located along 19 streams in Oklahoma, USA, between 1978 and 2008 (Appendix B). On average, each site was sampled 2.2 times a year for 21 years by Jimmie Pigg and Randy Parham of the Oklahoma Department of Environmental Quality (range = 12 – 23 yrs; Appendix B). Fishes were collected from wadable habitats with seines along ~200 m of shoreline for 1 hr during each sampling event. All specimens were preserved on site in 10% formalin and each species' mean annual biomass was quantified for each site to the nearest 10 mg. Because high or very low flow conditions during a given sampling event may have created potential sampling biases, annual fish community biomass was estimated by averaging species biomass from multiple collections each year (Appendix B). Only small-bodied species (< 200 mm maximum length; Miller and Robison 2004; Appendix D) were included in analyses because seines are inefficient at capturing adult large-bodied fishes.

We assumed sampling efficiency was constant across all years and sites, species did not vary in susceptibility of capture at each site, all species present at a site were captured, and species were not falsely reported as being present at a site. Sampling efficiency could potentially have been lower in larger streams, possibly

violating efficiency assumptions and artificially inflating community biomass variation at these sites. We quantitatively tested this possibility by including the effects of stream hydrology (which largely varied as a function of stream size, see Results below) as a possible driver of community stability (see Discussion below). Furthermore, lower sampling efficiency in larger, more species-rich streams would decrease community biomass stability in our dataset, a pattern opposite to theoretical expectations. Consequently, biomass stability in larger, more species-rich streams is likely under- not overestimated. Because of the geographic distances among collection locations, we also assumed fish populations varied independently among sites.

Species richness at each site was calculated as the mean number of fish species observed each year over the entire sampling period. To address potential sampling biases, we also scrutinized our species richness estimate at each site using individual-based rarefaction in EcoSim version 7 (Gotelli and Entsminger 2004). Estimates of rarefied species richness were based on 1000 individuals and 1000 iterations for each yearly collection and site. Two sites did not have any yearly collections of >1000 individuals and were dropped from the analysis. Log_{10} mean species richness and log_{10} mean rarefied species richness were highly correlated (Pearson correlation, $n = 33$; $r = 0.945$; $P < 0.001$), and the use of mean rarefied species richness opposed to mean number of actually observed species in subsequent analyses did not yield qualitatively different results (not shown). Based on a standardized sampling effort among sites, use of average biomass, and exclusion of large bodied fishes, our protocol represented an estimate of annual

standing biomass and temporal variation in biomass of small-bodied fishes from shallow, wadable habitats at each site.

Species richness of community/population stability

We defined community stability (S) at each site as the mean annual biomass relative to its standard deviation (μ/σ) over time (inverse of CV; Tilman 1999; Lehman and Tilman 2000; Tilman et al. 2006). Mean population stability for each community was calculated as the mean annual species biomass stability over the sampling period, averaged across all species in the community.

Separate stepwise multiple regressions were used to predict community stability and mean population stability using four independent variables: stream hydrology (PC axes I, II, and III) and species richness. Observed relationships between species richness and community stability may be confounded by correlations between species richness and environmental factors. Species richness often increases with stream size (i.e., flow variability, see stream hydrology results below; Angermeier and Schlosser 1989). Species richness positively and significantly correlated with stream hydrology (only the first PC axis, Pearson correlation, $n = 35$, $r = 0.37$, $P = 0.028$). We therefore interpreted partial correlation coefficients from variables that were not selected in the final models to assess their contribution to predicting each dependant variable.

Community stabilizing mechanisms

To test whether statistical averaging was driving a positive species richness-community stability relationship, a power function was fitted between each species' mean annual biomass (m) and their temporal variance of biomass (s^2). Because

statistical averaging effects will reduce the sum variance in more species-rich communities, we also tested this relationship using linear regression. Furthermore, because increased biomass evenness among species strengthens the effects of statistical averaging, we assessed the relationship between species richness and community evenness using linear regression. Mean community evenness was calculated from evenness of biomass among species during each year and site using Pielou's evenness index (J; Pielou 1966):

$$J = (H/H'),$$

where H is the Shannon diversity index and H' is the maximum possible H.

To assess potential overyielding effects on community stability, each community's species richness was regressed against mean total annual community biomass, whereby more diverse communities would be expected to have a higher total community biomass. Finally, to evaluate the covariance effect, sum covariance among species were made positive by adding a constant (60,000) to all data points, then square root transformed to approximate normality, and regressed against species richness. If competitive interactions were important in maintaining community stability, sum of covariance would be expected to become more negative as species richness increases.

Community structures

Because of high environmental variability and the mobile nature of fishes, species turnover within sites may be high. We tested whether species turnover in communities could be predicted with stream hydrology or species richness using stepwise linear regression. We assessed mean annual species turnover at each site

by quantifying presence/absence of species in each year and site (Diamond and May 1977; Meffe and Berra 1988). Species turnover was calculated as:

$$T = (C+E)/(S_1+S_2),$$

where T is species turnover, C is the number of species colonized, E is the number of species extirpated, and S_1 and S_2 are the number of species in each sample. T ranges from 0 (no turnover) to 1 (complete turnover).

To approximate normality, all variables were $\log_{10}$ transformed prior to analyses described above unless otherwise stated. Mean community turnover and evenness were arcsine-square-root transformed prior to analyses. All statistical analyses were performed in SPSS 16.0 (SPSS, Inc., Chicago, IL, USA). Sum covariances and sum variances were quantified using MATLAB 6.5 (The MathWorks, Natick, Massachusetts, USA).

Results

Stream hydrology

The first three PC axes explained 75.2 % of the variation in stream hydrology among sites (Appendix C). Along PC axis I (explaining 50.6 % of the variation), positive scores were associated with mean annual flow, date of minimum flow, and rise and fall rates; negative scores were associated with coefficient of variation in flow and number of zero flow days per year. The first PC axis predominantly reflected a gradient of stream discharge across our study area. In general, stream localities increased in size due to increased precipitation and greater drainage areas from west to east as they drain this region (Appendix E). There were no other obvious spatial correlations with the other two PC axes.

Species richness and community/population stability

In a regression model with stream hydrology (PC axes I, II, and III) and species richness as independent variables, species richness was the only variable retained in predicting community stability ($n = 35$, $F_{1,34} = 7.38$, $R^2 = 0.18$, $P = 0.01$; Fig. 1a). On average, total community biomass variation with 19 species was predicted to be 1.7 times less than the variation of biomass in communities with 5 species. None of the stream hydrology PC axes were significant enough to be included in the final model, but stream size (PC I) showed the strongest (and negative) association with community stability (Table 1). Conversely, PC I was the only variable retained when predicting population biomass stability ($n = 35$, $F_{1,34} = 23.59$, $R^2 = 0.42$, $P < 0.001$; Fig. 1b); population stability significantly declined in larger streams with less flow variability. Contrary to our prediction, the relationship between species richness and population stability was not significant (Table 1).

Community stabilizing mechanisms

When fitting a power function between population biomass (m) and variance in biomass (s^2), mean-variance scaled such that $z = 1.69$ ($n = 980$, $F_{1,977} = 39207.05$, $r^2 = 0.98$, $P < 0.001$; Fig. 2a). The power function fitted to the most abundant populations at each site (i.e., species that comprised at least 20% of the total biomass during any year and contributed the most biomass to communities) scaled by $z = 1.60$ ($n = 218$, $F_{1,216} = 1764.98$, $r^2 = 0.89$, $P <0.001$; Fig. 2b). Our data also indicated a significant inverse relationship between summed variances of species over time and species richness ($n = 35$, $F_{1,34} = 6.53$, $r^2 = 0.17$, $P = 0.015$; Fig. 2c). In

more species-rich communities, biomass was further spread out more evenly among species ($n = 35$, $F_{1,34} = 636$, $r^2 = 0.16$, $P = 0.017$; Fig. 3).

Mean annual community biomass did not increase in more species-rich communities ($n = 35$, $F_{1,34} = 2.09$, $P = 0.16$). In contrast, covariance among species significantly decreased in more species-rich communities ($n = 35$, $F_{1,34} = 12.23$, $r^2 = 0.27$, $P < 0.001$; Fig. 4a). However, only three of the 35 communities had negative sum covariance, and these communities were only moderately species rich (Fig. 4a).

Community structures

Stream hydrology presumably influenced the identity and persistence of species in each community during the sampling period. Community structure varied over time, and PC I was the only variable retained when predicting species turnover (i.e., higher species turnover in larger streams with less flow variability; $n = 35$, $F_{1,34} = 20.69$, $r^2 = 0.39$, $P < 0.001$; Fig. 5). Variables not selected in the final model only showed non-significant relationships with community turnover (Table 1.)

Discussion

Similar to other studies at smaller spatial and temporal scales, we found a positive relationship between species richness and community stability. In addition, community stability was not significantly related to stream hydrology. Conversely, population stability did not decrease in more species-rich communities, but varied as a function of stream size (PC I). Of the three possible mechanisms we investigated explaining the stabilizing effects of species richness on community stability, our data only supported the statistical averaging model.

Community stabilizing mechanisms

Several pieces of evidence suggest statistical averaging likely stabilized species-rich communities. First, the mean-variance scaling of population biomasses was greater than 1 both for all species and the most abundant species present at each site. Second, as predicted, sum variance of species decreased in more species-rich communities. Finally, as communities became more species-rich, biomass was distributed more evenly among species.

Because mean annual community biomass did not increase in more species-rich communities, the overyielding effect was not a major contributor in stabilizing community biomass. The overyielding effect is thought to occur when adding species increases the over-all niche space occupied and allows for conversion of more resources to biomass. However, the increase in richness across the species richness gradient was largely attributable to increases of species in the families Cyprinidae and Percidae (Fig. 6a). This phenomenon is common in freshwater fish communities, where species richness increases with additions of species in the same families rather than by adding species from new families (Fig. 6b; Matthews 1998). Because we assessed small-bodied fish communities and species of Cyprinidae and Percidae can be ecologically similar, the addition of species from the same family likely did not increase total niche occupancy as much as if species richness increased by additions of species from different families.

If the increased frequency of ecologically similar species (i.e., species in the same family, see Fig. 6b) in more species-rich communities increased competitive interactions, the decline in the sum covariance among species along the richness

gradient could have reduced the variation in annual community biomass in some communities. However, this was not a consistent trend across the species richness gradient, suggesting the covariance effect was not stabilizing communities. Only three of the 35 communities had a negative sum covariance, and the decline in sum covariance along the richness gradient was not due to more negative covariances but rather covariances nearing zero. Summed covariance nearing zero suggests populations in more species-rich communities did not increasingly covary as predicted if competitive interactions were strong, but to a certain extent fluctuated randomly relative to each other.

Competitive interactions for food resources, overall, may be relatively weak in this system because of where these fishes feed in the food web. The species investigated feed on invertebrates, algae, and detritus. Invertebrate production can be limiting for stream fishes, but food resources like detritus and algae are rarely, if ever, limiting (Moyle and Light 1996). Although we used a long-term data set, environmental conditions during this period could have been favorable for most species, and competitive interactions may have only been observed if communities had experienced "ecological crunches" (Weins 1977). If competitive or trophic interactions are relatively weak in this system (as suggested by weak interactions among the fishes investigated here), entire food webs may be stabilized similarly by low interaction strengths (McCann et al. 1998).

Although community stability was significantly associated with species richness, other environmental factors could have contributed to stabilizing species-rich communities. Species richness generally increases from west to east in

Oklahoma with the highest species richness occurring in the Ozark uplands in northeastern and the Ouachita mountain region and Red River in southeastern Oklahoma. Most urban centers in Oklahoma are found in central and north central regions of the state, with much lower population densities in east and southeast Oklahoma, which coincide with the highest fish species richness. Consequently, fish communities in these regions have likely experienced lesser habitat modifications compared to communities in central parts of the state. Therefore, community structures in these species-rich regions may have been kept more intact and experienced fewer human induced disturbances compared to more urbanized areas.

Effects of environmental variability and community structure

In spite of the fact that highly variable stream flows can increase the variation in the number of fish individuals present at a given site over time (Ross et al. 1985; Oberdorff et al. 2001), we found no evidence of stream flow variability influencing the variation in total biomass of fishes. This is perhaps because the streams we investigated were larger and less variable in annual flow compared to previously studied systems. Oberdorff et al. (2001) showed increased variation (CV) in several metrics of fish densities and population sizes in relation to increased CV of annual discharge of streams. However, the CV of annual discharge of our stream sites ranged from 1.3 to 12.09, far below the range of CV (roughly 30 - 75) in annual discharge Oberdorff et al. (2001) observed. Indeed, fishes subject to higher relative flow variability in smaller and especially intermittent streams experience more environmental stressors (e.g., low/high temperature, low oxygen,

drying) compared to larger streams with more stable flows (Schlosser 1990). In our system, stream hydrology likely had little influence on the variation in community biomass because of the relative contribution of stream flow versus other abiotic (e.g., habitat availability/suitability) or biotic (e.g., species richness) drivers of community biomass variation.

Although population stability did not decrease in more species-rich communities, relationships between population stability and stream hydrology and species turnover confounded this observation. Population biomass stability significantly decreased in larger and less variable streams. The negative association between stream size and flow variability is attributable to the averaging of tributary inflows in larger streams. We suggest the variation in population biomass over time could have been inflated in larger streams for several reasons. First, population stability could be reduced if species—albeit present—were more inconsistently collected in larger streams on a year-by-year basis. Because only wadable habitats were sampled, collection efficiency was likely higher in smaller streams with less deep-water habitats compared to larger streams. Larger streams also usually provide more heterogeneous habitats that may not have been represented in the 200 m of stream shore sampled at each site. Second, higher species turnover in larger and less variable streams could be attributable to vagrant species emigrating and immigrating in sample reaches. Lastly, because sampling occurred over a relatively long time period (i.e., up to 30 yrs), the lower population stability in larger streams may reflect long-term alteration of community structure by habitat modification. Because of the hierarchical nature of stream networks, downstream reaches likely

suffer accumulative effects of impoundments and water withdrawal in upper reaches. Although we cannot be certain, sampling efficiency could have been more variable in larger streams, thus lowering population stability in these habitats. Indeed, population and community stability were positively correlated (Pearson Correlation, $r = 0.55$, $P = 0.001$), and thus more variable sampling efficiency in larger streams possibly introduced artificial variation in community biomass in these habitats (evidenced by the non-significant negative trend between PC I and community stability; Table 1). If sampling efficiency reduced community stability in larger, more species-rich streams (species richness and PC I were slightly correlated), our estimate of community stability may actually represent a conservative estimate. Therefore, even if potential sampling efficiency biases affected stabilizing effects of species richness on annual variation in community biomass, we have likely underestimated the effects of species richness on community biomass stability in this system.

Community stability and conservation

Although consumer community stability across sites was relatively low compared to communities of primary producers (Tilman et al. 2006), the predicted insurance value of species in these communities suggests species richness can increase the constancy of annual biomass in higher trophic levels. Species richness effects on community biomass stability in higher trophic levels would be especially important for ecosystems with inverted biomass pyramids (e.g., lakes, marine systems; Odum 1971). Indeed, biomass available to detritivores and top predators can at least partially depend on the biomass of consumers, such as the fishes

investigated here. The trophic ecology of most freshwater fishes and their role in ecosystem processes are largely unknown. However, top down effects of fishes can physically structure habitats for aquatic invertebrates and influence primary production (Gelwick and Matthews 1992; Power 1992; Flecker and Taylor 2004). Fishes also can act as energy conduits to higher trophic levels (Steinmetz et al. 2003) and have profound effects on nutrient cycling in aquatic ecosystems (McIntyre et al. 2007; Schindler 2007; McIntyre et al. 2008).

The long-term effects of anthropogenically induced extirpations and altered community structures are unknown, but will likely have unforeseen consequences to ecosystem processes (Tilman et al. 1994). Based on the data presented here, species richness may influence the constancy of annual vertebrate community biomass, but species richness in this region and other aquatic systems will likely continue to be threatened by water withdrawal and development (Poff et al. 2007). Because statistical averaging effects were likely responsible for stabilizing communities in this system, these stabilizing effects could be reduced by not only by extirpation of species, but also by altered community structures (e.g., lower evenness). Although the ecosystem level effects of these fishes are relatively unknown, our data suggest species richness has community stabilizing effects at scales that are relevant to conservation.

Acknowledgements

This research was supported by funding from the Sam Noble Oklahoma Museum of Natural History (N.R.F.), and the Swiss National Science Foundation (M.T.). We thank J. Pigg and R. Parham and the numerous personnel involved in conducting field collections. A. Joern, E. Marsh-Matthews, W. Matthews, J.W. Moore, C. Vaughn, K. Winemiller, Ecomunch, ZEEB discussion group members at the University of Oklahoma, and anonymous reviewers made suggestions that greatly improved the manuscript.

Literature Cited

Angermeier, P. L and Schlosser, I. J. 1989. Species-area relationships for stream fishes. – Ecology 70: 1450–1462.

Balvanera, P. et al. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. – Ecol. Lett. 9: 1146–1156.

Cardinale, B. J. et al. 2002. Species diversity enhances ecosystem functioning through interspecific facilitation. – Nature 415: 426–429.

Cardinale, B. J. et al. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. – Nature 443: 989–992.

Cottingham, K. L. et al. 2001. Biodiversity may regulate the temporal variability of ecological systems. – Ecol. Lett. 4: 72–85.

Diamond, J. M. and May, R. M. 1977. Species turnover rates on islands: dependence on census interval. – Science 197: 266–270.

Doak, D. F. et al. 1998. The statistical inevitability of stability-diversity relationships in community ecology. – Am. Nat. 151: 264–276.

Dodds, W. K. et al. 2004. Life on the edge: the ecology of great plains prairie streams. – Bioscience 54: 205–216.

Dudgeon, D. et al. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. – Biol. Rev. 81: 163–182.

Emmerson, M. C. et al. 2001. Consistent patterns and the idiosyncratic effects of biodiversity in marine ecosystems. – Nature 411: 73–77.

Flecker, A. S. and Taylor, B. W. 2004. Tropical fishes as biological bulldozers: density effects on resource heterogeneity and species diversity. – Ecology

85: 2267–2278.

Frank, D. A. and McNaughton S. J. 1991. Stability increases with diversity in plant communities: empirical evidence from the 1988 Yellowstone drought. – Oikos 62: 360–362.

Gelwick, F. P. and Matthews, W. J. 1992. Effects of an algivorous minnow on temperate stream ecosystem properties. – Ecology 73: 1630–1645.

Gotelli, N. J. and G. L. Entsminger. 2004. EcoSim: null models software for ecology. Version 7. Acquired Intelligence Inc. & Kesey-Bear. Jericho, VT 05465. http://garyentsminger.com/ecosim/index.htm.

Hector, A. et al. 2010. General stabilizing effects of plant diversity on grassland productivity through population asynchrony and overyielding. – Ecology 91: 2213–2220.

Hooper, D. U. et al. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. – Ecol. Monogr. 75: 3–35.

Hughes, A. R. et al. 2007. Reciprocal relationships and potential feedbacks between biodiversity and disturbance. – Ecol. Lett. 10: 849–864.

Ives, A. R. and Carpenter, S. R. 2007. Stability and diversity of ecosystems. – Science 317: 58–62.

Jiang, L. and Pu, Z. 2009. Different effects of species diversity on temporal stability in single-trophic and multitrophic communities. – Am. Nat. 174: 651–659.

Lehman, C. L. and Tilman, D. 2000. Biodiversity, stability, and productivity in competitive communities. – Am. Nat. 156: 534–552.

Loreau, M. et al. 2001. Ecology - biodiversity and ecosystem functioning: current

knowledge and future challenges. – Science 294: 804–808.

Matthews, W. J. 1998. Patterns in freshwater fish ecology. – Chapman and Hall, Norwell, MA.

McCann, K. et al. 1998. Weak trophic interactions and the balance of nature. – Nature 395: 794–798.

McIntyre, P. B. et al. 2007. Fish extinctions alter nutrient recycling in tropical freshwaters. – Proc. Natl. Acad. Sci. USA 104: 4461–4466.

McIntyre, P. B. et al. 2008. Fish distributions and nutrient cycling in streams: can fish create biogeochemical hotspots? – Ecology 89: 2335–2346.

McNaughton, S. J. 1977. Diversity and stability of ecological communities: a comment on role of empiricism in ecology. – Am. Nat. 111: 515–525.

Meffe, G. K. and Berra, T. M. 1988. Temporal characteristics of fish assemblage structure in an Ohio stream. – Copeia 1988: 684–691

Miller, R. J. and Robison, H. W. II. 2004. The fishes of Oklahoma. – Univ. of Oklahoma Press.

Moyle, P. B. and Light, T. 1996. Biological invasions of fresh water: empirical rules and assembly theory. – Biol. Conserv. 78: 149–161.

Naeem, S. and Li, S. B. 1997. Biodiversity enhances ecosystem reliability. – Nature 390: 507–509.

Naeem, S. et al. 1994. Declining biodiversity can alter the performance of ecosystems. – Nature 368: 734–737.

Oberdorff, T. et al. 2001. Is assemblage variability related to environmental variability? An answer for riverine fish. – Oikos 93: 419–428.

Odum, E. P. 1971. Fundamentals of ecology. 3rd ed. Philadelphia: W.B. Saunders Company.

Petchey, O. L. et al. 2004. Species loss and the structure and functioning of multitrophic aquatic systems. – Oikos 104: 467–478.

Pielou, E. C. 1966. The measurement of diversity in different types of biological collections. – Jour. Theor. Biol. 13: 131–144.

Pimm, S. L. 1984. The complexity and stability of ecosystems. – Nature 307: 321–326.

Poff, N. L., et al. 2007. Homogenization of regional river dynamics by dams and global biodiversity implications. – Proc. Natl. Acad. Sci. USA 104: 5732–5737.

Power, M. E. 1992. Habitat heterogeneity and the functional significance of fish in river food webs. – Ecology 73: 1675–1688.

Richter, B. D. et al. 1996. A method for assessing hydrologic alteration within ecosystems. – Conserv. Biol. 10: 1163–1174.

Ross, S. T. et al. 1985. Persistence of stream fish assemblages: effects of environmental change. – Am. Nat. 126: 24–40.

Schindler, D. E. 2007. Fish extinctions and ecosystem functioning in tropical ecosystems. – Proc. Natl. Acad. Sci. USA 104: 5707–5708.

Schindler, D. E. et al. 2010. Population diversity and the portfolio effect in an exploited species. – Nature 465: 609–613.

Schlosser, I. J. 1990. Environmental variation, life history attributes, and community structure in stream fishes: implications for environmental management and

assessment. – Environ. Manage. 14: 621–628.

Seabloom, E. W. 2007. Compensation and the stability of restored grassland communities. – Ecol. App. 17: 1876–1885.

Srivastava, D. S. and Vellend, M. 2005. Biodiversity-ecosystem function research: is it relevant to conservation? – Annu. Rev. Ecol. Evol. S. 36: 267–294.

Steinmetz, J. et al. 2003. Birds are overlooked top predators in aquatic food webs. – Ecology 84: 1324–1328.

Tilman, D. 1999. The ecological consequences of changes in biodiversity: a search for general principles. – Ecology 80: 1455–1474.

Tilman, D. and Downing, J. A. 1994. Biodiversity and stability in grasslands. – Nature 367: 363–365.

Tilman, D. et al. 1994. Habitat destruction and the extinction debt. – Nature 371: 65–66.

Tilman, D. et al. 1998. Diversity-stability relationships: statistical inevitability or ecological consequence? – Am. Nat. 151: 277–281.

Tilman, D. et al. 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. – Nature 441: 629–632.

Troumbis, A. Y. and Memtsas D. 2000. Observational evidence that diversity may increase productivity in Mediterranean shrublands. – Oecologia 125: 101–108.

Valdivia, N. and Molis M. 2009. Observational evidence of a negative biodiversity-stability relationship in intertidal epibenthic communities. – Aquat. Biol. 4: 263–271.

Wiens, J. A. 1977. On competition and variable environments. – Am. Sci. 65: 590–597.

Table 1. Regression results of independent variables not included in the final model from stepwise regression predicting community stability, population stability, and community turnover using species richness and flow variability (PC I, II, and III) as independent variables. Each estimate is the standardized regression coefficient that would result if the variable were entered into the equation at the next step.

Dependant	Independent	Estimate	t	P	Partial r	Partial r^2
Community stability	PC 1	-0.312	-1.912	0.065	-0.320	0.102
	PC 2	-0.073	-0.433	0.668	-0.076	0.006
	PC 3	0.026	0.163	0.872	0.029	0.001
Population stability	Richness	0.239	1.717	0.096	0.290	0.084
	PC 3	-0.229	-1.776	0.085	-0.300	0.090
	PC 2	0.002	0.016	0.987	0.003	0.000
Community turnover	PC 2	-0.161	-1.179	0.247	-0.204	0.042
	PC 3	0.132	0.955	0.346	0.167	0.028
	Richness	-0.111	-0.743	0.463	-0.130	0.017

Figure 1**.** (a), Relationship between $\log_{10}$ species richness and $\log_{10}$ community stability (n = 35, $F_{1,34} = 7.38$, $R^2 = 0.18$, P = 0.01). (b), Relationship between stream hydrology (PC I) and $\log_{10}$ mean population stability (n = 35, $F_{1,34} = 23.59$, $R^2 = 0.42$, $P < 0.001$). Positive PC I scores associated with mean annual flow, date of minimum flow, and flow rise and fall rates; negative PC I scores associated with coefficient of variation of mean annual flow, and median number of days with no flow.

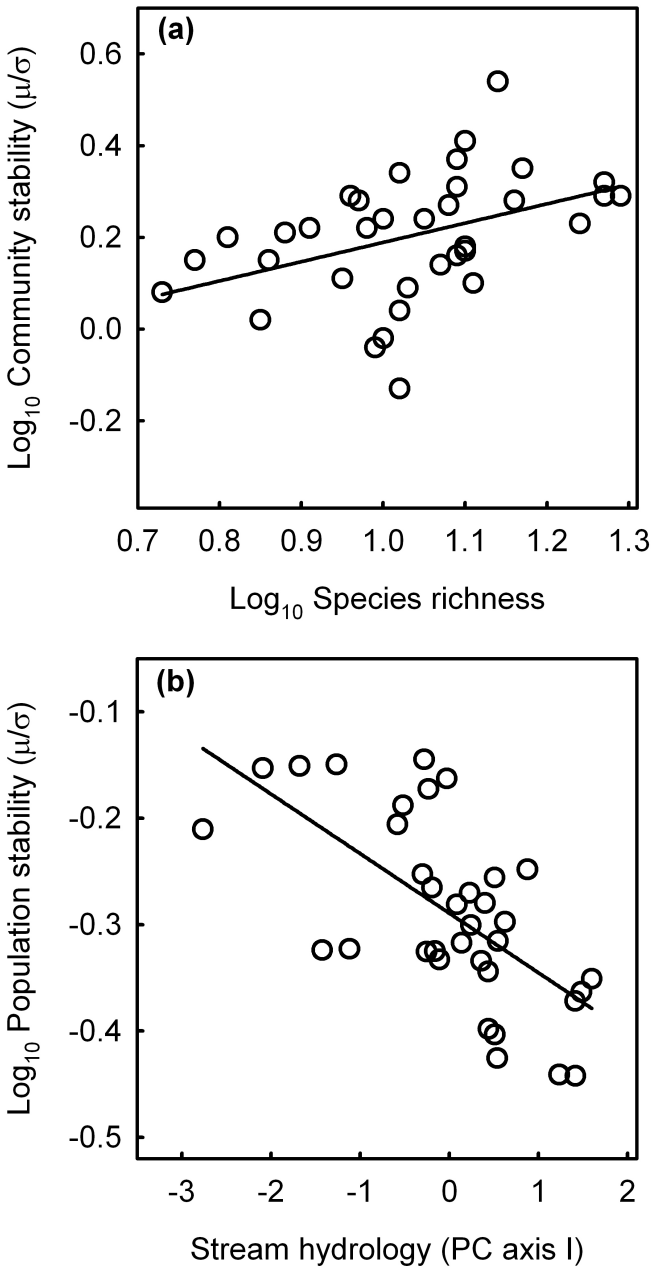


Figure 2. (a), Relationship between all species' mean annual biomass and their temporal variance ($s^2 = 5.95m^{1.69}$; n = 979, $F_{1,977} = 39207.05$, $r^2 = 0.98$, $P < 0.001$). The fitted lines are the power functions where z = 1 and z = 2 using the constant 5.95. Axes are $\log_{10}$ scaled to allow maximum separation of points. (b), Relationship between the most abundant species' mean annual biomass (i.e., species that comprised at least 20% of yearly biomass collections at each site) and their temporal variance ($s^2 = 8.70m^{1.60}$; n = 218, $F_{1,216} = 1764.98$, $r^2 = 0.87$, $P < 0.001$). Fitted lines are power functions where z = 1 and z = 2 using the constant 8.70. Axes are $\log_{10}$ scaled to allow maximum separation of points. (c), Relationship between species richness and sum variance of population biomass (n = 35, $F_{1,34} = 6.53$, $r^2 = 0.17$, $P = 0.015$).

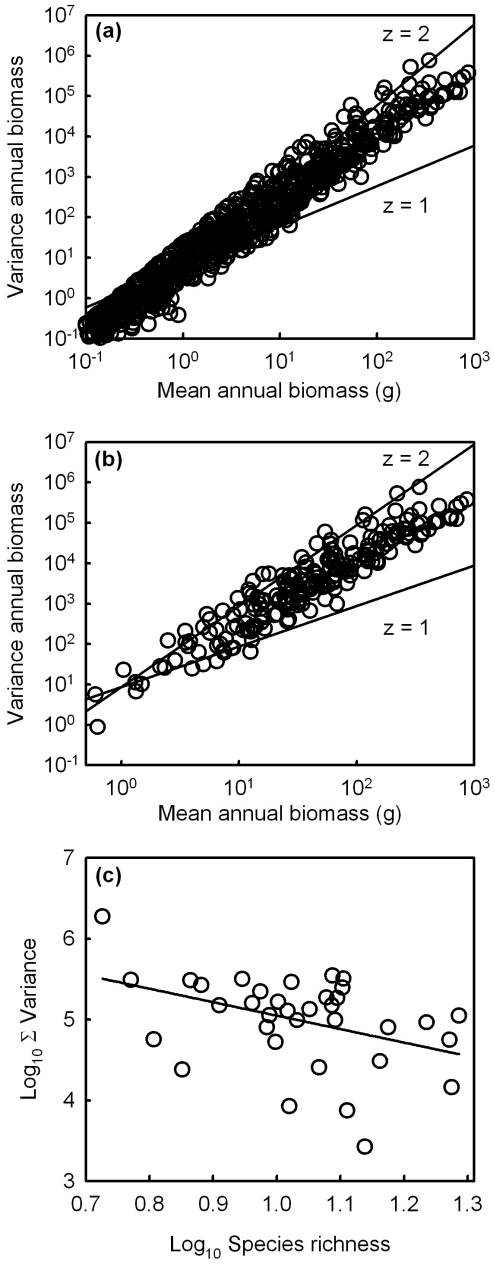


Figure 3. Relationship between $\log_{10}$ species richness and mean community evenness (J) (n = 35, $F_{1,34} = 6.36$, $r^2 = 0.16$, P = 0.017).

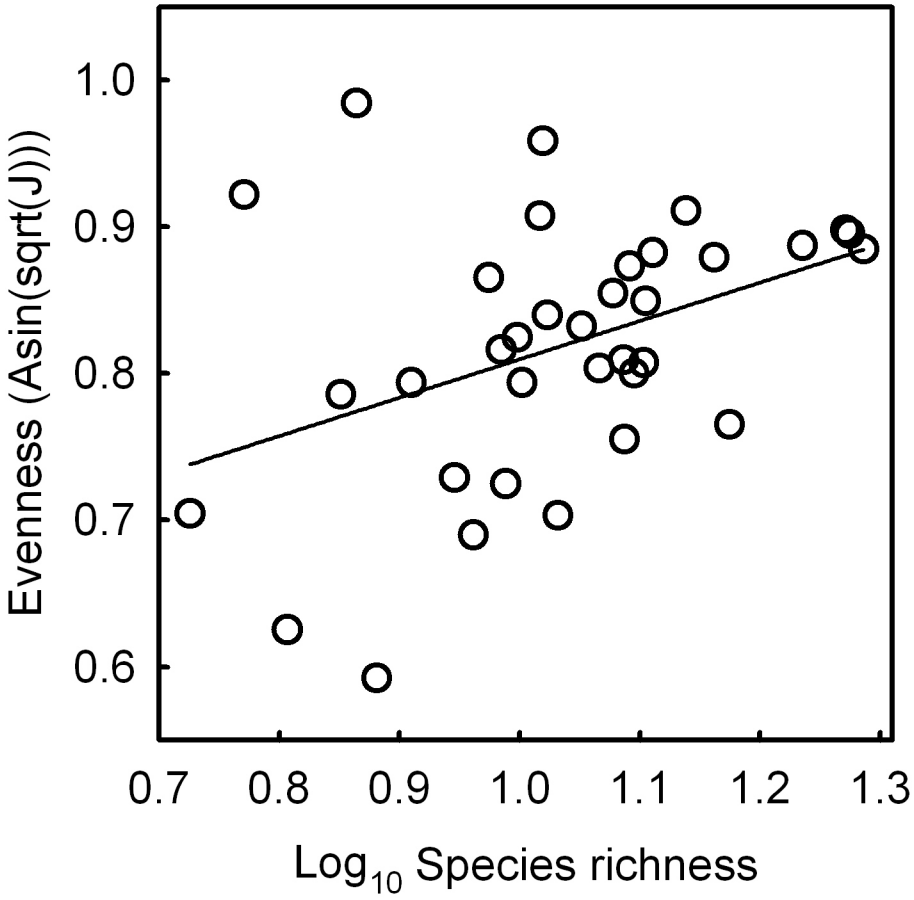


Figure 4. Relationship between $\log_{10}$ species richness and sum covariance (Square root(X + 60000)); (n = 35, $F_{1,34} = 12.23$, $r^2 = 0.27$, $P < 0.001$). The dashed line indicates zero covariance on untransformed data.

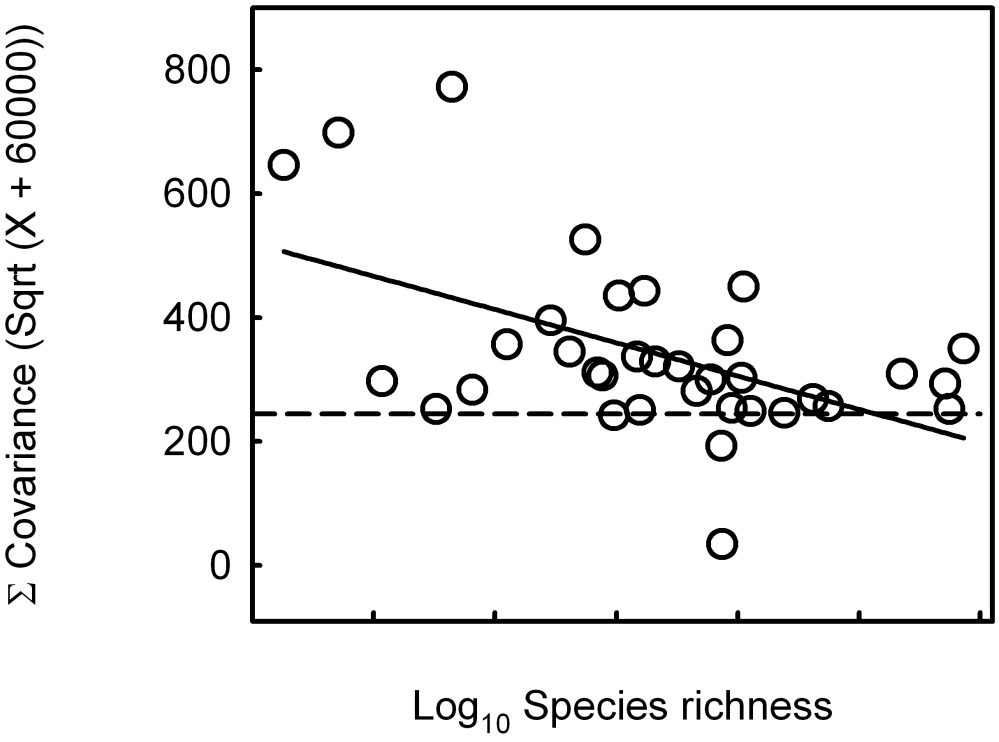


Figure 5. Relationship between stream hydrology (PC I) and mean species turnover in each community (n = 35, $F_{1,34} = 20.69$, $r^2 = 0.39$, $P < 0.001$). Positive PC I scores associated with mean annual flow, date of minimum flow, and flow rise and fall rates; negative PC I scores associated with coefficient of variation of mean annual flow, and median number of days with no flow.

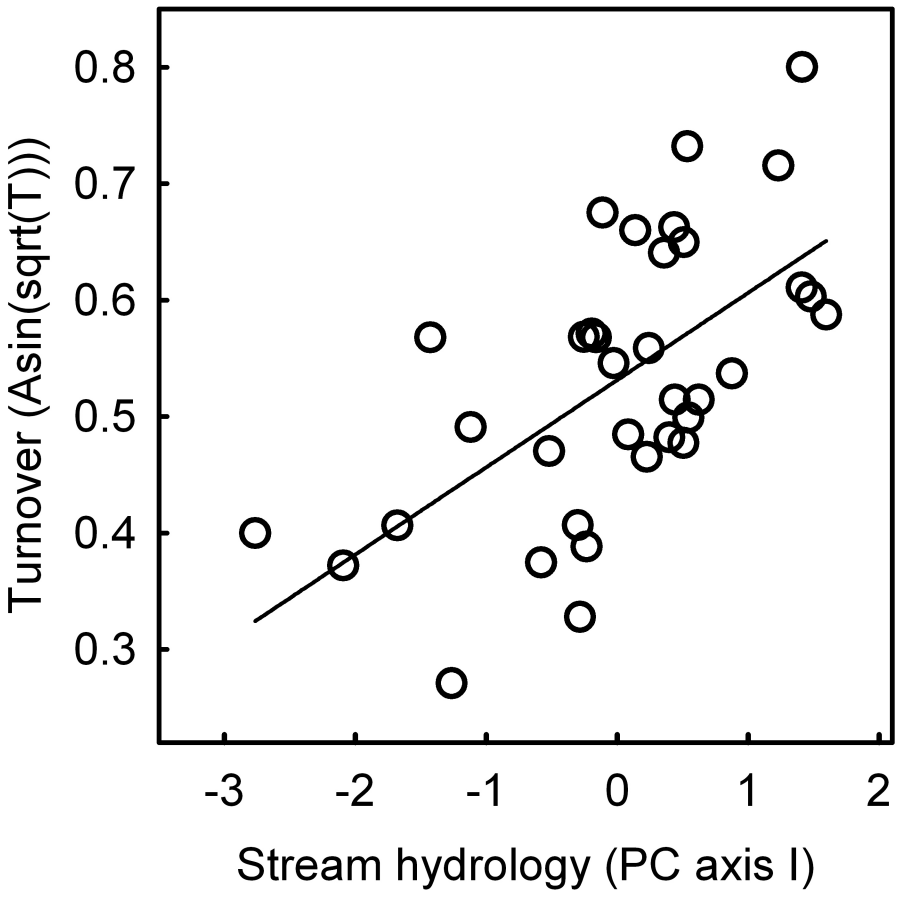
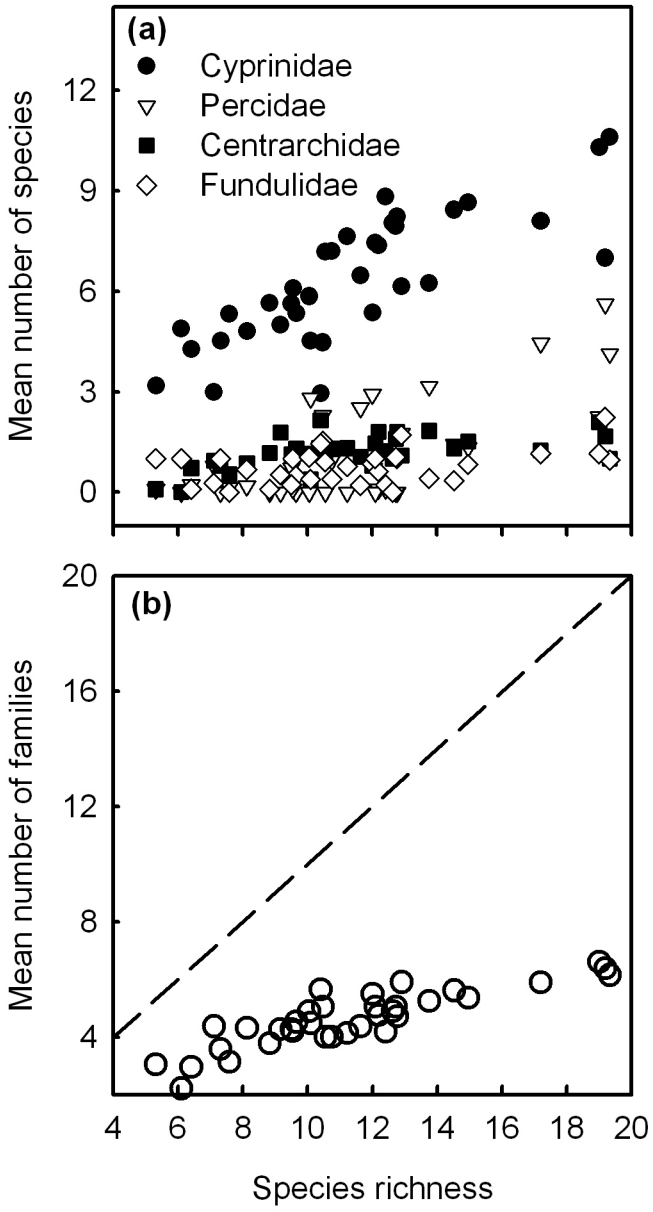
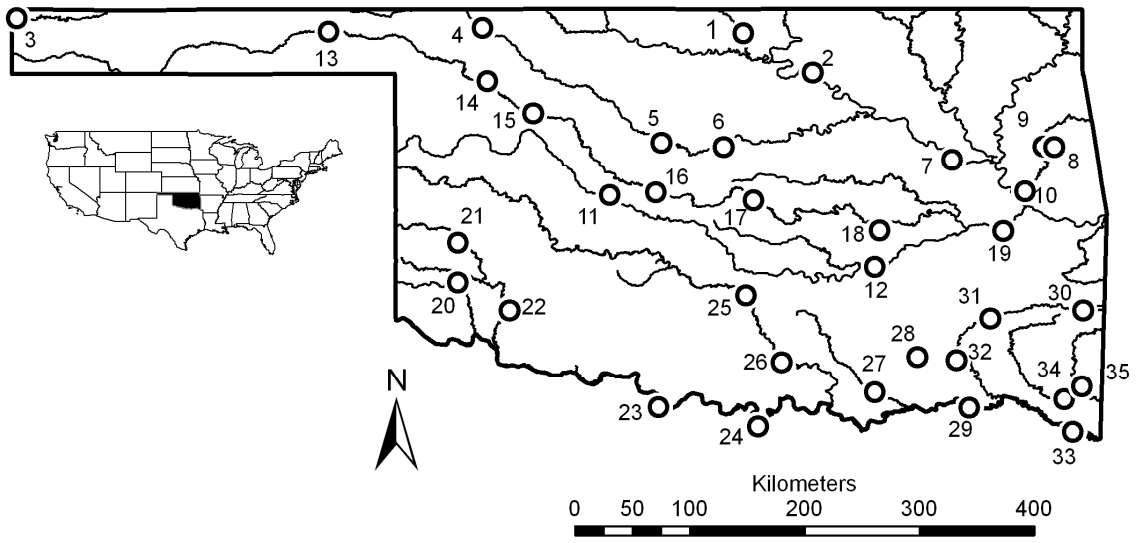


Figure 6. (a), Mean number of species in each family and mean number of species at each site over the sampling period. (b), Relationship between mean species richness and mean numbers of families present at each site over the sampling period. Dashed line represents a 1:1 relationship.



Appendix A
Localities of 35 fish communities monitored between 1978 and 2008 to assess the relationship between community species richness and community biomass stability. Site identification numbers are presented in Appendix B.



Appendix B

Collection site, latitude and longitude (DD), sampling period, number of times sampled, number of years sampled, years of flow data, and stream size site scores from PC I summarized using Principal Components Analysis (PCA) from the 35 sites in the central plains of North America.

Site ID	Location	Latitude (dd)	Longitude (dd)	Times sampled	Years sampled	Years of flow data	Stream hydrology PC I
1	Chikaskia River	36.81139	-97.27417	41	21	73	-0.23
2	Arkansas River	36.50417	-96.72806	56	23	84	1.30
3	Cimarron River	36.92667	-102.95861	38	20	59	-2.78
4	Cimarron River	36.85194	-99.31500	58	22	43	-1.28
5	Cimarron River	35.95167	-97.91417	42	22	36	0.01
6	Cimarron River	35.92056	-97.42556	50	23	66	0.20
7	Arkansas River	35.82083	-95.63861	53	23	37	2.05
8	Illinois River	35.92278	-94.92333	56	21	74	0.16
9	Baron Fork River	35.92111	-94.83833	25	15	61	-0.43
10	Illinois River	35.57306	-95.06861	57	22	72	0.91
11	Canadian River	35.54361	-98.31750	60	22	61	-0.44
12	Canadian River	34.97778	-96.24333	58	22	72	0.55
13	Beaver River	36.82222	-100.51889	31	19	72	-2.05
14	North Canadian R.	36.43667	-99.27806	52	21	73	-0.89
15	North Canadian R.	36.18333	-98.92083	27	16	63	-0.84
16	North Canadian R.	35.56306	-97.95722	56	20	78	-0.63
17	North Canadian R.	35.50028	-97.19361	59	23	41	0.00
18	North Canadian R.	35.26556	-96.20583	45	23	72	0.17
19	Canadian River	35.26222	-95.23694	38	21	71	1.74
20	Salt Fork Red River	34.85833	-99.50833	55	23	72	-1.75
21	North Fork Red River	35.16806	-99.50694	53	21	64	-1.49
22	North Fork Red River	34.63806	-99.10333	57	22	76	-0.61
23	Red River	33.87861	-97.93417	53	23	71	0.59
24	Red River	33.72778	-97.15972	45	20	73	0.78
25	Washita River	34.75472	-97.25111	38	22	72	0.20
26	Washita River	34.23333	-96.97556	48	23	81	0.57
27	Blue River	33.99694	-96.24083	32	20	73	-0.51
28	Muddy Boggy Creek	34.27139	-95.91194	31	12	72	0.03
29	Red River	33.87500	-95.50167	45	23	80	1.95
30	Kiamichi River	34.63833	-94.61250	56	22	44	-1.46
31	Kiamichi River	34.57472	-95.34056	38	20	29	0.27
32	Kiamichi River	34.24861	-95.60500	41	21	37	0.54
33	Red River	33.68389	-94.69417	57	21	38	2.06
34	Little River	33.94111	-94.75833	55	22	63	0.61
35	Mountain Fork	34.04167	-94.61972	37	20	82	0.71

Appendix C

Hydrologic parameter, parameter description, parameter transformation, and the first Principal Components Analysis (PCA) axis loading (percent of variation explained by each axis) of flow variation parameters quantified from USGS gauging stations at 35 stream sites in the central plains of North America.

Hydrologic parameter	Parameter description	Transformation	PC I (50.6 %)	PC II (13.9 %)	PC III (10.7 %)
Mean annual flow	Mean annual discharge (m^3/s)	$Log_{10}(X + 1)$	0.93	0.04	0.24
Number of zero days	Median number of zero flow days per year	$Log_{10}(X + 1)$	-0.82	0.20	0.20
Annual C.V.	Coefficient of variation in annual discharge	$Log_{10}(X + 1)$	-0.89	0.24	-0.14
Flow predictability	Flow predictability	Arcsine(Sqrt(X))	-0.43	0.40	0.48
Constancy/Predictability	Flow constancy/Flow predictability	Arcsine(Sqrt(X))	0.18	0.93	-0.17
Percent of floods in 60 day period	Percentage of floods that occur during a given 60 day period in all years	Arcsine(Sqrt(X))	-0.10	0.34	0.51
Base flow index	Ratio of base flow to total flow	Arcsine(Sqrt(X))	0.72	0.49	-0.16
Date of minimum flow	Julian date of minimum flow	$Log_{10}(X + 1)$	0.92	-0.20	-0.04
Date of maximum flow	Julian date of maximum flow	$Log_{10}(X + 1)$	-0.45	-0.21	0.67
Rise rate	Median of all positive differences between consecutive daily values	$Log_{10}(X + 1)$	0.90	-0.12	0.31
Fall rate	Median of all negative differences between consecutive daily values	$Log_{10}(X*-1 + 1)$	0.91	0.06	0.28
Number of reversals	Number of hydrologic reversals	$Log_{10}(X + 1)$	0.57	0.31	0.03

Appendix D

Small-bodied fishes included in analyses to assess the influence of species diversity on temporal community and population stability of 35 long-term stream sites in the central plains of North America, from 1978-2008. Species that comprised at least 20% of the biomass collected from each site and year are indicated with (*).

Family	Species	Family	Species
Petromyzontidae	*Ichthyomyzon castaneus*	Cyprinidae	*Notropis perpallidus*
			Notropis potteri
Clupeidae	*Dorosoma petenense**		*Notropis percobromis**
			*Notropis shumardi**
Cyprinidae	*Campostoma anomalum**		*Notropis stramineus**
	*Campostoma oligolopis**		*Notropis suttkusi*
	Cyprinella camura		*Notropis volucellus*
	*Cyprinella lutrensis**		*Opsopoeodus emiliae*
	*Cyprinella venusta**		*Phenacobius mirabilis**
	*Cyprinella whipplei**		*Phoxinus erythrogaster*
	*Dionda nubila**		*Pimephales notatus**
	Erimystax X-punctata		*Pimephales promelas**
	Hybognathus hayi		*Pimephales tenellus*
	Hybognathus nuchalis		*Pimephales vigilax**
	*Hybognathus placitus**		
	Hybopsis amblops	Aphredoderidae	*Aphredoderus sayanus*
	Hybopsis amnis		
	*Luxilus cardinalis**	Cyprinodontidae	*Cyprinodon rubrofluviatilis**
	*Luxilus chrysocephalus**		
	Luxilus cornutus	Fundulidae	*Fundulus blairae*
	*Luxilus pilsbryi**		*Fundulus notatus**
	Lythrurus fumeus		*Fundulus olivaceus**
	Lythrurus snelsoni		*Fundulus sciadicus*
	*Lythrurus umbratilis**		*Fundulus zebrinus**
	*Macrhybopsis aestivalis**		
	Macrhybopsis australis	Poecilidae	*Gambusia affinis**
	Macrhybopsis hyostoma		
	*Notemigonus crysoleucas**	Atherinopsidae	*Labidesthes sicculus**
	*Notropis atherinoids**		*Menidia beryllina**
	Notropis atrocaudalis		
	*Notropis bairdi**	Cottidae	*Cottus carolinae*
	*Notropis blennius**		
	*Notropis boops**	Centrarchidae	*Centrarchus macropterus*
	*Notropis buchanani**		*Lepomis auritus*
	Notropis emiliae		*Lepomis humilis**

Appendix D (Continued)

Family	Species	Family	Species
Cyprinidae	*Notropis girardi**	Centrarchidae	*Lepomis marginatus**
	*Notropis greenei**		*Lepomis megalotis**
	Notropis hubbsi		*Lepomis punctatus**
	Notropis ortenburgeri		*Lepomis symmetricus*
Percidae	*Ammocrypta clara*		
	Ammocrypta vivax		
	Crystallaria asprella		
	Etheostoma asprigene		
	Etheostoma blenniodes		
	Etheostoma chlorosomum		
	Etheostoma collettei		
	Etheostoma cragini		
	Etheostoma flabellare		
	Etheostoma gracile		
	Etheostoma histrio		
	Etheostoma microperca		
	Etheostoma nigrum		
	Etheostoma proeliare		
	Etheostoma punctulatum		
	*Etheostoma radiosum**		
	*Etheostoma spectabile**		
	Etheostoma stigmaeum		
	Etheostoma whipplei		
	Etheostoma zonale		
	*Percina caprodes**		
	Percina copelandi		
	Percina macrolepida		
	Percina maculata		
	Percina pantherina		
	Percina phoxocephala		
	Percina sciera		
	Percina shumardi		
Elassomatidae	*Elassoma zonatum*		

Appendix E

Principal Components Analysis (PCA) axis I loadings summarizing stream size across the study area. Positive PC I scores associated with mean annual flow, date of minimum flow, rise and fall rates; negative PC I scores associated with coefficient of variation in annual flow, and median number of days with no flow.

