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EFFECTS OF IMPOUNDMENTS ON THE COMMUNITY ASSEMBLAGE AND GENE FLOW OF STREAM CRAYFISHES

A Dissertation
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for the degree of Doctor of Philosophy
in the Department of Biological Sciences
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By

ZANETHIA CHOICE BARNETT

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ABSTRACT

Dams and their impoundments block movement of stream organism and change stream physiochemical properties, which subsequently changes biological assemblages and creates barriers to gene flow. While changes in species assemblages and gene flow have been assessed for numerous impoundments and stream organisms, no study has assessed the effects of large impoundments on crayfish assemblages and population genetic diversity and connectivity. I examined the physiochemical, biological, and genetic effects of impoundments on crayfishes. Between May 2015 and August 2017, I sampled multiple sites up- and downstream of three impounded streams, and along the lengths of two unimpounded streams, in the Bear Creek and Cahaba River drainages in Alabama, USA. First, I assessed the most effective sampling methods for collecting crayfishes in high gradient southern Appalachian streams. A combination of kick seining and electroshocking were most effective at collecting crayfishes, with higher species richness and decreased sampling biases when using both methods. Once effective methods were established, I assessed the effects of impoundments and their subsequent changes to crayfish assemblages and their habitats. Impoundments altered crayfish assemblages up- and downstream of impoundments. Crayfish abundances and species diversity were lower in impounded than unimpounded streams. Assemblages up- and downstream of impoundments were similar, but in unimpounded streams, gradual shifts in dominant species occurred from up- to downstream. Assemblage differences between impounded and unimpounded streams were associated with more stable temperature and flow regimes, decreases in crayfish refuge habitats (i.e., aquatic vegetation, interstitial space), and increased abundances of predatory fishes in impounded streams. Nonetheless, with distance downstream of impoundments, crayfish assemblages began to recover and resemble unimpounded stream assemblages. Impoundments also impacted gene flow and genetic structure of crayfishes. Impounded streams' crayfish populations were genetically isolated, and unidirectional downstream gene flow, or no gene flow, was detected between up- and downstream populations. The degree of impact of impoundments on gene flow was species-specific, with intrinsic biological and life history characteristics, such as dispersal ability and physiological tolerance, determining the degree of impact. With already declining crayfish populations, decreases in species and genetic diversity due to impoundments can decrease the persistence of hundreds of crayfish species in thousands of impounded streams. These changes in crayfish populations can cause cascading effects throughout stream ecosystems by altering the numerous ecosystem services provided by crayfishes.

DEDICATION

This work is dedicated to my grandmothers, Regenia Peterson and Mary Choice, who were two of the most tenacious women I knew. They showed me what it meant to work your hardest and strive for excellence despite all adversity. Their motivation to defeat the odds was passed on to me, and because of them, I can take advantage of opportunities that they didn’t even dare to dream. Thus, this dissertation is a product of their struggles to pursue their dreams when there were little to no opportunities for African Americans or women.

LIST OF ABBREVIATIONS AND SYMBOLS

μ	Mutation rate
π	Nucleotide diversity
θ	Mutation-scaled effective population size
AFS	American Fisheries Society
AIC_c	Corrected Akaike information criterion
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
ARC	Appalachian Regional Commission
CAP	Canonical analysis of principal coordinates
CBHR	Center of Bottomland Hardwood Research
COI	Cytochrome oxidase subunit I
CPUE	Catch per unit effort
D16	particle sizes the 16% of particles were smaller than
D50	Mean particle size
D84	particle size that 84% of particles were smaller than
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
ERDAS	Earth Resources Data Analysis System
F_{CT}	Proportion of genetic variance explained by difference among groups
F_{ST}	Haplotype frequency-based genetic differentiation

GIS	Geographic information system
h	number of haplotypes
*h*d	Haplotypic diversity
IBD	Isolation by distance
K	Number of genetic groups
LDA	Linear discriminant analyses
LME	Linear mixed-effect repeated-measures
LOO	Leave-one-out
LWD	Large woody debris
m	Migration rate
M	Mutation-scaled immigration rate
MEGA	Molecular evolutionary genetic analysis
mtDNA	Mitochondrial deoxyribonucleic acid
N_e	Effective population size
NID	National Inventory of Dams
NMDS	Non-metric multidimensional scaling
NOAA	National Oceanic and Atmospheric Administration
NRCS	Natural Resource Conservation Service
PCR	Polymerase chain reaction
PERMANOVA	Permutational multivariate analysis of variance
POCL	Postorbital carapace length

PRIMER	Plymouth Routines in Multivariate Ecological Research
Rkm	River kilometer
RVI	Relative variable importance
S_{est}	Average number of species estimated in a sample
SAMOVA	Spatial analysis of molecular variance
SD	Standard deviation
SWD	Small woody debris
TVA	Tennessee Valley Authority
UM	University of Mississippi
USA	United States of America
USDA	United States Department of Agriculture
USFS	United States Forest Service
USGS	United States Geological Survey

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CHAPTER I: INTRODUCTION

Rapid human population growth has led to increased water demand and flow regulation for human consumption, agriculture, industry, and flood control. Consequently, freshwater systems are considered our most imperiled ecosystems due, in part, to anthropogenic streamflow modifications (i.e., water regulation by impoundments) (Richter et al. 1997, Carpenter et al. 2011). Numerous concepts (e.g., River Continuum Concept, Serial Discontinuity Concept, Flood Pulse Concept, River Ecosystem Synthesis) describe the importance of flow regimes, stream geomorphic integrity, and connectivity in maintaining stable and biodiverse aquatic ecosystems (Vannote et al. 1980, Ward and Stanford 1983, Junk et al. 1989, Thorp et al. 2006). Nevertheless, there are only 42 free-flowing rivers longer than 200 km long in the contiguous United States (Benke 1990). The National Inventory of Dams (NID) documents approximately 87,000 large (> 15 m high) or hazardous (i.e., high risk of failure) impoundments in the USA (McAllister et al. 2001, NID 2013). Approximately 25% of these impoundments are in the southeastern USA, a freshwater biodiversity hotspot with over 530 freshwater animal species (i.e., crayfishes, snails, mussels, fishes and turtles) endemic to the region (Lydeard and Mayden 1995, Taylor et al. 2007, Noss et al. 2015). Understanding the effects of impoundments on aquatic habitats is essential to conserving freshwater ecosystems.

1.1 Physiochemical effects

When a river is dammed, an upstream segment is converted from lotic to lentic habitat, natural flow variability is greatly reduced downstream, and most of the river's important ecological processes (e.g., nutrient cycling, primary production) and biological make-up (e.g., species composition) are affected by these changes (Baxter 1977, Watters 1996, Cumming 2004). Thus, impoundments dramatically alter stream physiochemical properties including flow and temperature regimes, channel geomorphology, and water chemistry (Baxter 1977). The impacts of these changes depend on impoundment size, physiographic setting, location within the drainage, and location along the stream. In addition, the cumulative effects of these hydrological alterations cause ripple effects on biological assemblages and stream ecosystem functions (Carlisle et al. 2010).

The magnitude, frequency, duration, timing, and rate of change of flows dictate the structure and function of stream ecosystems, impacting water quality, energy sources, physical habitats, and biotic interactions (Poff et al. 1997, Poff and Zimmerman 2010). Impoundments, by design, alter the natural flow regime, with the degree of impact dependent on the impoundment type (Poff et al. 1997). Storage impoundments typically increase the duration of minimum flow events downstream while decreasing the number of maximum flow events (Jansson et al. 2000, Kabat et al. 2004). Reducing extreme high-water levels minimizes flooding, an important subsidy between a river and its adjoining floodplain (Benke et al. 2000), which decreases nutrient exchange and stream organism habitat availability (Welcomme 1975, Junk and Wantzen 2004). Increases in the duration of minimum flows also cause changes in water chemistry and nutrient loads, which can consequently lead to algal blooms and fish kills (Wright 1967, Nilsson et al. 1997, Singer and Gangloff 2011).

Within the impounded zone, a stream's natural current velocity is greatly reduced, decreasing the natural transport of sediment downstream (Baxter 1977). This decrease in velocity not only affects the impounded zone, but also reduces velocity in upstream river segments. With decreased velocity and reduced peak flows, streams lose their normal "cleaning mechanisms" and increased siltation occurs, especially in streams with unregulated tributaries (Ward 1976). Sediment deposition also occurs in the stream bed, stream margins, and interstitial spaces as flow decreases in sections upstream of impoundments (Baxter 1977, Wood and Petts 1994, Graf 2005, Hu et al. 2009). With increased sedimentation upstream, there is no replacement of lost sediment downstream of impoundments. Additionally, settling of sediment from the water column causes impoundments to discharge clearer water (i.e., water with low amounts of sediment), creating coarser stream beds and increased stream channel erosion, subsequently increasing channel widths downstream (Baxter 1977, Chien 1985, Wood and Petts 1994, Gordon et al. 2004, Graf 2005). Fewer bars and shallow water habitats are also commonly found in tailwaters, causing them to become less geomorphically complex (Poff et al. 1997, Graf 2006). Reduction of current velocity in impounded sections also causes depth stratification, resulting in a colder, less oxygenated hypolimnetic zone relative to surface waters (Baxter 1977, Hart et al. 2002). Thus, water released from the hypolimnion is generally colder, more nutrient rich, and often lower in oxygen compared to surface water (Voelz and Ward 1990).

1.2 Biotic effects

Impoundments alter faunal distributions and food availability, which can lead to dramatic shifts in community structure (Watters 1996, Cumming 2004, McLaughlin et al. 2006). Impoundment managers set the timing of minimum and maximum flow events, creating a more predictable flow regime downstream (Graf 2006) that alters life cycles of numerous freshwater organisms' (Junk et al. 1989, Naesje et al. 1995). For example, the natural flow regime initiates spawning for many fishes (Montgomery et al. 1983, Nesler et al. 1988) and impacts chemical signals used by crayfishes to detect predators, prey, and mates (Mead 2008). Additionally, long durations of low flows upstream of impoundments cause high siltation and sedimentation, smothering macrophytes, and filling of interstices (Ward 1976), consequently eliminating key benthic organism habitats (Peay 2003). Erratic flow events (i.e., abrupt changes in high and low flows) created by impoundments also cause species-poor macroinvertebrate communities downstream of impoundments (Ward 1976, Munn and Brusven 1991, Poff et al. 1997).

Changes in stream thermal regimes impact stream organisms, with colder and warmer waters causing a decline and increase, respectively, in productivity, reproduction, and growth rates of warm-water species (Carmona-Osalde et al. 2004, Haxton and Findlay 2008). Temperature increases also increase bioenergy exchange within a system, causing organisms to eat more food to maintain growth and survival at a higher metabolic rate (Wotton 1994, 1995). Temperature fluctuations cause false perceptions of seasonal changes, prompting various life events (e.g., mating, ovipositing) to occur before they would naturally occur (Lehmkuhl 1974, Ward and Stanford 1982), and influencing development (Voelz and Ward 1990).

The lentic conditions created upstream of impoundments increases the abundance of lentic species in impounded relative to unimpounded streams. In addition, impoundments are also often stocked with game fishes, thereby increasing the rate of biotic change within systems (Carpenter et al. 2011). Lentic conditions and stocking of fishes cause streams connected to impoundments to become increasingly vulnerable to invasion by lentic species (Pringle 1997, Johnson et al. 2008). Lentic species have caused extirpation of lotic species upstream of impoundments (Winston et al. 1991, Roghair et al. 2016), consequently impacting numerous ecosystem processes.

1.3 Genetic effects

Besides physiochemical and biological changes, barrier effects are one of the most serious impacts of impoundments. Impoundments can prevent dispersal of aquatic organisms in a stream system by physically blocking movement. The loss of longitudinal and lateral connectivity can lead to isolation of populations, failed recruitment, and local extinction (Bunn and Arthington 2002). Isolated populations may be found within both up- and downstream sections due to the barrier effect and patchiness of suitable habitats caused by impoundments. This isolation can be detrimental to small populations through demographic, environmental, and genetic stochasticity (Morita and Yamamoto 2002). Habitat fragmentation can cause loss of genetic diversity, inbreeding depression, and may hinder a population's ability to adapt to ecological perturbations (Charlesworth and Charlesworth 1987, Vandergast et al. 2006), making it more susceptible to local extinction (MacArthur and Wilson 1967, Lande 1988, Pringle 1997).

1.4 Study goals

Changes caused by impoundments to stream physiochemical, biological, and genetic properties impact stream ecosystems. Stream ecosystem changes will depend on how key organisms are impacted by impoundments. While impoundment effects on numerous organisms have been assessed (Gherke et al. 2002, McGregor and Garner 2003, Yamamoto et al. 2004, Kelly and Rhymer 2005, Alp et al. 2012), only one published study has assessed the impacts of impoundments and its physiochemical changes on crayfish assemblages (Adams 2013). This previous study focused on the effects of small impoundments on crayfish assemblages. No published study has assessed the impacts of large impoundments on crayfish assemblages or the genetic effects of impoundments on crayfishes.

Understanding the effects of impoundments on crayfishes is important because crayfishes serve as stream ecosystem engineers (Creed and Reed 2004) through their ability to process detritus, consume macrophyte biomass, manipulate and mobilize substrate, and serve as prey for or predators of numerous aquatic animal species (Chamber et al. 1990, Holdich 2002). Crayfishes' roles as prey and predators also influence behavior and biomass of some fishes and amphibians (Guan and Wiles 1997, Dorn and Mittelbach 2004). The goal of this dissertation was to examine the physiochemical, biological, and genetic effects of impoundments on crayfishes. Because quantitative sampling methods are not well established for crayfishes (Barnett and Adams 2018, Budnick et al. 2018), and no study has assessed sampling method accuracy when collecting crayfishes and fishes simultaneously, I also evaluated sampling method biases and efficiencies. My specific objectives were to 1) evaluate the effectiveness of three sampling methods for collecting crayfishes and fishes in southern Appalachian streams, 2) assess the relationships between crayfish assemblages and stream abiotic and biotic factors altered by impoundments, and 3) characterize and compare the spatial genetic structure and genetic diversity of two abundant and widespread crayfish species in impounded and unimpounded streams.

To achieve these objectives, I sampled and characterized biotic and abiotic factors multiple times over three years (2015–2017) in two unimpounded streams and three streams impounded by relatively large dams, in the Southern Appalachian region of the southeastern USA. This region is the center of crayfish diversity (Crandall and Buhay 2007) and contains numerous impoundments (Morse et al. 1993, NID 2013). This assessment will help scientists and managers understand crayfishes' responses to alterations caused by impoundments, as well as inform future management and restoration efforts.

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CHAPTER II:

NOT ALL METHODS ARE CREATED EQUAL: ASSESSMENT OF SAMPLING METHODS FOR CRAYFISHES AND FISHES IN SOUTHERN APPALACHIAN STREAMS

Abstract

Effective sampling methods are needed to accurately assess stream crayfish and fish distributions and assemblage structures. We assessed and compared the effectiveness of three sampling methods (kick seining, electrofishing, and nest trapping) to collect crayfishes in wadeable streams of the southern Appalachian Mountain region of Alabama, USA. Using the same methods, we simultaneously collected stream fishes, which as predators can be an important influence on crayfish assemblage structure. For crayfishes, kick seining was the single most effective sampling method, collecting the highest species richness and the most individuals. However, we determined that combining both kick seining and electrofishing would decrease the number of sites (31–68% fewer sites, sampling 1.5–5.1 rkm) needed to accurately assess crayfish richness. Double-pass electrofishing was also more effective than single-pass electrofishing for estimating crayfish richness. Nest traps were the least effective crayfish sampling method, collecting 20%–67% ($\bar{X}$ = 52%) of species from streams. For 9 of the 13 species collected, no differences in crayfish sizes and sex ratios were detected between sampling methods. In the four species with differences, electrofishing collected longer crayfishes and more females than kick seining. Crayfish catchability by electrofishing was higher in streams with higher conductivities, longer crayfishes, higher water temperatures, and lower percentages of adult males. For fishes, electrofishing was the most effective sampling method, collecting the most individuals and most effectively assessing fish species richness (N = 87) in all sampled streams. Electrofishing for fishes was more effective in streams with smaller substrate sizes, higher width to depth ratios, warmer waters, and lower conductivities. Nest traps were the least effective fish sampling method, collecting 9% of species from each drainage. We conclude that using a combination of kick seining and electrofishing is best for assessing stream fish and crayfish assemblages,

simultaneously, which can improve management, biomonitoring, and understanding of the complex relationships of two important taxonomic groups.

2.1 Introduction

Using effective sampling methods is vital for study designs but can be challenging when sampling in lotic environments with variable habitat parameters (i.e., depth, discharge, and substrate). Unlike for many stream organisms (e.g., mussels [Huang et al. 2011, Haag et al. 2012], aquatic insects [Peckarsky 1984, Rosenberg et al. 1997, Stark et al. 2001], and fishes [Kushlan 1974, Jackson and Harvey 1997, Bonar et al. 2009]), quantitative sampling methods are not well established for crayfishes (Barnett and Adams 2018, Budnick et al. 2018). Yet, numerous studies simultaneously (using the same method) assess abundance and diversity of crayfishes and other stream organisms (Degerman et al. 2007, Dorn 2008), which could lead to erroneous conclusions. Furthermore, we must establish quantitative methods and evaluate methodological biases and efficiencies to accurately incorporate crayfishes into ecosystem management decisions and maintain their ecological functions (Black 2011, Legendre and Legendre 2012).

Active (e.g., electroshocking, dip netting) and passive sampling (e.g., trapping) methods are used to assess stream crayfish assemblages, but few standard method recommendations (i.e., using methods to sample particular habitat types or assess specific research questions) have been made (Engelbert et al. 2016, Larson and Olden 2016, Budnick et al. 2018). The effectiveness of sampling methods is influenced by the characteristics and spatial extent of the habitat sampled, the abundance, species diversity, and distribution of crayfishes, and their species-specific behavior (e.g., mobility, avoidance, hiding) or conspicuousness (size, coloration). Thus, sampling method comparison studies generally focus on one target species (Olsen et al. 1991, Rabeni et al. 1997, Alonso 2001, Gladman et al. 2010, Reid and Devlin 2014, Williams et al. 2014). While streams in many geographic regions are occupied by only one crayfish species, regions such as the southeastern U.S. possess diverse crayfish assemblages (Richman et al. 2015). Thus, methods are needed to effectively collect and accurately represent abundances and distributions of multiple co-occurring crayfish species (DiStefano 2000, Larson et al. 2008, Engelbert et al. 2016, Budnick et al. 2018). Few studies have compared the effectiveness of crayfish sampling methods in species-rich streams, and results have been inconsistent across studies (Price and Welch 2009, Engelbert et al. 2016, Budnick et al. 2018). Among these studies, sampling biases were noted in size, sex, and species sampled, with biases varying by method, region and habitat. To reduce biases, direct comparisons of sampling methods and integration of complementary methods in heterogeneous, species-diverse stream habitats are needed (Barnett and Adams 2018, Budnick et al. 2018).

Crayfishes and fishes can have strong interactions including mutual predation and competition for prey and shelter (Stein 1977, Rahel and Stein 1988, Englund and Krupa 2000, Reynolds 2011). These interactions affect crayfish and fish distributions, densities, behavior, assemblage diversity, and size structure (Stein and Magnuson 1976, Rahel and Stein 1987, Garvey et al. 1994, Dorn and Mittelbach 1999, Keller and Moore 1999). To further understand these relationships, sampling methods are needed that accurately assess crayfish and fish assemblage structures simultaneously (Kusabs et al. 2018).

In the present study we examined the effectiveness of three commonly used methods, electrofishing, kick seining, and nest trapping (Bechler et al. 2014), individually and combined for sampling crayfishes and fishes in southern Appalachian streams. Although the effectiveness of different stream crayfish sampling methods has been assessed previously (Price and Welch 2009, Williams et al. 2014, Engelbert et al. 2016, Budnick et al. 2018), these methods have not been directly compared. Additionally, no studies have used multiple passes to assess the efficiency of electrofishing for crayfishes in streams with more than one crayfish species (Bernardo et al. 1997, Rabeni et al. 1997, Alonso 2001). Three studies assessed crayfish sampling methods in the southeastern USA (Price and Welch 2009, Barnett and Adams 2018, Budnick et al. 2018), but none of these studies were conducted in the southern Appalachian region, the northern hemisphere center of crayfish diversity (Crandall and Buhay 2008). This region consists of rocky, mountainous streams that are very different than the fine, silty/clay substrates in most southeastern (Coastal Plain) streams (Williams and Amatya 2016). Likewise, no studies have assessed the accuracy of different stream sampling methods for crayfishes and fishes simultaneously, even though many research studies target both faunal groups (Englund 1999, Usio and Townsend 2000, Degerman et al. 2007). To address these knowledge gaps, we 1) compared stream crayfish species richness, catch per unit effort (CPUE), sex ratios, and sizes between kick seining and electrofishing; 2) compared fish species richness and CPUE between kick seining and electrofishing; 3) assessed how environmental factors influenced the effectiveness of kick seining and electrofishing for crayfishes and fishes; 4) determined the number of sites needed to accurately assess crayfish and fish species richness by electrofishing, kick seining, and nest trapping; and 5) assessed electrofishing efficiency for crayfishes via multi-pass surveys. Our findings will benefit stream management and biomonitoring by improving the understanding of the effectiveness of crayfish sampling methods in species rich habitats, and the benefits and biases associated with simultaneously collecting crayfishes and fishes.

2.2 Methods

2.2.1 Study Area

We sampled crayfishes and fishes in five streams in the Bear Creek (Tennessee River Basin) and Cahaba River (Mobile River Basin) drainages in the southern Appalachian region of Alabama, USA (Figure 2.1). Both drainages were valuable ecological resources due to highly diverse aquatic faunal communities and numerous imperiled species (Allen 2001, McGregor and Garner 2003, Philip and Johnston 2004). All streams sampled were wadeable, perennial streams, with distinct pool-riffle complexes and channel widths ranging from 3–30 m. Streams were typical of the rocky, mountainous streams found throughout the southern Appalachian region. Surrounding land uses were predominantly forest intermixed with pasture, row crops and poultry production in the Bear Creek drainage and forest, low-density residential, medium intensity commercial, and pasture and row crops in the Cahaba River drainage (Thom et al. 2013).

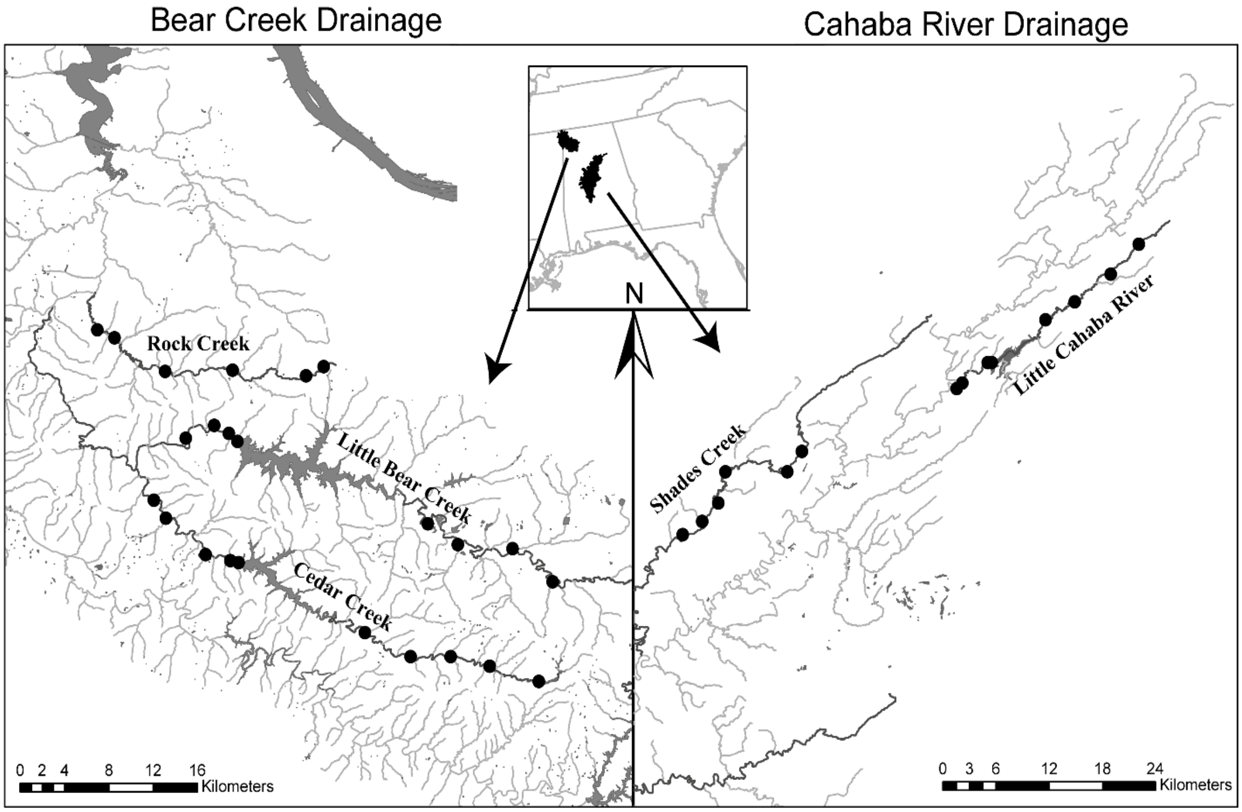

Figure 2.1. Map of Bear Creek and Cahaba River drainages, Alabama, with collections sites represented by circles. Inset shows drainage locations within the southeastern United States.

2.2.2 Method comparison sampling.

Among all five streams, 38 sites were sampled: 24 in the Bear Creek drainage and 14 in the Cahaba River drainage (Table 2.1). We selected sites at set intervals along both impounded and unimpounded streams, with six to ten sites per stream. If a predetermined location was inaccessible, we sampled the closest accessible site. Sites covered on average 38.6 km of stream length (distance between furthest up- and downstream sites). We sampled in the spring/summer (“spring”; May–July) and fall/winter (“fall”; September–December) of 2015–2017, hereafter “sampling rounds”.

Table 2.1. Number of sites sampled by each sampling method during each seasonal sampling round. A dashed line indicates that the method was not used in that sampling round. E-fishing = electrofishing.

Drainage	Stream	Spring 2015	Summer 2015	Fall 2015	Spring 2016	Summer 2016	Fall 2016	Spring 2017	Summer 2017
Bear Creek	Cedar Creek								
	E-fishing and kick seining	8	--	8	--	--	--	--	--
	Nest trap	--	--	8	--	--	--	--	--
	Multi-pass e-fishing	--	2	--	--	--	--	--	2
	Little Bear Creek								
	E-fishing and kick seining	10	--	10	--	--	10	10	--
	Nest trap	--	--	--	--	--	10	10	--
	Multi-pass e-fishing	--	2	--	--	--	--	--	2
	Rock Creek								
	E-fishing and kick seining	4	--	6	--	--	6	6	--
	Nest trap	--	--	6	--	--	6	6	--
	Multi-pass e-fishing	--	1	--	--	--	--	--	3
Cahaba River	Little Cahaba River								
	E-fishing and kick seining	--	--	--	6	--	8	8	--
	Nest trap	--	--	--	6	--	8	8	--
	Multi-pass e-fishing	--	--	--	--	2	--	--	2
	Shades Creek								
	E-fishing and kick seining	--	--	--	3	--	6	6	--
	Nest trap	--	--	--	3	--	6	6	--
	Multi-pass e-fishing	--	--	--	--	1	--	--	3

At each site, we sampled a linear reach 30 times the wetted width, unless wetted widths were less than 6 m or greater than 16 m, in which case minimum (200 m) or maximum (500 m) reach lengths were sampled (Simon 2004). We sampled reaches by kick seining, electrofishing, and nest trapping. Because kick seining and electrofishing are more effective in shallow flowing segments of streams (Larson and Olden 2016), only riffle and run habitats with maximum depths ≤ 1 m (≥ 85% of ech reach) were kick seined and electrofished. Additionally, we sampled pools with nest traps to prevent traps from filling with sand and trap loss due to sediment movement and dislodging in flowing waters (Bechler et al. 2014). We divided each reach equally into two subreaches. Kick seining occurred in downstream subreaches and electrofishing in upstream subreaches to prevent any negative impacts to electrofishing through decreased water clarity downstream of kick seined areas. In both subreaches, we sampled pool macrohabitats with nest traps. We classified macrohabitats based on channel characteristics and stream velocity (Bisson et al. 1982).

For comparison of sampling methods, using two personnel crews, we made one pass of each subreach simultaneously. Sampling effort was partitioned between macrohabitats (riffle/run) based on the percentage of each macrohabitat within subreaches. We conducted 20 kick seines every 100 m (Simon 2004) using a seine 2.6 m long x 1.6 m high with 3-mm mesh, sampling, on average, 15% of the subreach. One person kicked, disturbing the substrate in a 2 m long x 1.5 m wide plot (measured with strings attached to seine brails) immediately upstream of the seine. Large rocks were lifted while kicking, and the seine was lifted immediately after kicking was completed. After each kick, the seine was moved diagonally (from bank to bank) through the subreach (at least 2 m away from area previously kicked) and spaced to cover the length of the subreach to ensure sampling throughout the entire subreach and all riffle and run habitat types. Two to three people alternated kicking within each subreach. We conducted single pass electrofishing (effort = 0.4 sec/m^2) with two dip netters (42 cm diameter net with 3 mm mesh), using a Smith-Root backpack electrofisher (model 12A programmable output wave, battery-powered electrofisher set at 50–60 Hz, 4–5 ms pulse width, 300–400 V; Vancouver, Washington) with a circular anode covered with 3 mm meshed netting. Electrofishing settings were adjusted at each site. We based electrofishing effort on the time necessary to sample riffle/run macrohabitats during preliminary sampling in several sites. To standardize efforts, electrofishing time was calculated before sampling ($\bar{X}$ = 729 sec; range = 216-1801 sec), and once electrofishing time was reached sampling was concluded (mean area sampled = 70% of subreach). We adequately sampled all habitat types within a subreach, sampling stream banks and mid-channels.

Nest traps, like those in Bechler et al. (2014), were set in up to five pools per reach ($\bar{X}$ = 3 pools/reach). If a reach had five or fewer pools, all were sampled; if it had more, five were randomly chosen. Traps were 30 cm long and constructed from 5 cm diameter polyvinyl chloride (PVC) irrigation pipes with three drain holes (1 cm diameter) 7 cm apart along the top and bottom, and irrigation drain caps attached to each end. A semicircular opening (5.0 cm wide x 2.2 cm tall) in one cap allowed organisms to enter and leave the trap. This cap was attached with an eye bolt and nut for easy removal when checking the trap, and the other cap was glued on. To provide habitat within traps, stream substrate was placed in the lower half of traps. We placed nest traps in crevices under large rocks, roots, and fallen debris. Nylon parachute cords were tied to eye bolts on nest traps and secured to stable objects (e.g., root, metal stake in bank). Parachute cords also allowed traps to be easily retrieved from crevices in a vertical position so that organisms could not escape through the entrance hole. After retrieving traps, eye bolt and entrance cap were detached, all organisms removed from trap, trap reassembled, and placed back into crevice. The number of traps in each pool was determined by the size of the pool and number of available crevices, with two to six traps per pool ($\bar{X} = 4$ traps/pool). Traps were set for at least a week before sampling and checked during every sampling round (i.e., spring and fall). Traps were not removed until the completion of the study (deployed for up to 24 months).

For all collections, we recorded crayfish and fish species and life stage (i.e., adult, juvenile). For crayfishes, we also recorded sex, adult reproductive form (form I male [reproductive], form II male [nonreproductive], female [without eggs], and ovigerous female [bearing eggs]), and postorbital carapace length (POCL). Most crayfishes and fishes were identified, measured, and released in the subreach of capture; all others were preserved in 5% formalin (fishes) or ≥ 70% ethanol (crayfishes) for further laboratory analyses.

2.2.3 Environmental sampling

Environmental sampling quantified channel and substrate characteristics and water quality (Table 2.2). During each sampling round, we measured channel characteristics (wetted width, depth, and percent canopy cover) at four evenly spaced transects, ranging from 50 to 125 m apart, within each reach (2 locations in each subreach). Depth was measured midchannel and 10 cm from right and left edges. Canopy cover was also measured mid-channel with a convex spherical densiometer. Streambed composition across the bankfull channel width was analyzed using pebble count procedures (Wolman 1954, Harrelson et al. 1994) once per year. Data were collected from at least ten diagonal transects (five per subreach) with ten points equally spaced along each transect. The first transects began along stream banks at either the furthest up- or downstream point within the reach. At each of the ten points, we blindly chose and measured one pebble and sample of woody debris, if present, at tip of the boot, and averaged measurements for each subreach. Between each of the ten points, we visually estimated percentages of the streambed covered by vegetation and small woody debris (SWD, < 10 cm diameter) (Bain and Stevenson 1999), and counted large woody debris (LWD, ≥ 10 cm diameter). Three substrate metrics were derived from pebble counts from each subreach: the median particle size (D50), and the particle sizes that 16% (D16) and 84% (D84) of particles were smaller than (Olsen et al. 2005). Before sampling, we measured water quality parameters (water temperature, conductivity, dissolved oxygen [DO], and pH) at one location within each site with a Hydrolab Quanta (Hach-Hydrolab, Loveland, Colorado). We calibrated the Hydorolab before each sampling round for all parameters and daily for DO.

Table 2.2. Median (and standard deviation [SD]) values for environmental parameters from spring and fall sampling. *N* = total sites sampled; DO = dissolved oxygen; D16 = size (mm) that 16% of particles were smaller than; D84 = size (mm) that 84% of particles were smaller than; SWD = percent small woody debris; LWD =number of pieces of large woody debris.

	Little Bear	**Cedar**	**Rock**	**Little Cahaba**	**Shades**
Stream length (km)	80	82	33	45	87
Site length	0.17 (0.5)	0.22 (0.1)	0.12 (0.5)	0.17 (0.1)	0.19 (0.5)
Spring					
N	20	8	10	14	9
Water temperature (°C)	22.04 (3.00)	25.26 (1.82)	22.13 (1.06)	23.98 (2.21)	25.57 (0.90)
DO (mg/l)	7.52 (0.60)	6.75 (0.42)	6.91 (0.90)	6.76 (0.80)	6.24 (0.32)
Conductivity (µS/cm)	104.7 (51.8)	340.0 (83.8)	175.9 (70.5)	288.1 (71.0)	235.4 (30.8)
pH	7.37 (0.35)	7.49 (0.30)	7.14 (0.36)	8.14 (0.70)	7.33 (0.50)
Wetted width (m)	10.8 (3.2)	14.0 (4.2)	6.9 (4.2)	11.5 (4.0)	13.4 (2.7)
Depth (cm)	20.6 (9.1)	25.7 (15.5)	16.9 (16.5)	21.0 (7.8)	33.4 (18.0)
Width to depth ratio	0.48 (0.24)	0.59 (0.61)	0.33 (0.55)	0.58 (0.36)	0.40 (0.14)
D16	2.2 (16.7)	4.7 (26.8)	3.2 (445.5)	3.5 (45.4)	2.0 (21.9)
D84	98.0 (656.6)	64.3 (854.0)	63.7 (900.3)	300.5 (895.4)	995.8 (965.0)
Aquatic vegetation (%)	9.8 (9.1)	15.7 (9.3)	19.8 (13.3)	11.1 (20.1)	11.0 (8.1)
Canopy cover (%)	51.5 (24.4)	68.6 (12.6)	64.4 (22.9)	54.2 (21.5)	44.9 (22.7)
SWD	5.2 (2.9)	5.8 (2.8)	6.8 (2.2)	5.9 (3.8)	9.8 (5.9)
LWD	5.0 (7.5)	8.5 (4.8)	5.0 (5.3)	4.0 (4.8)	11.5 (7.5)
Fall					
N	20	8	12	8	6
Water temperature (°C)	20.67 (1.97)	19.95 (1.15)	18.22 (5.16)	23.11 (3.76)	23.79 (1.75)
DO (mg/l)	7.65 (0.68)	6.23 (0.92)	5.39 (3.17)	6.65 (1.53)	5.15 (0.69)
Conductivity (µS/cm)	99.8 (39.4)	334.0 (93.7)	194.4 (124.0)	331.8 (84.6)	353.0 (32.8)
pH	7.42 (0.31)	7.64 (0.21)	7.44 (0.42)	7.84 (0.31)	7.64 (0.07)
Wetted width (m)	9.3 (2.9)	13.5 (3.5)	6.5 (3.8)	10.8 (4.3)	11.0 (2.8)
Depth (cm)	15.7 (7.5)	16.6 (18.1)	15.5 (18.2)	18.0 (9.4)	20.7 (9.4)
Width to depth ratio	0.62 (0.52)	0.65 (0.54)	0.34 (0.48)	0.73 (0.44)	0.50 (0.15)
D16	2.8 (26.4)	4.7 (26.8)	2.5 (24.4)	8.3 (40.8)	1.3 (16.3)
D84	121.8 (750.3)	81.9 (842.8)	45.4 (788.1)	309.1 (894.4)	917.4 (1004.0)
Aquatic vegetation (%)	13.3 (9.7)	15.7 (9.3)	31.7 (12.0)	11.8 (21.8)	13.7 (6.2)
Canopy cover (%)	46.4 (19.3)	35.6 (16.1)	49.3 (14.8)	57.3 (24.9)	71.8 (22.3)
SWD	7.6 (2.6)	5.8 (3.0)	9.2 (3.6)	8.8 (4.1)	13.5 (6.0)
LWD	7.0 (6.7)	8.5 (4.8)	5.0 (5.6)	6.5 (4.5)	18.0 (8.1)

2.2.4 Multi-pass electrofishing sampling

To estimate electrofishing efficiency for collecting crayfishes, we conducted multi-pass electrofishing surveys. Surveys took place in summer (July and August) 2015–2017 at twenty sites previously sampled (during method comparison sampling; Table 2.1). We isolated sections (≥ 3 times section's wetted width) 30–105 m long with block nets (5-mm mesh seines) to prevent organisms from leaving sites. Each section consisted of both riffle and run macrohabitats. A minimum of three successive full pass depletion efforts were made in each section. Electrofishing time was standardized, as above, ($\bar{x}$ = 224 sec; range = 120–403 sec), and effort remained constant for each pass. In 2017, if total crayfishes collected did not decrease from the second to third pass, we conducted a fourth pass. Immediately after each pass, crayfish statistics were recorded as above, and most crayfishes were released outside of the blocked section. We preserved crayfishes not released in the field as above for further laboratory analyses.

To understand what environmental factors impacted electrofishing efficiency, we measured water quality parameters and channel characteristics at four equidistant locations 10 to 68 m apart, as described above (Appendix A). We calculated stream discharge (m^3/s) using the transect method (Harrelson et al. 1994) with a Marsh-McBirney Flo-Mate 2000 and topsetting rod (Hach, Loveland, Colorado) at one location per site.

2.2.5 Data analyses

Data analyses consisted of four main components. First, we identified differences in collections of crayfishes and fishes assemblages, separately, between kick seining and electrofishing. Next, for assemblages that were different between methods, we used models to relate differences to stream environmental characteristics. For all sampling methods, we compared the sampling effort needed by methods singly versus combined to accurately estimate crayfish and fish species richness within streams. Finally, we determined electrofishing efficiency for collecting crayfishes. For all models, histograms of model residuals did not depart from normality.

2.2.5.1 Method comparison analyses.

We compared crayfish and fish species richness and CPUE (*N*/100 m^2 [total area within subreach]) between electrofishing and kick seining, and compared methods effectiveness in each macrohabitat, stream, and season. Because we used nest traps in a different macrohabitat type (pools), nest trap captures were not statistically compared to results from other methods. We excluded from analyses age-0 individuals that were not identifiable to species. We calculated $\log_e$ CPUEs (+0.001) of the most widespread crayfish species (present in ≥ 35% of sites) and total fish CPUE. We compared species richness (response variable) between sampling methods and methods interactions with streams, seasons, and macrohabitats (fixed effects) using repeated-measures ANOVA models, with site as a random effect. In the same manner, we compared CPUEs (response variables) between sampling methods. Only sampling method or its interactions (e.g., methods interaction with streams) were interpreted to evaluate differences between the effectiveness of sampling methods in each macrohabitat, stream, and season. If significant interactions were detected between sampling methods and streams, indicating sampling method effectiveness differed among streams, we investigated how stream habitat characteristics impacted the effectiveness of sampling methods using linear mixed-effect repeated-measures models (LME; See Method, Analyses of environmental effects section).

Analyses were performed with the *lmerTest* package (Kuznetsova et al. 2015) in R software version 3.4.2 (R Core Team 2013), using Tukey's HSD post-hoc tests for comparing means.

We compared lengths and age class estimates of the most abundant crayfish species ($N >$ 25 individuals) between electrofishing and kick seining. We compared crayfish $\log_e$ POCLs (response variable), separately for each abundant species, between sampling methods as in the above species richness comparisons. We also compared crayfish age-class estimates (i.e., age-0, age-1) between kick seining and electrofishing collections. We estimated the number of age-classes (all sites within a drainage combined), separately for spring and fall collections, using mixed distribution analysis (*flexmix* R package) of the length-frequency data (France et al. 1991, Leisch 2014, Barnett et al. 2017). Because of low numbers of *Cambarus striatus* collected within the Cahaba River drainage, age classes were not analyzed for individuals in this drainage. We used a maximum of four age classes for each species (Weagle and Ozburn 1972, Page 1985), except *C. striatus* for which seven groups were used (Camp et al. 2011). We ran models with 1,000 iterations, used integrated completed likelihoods to select the best models (Biernacki et al. 2000), and compared model results.

For the most abundant crayfish species within each drainage, we compared differences in ratios of adult reproductive forms (i.e., form I males, form II males, and females) between kick seining and electrofishing collections, macrohabitats, and seasons using a log-linear model computed with the *stats* package (R Core Team 2013) in R. Data were analyzed separately for each drainage and species. We pooled data across streams and years because we identified only small variations in adult sex ratios. In the Cahaba River drainage, sites were sampled twice in the spring and once in the fall. Thus, to keep sampling efforts balanced between seasons, sites sampled in spring 2016 (6 in Little Cahaba River and 3 in Shades Creek; Table 2.1) were excluded. Because the focus of this model was to assess differences in ratios of crayfish adult reproductive forms between sampling methods across macrohabitat and season, only sampling method or its interactions (e.g., method interaction with macrohabitat) were interpreted.

2.2.5.2 Analyses of environmental effects

To understand if stream environmental characteristics impacted the effectiveness of collecting crayfishes, we created models investigating the relationship between sampling methods and environmental characteristics. This relationship was only investigated for crayfish parameters in models with significant interactions (methods interacted with streams) in the "method comparison" section. Interactions indicated that methods effectiveness may be driven by stream characteristics. We constructed separate LME models for kick seining and electrofishing to infer whether stream environmental characteristics had different influences on each method. In the models, the crayfish parameter was the response variable, and site was the random effect. Independent variables included season, $\log_e$ transformed stream characteristics, $\log_e$ transformed water quality parameters (water temperature, conductivity, and DO), pH, and substrates sizes. Because only two macrohabitat types were kick seined and electrofished, only one macrohabitat percentage (riffles) was used in models. Models were fit with maximum likelihood estimations. We used the *MuMIn* R package (Barton and Anderson 2002) to analyze all possible models. Model selection was based on corrected Akaike information criterion (AIC_c), because sample sizes were small relative to the number of estimated parameters (Burnham and Anderson 2004). We compared alternative models by weighting their level of data support (Hurvich and Tsai 1989), with delta AIC_c values ≤ 2 representing the best-supported models. We calculated relative variable importance (RVI) scores for each predictor variable, based on variables appearance in the AIC_c-best models. Predictors with RVI > 0.5 were considered most important. To assess the fit of each model, we calculated marginal R^2s, the proportion of variance explained by the fixed effects, and conditional R^2s, the proportion of variance explained by the fixed and random effects (Nakagawa and Schielzeth 2013, Johnson 2014).

To understand if stream environmental characteristics impacted the effectiveness of sampling methods for fish collections, we created LME models as above. This relationship was only investigated for fish parameters in models with significant interactions (methods interacted with streams) in the "method comparison" section. In the models, the fish parameter was the response variable, and site was the random effect. Independent variables included season, $\log_e$ transformed stream characteristics, $\log_e$ transformed water quality parameters, pH, and substrate sizes. We used the same approach to model fitting and selection as used for crayfish LME models.

2.2.5.3 Stream species richness estimations

We compared the sampling effort needed by methods singularly versus combined to accurately estimate crayfish and fish species richness within streams. We estimated the rate of species accumulation as a function of the number of sites sampled for each method, separately, and for kick seining and electrofishing combined. Combined methods sampled double the area at each site relative to single methods. To account for the differences in area sampled, we also compared stream length (rkm) needed per site to accurately estimate species richness between methods. We extrapolated stream species composition (counts of individuals and species in each collection) to estimate species richness for 100 sites with the Chao-1 method (Chao 1984) using

EstimateS 9.1.0 (Colwell 2013). The Chao-1 method estimates species richness by extrapolating the probability of undetected species within each site from the number of rare species captured (i.e., singletons). Collections were randomly reordered and resampled 100 times within the program (Colwell 2013, Engelbert et al. 2016) and given an S_{est}-value and 95% confidence intervals. S_{est}-values were the average number of species estimated in a sample during the 100 resampling events. We used S_{est}-values to determine species accumulation in each stream (Engelbert et al. 2016).

2.2.5.4 Electrofishing efficiency analyses

For multi-pass electrofishing data, we estimated each crayfish species' catchability (probability of collecting all individuals) and species density (number of individuals/m^2) for each site using the maximum weighted likelihood method (Carle and Strub 1978). This maximum likelihood algorithm assumes a constant catchability and constant effort in each pass and was selected because of its statistical robustness. We tested the constant probability of capture assumption using a chi-squared based statistic (Seber 1982). We ran all analysis with the *FSA* package (Ogle et al. 2018) in R.

To estimate the number of electrofishing passes needed to accurately assess species richness, we compared differences between numbers of species collected by each pass using a repeated measures ANOVA model. In the model, number of species collected was the response variable, pass and stream were independent variables, and site was the random effect. Interaction of pass and stream was included in the model. Analyses were performed with the *lmerTest* package in R, using Tukey's HSD post-hoc tests for comparing means.

We created LME models to understand if crayfish assemblages and stream environmental characteristics impacted the catchability of crayfishes when electrofishing. We fit LME models with maximum likelihood estimations to compare catchability between channel characteristics, water quality parameters, crayfish species, median crayfish size, and percentages of adults and adult males. In the model, crayfish catchability was the response variable, and collinearity between sites sampled within a stream was accounted for by treating stream as a random effect. We included interactions between crayfish species and crayfish parameters (i.e., crayfish size, percentages of adults, and percentages of males) in the full model. Model selection, based on AIC_c values, was carried out as described above.

2.3 Results

We collected 13 crayfish species (Table 2.3) and 87 fish species (Appendix B). Eighty-eight percent of the crayfish species known from both drainages, and 64% and 36% of the fish species known from the Bear and Cahaba River drainages, respectively, were collected. We collected crayfishes and fishes from all sites, with a maximum of five crayfish species and 29 fish species in a single collection. The five most abundant crayfish species, constituting 97% of total collections, were *C. striatus*, *Faxonius erichsonianus*, *F. validus*, *F. virilis,* and *F. compressus*, with the first four also being the most widespread.

Table 2.3. Number of crayfishes captured by sampling methods in Bear Creek and Cahaba River drainages during seasonal sampling, with total number of sites (*N*) containing each species indicated. Numbers indicate individuals captured in spring and fall (spring/fall) sampling rounds. E-fishing = electrofishing

Drainage	Crayfish (*N*)	E-fishing	Kick seine	Nest trap	Total
Bear Creek (24 sites)	*Lacunacambarus aff. diogenes* (6)	0/2	6/11	0/1	20
	C. striatus (12)	14/25	61/28	2/2	132
	Faxonius compressus (6)	18/11	35/55	2/0	121
	F. erichsonianus (22)	98/78	221/274	1/11	683
	F. etnieri (1)	4/2	8/0	0/0	14
	F. validus (24)	489/128	422/185	1/9	1,234
	Procambarus hayi (1)	2/0	0/0	0/0	2
Cahaba River (14 sites)	*C. acanthura* (1)	0/0	1/0	0/0	1
	C. coosae (4)	4/2	14/2	0/0	22
	C. striatus (4)	1/0	7/1	1/0	10
	F. erichsonianus (9)	23/20	153/63	1/0	260
	F. spinosus (1)	0/0	1/1	0/0	1
	F. virilis (13)	70/8	198/46	5/9	336
	P. acutus (3)	0/8	3/0	1/0	4
	P. clarkii (6)	3/5	2/2	0/0	12
Total		726/667	1,132/667	14/32	2,852

2.3.1 Method Comparisons

2.3.1.1 Species richness and CPUE comparisons

Kick seining, electrofishing, and nest trapping collected crayfishes in 97% (*N* = 1,799 individuals), 89% (*N* = 1,007 individuals), and 39% (*N* = 33 individuals) of collections, respectively. Of the species collected, 92%, 85%, and 54% of species were collected by kick seining, electroshocking, and nest trapping, respectively. Sixty percent of traps, across all sampling dates, were recovered, resulting in 418 traps examined, of which 11% (*N* = 47) were occupied by crayfishes. No trap was occupied by more than one crayfish.

Crayfish species richness was 30% higher in kick seining than electrofishing collections (ANOVA $F_{1,392} = 22.26$, $P < 0.001$). We identified no difference in crayfish CPUE between kick seining and electrofishing for any species (all P values > 0.05) except *F. erichsonianus.* The differences in *F. erichsonianus* CPUE between kick seining and electrofishing collections were inconsistent across macrohabitat (Figure 2.2A; ANOVA $F_{1,290} = 7.36$, $P < 0.01$) and stream (Figure 2.2B; $F_{4,290} = 4.38$, $P < 0.01$), but overall, for all streams and macrohabitats, *F. erichsonianus* CPUEs were two times higher from kick seining than electrofishing (ANOVA $F_{1,291} = 13.33$, $P < 0.001$).

Kick seining and electrofishing collected fishes in 99% of collections, and nest trapping collected fishes in 9% of collections. Five percent of nest traps were occupied by fishes. Of the species collected, 87% were captured by kick seining, 100% by electrofishing, and 9% by nest trapping. Larger-bodied centrarchids and minnows were more vulnerable to electrofishing (61% of individuals collected by electrofishing), and small-bodied benthic fishes were more vulnerable to kick seining (28% of individuals collected by kick seining). Seventy-one percent of fish species not collected by kick seining were larger-bodied pelagic fishes. Additionally, cavity-spawners dominated the fishes caught in nest traps (67% of individuals collected). Fish CPUE was three times higher in electrofishing than kick seining collections (ANOVA $\mathrm{F}_{1,392} = 8.31$, $P < 0.001$). The differences in fish species richness were inconsistent across streams (Figure 2.2C; $F_{4,392} = 3.56$, $P < 0.01$); however, overall 40% more species were collected when electrofishing than kick seining (ANOVA $F_{1,393} = 82.80$, $P < 0.001$).

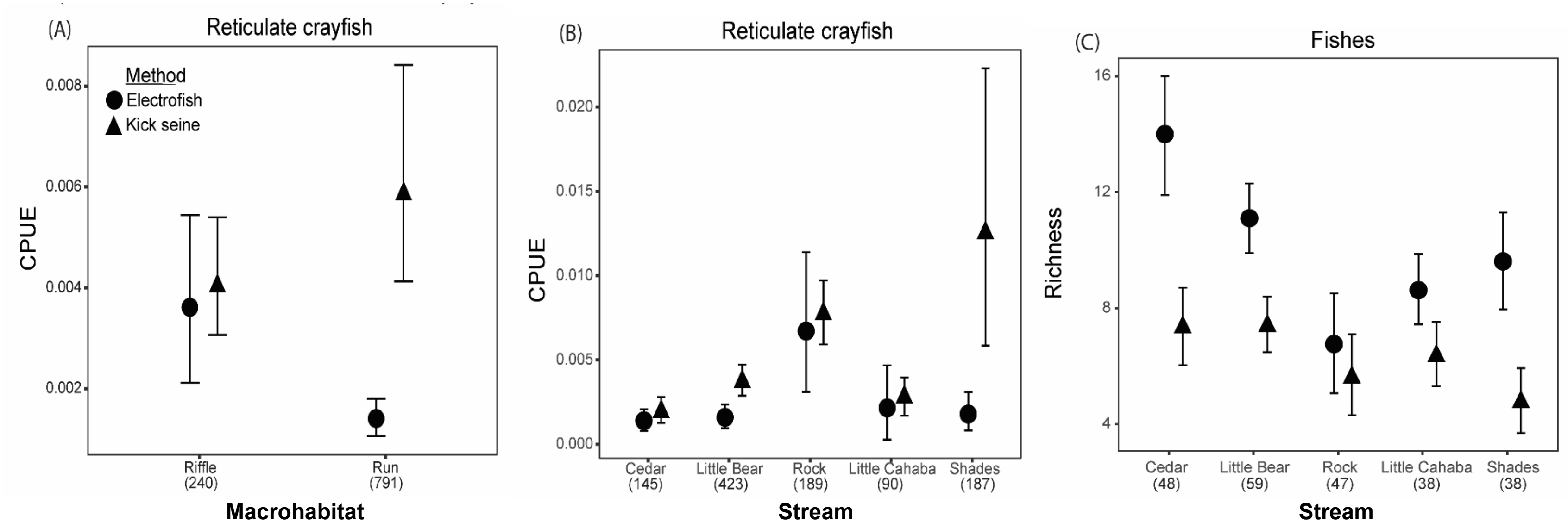

Figure 2.2. Sampling method comparisons of mean catch per unit effort (CPUE; *N*/100 m^2) (A, B) and richness (C) of reticulate crayfish and fishes, respectively, among macrohabitats and streams. Numbers in parentheses along x-axes indicate the number of individuals (A, B) or species (C) collected. Whiskers indicate 95% CI. Log$_e$ transformed data were used in analyses. Only relationships with significant interactions in repeated measures ANOVA models are displayed.

2.3.1.2 Crayfish size, age class, and reproductive form comparisons

Crayfish sizes ranged from 3.2–37.0 mm POCL ($\bar{X}$=11.8 mm) in kick seine, 3.8–52.9 mm ($\bar{X}$= 13.0 mm) in electrofishing, and 5.5–33.9 mm ($\bar{X}$= 17.6 mm) in nest trap collections. Neither kick seining nor electrofishing consistently collected larger or smaller crayfishes (P values < 0.05), with stream, macrohabitat and season impacting sizes collected (Figure 2.3). Nonetheless, electrofishing collected crayfishes of similar sizes or larger than kick seining in each stream and season.

We estimated two age classes for all species except *C. striatus* (Appendix C). Kick seining and electrofishing age class estimates were similar for all species except *F. erichsonianus* and *F. virilis* in Cahaba River drainage fall collections (Appendix C), where low numbers of crayfishes were collected by electrofishing ($N < 25$). In both kick seining and electrofishing collections, growth of age-0 crayfishes were documented from spring to fall for all species except *C. striatus*, with larger age-0 crayfishes collected later in the year (Appendix C).

Ratios of adult reproductive forms did not differ between sampling methods for most crayfishes. Females were the most abundant adult reproductive form collected ($N = 676$), consisting of 57% of total adult collections. No ovigerous females or females with young were collected. Form I male collections increased during fall sampling for each species and sampling method. Because of low numbers of *C. striatus* form I males ($N = 2$) collected in the Bear Creek drainage, these individuals were not included in analyses. No differences were detected in ratios of reproductive forms between kick seining and electrofishing collections for any species (X^2, all $P > 0.05$) except *F. virilis*. Female *F. virilis* were 2.7 times more likely to be collected than form I or II males when electrofishing ($X^2_2 = 4.08$, $P = 0.05$), but no differences were detected among reproductive forms in kick seining collections ($X^2_2 = 0.01$, $P = 0.91$).

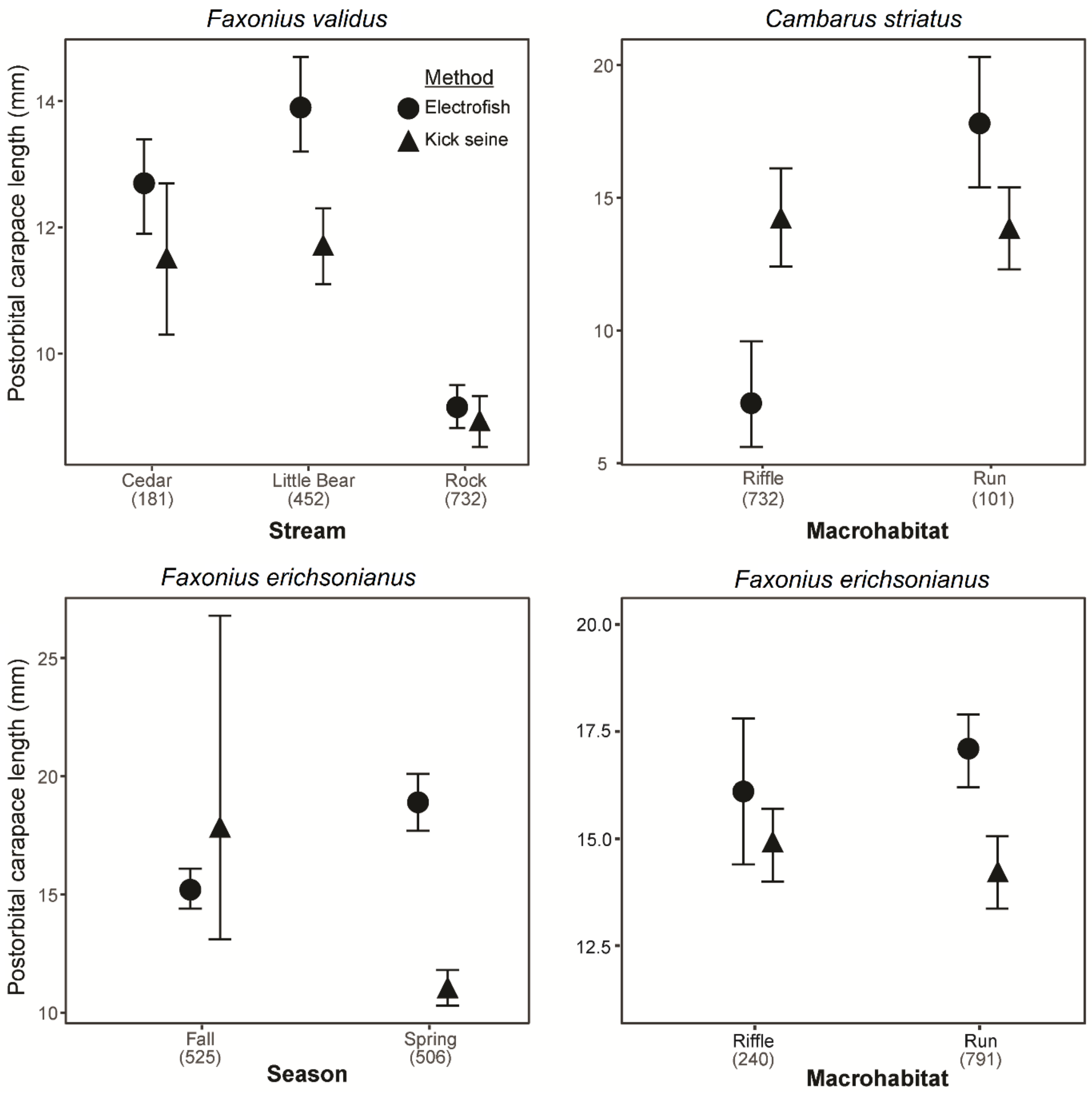

Figure 2.3. Sampling method comparisons of crayfish postorbital carapace lengths (± 95% CI) among streams (*N*; number of individuals), macrohabitats (*N*), and seasons (*N*). Log_e transformed data were used in analyses. Only relationships with significant interactions in repeated measures ANOVA models are displayed.

2.3.2 Environmental Effects

Kick seining and electrofishing effectiveness varied by stream for *F. erichsonianus* CPUE and fish richness, indicating that stream environmental factors impact methods effectiveness. The CPUE of *F. erichsonianus* was higher when kick seining in streams with greater percentages of aquatic vegetation and smaller particle sizes, as well as when electrofishing in cooler streams (Table 2.4). Fixed effects explained 4–11% of the variation in the dependent variable, indicating that other unmeasured variables may be important in the effectiveness of each method. Fish species richness was positively correlated with water temperature and negatively correlated with particle size for both sampling methods (Table 2.4). Fish species richness was higher in streams with higher width to depth ratios and lower conductivities when electrofishing, and higher in streams with lower DO and SWD when kick seining (Table 2.4).

Table 2.4. Results from linear mixed-effect repeated-measure models within two AIC_C units of the best model. Models show estimates for stream environmental variables that best explain sampling method effectivness of collecting *F. erichsonianus* CPUE and total fish richness across all sites and drainages (negative estimates indicate a negative correlation with method effectiveness). Only estimates of important variables (relative variable importance [RVI] > 0.50), averaged across models, are displayed. M-R^2 = marginal R^2 of important variables; C-R^2 = conditional R^2 of important variables; N = number of models within two AIC_C units of the best model; SE = standard error; RVI = relative variable importance (parameters with RVI of 1.00 were included in all of the best models); D50 = median particle size (mm); Vegetation = percent aquatic vegetation. All other abbreviations as in Table 2.2.

Model	M-R^2	C-R^2	N	Estimate	SE	RVI	Model	M-R^2	C-R^2	N	Estimate	SE	RVI
F. erichsonianus							***F. erichsonianus***						
Kick seine CPUE	0.11	0.53	40				**Electrofish CPUE**	0.04	0.28	15			
D50				-0.193	0.106	0.70	Water temp (°C)				-0.391	0.188	0.89
Vegetation				0.256	0.135	0.70	D84						0.41
Water temp (°C)						0.49	LWD						0.20
SWD						0.36	pH						0.19
D84						0.18	Width to depth ratio						0.12
Conductivity (µS/cm)						0.10	Canopy cover (%)						0.10
D16						0.08	D50						0.05
pH						0.06	SWD						
Canopy cover (%)						0.04							
Width to depth ratio						0.02							
LWD						0.02							
Fish							**Fish**						
Kick seine richness	0.30	0.57	21				**Electrofish richness**	0.21	0.62	11			
DO				-0.705	0.270	1.00	D50				-0.096	0.034	1.00
SWD				-0.521	0.145	1.00	Water temp (°C)				0.557	0.223	1.00
Water temp (°C)				0.843	0.289	1.00	Width to depth ratio				0.472	0.204	1.00
D50				-0.136	0.062	0.97	Conductivity (µS/cm)				-1.137	0.662	0.58
D84				-0.109	0.054	0.80	SWD						0.49
pH						0.46	pH						0.22
LWD						0.28	LWD						0.14
Conductivity (µS/cm)						0.23	Canopy cover (%)						0.08
Canopy Cover (%)						0.20	Vegetation						0.07
Riffles (%)						0.16							
D16						0.03							

2.3.3 Stream Richness Estimations

We collected 86–100% of crayfish species estimated by the Chao-1 method in each stream (Figure 2.4). Nest traps collected significantly fewer crayfishes per stream (20–67% of species) than other methods (Figure 2.4). Species richness accumulation curves were not significantly different among electrofishing, kick seining, and the two methods combined (overlapping confidence intervals). Nonetheless, species richness accumulation curves for kick seine alone and combined methods rose at a faster rate than electrofishing and nest trapping curves in all streams (Figure 2.4). To collect the maximum species estimated, combined methods required sampling 1.5–5.1 rkm (3–6% of total stream length) and 31–68% fewer sites (6–15 sites) than electrofishing or kick seining alone.

We collected 68–92% of fish species estimated by the Chao-1 method (Figure 2.5). Nest traps caught 0–2 fish species in each stream, so species richness accumulation curves were not estimated for this method. Species richness accumulation curves were not distinguishable between electrofishing and a combination of kick seining and electrofishing for all streams (Figure 2.5). Kick seining collected significantly less fish than electrofishing in all streams except Rock Creek and Little Cahaba River (Table 2.2). Electrofishing was the most effective single method at capturing all species in all streams except Rock Creek, requiring 14–61 sites (2.6–10 rkm [sampling 4–13% of the stream]) to capture 100% of species. Kick seining was the most effective single method in Rock Creek, needing 38 sites (4.4 rkm [sampling 14% of the stream]) to capture 100% of species.

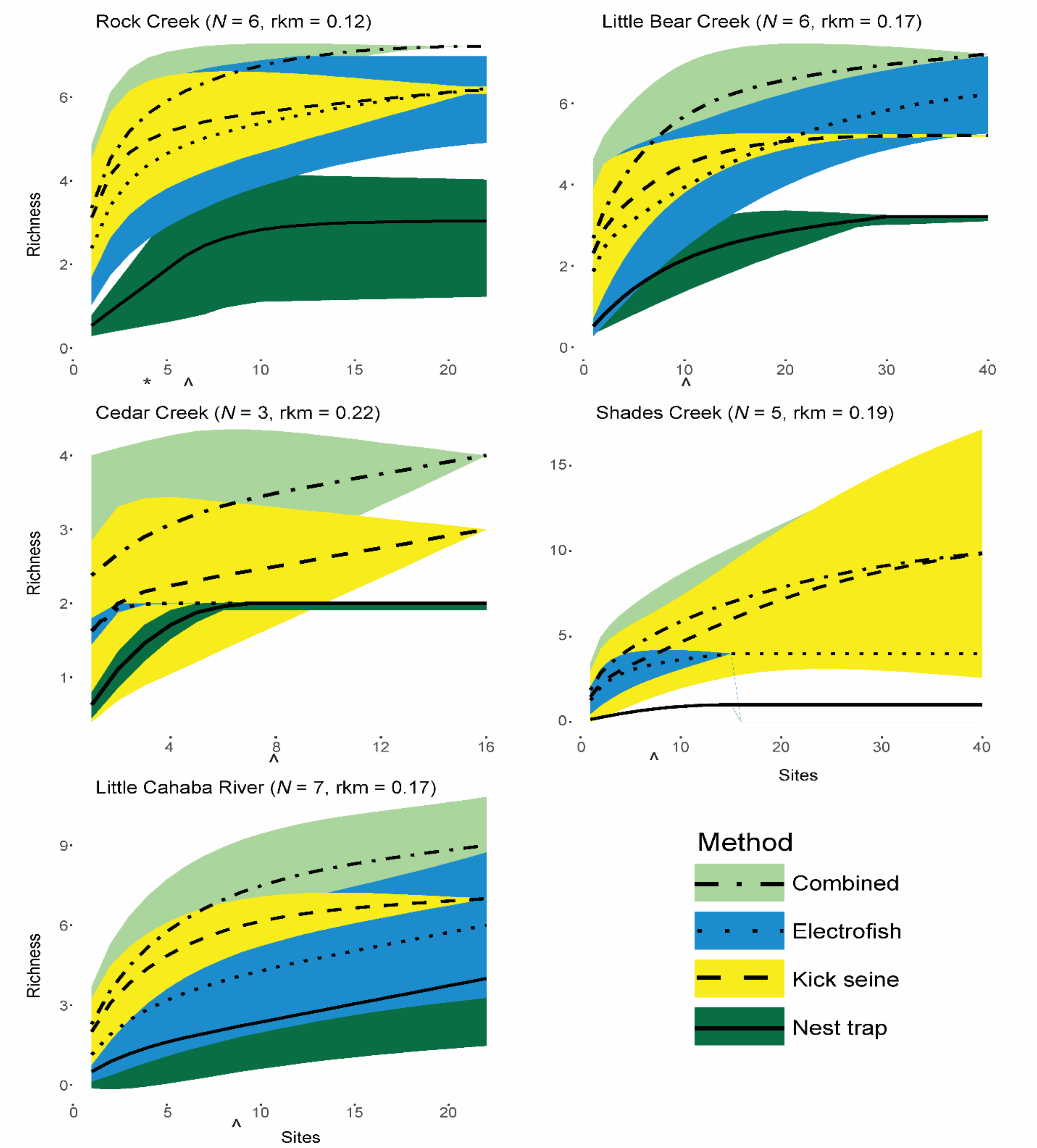

Figure 2.4. Species accumulation curves (Chao 1984), calculated to assess sampling effort (number of sites) needed to accurately estimate crayfish richness within streams. Colored polygons represent 95% confidence intervals. With combined gears (kick seining and electrofishing together), we sampled double the area and expended twice the effort relative to single gears at each site. *N* = number of species collected in each stream; rkm = average site length (km) for single sampling method; carets indicate number of sites sampled during study; asterisks indicate number of sites sampled during first sampling round (if different from other rounds).

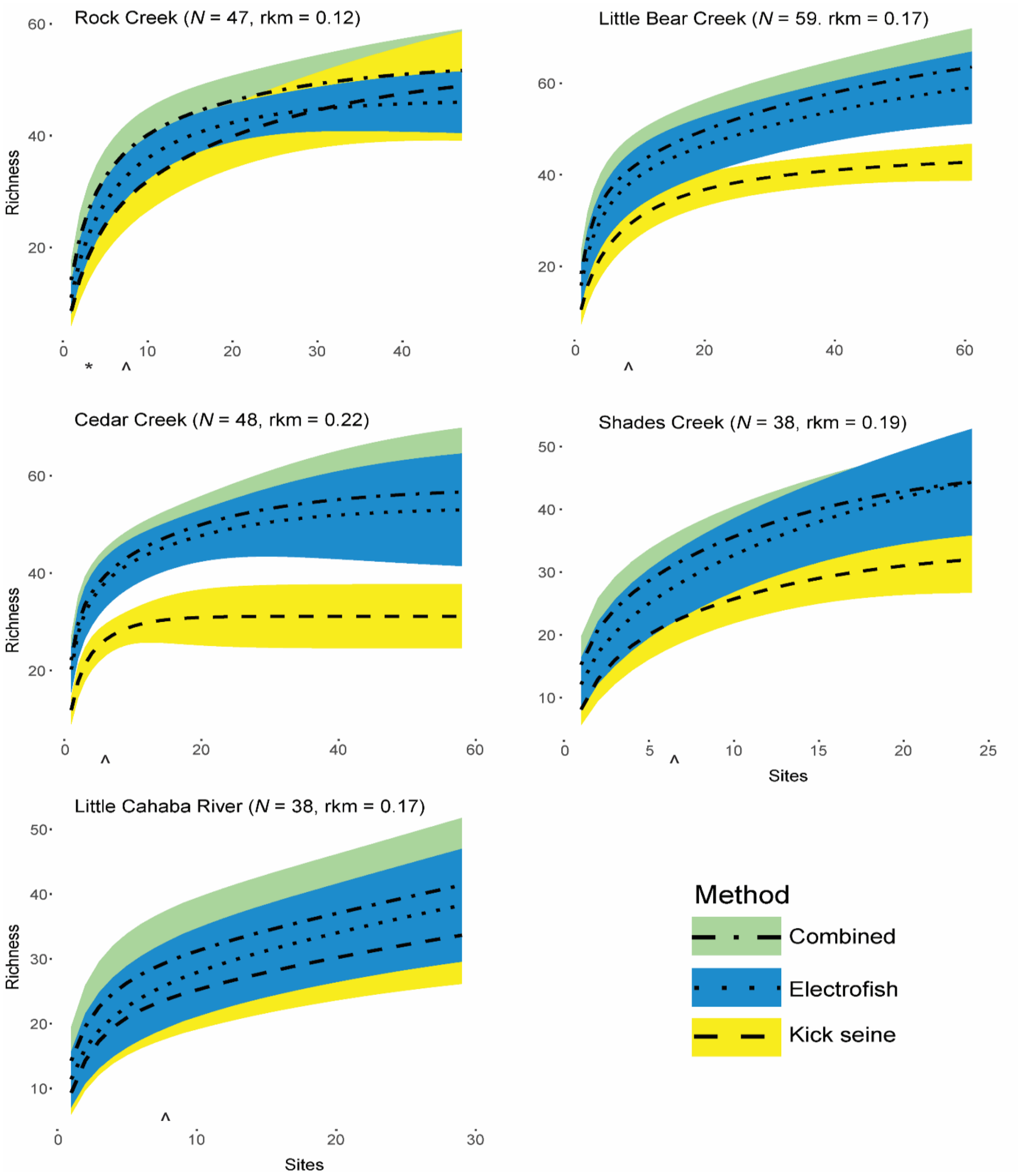

Figure 2.5. Species accumulation curves (Chao 1984), calculated to assess sampling effort needed to estimate fish richness within streams. Colored polygons represent 95% confidence intervals. With combined gears (kick seining and electrofishing together), we sampled double the area and expended twice the effort relative to single gears at each site. Abbreviations follow Figure 2.4.

2.3.4 Electrofishing Efficiency

We collected 510 crayfishes (5 species) in 18 multi-pass electrofishing surveys. Collections ranged from 1–99 crayfish per survey ($\bar{X}$= 28/survey), with section density estimates ranging from 0.00–2.69/m^2 ($\bar{X}$= 0.15/m^2). On average, we captured 73% ± 5% of individuals estimated within populations during multi-pass surveys, with 34% catchability. Catchability averaged 37% ± 4% for *F. virilis*, 29% ± 6% for *F. validus,* and 37% ± 5% for *F. erichsonianus*. We collected *C. striatus* and *F. compressus* at one and two sites, respectively. Catchabilities were 44% for *C. striatus* and 30% and 50% for *F. compressus*. Catchability was the same for each pass during most surveys, but 8–42% ($\bar{X}$= 21%) of sites for each species had a lower catchability for pass 1 than other passes. Species richness also increased after the first pass (comparisons by ANOVA models of pass 1 with passes 2, 3, and 4, all P values < 0.05), with a difference not detected among subsequent passes (comparisons of passes 2, 3, and 4, all P values > 0.05).

Water quality and crayfish size and sex were correlated with catchability. Because *C. striatus* and *F. compressus* were collected at few (≤ 3) sites, they were excluded from the catchability model. Catchability was positively correlated with conductivity, crayfish size, and water temperature and negatively correlated with percent adult males (Table 2.5).

Table 2.5. Estimates of water quality, crayfish size, and percent males that best explain catchability (Carl and Strub 1978) of crayfish during multi-pass electrofishing surveys. Results include variables from models within two AICc units of the best model. Only estimates of important variables (RVI > 0.50), averaged across models, are displayed. Abbreviations follow Table 2.4.

Model	M-R^2	C-R^2	N	Estimate	SE	RVI
Catchability	0.45	0.83	4			
% Adult males				-0.041	0.012	1.00
Average crayfish size (mm)				0.187	0.061	1.00
Water temperature (°C)				0.825	0.259	1.00
Conductivity (µS/cm)				0.780	0.336	0.80
DO						0.43

2.4 Discussion

Sampling methods should be selected based on study objectives, targeted faunal groups, and effectiveness of methods in habitat types sampled (Bonar et al. 2009, Parkyn 2015). We compared the effectiveness of three crayfish and fish sampling methods in rocky, high gradient streams in the southern Appalachian Mountains. Like previous studies assessing crayfish sampling methods (Barnett and Adams 2018, Budnick et al. 2018), combining methods was also most effective when assessing both crayfishes and fishes. Combining methods can offset sampling biases (e.g., sex, habitat, size), providing more accurate, robust data (Barnett and Adams 2018, Budnick et al. 2018).

For crayfishes, kick seining collected the greatest number of individuals and was the most effective single sampling method for documenting species richness. However, combining both kick seining and electrofishing decreased the number of sites needed to assess crayfish species richness. Because neither electrofishing nor kick seining collected all species present within all sampled streams, using both methods simultaneously increased the number of species collected while sampling fewer sites. Sampling effort needed to assess crayfish species richness has been evaluated for kick seining (Engelbert et al. 2016) and electrofishing (Budnick et al. 2018), but not the two methods combined. Among the individual methods, kick seining required the fewest sites to assess crayfish species richness in this study. Nonetheless, 2.5 times more sites were required to assess species richness when using kick seining in this study than in Missouri (Engelbert et al. 2016), an area with comparable crayfish species richness, but much higher densities (Missouri common species densities $> 0.5/\text{m}^2$; current study common species densities $> 0.01/\text{m}^2$). In addition, Engelbert et al. (2016) only sampled sites containing diverse mesohabitats (i.e., woody debris, emergent vegetation), factors that were positively correlated with crayfish CPUE when kick seining and could have increased the likelihood of crayfish capture. In Missouri streams, the precision of kick seining was comparable to that of quadrat sampling (Williams et al. 2014) and provided a repeatable and statistically supported tool to assess stream crayfish species richness (Engelbert et al. 2016). Additionally, quadrat sampling is the only stream crayfish sampling method with known efficiencies (Distefano et al. 2003, Larson et al. 2008).

Unlike in Louisiana (Budnick et al. 2018) and South Carolina (Price and Welch 2009), where electrofishing most often collected all species detected (11 and 5 species, respectively), when compared with kick seining in the current study, electrofishing was not effective in evaluating crayfish species richness (13 species). Electrofishing collected only the two most dominant species in 72% of collections where crayfishes were present. Electrofishing ineffectiveness in the current study may be largely associated with the larger, cobble substrate in southern Appalachian streams compared to smaller, silty/clay substrate in coastal plain streams (Zhao et al. 2006, Wohl et al. 2011, Budnick et al. 2018).

Crayfish sizes, age classes (with the exception of two species), and growth were comparable between kick seining and electrofishing. Although collections of small crayfishes often vary by sampling methods (Parkyn et al. 2011, Barnett and Adams 2018), sizes were also comparable between methods for *F. compressus*, a small crayfish with a maximum length (51.0 mm) about half the size of other species collected (Taylor and Schuster 2004). Likewise, Price and Welch (2009) collected similar sized crayfishes by electrofishing and seining in South Carolina. In the current study, both methods also collected age-0 crayfishes. Growth of age-0 crayfishes (i.e., increased length from spring to fall), although not statistically analyzed, was assessed through length-frequency analyses. Growth was observed in both kick seining and electrofishing collections, indicating that both methods can be used assess seasonal changes in juvenile crayfish composition. The present study is the first comparison of kick seining and electrofishing assessment of seasonal changes in age-0 crayfishes. Because temporal changes of age-0 crayfishes' abundance and growth should be expected (Brewer et al. 2009), understanding the effectiveness of methods across seasons is essential.

Sex biases have been observed for crayfishes collected by the three sampling methods (Alonso 2001, Price and Welch 2009, Hightower and Bechler 2013, Bechler et al. 2014, Reid and Devlin 2014); nonetheless, biased sex ratios do not necessarily indicate biased methods (Barnett and Adams 2018). For all methods, most collections were female dominated, with sex ratio differences between methods detected for only one species. Thus, sex ratios may not be 1:1 for most species.

In the Cahaba River drainage, *F. virilis*, an introduced species, was the most abundant and widespread species. Its native range is largely confined to the upper Mississippi River and Great Lakes drainages (Hobbs 1959, Schwartz et al. 1963, Hamr 2002). *Faxonius virilis* was the only species in this study for which sex ratios and age class estimates differed between electrofishing and kick seining collections. Kick seining age-class and sex ratio estimates (2 age classes; 1.4 adult sex ratio) were more similar to what is known for this species (2-3 age classes; ≥ 1.0 adult sex ratio) (Momot 1967, Momot and Gowing 1972) than electrofishing estimates (1-2 age classes, 0.5 adult sex ratio). Furthermore, kick seining would be a less biased method for monitoring *F. virilis*.

Stream vegetation, wood, and the interstitial spaces between and under rocks are often the dominant habitats used by stream crayfishes (Rabeni 1985, Parkyn and Collier 2004, Parkyn et al. 2009), and thus, they impact sampling method effectiveness. Unlike electrofishing collections in the current study, kick seining collected higher CPUEs of crayfishes at sites with higher percentages of aquatic vegetation and smaller median particle sizes. Conversely, in Oklahoma, kick seining collected higher CPUEs of crayfishes in non-vegetated, shallow (mean depth = 12 cm) streams (Williams et al. 2014). Furthermore, the differences in effectiveness of kick seining between vegetated and non-vegetated streams may be dependent on stream depth (mean depth in current study 20 cm), with kick seining in vegetated areas of deeper streams more effective than shallow streams. The effectiveness of kick seining may also be dependent on substrate size, with larger substrate (i.e., cobbles and boulders) providing more interstitial space for crayfishes, but also more difficult to move and kick through than very small substrate. The mean particle size kick seined, in this study, was large cobble (11.5 cm), and kick seining was more efficient in habitats with smaller substrate.

To increase the efficiency of electrofishing and accurately assess stream species abundance, more than one electrofishing pass may be needed (Kennard et al. 2006). Crayfishes often become more susceptible to capture after being disturbed during the first electrofishing pass (Reid and Devlin 2014), and with increasing stream widths a smaller proportion of the stream channel is often covered by a single electrofishing pass. Thus, conducting more than one pass when sampling can increase catchability and give more accurate population estimates. Two electrofishing passes may be efficient for accurately assessing crayfish species richness, because species richness did not differ between the second and subsequent passes in this study. Crayfish catchability in this study (34%) was less than previously reported catchabilities of 60% (Alonso 2001) and 52% (Gladman et al. 2010). Lower catchability may be attributed to larger streams sampled, larger rocks, macrohabitat differences, and lower conductivities (Penczak and Rodriguez 1990, Paller 1995, Alonso 2001, Gladman et al. 2010). Catchability was higher when more large crayfishes (Zalewski and Cowx 1990, Alonso 2001) and fewer adult males were present. This may be due to males' ability to out-compete juveniles and adult females for shelter (Rabeni 1985, Nakata and Goshima 2003), making them harder to dislodge and collect when electrofishing (Portt et al. 2006, Gladman et al. 2010). Catchability was also higher in warmer waters. Ectothermic organisms are often more active and excitable in warmer temperatures, making them easier to catch (Somers and Stetchy 1986, SFCC 2007).

Fish species richness and CPUEs were higher in electrofishing than kick seining collections. Nonetheless, only 50% of known fish species were collected from drainages. Fish species compositions often play an important role in the effectiveness of sampling methods, with small-bodied benthic fishes more susceptible to kick seining and larger-bodied pelagic fishes more susceptible to electrofishing (Matthews and Marsh-Matthews 2017). Additionally, areas sampled also impact the species collected. We sampled habitats with depths ≤ 1 m, which limited our collections to shallow water species. Nonetheless, all fish species detected in the study were collected when electrofishing. Electrofishing was more efficient at sites with lower conductivities (range 46–538 μS/cm; Table 2). Although electrical currents are more readily transmitted in highly conductive waters, higher wattages (i.e. > 500 w; more than the maximum output of backpack electrofishers) are needed to stun fishes in highly conductive waters, indicating a nonlinear relationship, with electrofishing ineffective in streams with very high and very low conductivities (SFCC 2007).

Macrohabitats are often partitioned among crayfish and fish species and size classes (DiStefano et al. 2003). In the current study, nest traps sampled pools, and electrofishing and kick seining sampled riffles and runs, yet species collected in nest traps were also collected by kick seining and electrofishing. As in other nest trap samples (Bechler et al. 1990, 2014), cavity-spawners dominated the fishes caught in nest traps, and the most abundant crayfish species in nest traps represented the most abundant crayfishes within the stream system. Additionally, smaller crayfishes were collected by nest traps than by kick seines in Georgia streams (Bechler et al. 2014), whereas larger crayfishes were collected by nest traps than by kick seines and electrofishers in the current study. Higher percentages of form I males (20%) were also collected by nest traps in this study than in Georgia streams (5%; Bechler et al. 2014).

Nest traps in this study were not as efficient as nest traps in Georgia streams (Bechler et al. 2014), with occupancy rates four times higher in Georgia. Nest traps in Georgia streams also collected up to five crayfish per trap, as well as fishes and crayfishes simultaneously in traps (Bechler et al. 2014). On one occasion, we collected two fish (black madtoms, *Noturus funebris*) from one nest trap. Differences in nest trap effeciecncy between studies could possibly represent differences in crayfishes' and fishes' use of macrohabitats, with runs sampled in Georgia streams (Bechler et al. 2014) and pools sampled in the present study. Future studies using methods adequate for all macrohabitat types are needed to assess differences in crayfish macrohabitat use.

Duration of trap deployment impacted the number of traps recovered, with a 50% decrease in recovery when traps were deployed for more than 12 months (Bechler et al. 2014). Lower recovery rates may be due to displacement of traps during heavy winter and early spring rains. The removal and resetting of traps between fall and spring sampling may increase the number of traps recovered.

Species distribution will vary throughout a stream system due to variation in stream order, substrate composition, and habitat along a stream length (Vannote et al. 1980). To help ensure accurate assessments of species richness, estimations of stream sampling length (reach length) and number of sampling sites from this study can be used on similar stream types within the region. Because sampling methods that were most efficient at collecting crayfish versus fish species richness differed, a combination of kick seining and electrofishing methods is recommended for accurate sampling of both taxa. Sampling both taxa simultaneously will decrease the time needed to conduct separate surveys and assess research questions that cannot be answered when sampling at different times. However, simultaneously sampling for both crayfishes and fishes when electrofishing can be more difficult than sampling for one taxon, due to differences in responses to sampling methods. Unlike most fishes that float to the water's surface after coming into the electrofisher's electrical field, crayfishes often erratically move through the water column trying to escape the electrical field or remain stunned at the bottom of the stream (Burba 1993, Westman et al. 1978). Thus, collectors need to focus on numerous parts of the water column to ensure collection of both taxa, which could reduce collection accuracy. Nonetheless, because both taxa use similar habitats and can be disturbed (i.e., dislodged from habitat) when sampling for one taxon, conducting separate surveys within the same sites will likely create biased samples (i.e., decreased collections in habitats previously sampled) and lead to inaccurate conclusions. Likewise, using one sampling method to sample both taxa will also lead to biased samples for at least one of the taxa.

While kick seining and electrofishing were more effective than nest traps at collecting crayfishes and fishes, they both come with drawbacks. Kick seining in rocky, highly vegetated streams is time- and energy-intensive, and fewer sites can be sampled during a day by kick seining (average = 2 sites/ day; ≤ 100 kick seines/day) than electrofishing. Electrofishing also sampled more of each subreach ($\bar{X}$= 70%) than kick seining ($\bar{X}$= 15%) in either the same or less time. Kick seining and electrofishing cause more harm to organisms than nest trapping due to possibly crushing organisms, dislodging mussels, and disrupting habitats when kick seining (Larson and Olden 2016), as well as crayfish chelae loss, fish bruising, and broken backs when electrofishing (Westman et al. 1978, Alonso 2001; Snyder 2003, Miranda and Kidwell 2010). We did not record such injuries but sometimes observed them during sampling.

Understanding the effectiveness of different sampling methods in evaluating stream communities is key to accurate species assessments and informed stream ecosystem management. Using sampling methods that accurately assess the study question is vital. For example, it is best to use both kick seining and electrofishing if the study goal is to analyze stream crayfish species richness, or use electrofishing to analyze fish species richness. Understanding the effectiveness of sampling methods in various habitat types, such as kick seining in streams with aquatic vegetation or electrofishing in streams with smaller substrates, and simultaneously sampling more than one faunal group can reduce sampling effort and cost. Using kick seining and electrofishing methods in tandem to assess fish and crayfish populations will contribute to a better understanding of stream community structures.

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APPENDIX A

TRIPLE PASS ELECTROFISHING ENVIRONMENTAL PARAMETERS

Appendix A. Median (SD) values for environmental parameters from triple pass electrofishing surveys. Four sites were sampled in each drainage. Abbreviations as in Table 2.2.

	Little Bear	**Cedar**	**Rock**	**Little Cahaba**	**Shades**
Water temperature (°C)	21.48 (2.75)	25.83 (1.76)	22.24 (1.74)	23.43 (2.48)	26.68 (1.03)
DO (mg/l)	8.00 (0.18)	6.98 (1.17)	4.95 (1.78)	6.67 (0.18)	6.12 (0.95)
Conductivity (µS/cm)	96.5 (54.7)	272.0 (85.0)	160.8 (52.9)	326.7 (86.3)	211.0 (29.7)
pH	7.54 (0.13)	7.79 (0.85)	7.38 (0.41)	7.62 (0.03)	7.35 (0.14)
Wetted width (m)	6.6 (2.5)	10.4 (3.5)	6.9 (1.3)	11.3 (5.1)	11.9 (2.9)
Depth (cm)	9.9 (5.1)	16.5 (5.7)	11.3 (8.7)	18.8 (12.2)	15.7 (1.7)
Width to depth ratio	0.67 (0.38)	0.76 (0.18)	0.54 (0.51)	0.80 (0.47)	0.69 (0.19)
D16	1.1 (1.2)	7.7 (5.7)	1.4 (16.1)	21.7 (32.6)	0.2 (0.9)
D84	1,300.4 (1,000.1)	249.3 (932.0)	48.8 (978.8)	180.3 (942.4)	1,021.7 (1,137.0)
Aquatic vegetation (%)	12.0 (13.2)	17.3 (9.3)	16.0 (11.7)	11.7 (3.3)	7.9 (8.2)
Canopy cover (%)	63.9 (20.9)	59.7 (17.2)	56.1 (20.7)	61.1 (10.2)	77.4 (20.9)
Discharge (m^3/s)	2.87 (1.25)	7.51 (3.91)	0.19 (0.55)	11.80 (11.70)	10.56 (8.30)

APPENDIX B

FISH SPECIES

Appendix B. Total numbers of each fish species (number of sites containing species) collected in the Bear Creek (A; 24 sites) and Cahaba River (B; 14 sites) drainages and sampling method listed in descending order of abundance.

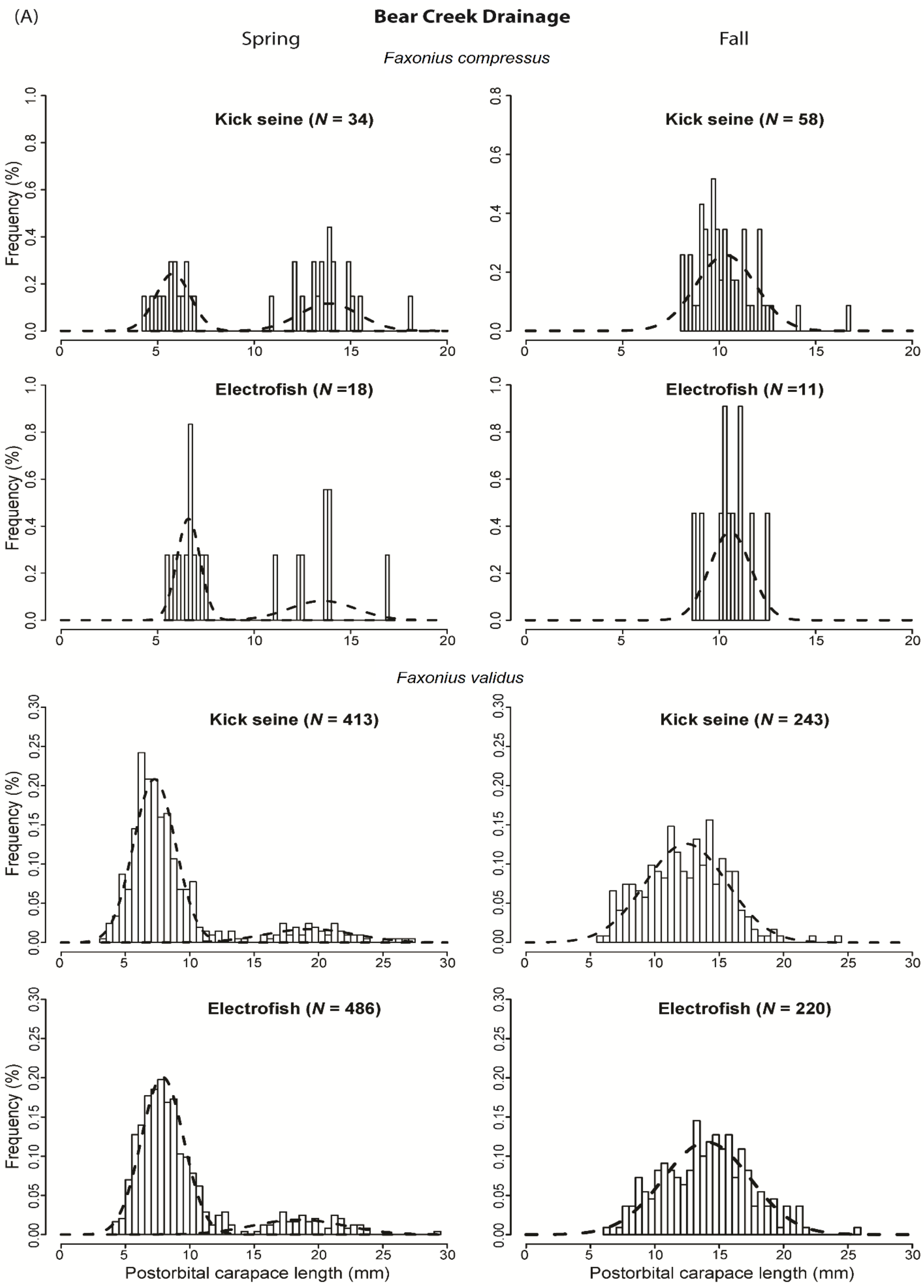
(A)	Fish (*N*)	Electrofish	Kick seine	Nest trap	Total
	Largescale Stoneroller *Campostoma oligolepis* (23)	2,994	623	0	3,617
	Redline Darter *Etheostoma rufilineatum* (21)	2,135	583	0	2,718
	Snubnose Darter *Etheostoma simoterum* (18)	747	921	0	1,668
	Striped Shiner *Luxilus chrysocephalus* (22)	475	366	0	841
	Blackside Snubnose Darter *Etheostoma duryi* (23)	338	456	0	794
	Banded Sculpin *Cottus carolinae* (17)	534	146	1	681
	Rainbow Darter *Etheostoma caeruleum* (21)	342	212	0	554
	Longear Sunfish *Lepomis megalotis* (21)	436	70	0	506
	Greenside Darter *Etheostoma blennioides* (15)	382	79	0	461
	Northern Hogsucker *Hypentelium nigricans* (22)	333	109	0	442
	Stripetail Darter *Etheostoma kennicotti* (21)	206	198	0	404
	Creek Chub *Semotilus atromaculatus* (17)	138	243	0	381
	Bigeye Chub *Hybopsis amblops* (9)	89	186	0	275
	Scarlet Shiner *Lythrurus fasciolaris* (16)	71	200	0	271
	Bluegill *Lepomis macrochirus* (23)	234	22	0	256
	Blackspotted Topminnow *Fundulus olivaceus* (22)	171	44	0	215
	Bluntnose Minnow *Pimephales notatus* (18)	79	92	0	171
	Rock Bass *Ambloplites rupestris* (19)	164	4	0	168
	Common Logperch *Percina caprodes* (18)	126	41	1	168
	Green Sunfish *Lepomis cyanellus* (19)	152	9	2	163
	Whitetail Shiner *Cyprinella galactura* (15)	92	62	0	154
	Brook Silverside *Labidesthes sicculus* (9)	20	131	0	151
	Black Redhorse *Moxostoma duquesnei* (15)	67	64	0	131
	Northern Studfish *Fundulus catenatus* (16)	59	41	0	100
	Western Mosquitofish *Gambusia affinis* (15)	47	41	0	88
	Largemouth Bass *Micropterus salmoides* (17)	48	35	0	83
	Spotted Bass *Micropterus punctulatus* (19)	65	15	0	80
	Mimic Shiner *Notropis volucellus* (9)	71	9	0	80
	Blueside Darter *Etheostoma jessiae* (9)	32	44	0	76
	Spotfin Shiner *Cyprinella spiloptera* (9)	58	16	0	74
	Rosyside Dace *Clinostomus funduloides* (6)	21	29	0	50
	Weed Shiner *Notropis texanus* (6)	36	11	0	47
	Dusky Darter *Percina sciera* (8)	34	11	0	45
	Golden Redhorse *Moxostoma erythrurum* (10)	23	18	0	41
	Bigeye Shiner *Notropis boops* (6)	33	6	0	39
	Slender Madtom *Noturus exilis* (7)	24	12	0	36
	Black Madtom *Noturus funebris* (6)	24	5	4	33
	Brindled Madtom *Noturus miurus*(7)	12	17	2	31

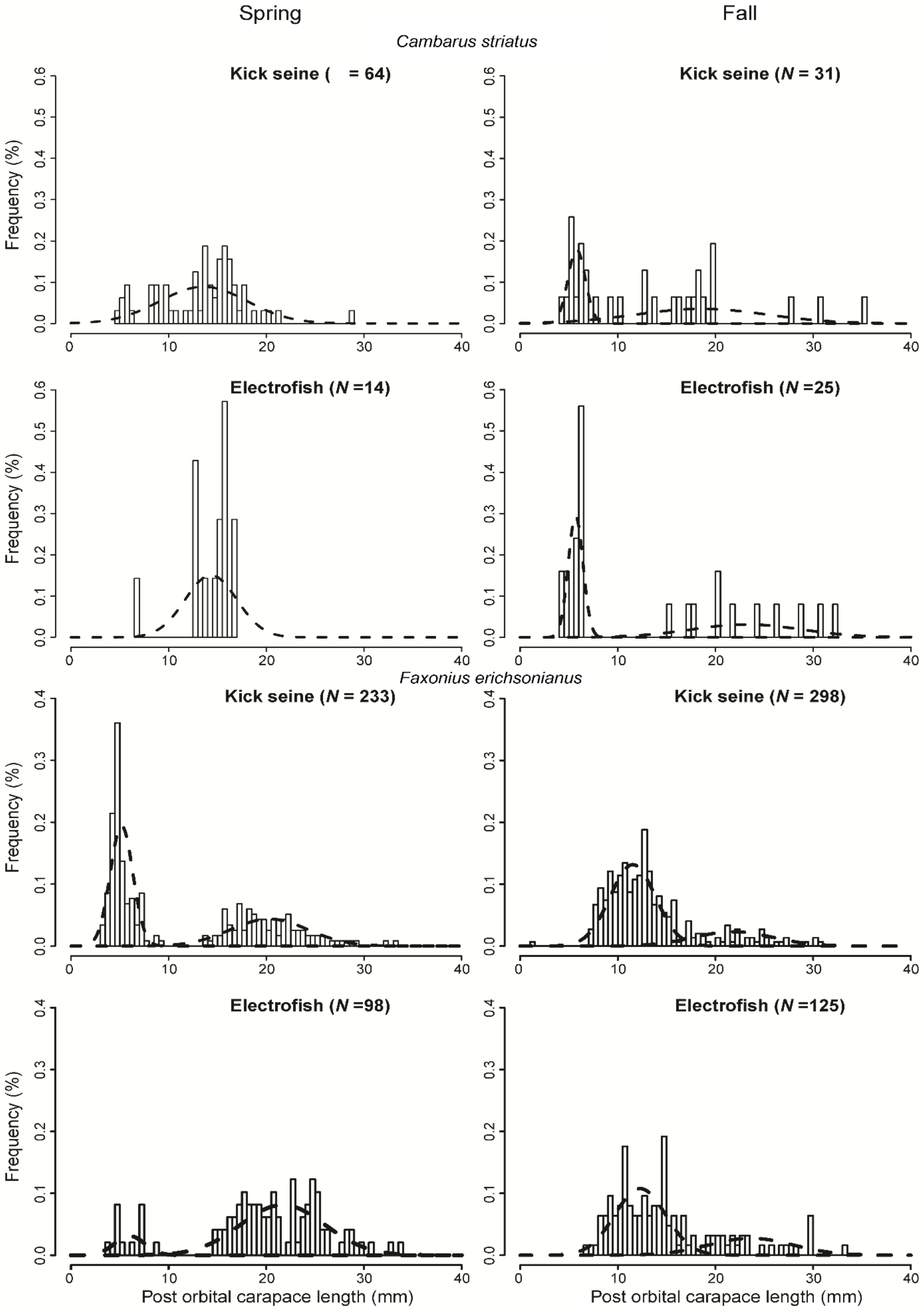
(A) Fish (*N*)	Electrofish	Kick seine	Nest trap	Total
Bullhead Minnow *Pimephales vigilax* (8)	22	7	0	29
Johnny Darter *Etheostoma nigrum* (7)	10	11	0	21
Yellow Bullhead *Ameiurus natalis* (9)	13	0	1	14
Channel Catfish *Ictalurus punctatus* (5)	8	5	0	13
Dollar Sunfish *Lepomis marginatus* (7)	5	6	0	11
Warmouth *Lepomis gulosus* (4)	4	7	0	11
Western Creek Chubsucker *Erimyzon claviformis* (5)	8	2	0	10
Redfin Pickerel *Esox americanus* (3)	6	2	0	8
Blackside Darter *Percina nigrofasciata* (3)	4	3	0	7
Silver Redhorse *Moxostoma anisurum* (4)	5	1	0	6
Bluehead Chub *Nocomis leptocephalus* (3)	4	2	0	6
Pirate Perch *Aphredoderus sayanus* (2)	3	2	0	5
Spotted Sucker *Minytrema melanops* (4)	4	0	0	4
Least Brook Lamprey *Lampetra aepyptera* (2)	4	0	0	4
White Crappie *Pomoxis annularis* (2)	4	0	0	4
Longnose Gar *Lepisosteus osseus* (3)	2	2	0	4
Flathead Catfish *Pylodictis olivaris* (3)	3	0	0	3
Threadfin Shad *Dorosoma petenense*(2)	1	2	0	3
Telescope Shiner *Notropis telescopus* (2)	1	2	0	3
Common Carp *Cyprinus carpio* (1)	3	0	0	3
Golden Shiner *Notemigonus crysoleucas* (2)	2	0	0	2
Redear Sunfish *Lepomis microlophus* (2)	2	0	0	2
Gilt Darter *Percina evides* (1)	2	0	0	2
Smallmouth Buffalo *Ictiobus bubalus* (1)	1	0	0	1
Gizzard Shad *Dorosoma cepedianum* (1)	1	0	0	1
Mobile Logperch *Percina kathae* (1)	1	0	0	1
Highland Shiner *Notropis micropteryx* (1)	1	0	0	1
Redspotted Sunfish *Lepomis miniatus* (1)	1	0	0	1
Freckled Madtom *Noturus nocturnus* (1)	1	0	0	1
(B) Largescale Stoneroller *Campostoma oligolepis* (14)	477	274	0	751
Longear Sunfish *Lepomis megalotis* (14)	587	112	1	700
Banded Sculpin *Cottus carolinae* (6)	377	60	0	437
Tricolor Shiner *Cyprinella trichroistia* (13)	112	295	0	407
Bluegill *Lepomis macrochirus*(14)	355	38	0	393
Blackbanded Darter *Percina nigrofasciata* (14)	241	66	0	307
Alabama Hogsucker *Hypentelium etowanum* (14)	162	53	0	215
Alabama Shiner *Cyprinella callistia* (12)	61	149	0	210
Redspotted Sunfish *Lepomis miniatus* (11)	158	20	1	179
Green Sunfish *Lepomis cyanellus* (11)	126	37	2	165
Western Mosquitofish *Gambusia affinis* (12)	40	101	0	141

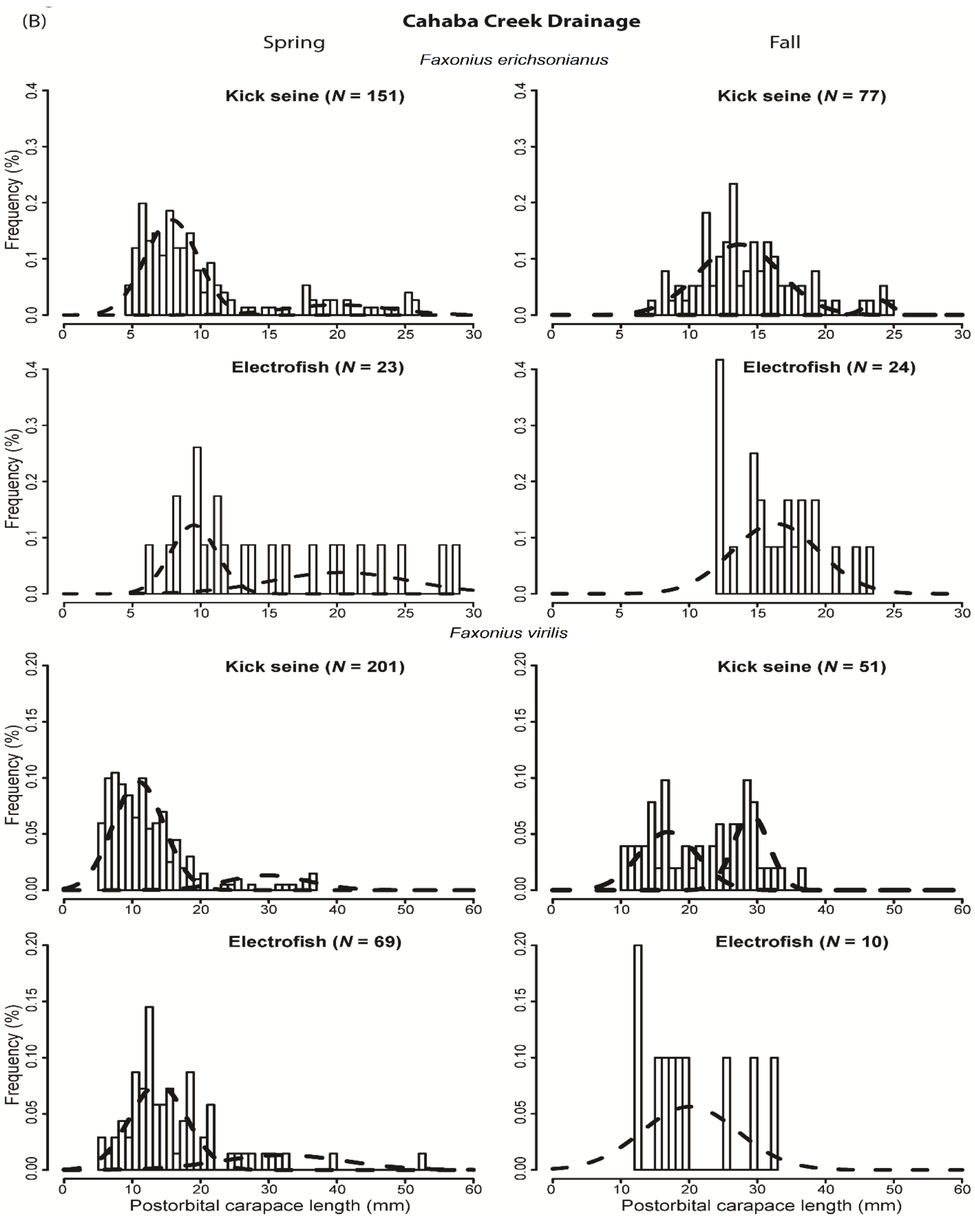
(B)	Fish (*N*)	Electrofish	Kick seine	Nest trap	Total
	Silverstripe Shiner *Notropis stilbius* (9)	23	114	0	137
	Alabama Darter *Etheostoma ramseyi* (4)	76	65	0	136
	Alabama Bass *Micropterus henshalli* (13)	53	46	0	96
	Blackspotted Topminnow *Fundulus olivaceus* (13)	39	33	0	72
	Cahaba Bass *Micropterus cahabae* (8)	47	17	0	64
	Mobile Logperch *Percina kathae* (9)	46	16	0	62
	Speckled Darter *Etheostoma stigmaeum* (10)	8	52	0	60
	Creek Chub *Semotilus atromaculatus* (6)	16	25	0	41
	Blacktail Shiner *Cyprinella venusta* (6)	25	14	0	39
	Warmouth *Lepomis gulosus* (8)	29	3	1	33
	Blackside Darter *Percina maculata* (2)	27	1	0	28
	Redspot Darter *Etheostoma artesiae* (4)	17	5	0	22
	Rock Darter *Etheostoma rupestre* (4)	18	3	0	21
	Clear chub *Hybopsis winchelli* (2)	17	1	0	18
	Emerald Shiner *Notropis atherinoides* (3)	1	16	0	17
	Largemouth Bass *Micropterus salmoides* (9)	10	4	0	14
	Blacktail Redhorse *Moxostoma poecilurum* (3)	10	1	0	11
	Redear Sunfish *Lepomis microlophus* (5)	9	0	0	9
	Mimic Shiner *Notropis volucellus* (3)	1	6	0	7
	Yellow Bullhead *Ameiurus natalis* (5)	5	1	0	6
	Dollar Sunfish *Lepomis marginatus* (3)	5	1	0	6
	Striped Shiner *Luxilus chrysocephalus* (3)	3	3	0	6
	Greenbreast Darter *Etheostoma jordani* (2)	6	0	0	6
	Riffle Minnow *Phenocobius catostomus* (3)	1	3	0	4
	Mountain Shiner *Lythrurus lirus* (2)	2	2	0	4
	Channel Catfish *Ictalurus punctatus* (2)	2	1	1	4
	Black Redhorse *Moxostoma duquesnii* (2)	1	2	0	3
	Flathead Catfish *Pylodictis olivaris* (2)	1	1	0	2
	Spotted Sucker *Minytrema melanops* (1)	2	0	0	2
	Golden Shiner *Notemigonus crysoleucas* (1)	1	0	0	1
	Golden Redhorse *Moxostoma erythrurum* (1)	1	0	0	1
	Chain Pickerel *Esox niger* (1)	1	0	0	1
	Blackstripe Topminnow *Fundulus notatus* (1)	1	0	0	1
	Dusky Darter *Percina sciera* (1)	1	0	0	1

APPENDIX C

POSTORBITAL CARAPACE LENGTH-FREQUENCY CHARTS


(A)
Bear Creek Drainage
Spring
Fall
Faxonius compressus
Kick seine (N = 34)
Kick seine (N = 58)
Electrofish (N =18)
Electrofish (N =11)
Faxonius validus
Kick seine (N = 413)
Kick seine (N = 243)
Electrofish (N = 486)
Electrofish (N = 220)
Frequency (%)
Postorbital carapace length (mm)
0.0
0.2
0.4
0.6
0.8
1.0
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0
5
10
15
20
25
30


Spring
Fall
Cambarus striatus
Kick seine (= 64)
Kick seine (N = 31)
Electrofish (N =14)
Electrofish (N =25)
Faxonius erichsonianus
Kick seine (N = 233)
Kick seine (N = 298)
Electrofish (N =98)
Electrofish (N =125)
Frequency (%)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
10
20
30
40
Post orbital carapace length (mm)



Appendix C. Postorbital carapace length-frequency charts for kick seining and electrofishing collections of the most abundant crayfish species in the Bear Creek (A) and Cahaba River (B) drainages. Dashed lines represent age classes estimated using mixed distribution analyses with peaks at age class median lengths.

CHAPTER III:

IMPACTS OF IMPOUNDMENTS ON STREAM CRAYFISHES: EFFECTS OF ABIOTIC FACTORS AND FISH PREDATORS

Abstract

Flow alterations by dams impact up- and downstream physiochemical properties, leading to drastic shifts in stream biotic assemblages. Effects of dams and their subsequent impoundments have been examined across a range of faunal groups, but only one published study has assessed impacts on crayfishes. Thus, we assessed the effects of dams and impoundments on crayfish assemblages in streams fragmented by dams at least 30 times larger than dams previously studied. We sampled crayfishes and measured environmental variables at multiple up- and downstream sites in three impounded and two unimpounded streams in the Bear Creek and Cahaba River drainages, Alabama, USA. Crayfish assemblages differed between impounded and unimpounded streams in both drainages. We detected greater assemblage differences down- than upstream of impoundments and greater differences in the Bear Creek than Cahaba River drainage. In downstream sections of both drainages, adults were smaller and catch per unit effort (*N*/100 m^2) was higher in unimpounded than impounded streams. In unimpounded streams, crayfish assemblage structure gradually shifted, with the dominant species changing with distance downstream. Conversely, impounded streams' assemblage structures did not differ between up- and downstream sections. For example, two species dominated all sites in impounded streams in the Bear Creek drainage. Although assemblage structure was similar in sections up- and downstream of impoundments, finer scale assessments showed increases in the abundance of rare species with distance downstream of impoundments, creating more diverse assemblages (i.e., species recovery) with distance downstream. Flow and temperature regimes, percent aquatic vegetation, substrate sizes, and fish predators discriminated between impounded and unimpounded streams and were correlated to crayfish assemblage

differences. We conclude that impoundments played a substantial role in shaping stream crayfish assemblages, with possible ripple effects on system functional attributes.

3.1 Introduction

Flowing water is the defining characteristic of streams (Poff et al. 1997). Yet, over half of the world's rivers have seen changes in the magnitude and timing of flows due to water usage and water regulation by impoundments (McAllister et al. 2001). Flow alterations by impoundments impact streams' physiochemical properties including modifications to temperature regimes, water chemistry, channel geomorphology, and floodplain connectivity, both within the impounded area and in connecting streams (Ward 1976, Baxter 1977, Chien 1985, Voelz and Ward 1990, Wood and Petts 1994, Gordon et al. 2004, Graf 2006). The magnitude and consequences of these effects depend on impoundment size, physiographic setting, location within the drainage, and location along the stream (Ward and Stanford 1979, Poff and Hart 2002). Changes to streams' physiochemical characteristics are documented in nearly all studies of impounded stream properties (Palmer and O'Keeffe 1990, Arnwine et al 2006, Gangloff et al. 2011). Understanding the threats facing aquatic organisms due to these changes is key to conserving biodiversity.

Impoundments also alter species' distributions and food availability, leading to dramatic shifts in ecosystem or community properties (Watters 1996, Cumming 2004, McLaughlin et al. 2006). A stream's flow regime is a major determinant of aquatic species distributions and life histories (Naesje et al. 1995, Fausch and Bestgen 1997, Mims and Olden 2012), with many organisms' life events (e.g., spawning, larval survival, growth patterns, and recruitment) synchronized with the natural flow regime. Consequently, due in part to flow modifications from impoundments, streams are considered our most imperiled ecosystems (Bunn and Arthington 2002). Documented changes to stream biota due to impoundments include increases in invertebrate biomass, decreases in mussel biomass, decreases in the diversity of numerous stream taxa, and changes in the dominant species and dispersal of invertebrates and fishes (Cadwallader 1978, Mackay and Waters 1986, Gherke et al. 2002, McGregor and Garner 2003, Gangloff et al. 2011). In addition, biological responses to impoundment alterations are often correlated to distance from impoundments (Ward and Stanford 1983). For example, fish, macroinvertebrate, and mussel species assemblages and biomass often become increasingly similar to pre-impoundment assemblages with increasing distance from impoundments, both up- and downstream (Penczak et al. 1984, Voelz and Ward 1990, McGregor and Garner 2003, Phillips and Johnston 2004).

Although effects of impoundments have been examined across a range of faunal groups, only one published study has assessed their impacts on crayfishes (Adams 2013). That study showed assemblage and reproductive timing differences, as well as decreased abundances of some crayfish species in streams with small impoundments. No study has assessed the effects of large impoundments (dams > 15 m high) on crayfishes. Crayfishes play an important role in stream ecosystem trophic processes by processing detritus, altering the composition of macrophytes and substrate, and transferring energy to a variety of predators including fish, birds, and other crayfishes (Chambers et al. 1990, Hanson et al. 1990, Momot 1995, Rabeni et al. 1995, Statzner et al. 2003, Stenroth and Nystrom 2003). Yet, crayfish populations are declining worldwide, with 48% of North American crayfish species threatened (Taylor et al. 2007) and endangerment rates rapidly increasing (Richman et al. 2015). Alterations to the physiochemical make-up and community structure of streams through flow regulations may directly affect the diversity, abundance, and ecosystem functions of crayfishes, creating ripple effects throughout freshwater and riparian ecosystems. Furthermore, understanding the effects of impoundments and conserving crayfishes are essential to management and protection of riverine biodiversity.

Alabama (USA) is a freshwater biodiversity hotspot, supporting 60% of North America's native mussel species, 43% of native freshwater snails, 38% of native fishes, and 24% of native crayfishes, many of which are endemic to the southeastern USA (Lydeard and Mayden 1995, Crandall et al. 2000). In addition, northern Alabama is in the southern Appalachian Mountains (ARC 2009), the global center of crayfish diversity (Crandall and Buhay 2008). Although Alabama supports rich freshwater ecosystems, it contains more than 2,000 large dams (NID 2013), and declines in species richness and diversity are occurring at an alarming rate (Warren and Burr 1994).

In the present study, we assessed the effects of impoundments on crayfish assemblages in Alabama streams fragmented by dams at least 30 times larger than dams previously studied (Adams 2013). To do this, we compared crayfish assemblages at multiple locations in adjacent impounded and unimpounded streams and assessed relationships between crayfish assemblages and abiotic and biotic factors. We addressed five questions (Table 3.1): 1) Are crayfish assemblages different between impounded and unimpounded streams? 2) Do differences in abiotic and biotic factors between impounded and unimpounded streams explain assemblage differences? 3) Do crayfish assemblages in impounded streams show concordant responses to impoundments across drainages and years? 4) Does land use differ between drainages and years, and are those differences consistent with assemblage differences between drainages and years? and 5) Do impounded streams' crayfish assemblages change with distance from impoundments?

Table 3.1. Research questions, statistical analyses, and expected results. PERMANOVA = permutational multivariate analysis of variance; ANOVA = analysis of variance; LME = linear mixed-effect model; CPUE = catch per unit effort.

Research Question	Statistical analyses	Expected result if impoundments impacted crayfish assemblages
Question 1: Are crayfish assemblages different between impounded and unimpounded streams?	PERMANOVA/ ANOVA/ LME	Similar crayfish assemblages in up- and downstream sections of impounded streams, but differences between sections in unimpounded streams. Greater crayfish CPUEs in unimpounded than impounded streams Larger crayfishes and fewer juveniles in downstream sections of impounded streams
Question 2: Do differences in abiotic and biotic factors between impounded and unimpounded streams explain assemblage differences?	Linear discriminant analyses	Stream and biotic characteristics discriminate between impounded and unimpounded streams
	Distance based linear models	Variables that discriminate between impounded and unimpounded streams will also explain differences between impounded and unimpounded stream crayfish assemblages
Question 3: Do crayfish assemblages in impounded streams show concordant responses to impoundments across drainages and years?	Qualitatively examined	No differences in crayfish assemblages between drainages and years
Question 4: Does land use differ between drainages and years, and if so, are differences consistent with assemblage differences between drainages and years?	PERMANOVA	Historical and current land use does not differ between drainages or years.
Question 5: Do impounded streams' crayfish assemblages change with distance from impoundments?	PERMANOVA/ ANOVA/LME	Impounded streams' crayfish abundances and species richness will increase with distance up- and downstream from impoundments, becoming similar to unimpounded stream assemblages

3.2 Methods

3.2.1 Study Area

We sampled flowing sections of impounded and unimpounded streams in the Bear Creek (Tennessee River Basin) and Cahaba River (Mobile River Basin) drainages, Alabama, USA (Figure 3.1). Both drainages have highly diverse aquatic faunal communities and numerous imperiled species (Allen 2001, McGregor and Garner 2003, Philip and Johnston 2004). In each drainage, impounded and unimpounded streams were in the same physiographic regions, were comparable widths, and had distinct pool-riffle complexes. Streams in the Bear Creek drainage were in the Fall Line Hills physiographic province in Franklin and Colbert counties. Streams in the Cahaba River drainage were in the Ridge and Valley physiographic province in St. Clair, Shelby and Jefferson counties.

We sampled two impounded (Little Bear and Cedar creeks) and one unimpounded (Rock Creek) stream in the Bear Creek drainage, and one impounded (Little Cahaba River) and one unimpounded stream (Shades Creek) in the Cahaba River drainage. Impounded streams each had one earthen storage dam with hypolimnetic releases. Dams were 17–29 m high, creating 425–1700 ha impoundments (Appendix D). Little Bear and Cedar creek impoundments were built in 1975 and 1979, respectively, and used for flood control. Little Cahaba River impoundment (Purdy Lake) was built in 1911 and used for water usage. From November until February and during heavy rain events water was released from 19.5 and 23.2 m below full pool levels in Little Bear and Cedar creeks, respectively. In Little Cahaba River, when water flow in the river was too low to meet water usage demands, water was released from two valves 11.0 and 15.5 m below Purdy Lake's full pool level.

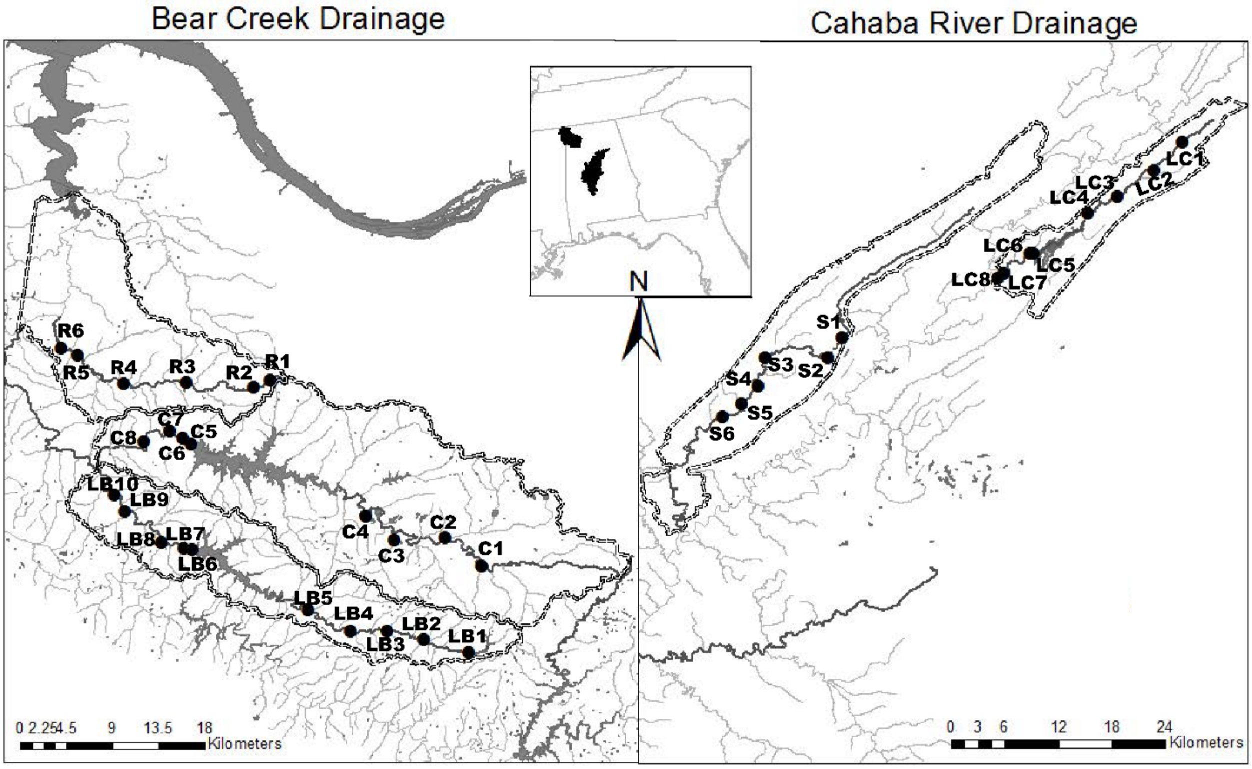


Figure 3.1. Map of Bear Creek and Cahaba River drainages, Alabama, with collection sites represented by labeled circles. Sites are numbered in ascending order from up- to downstream, with letters representing stream names (R = Rock Creek, C = Cedar Creek, LB = Little Bear Creek, S = Shades Creek, and LC = Little Cahaba River). Dashed lines delineate each stream's watershed. Inset shows drainage locations within the southeastern Unites States, with the Bear Creek drainage in the northwest corner and the Cahaba River drainage in the center of Alabama.

3.2.2 Phase 1 sampling

The study was conducted in two phases to assess differences between impoundment effects within and between drainages. In phase one, we sampled all streams in the Bear Creek drainage, two impounded (Little Bear and Cedar creeks) and one unimpounded (Rock Creek), to assess crayfish assemblage differences within a drainage. We sampled 24 sites in the spring/summer ("spring"; May–July) and fall/winter ("fall"; September–December) of 2015 (Appendix D). We visited each site once per seasonal sampling round. We sampled three to five sites in up- and downstream sections of streams, with all sites up- or downstream of impoundments grouped together. We selected sites at set intervals, based on stream length, up- and downstream of impoundments and mimicked these selections in the unimpounded stream. If a predetermined location was inaccessible, we sampled the closest accessible site. At each site, we sampled a linear reach 30 times the wetted width or minimum and maximum reach lengths of 200 m and 500 m (Simon 2004). Reach lengths remained constant across seasons unless dry sections shortened a reach (Adams 2013).

We divided each reach equally into two subreaches and electrofished and kick seined riffle and run macrohabitats (Bisson et al. 1982). Using two personnel crews, we simultaneously electrofished upstream subreaches and kick seined downstream subreaches. Only riffles and runs with maximum depths ≤ 1 m were sampled due to the ineffectiveness of our sampling methods in deeper waters. Sampling efforts in each site were partitioned between macrohabitats in proportion to the percent stream length of each macrohabitat. We conducted single pass electrofishing (effort = 0.4 sec/m^2), using a backpack electrofisher (Smith-Root model 12-A; Vancouver, Washington) with two dip netters (3 mm mesh nets). We based efforts on the time necessary to sample macrohabitats during preliminary sampling in several sites. We kick seined 20 plots, 2 m long x 1.5 m wide, every 100 m (Simon 2004) using a 2.6 m long x 1.6 m high seine with 3-mm mesh. One person kicked and lifted large rocks while kicking to disturb the substrate immediately upstream of the seine. We lifted the seine immediately after kicking. Two to three people alternated kicking. Each kick seine plot was at least 2 m away from any previously sampled plot, and plots were spaced throughout the subreach.

For all crayfishes captured, we recorded species, life stage (i.e., adult, juvenile), and postorbital carapace length (POCL). We recorded fish species and total lengths of predator fishes (see below). Most fishes and crayfishes were released in the reach after processing, and all others were preserved in 5% formalin (fish) or ≥ 70% ethanol (crayfish) for further laboratory analyses. We designated fish species as potential crayfish predators (hereafter, "predatory fish") if the FishTraits database (Frimpong and Angermeier 2009) indicated that they eat other fishes, crayfishes, crabs, frogs, amphibians, or macroinvertebrates. We also considered all basses and catfishes (Appendix E) top predators because greater than 40% of their diets are composed of crayfishes (Dorn and Mittelbach 1999).

3.2.3 Phase 2 sampling

In phase two (fall 2016 and spring 2017), we sampled 16 and 14 sites in the Bear Creek and Cahaba River drainages, respectively, to assess crayfish assemblage differences between drainages. Both Cahaba River and two Bear Creek drainage streams (unimpounded: Rock Creek and impounded: Little Bear Creek) were sampled (Appendix D). Little Bear Creek was chosen to represent Bear Creek drainage impounded streams because of its dam's similarity to Little Cahaba River dam (Cahaba River drainage impounded stream), with similar heights and impounded areas. We selected sites in the Cahaba River drainage as in phase 1. Sites previously sampled in Rock and Little Bear creeks and Cahaba River drainage sites were sampled as previously stated in phase 1.

3.2.4 Predatory fish biomass sampling

To estimate predator fish biomass, we conducted multi-pass electrofishing surveys in July and August 2015–2017. We isolated stream sections 30–105 m long (≥ 3 times the sections' wetted width) with block nets (5-mm mesh seines) at 20 of the sites described above. We made three-pass depletion efforts in each section, electrofishing 0.4 sec/m^2. Immediately after each pass, we recorded species, total length, and weight of each predator fish and released most fishes outside of the blocked section. We preserved fishes not identified in the field in 5% formalin.

3.2.5 Environmental Sampling

During all above sampling efforts, we quantified channel and water quality characteristics at each site. We measured channel characteristics (wetted width [m], depth [cm], and canopy cover [%]) at four evenly spaced transects during each sampling round. Stream depth was measured at the midpoint of streams and 10 cm away from right and left edges. Canopy cover was measured mid-channel with a convex spherical densiometer. We averaged channel characteristics across subreaches. Before sampling began at each site, we measured water quality parameters (Table 3.2) with a Hydrolab Quanta (HACH-Hydrolab, Loveland, CO) at one location. Before each sampling round, the Quanta was calibrated for all parameters except dissolved oxygen (DO). We calibrated DO daily. We recorded hourly water temperatures with iButton data loggers (Maxim Integrated, San Jose, CA) in the Bear Creek and Cahaba River drainages from September 17, 2015 and June 14, 2016, respectively, until completion of study. We also calculated stream discharge (m^3/s) using the transect method (Harrelson et al. 1994) with a Marsh-McBirney Flo-Mate 2000 and topsetting rod (Hach, Loveland, CO) at one location per site. Using Wolman pebble count procedures (Wolman 1954, Harrelson et al. 1994), we analyzed streambed composition across the bankfull channel width. We measured one pebble (mm) and sample of woody debris (mm), when present, blindly selected at our boot tip at a minimum of 100 points distributed among at least ten diagonal transects (five per subreach), with ten points equally spaced along each transect. The first transect began along the stream bank at the furthest up- or downstream point within each site. Between adjacent sampling points, we visually estimated the percentage of streambed covered by vegetation or small woody debris (SWD, < 10 cm diameter) (Bain and Stevenson 1999) and counted large woody debris (LWD, ≥ 10 cm diameter). We averaged streambed composition across subreaches.

Table 3.2. Stream and biotic characteristics used in linear discriminant analyses and environmental model selection for phase 1 and 2 comparisons. ** = characteristics only used in phase 2 comparisons due to unavailable data in phase 1. Max = maximum; Min = minimum; Temp = temperature.

Stream characteristics		Definition
Temperature	Max. water temp. (°C)	Max. spring and fall water temperatures
	Min. water temp.	Min. spring and fall water temperatures
	Temp. variation	Coefficients of variation for spring and fall water temperatures
Channel	Width to depth ratio	Ratio of mean wetted width to mean depth
	Canopy cover	Mean percent canopy cover
Bed composition	D50 (mm)	Median particle size
	D16 (mm)	Particle size at which 16% of particles were smaller
	D84 (mm)	Particle size at which 84% of particles were smaller
	Aquatic vegetation	Mean percent cover of aquatic vegetation
	Small woody debris (SWD)	Mean percent cover of SWD; < 10 cm diameter
	Large woody debris (LWD)	Number of pieces of LWD; ≥ 10 cm diameter
	Wood size (mm)	Mean wood size
	Riffle	Percent of site length containing riffles
Water quality	Conductivity (µS/cm)	Conductivity on sample day
	Dissolved oxygen (mg/l; DO)	DO on sample day
	pH	pH on sample day
	Turbidity (NTU)	Turbidity on sample day
**Flow	Max. discharge (m^3/s)	Max. spring and fall discharge
	Min. discharge	Min. spring and fall discharge
	Discharge variation	Coefficients of variation for spring and fall discharge
	Flashiness	Richards-Baker flashiness index: the sum of the absolute values of day-to-day changes in mean daily flow divided by total discharge during spring and fall
	Peak flow day	Julian day during spring and fall with greatest discharge
	Days zero flow	Number of days during spring and fall with no flow
Biotic	Fish abundance	Number of fish collected per 100 m^2
	Predator fish abundance	Number of predator fish collected per 100 m^2
	Predator fish biomass	Total and mean wet weight (g) of all predator fish
	Top predator fish biomass	Total and mean wet weight (g) of all top predator fish

3.2.6 Drainage land use

We used imagery from Landsat 8 Operational Land Imager to quantify recent land use for each stream's watershed (Figure 3.1). Satellite imagery from July 2014 and August 2015 (1 year previous to phase 1 and 2 sampling, respectively) were downloaded from the United States Geological Survey (USGS) Earth Explorer online remote sensing database (https://earthexplorer.usgs.gov) for the Bear Creek (path/row: 21/36, July 16, 2014 and August 27, 2015) and Cahaba River (path/row: 22/37, August 20, 2015) drainages. Images from these months were downloaded to ensure proper classifications of agricultural lands. No cloud cover was present in watershed areas in the images. We defined the predominant land use within 30 m x 30 m blocks as forest, water, agriculture, barren, or developed. Agriculture classifications included agricultural lands and grasses (i.e., lawns or pastures). Images were classified using supervised classifications in Earth Resources Data Analysis System (ERDAS) Imagine v. 16 (Hexagon Geospatial, Madison, AL). We merged 10–20 training classes for each land use classification and used maximum likelihood calculations (Foody et al. 1992, Jia et al. 2011) to classify images. To assess the accuracy of classifications, 120 randomly selected stratified points were overlain on the original Landsat images and visually classified then compared to land uses from the classified image. The overall classification accuracy (mean = 93%) and Kappa statistics (mean = 0.87) (Appendix F) showed that supervised classifications were suitable for this assessment (Congalton and Green 1999, Tso and Mather 2001).

We used 1:20,000 aerial photography (400 dpi) from the University of Alabama's cartographic research laboratory to quantify historical land use for each stream's watershed. Bear Creek drainage photographs were from October 31, 1970 (Rock Creek), November 12, 1971 (Little Bear Creek), and November 16, 1971 (Cedar Creek). Cahaba River drainage photographs were from October 29, 1960 (Little Cahaba River) and November 15, 1960 (Shades Creek). Photographs covered ≥ 94% of watersheds. We visually defined land use, as above, using ArcGIS v. 10.5.1 (ESRI, Redlands, CA).

We used 11-digit hydrological unit boundaries of each watershed (USGS 2015) to subset watersheds from each classified image. Attribute tables from subset images were used to quantify the percentage of each land use within a watershed.

3.2.7 Data Analyses

Our research questions addressed effects of impoundments and subsequent stream characteristic and biotic changes on crayfish assemblages. Table 3.1 summarizes the suite of analyses addressing each research question and expected outcomes if impoundments impact crayfish assemblages. To assess differences in crayfish assemblages between impounded and unimpounded streams, we used both univariate and multivariate split-plot designs, with impounded and unimpounded streams as whole plots and sections up- and downstream as split plots. In phase 1, we assessed differences between each stream to understand within-drainage changes. In phase 2, we assessed differences between impounded and unimpounded streams to understand the consistency of differences in impounded versus unimpounded streams between drainages. We combined electrofishing and kick seining data for all analyses. For all univariate models, histograms of model residuals did not depart from normality, and dispersion did not vary among groups in multivariate models.

3.2.7.1 Assemblage comparisons

We summarized crayfish assemblages in three ways (assemblage structure, catch per unit efforts [CPUE], and adult sizes) to assess assemblage differences between impounded and unimpounded streams.

3.2.7.1.1 Assemblage structure comparisons

First, we used permutational multivariate analysis of variance (PERMANOVA) to test if assemblage structure (i.e., matrices of the CPUE [N/100 m^2] of each crayfish species) differed between impounded and unimpounded streams. We constructed separate models for each study phase, and square-root transformed CPUEs to change the relative emphasis of the anlysis on rare versus more abundant species (Anderson et al. 2008). We assembled Bray-Curtis similarity matrices comparing assemblage structure between each site and conducted two-dimensional non-linear ordination with multidimensional scaling (NMDS; Clarke 1993) to visualize species structure differences. For phase 1, we used PERMANOVAs to test responses of assemblage structures (response variable) to three fixed effects: streams, stream sections (up/downstream), and seasons (spring/fall). To account for repeated samples at each site, site was a random effect. Interactions of streams with stream sections and seasons were included in models. To evaluate assemblage structure differences between stream sections and seasons among streams, we only interpreted stream or its interactions. We futher analyzed all significant interactions with pairwise PERMANOVA comparisons. We used the PERMANOVA add-on (Anderson et al. 2008) in the software Plymouth Routines in Multivariate Ecological Research (PRIMER) 7.0 (Quest Research Limited, Auckland, New Zealand) to analyze data, with 9999 permutations of residuals in both the main tests and post-hoc pairwise comparisons.

For phase 2, we used PERMANOVAs to compare crayfish assemblage structure (response variable) among four fixed effects: stream types (impounded/unimpounded), drainages (Cahaba/Bear), stream sections, and seasons, with site as a random effect. Interactions of stream types with drainages, stream sections, and seasons were included in models. Only stream type or its interactions were interpreted to evaluate assemblage structure differences between drainages, seasons, and stream sections among impounded and unimpounded streams. We analyzed all main tests and post-hoc pairwise PERMANOVA comparisons with PRIMER.

3.2.7.1.2 Adult and juvenile CPUE comparisons

Second, to assess differences between impounded and unimpounded streams' adult and juvenile CPUE, separately, we compared $\log_e$ CPUE (+0.001) of all species combined using linear mixed-effect repeated-measures (LME) models. Data was log transformed to meet normality assumptions for maximum likelihood tests. We used the same fixed and random effects described above in PERMANOVA models for phase 1 and 2 comparisons to assess CPUEs in LME models. Analyses were performed with the *lmerTest* package (Kuznetsova et al. 2015) in R software version 3.4.2 (R Core Team 2013), using Tukey's HSD post-hoc tests for comparing means.

3.2.7.1.3 Adult size comparisons

Third, to assess differences in adult crayfish sizes between impounded and unimpounded streams, we compared sizes of small and large adults, separately, using analysis of variance (ANOVA) models. Small and large adult sizes were defined as the 25th and 75th percentiles of POCLs, respectively, for all species combined across seasons. For phase 1, we compared adult sizes among streams and streams' interaction with stream sections. For phase 2, we compared adult sizes between stream types, drainages, and stream sections. Interactions of stream type with drainage and stream section were included. To evaluate how drainages and stream sections differ among impounded and unimpounded streams, we only interpreted stream type or its interactions. Analyses were performed with the *stats* package (R Core Team 2013) in R, using Tukey's HSD post-hoc tests for comparing means.

3.2.7.2 Stream and biotic characteristic comparisons

We used linear discriminant analyses (LDA) to identify stream and biotic characteristics (Table 3.2) that best separated impounded and unimpounded stream sections. For phase 1 analyses, we averaged channel characteristics, streambed composition, and water quality parameters measurements from spring and fall sampling, separately (Table 3.2). We also calculated spring (March 20 – June 21) and fall (September 22–December 21) stream temperature characteristics and estimated fish abundance (N/m^2) and biomass (g, wet weight) as described in Appendix G. All stream and biotic variables were $\log_e$ (variable + 1) transformed and normalized so that variables had comparable, dimensionless scales. We assembled Euclidean distance matrices between each site's stream and biotic characteristics. To discriminate groups (up and downstream sections of impounded and unimpounded streams), canonical analysis of principal coordinates (CAP) was performed in PRIMER, and stream and biotic characteristics highly correlated (> 30 %) with eigenvalues one and two were identified. We visualized group differences via ordination plots and quantified separation among groups using leave-one-out (LOO) allocation success.

For phase 2 analyses, we used parameters listed above in phase 1 stream and biotic characteristic analyses, as well as discharge characteristics (calculations as in Appendix G) in LDA to analyze differences between up and downstream sections of impounded and unimpounded streams. We used CAP as described in phase 1 comparisons to discriminate between groups and visualize stream characteristics that differed.

To identify if stream and biotic characteristics that discriminated impounded and unimpounded streams also impacted crayfish assemblages, we modeled the relationship of crayfish assemblages to stream and biotic characteristic for phases 1 and 2, separately. Only crayfish assemblage variables and stream abiotic and biotic characteristics with differences between impounded and unimpounded streams were analyzed.

Multivariate distance based linear models (McArdle and Anderson 2001) were used to measure the strength and significance of the relationships between crayfish assemblage structure and stream and biotic predictor variables (Table 3.2). Crayfish CPUEs were square root transformed and Bray-Curtis similarity matrices between each site's species structure assembled. All stream and biotic variables were $\log_e$ (variable + 1) transformed and normalized. Stream and biotic variables were fitted conditionally to find the best combinations of predictor variables based on 9999 permutations of the residuals. Because sample sizes were small relative to the number of estimated parameters, model selection was based on Akaike information criterion (AIC_c; Burnham and Anderson 2004). Stream and biotic characteristics associated with assemblage structure variation were plotted as vectors in NMDS ordinations. Analyses were performed using PRIMER. Additionally, LME models were used to assess the relationships between univariate crayfish assemblage measures (i.e., size, CPUE) and stream and biotic variable. LME models were fited with maximum likelihood estimations using the *lmerTest* package in R. In the models, the assemblage measure was the dependent variable and site was the random effect.

3.2.7.3 Assessment of land use differences among drainages and years

We used PERMANOVAs to identify differences between 1) recent (2015) and historical (1960–1971) land use within each drainage, 2) recent land use (2015) between drainages, and 3) 2014 and 2015 land use within the Bear Creek drainage. Each watershed's land use percentages were calculated and $\log_e$ (variable + 1) transformed. We assembled Euclidean distance matrices between land uses of each watershed. To identify if any major land use change occurred in the watershed other than the installation of impoundments, we compared differences between recent and historical land use for each watershed. If crayfish assemblage differences were inconsistent between each drainage, we assessed if recent land use also differed between drainages. We also compared 2014 and 2015 land use within the Bear Creek drainage to assess crayfish assemblage yearly differences. If we identified differences from PERMANOVA analyses, we used distance based linear models (as explained above) to assess what land use types best explained the differences. These land use types were used to qualitatively assess inconsistent assemblage differences between drainages and years. All analyses were performed in PRIMER.

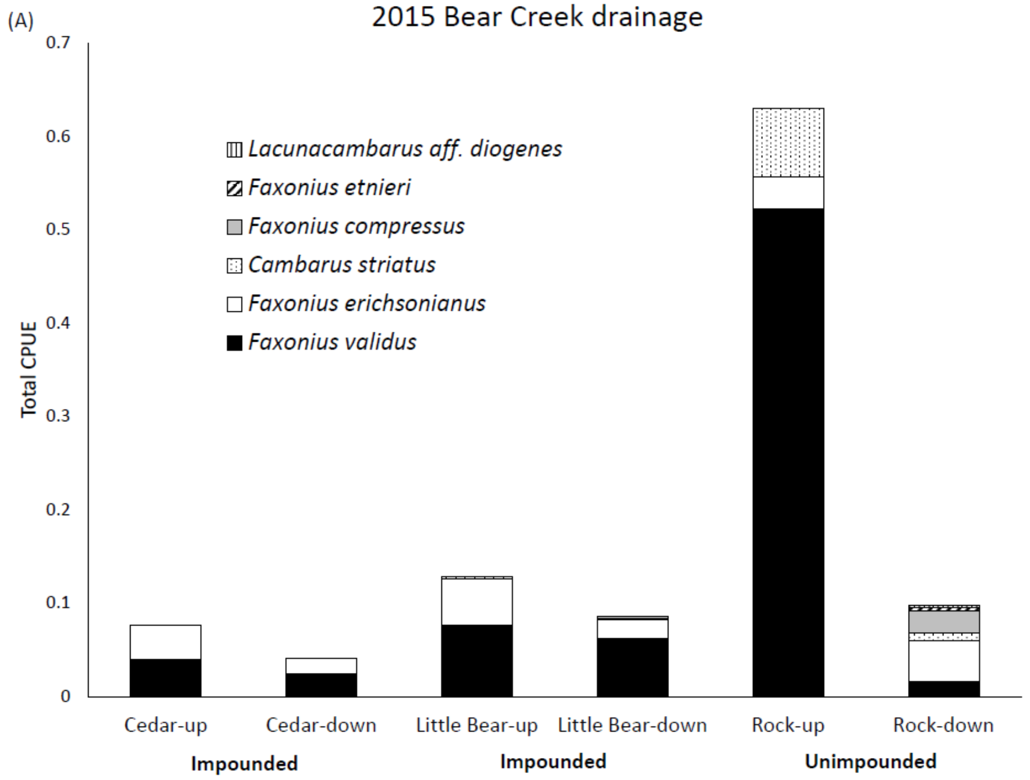
3.2.7.4 Assemblage changes with distance from impoundments

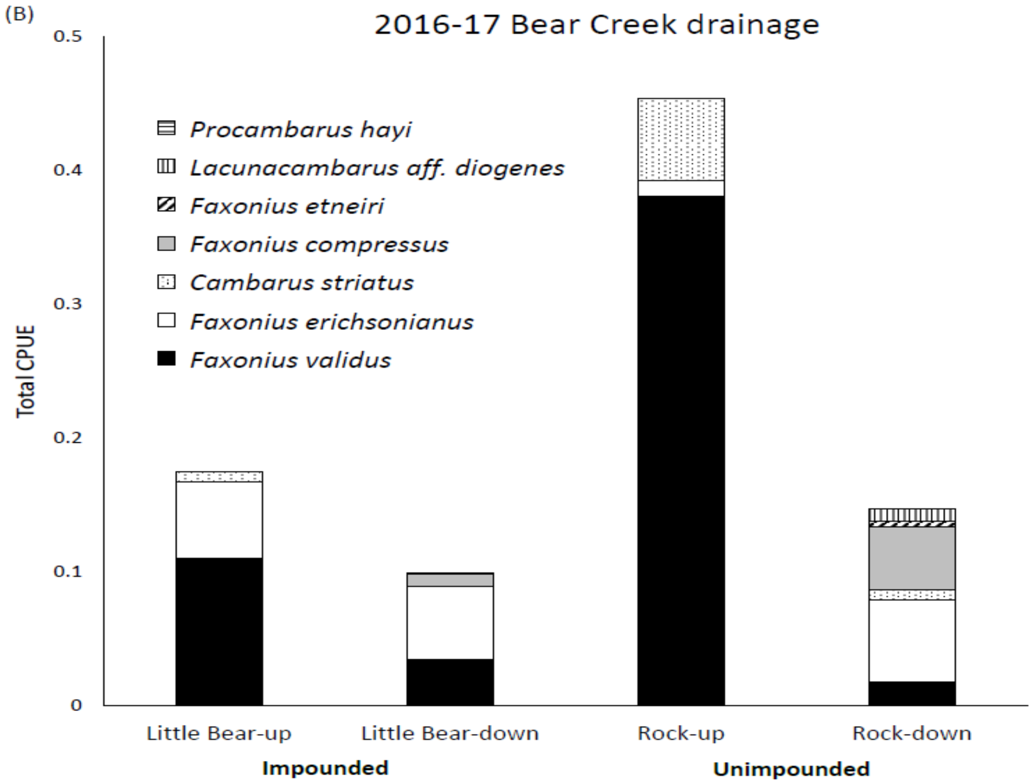
To test if crayfish assemblages changed with distance away from impoundments (hereafter distance) we compared assemblage structure changes within impounded streams using PERMANOVAs. All CPUEs were square root transformed and Bray-Curtis similarity matrices between each site's species structure assembled. We compared assemblage structure differences within each stream section to distances from impoundment (Appendix D). Because only categorical variables can be used in PERMANOVA, we used dummy variables to categorize distance, with one representing sites adjacent to impoundments and numbers increasing with distance from impoundments. To distinguish between up- and downstream assemblage structure changes, interactions of stream section and distance were included in models. Site was a random effect to account for repeated sampling of sites. Only distance or its interaction was interpreted. Data was analyzed in PRIMER with Monte Carlo simulations to estimate distribution under the null hypothesis (i.e., no difference between crayfish assemblage structure with distance away from impoundments) for the main tests and pairwise PERMANOVA comparisons (Anderson et al. 2008).

3.3 Results

3.3.1 Assemblage comparisons

We collected 13 crayfish species total (Figure 3.2), with seven and eight species in the Bear Creek and Cahaba River drainages, respectively. Crayfishes were collected in 97% of sites, with CPUE (N/100 m^2) ranging from 0.0 to 20.0. *Faxonius validus* and *F. erichsonianus* were the most abundant (≥ 30% of individuals collected) and widespread species (present in > 90% of sites) in the Bear Creek drainage. The most abundant and widespread species in the Cahaba River Drainage was *Faxonius virilis*, an introduced species native to the upper Mississippi River and Great Lakes drainages (Hobbs 1959, Schwartz et al. 1963, Hamr 2002).


(A)
2015 Bear Creek drainage
Total CPUE
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Lacunacambarus aff. diogenes
Faxonius etnieri
Faxonius compressus
Cambarus striatus
Faxonius erichsonianus
Faxonius validus
Cedar-up
Cedar-down
Little Bear-up
Little Bear-down
Rock-up
Rock-down
Impounded
Impounded
Unimpounded


(B)
2016-17 Bear Creek drainage
Total CPUE
0.5
0.4
0.3
0.2
0.1
0
Procambarus hayi
Lacunacambarus aff. diogenes
Faxonius etneiri
Faxonius compressus
Cambarus striatus
Faxonius erichsonianus
Faxonius validus
Little Bear-up
Little Bear-down
Rock-up
Rock-down
Impounded
Unimpounded

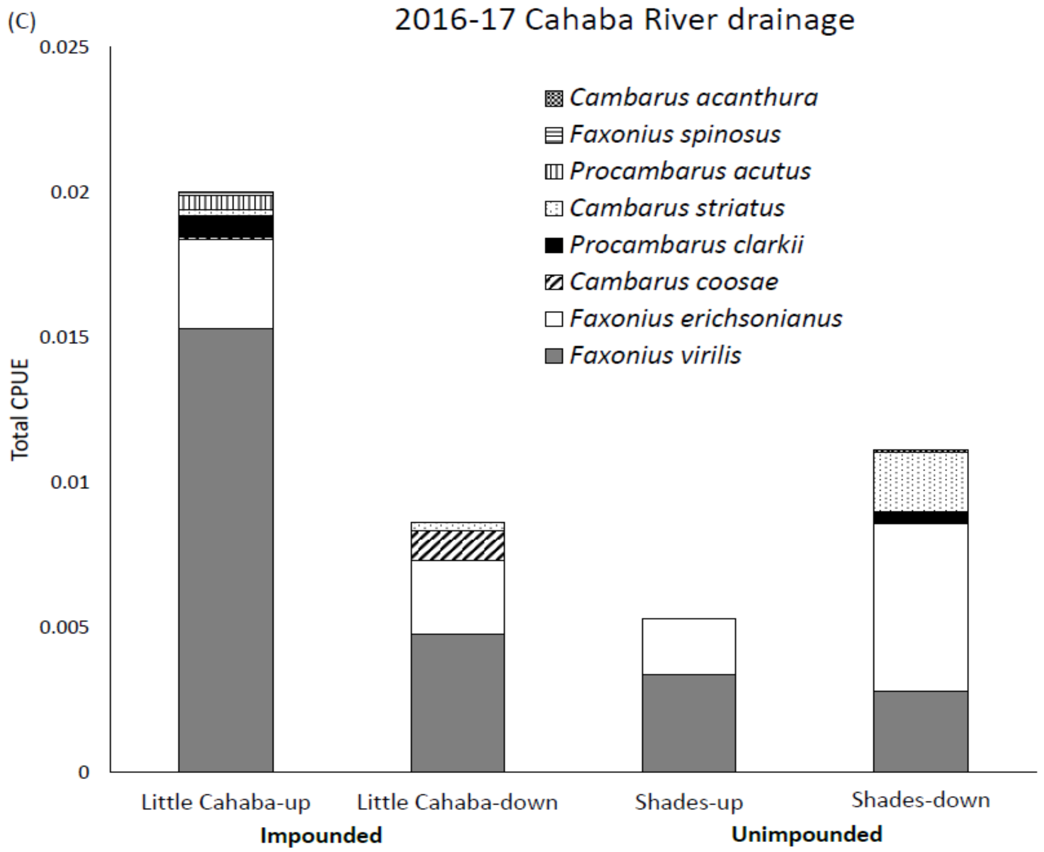


Figure 3.2. Total crayfish catch per unit effort (CPUE) in up- and downstream sections of impounded and unimpounded streams during phase 1 (2015; A) and 2 (2016-17; B-C) sampling. Up = upstream; Down = downstream.

3.3.1.1 Assemblage structure comparisons

In phase 1, differences in assemblage structure between up- and downstream sections were inconsistent across streams (Figure 3.3A; PERMANOVA $F_{2,34} = 4.09$, $P < 0.01$). Up- versus downstream assemblages differed in Rock Creek (unimpounded stream; $t_{1,6} = 2.28$, $P = 0.02$) but not in the two impounded streams. In addition, both Rock Creek assemblages differed from Little Bear (upstream: $t_{1,12} = 2.91$, $P < 0.01$; downstream: $t_{1,10} = 2.77$, $P < 0.01$) and Cedar (upstream: $t_{1,10} = 3.39$, $P < 0.01$; downstream: $t_{1,8} = 4.20$, $P < 0.01$) creek assemblages. Two species dominated all sites in Little Bear and Cedar creeks, while assemblage structure gradually changed with distance downstream in Rock Creek (Figure 3.2A).

In phase 2, differences in assemblage structure between up- and downstream sections were inconsistent between impounded and unimpounded streams (Figure 3.3B; PERMANOVA $F_{1,44} = 5.00$, $P < 0.01$) and drainages (Figure 3.3B; PERMANOVA $F_{1,44} = 5.52$, $P < 0.01$). As in phase 1, up- versus downstream assemblages differed in unimpounded ($t_{1,16} = 3.79$, $P < 0.01$) but not in impounded ($t_{1,28} = 1.23$, $P = 0.22$) streams (Figure 3.2). However, assemblage structures differed between impounded and unimpounded streams in only the Bear Creek drainage (Figure 3.3B: Bear Creek $t_{1,24} = 2.79$, $P = 0.001$; Cahaba River $t_{1,20} = 1.36$, $P = 0.16$).

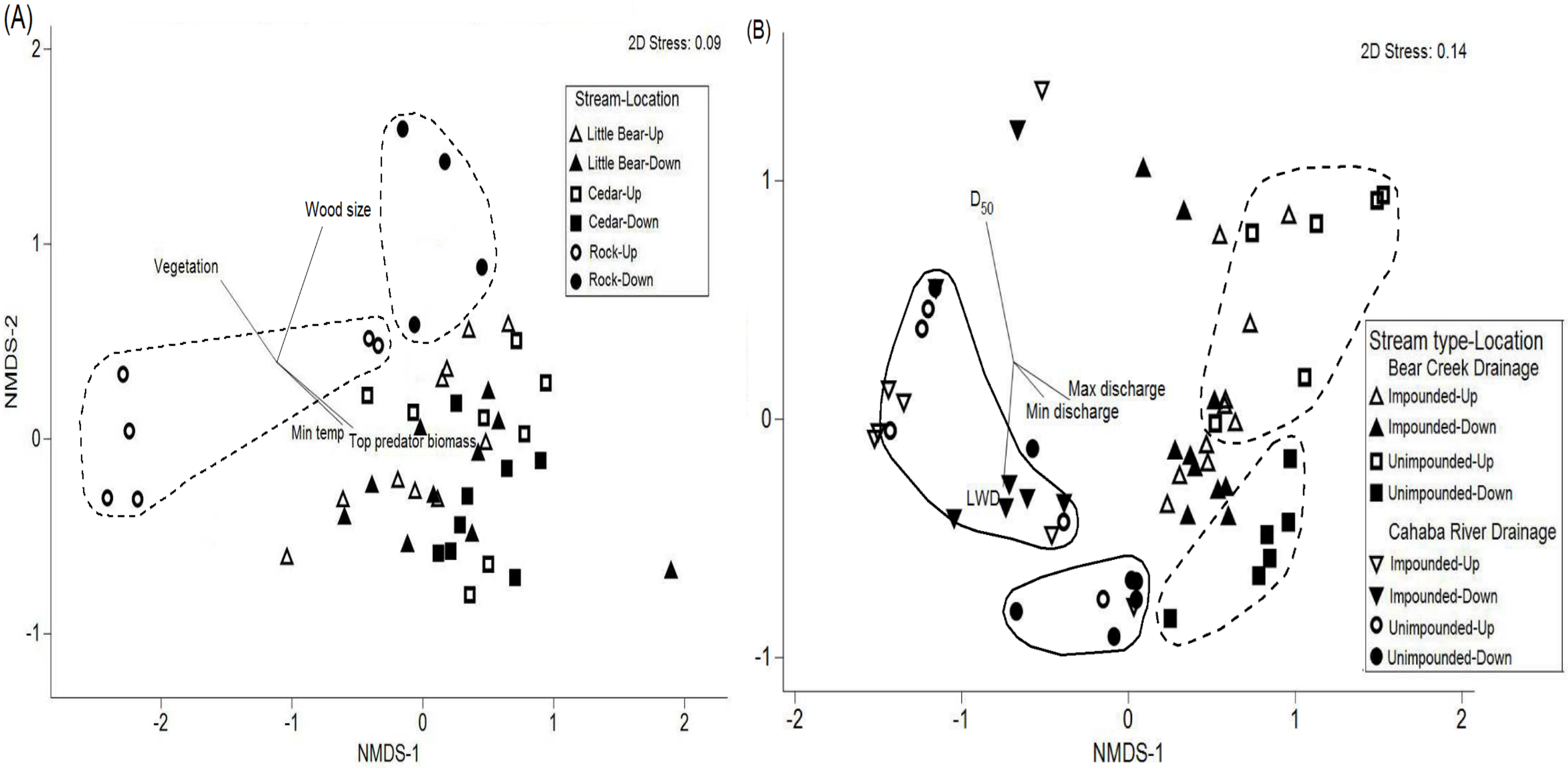


Figure 3.3. Assemblage structure ordinations (NMDS) of phase 1 (A) and 2 (B) comparisons using Bray-Curtis distance measures, with symbols representing sites from each stream (A) or stream type (B) and location within streams (up- or downstream). Note that some symbols overlap. Sites were ordinated by species catch per unit efforts and plotted with an overlay of habitat variables that discriminated between impounded and unimpounded streams and were strongly correlated with the ordination (see stream and biotic characteristic results). Habitat vectors show the relative association and magnitude of correlation for each variable. Dashed (Bear Creek drainage) and solid (Cahaba River drainage) polygons enclose points from up- and downstream sections of streams that differed from each other (PERMANOVA analyses $P < 0.05$, see Phase 1 and 2 comparison results). Up = upstream; Down = downstream; Vegetation = percent aquatic vegetation; Min temp = minimum water temperature (°C); D50 = median particle size (mm); LWD = number of pieces of large woody debris; Max discharge = maximum discharge (m^3/s); Min discharge = minimum discharge (m^3/s).

3.3.1.2 Adult and juvenile CPUE comparisons

In phase 1, adult and juvenile CPUEs averaged 0.4 (range 0.0–2.3) and 0.8 (range 0–11.6), respectively. Differences in juvenile CPUE between up- and downstream sections were inconsistent across streams (LME $F_{2,19} = 6.04$, $P < 0.01$). More juveniles were collected upstream in Rock Creek than in any other stream section (Figure 3.4A). More juveniles were also collected downstream in Rock Creek than downstream in Cedar Creek ($t_{1,24} = -3.19$, $P = 0.04$; Figure 3.4A). For adult CPUEs, more adults were collected in Rock Creek than in any other stream ($F_{2,20} = 11.31$, $P < 0.001$).

During phase 2, CPUEs averaged 0.4 (range 0.0–1.6) and 0.5 (range 0.0–8.5) for adults and juveniles, respectively. Differences in adult and juvenile CPUEs between impounded and unimpounded streams were inconsistent across drainages (adults: LME $F_{1,23} = 6.45$, $P = 0.02$ Figure 3.5C; juveniles: $F_{1,26} = 12.63$, $P < 0.01$, Figure 3.5A). Adult and juvenile CPUE was higher in unimpounded than impounded streams in the Bear Creek (adults: $t_{1,29} = -3.05$, $P = 0.02$; juveniles: $t_{1,30} = -4.32$, $P < 0.001$), but not the Cahaba River drainage (juveniles: $t_{1,22} = 0.64$, $P = 0.92$; adults: $t_{1,18} = 0.46$, $P = 0.97$). Differences in adult CPUEs between impounded and unimpounded streams were also inconsistent across stream sections ($F_{1,22} = 4.33$, $P = 0.04$, Figure 3.5D). More adults were collected in downstream sections of unimpounded than impounded streams ($t_{1,22} = -2.84$, $P = 0.04$), but upstream sections did not differ between impounded and unimpounded streams ($t_{1,23} = 0.62$, $P = 0.93$).

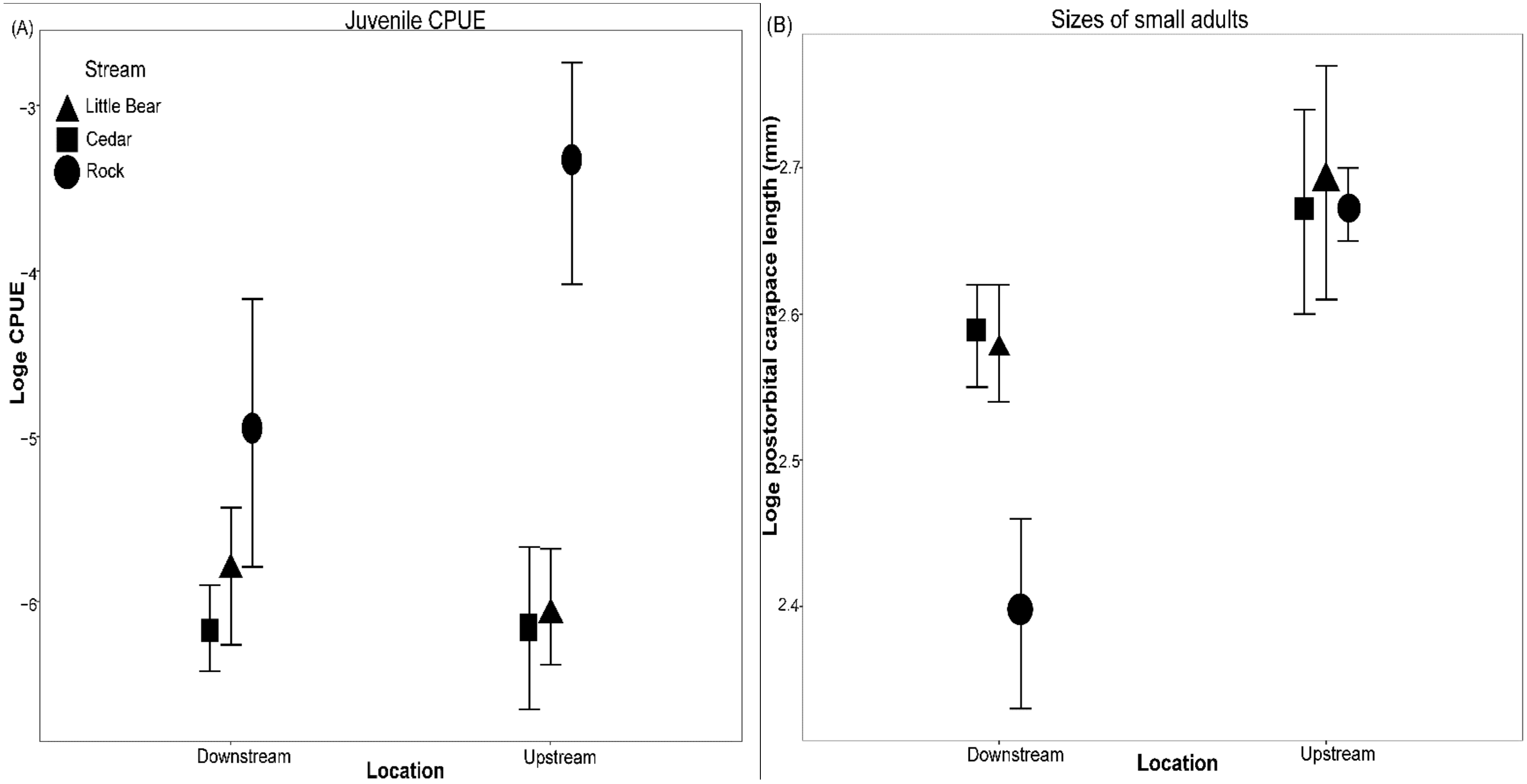


Figure 3.4. Phase 1 comparisons of juvenile crayfish catch per unit effort, CPUE, (A) and sizes of small adult crayfishes, 25th percentile of postorbital carapace lengths, (B) ± 95% CI between up- and downstream sites. Only relationships with significant interactions in LME and ANOVA analyses displayed.

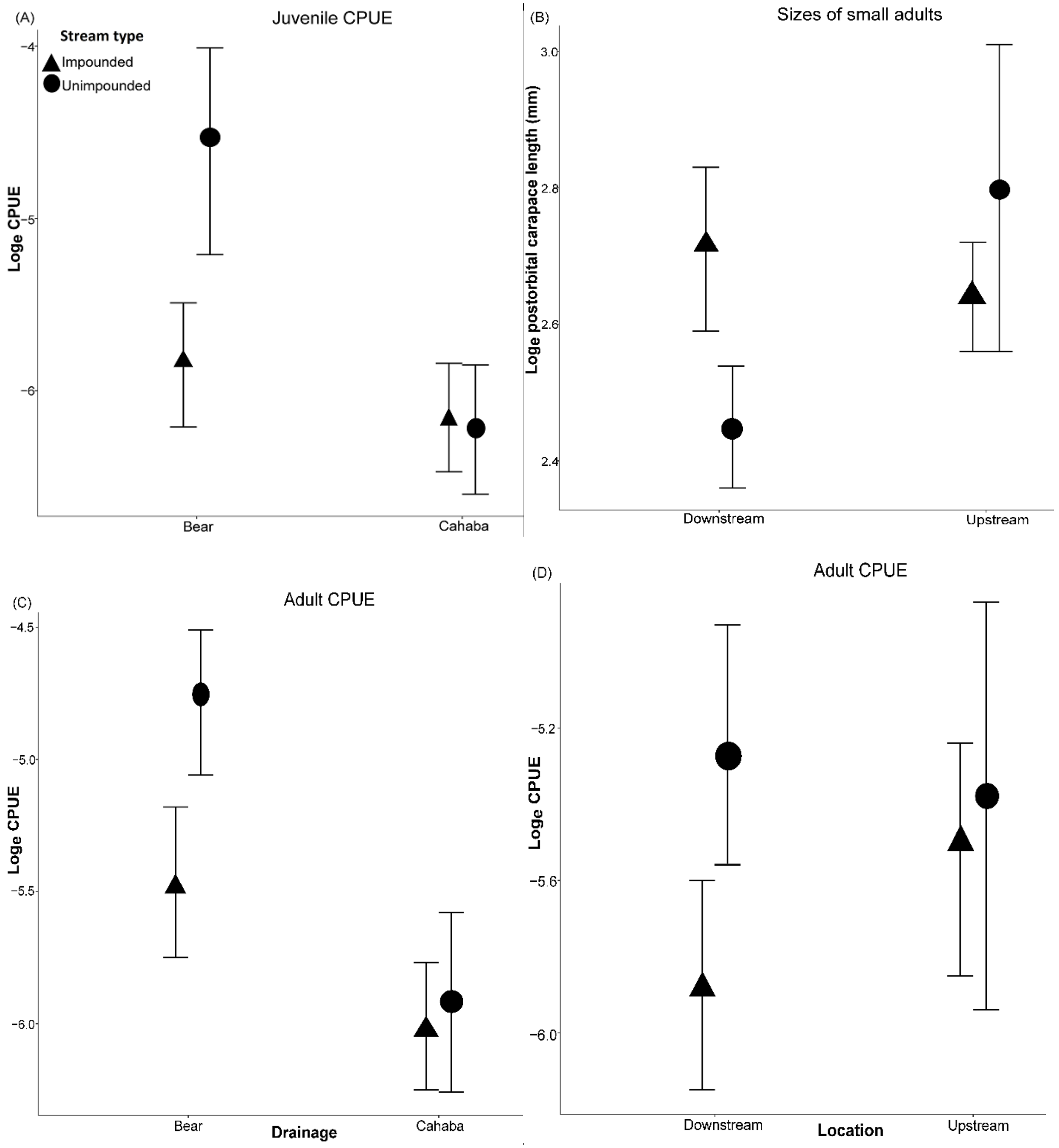


Figure 3.5. Phase 2 comparisons of juvenile (A) and adult (C, D) crayfish catch per unit effort (CPUE) and sizes of small adult crayfishes (B; 25th percentile of postorbital carapace lengths) between drainages (A, C) and up- and downstream sections (B, D) of impounded and unimpounded streams. Error bars indicate 95% CI. Only relationships with significant interactions in phase 2 LME and ANOVA comparisons displayed.

3.3.1.3 Adult size comparisons

Sizes of small (25th percentile) and large (75th percentile) adults averaged 14.1 mm POCL (range 9.7–24.9 mm) and 19.7 (12.5–34.4), respectively, during study phases 1 and 2. Differences in small adult sizes between up- and downstream sections were inconsistent across streams (ANOVA Phase 1 $F_{2,18} = 3.75$, $P = 0.04$; Phase 2 $F_{1,23} = 7.19$, $P = 0.01$). Adults were smaller down- than upstream in unimpounded, but not in impounded streams (Phase 1 Figure 3.4B; Phase 2 Figure 3.5B). Sizes of large adults did not differ between streams (Phase 1 $F_{2,18} = 2.06$, $P = 0.16$; Phase 2 $F_{1,23} = 1.06$, $P = 0.32$).

3.3.2 Stream and biotic characteristic comparisons

In phase 1, one biotic and five abiotic stream characteristics best explained differences between Bear Creek drainage impounded and unimpounded streams (Figure 3.6A; Table 3.3). Unimpounded upstream assemblages and all impounded stream assemblages were separated along CAP axis 1 (Eigenvalue CAP 1 = 0.91), while axis 2 separated assemblages upstream of impoundments from downstream of impounded and all unimpounded stream section assemblages (Eigenvalue CAP 2 = 0.85). Total LOO allocation success was strong (89%), indicating that biotic and stream characteristics successfully discriminated between impounded and unimpounded streams. Unimpounded stream sites had larger woody debris and greater temperature variation, percent vegetation, and turbidity, while impounded streams had higher minimum temperatures, greater top predator biomass, and larger substrates (Figure 3.6A).

In phase 1, four of the variables that discriminated between impounded and unimpounded streams were also correlated with crayfish assemblage structure. Average size of woody debris, minimum temperature, top predator biomass, and percent aquatic vegetation were correlated to assemblage structure, explaining 34% of assemblage variation (Figure 3.3A). A combination of these variables was also correlated with juvenile and adult CPUEs, as well as sizes of crayfishes collected (Table 3.4).

In phase 2, one biotic and six abiotic stream characteristics discriminated between impounded and unimpounded streams (Figure 3.6B; Table 3.3). Assemblage structures downstream of impoundments were separated from all assemblages in unimpounded streams along CAP axis 1 (Eigenvalue CAP 1 = 0.93). Discharge variation was higher in unimpounded streams while top predator biomass and substrate size was larger in downstream sections of impounded streams (Figure 3.6B). Assemblage structures upstream of impoundments were separated from all assemblages in unimpounded streams along CAP axis 2 (Eigenvalue CAP 2 = 0.82). Unimpounded streams had lower minimum and maximum discharge and higher turbidity and LWD than sections upstream of impoundments (Figure 3.6B). Total LOO allocation success was strong (88%), indicating that biotic and stream characteristics successfully discriminated between impounded and unimpounded streams.

Four of the variables that discriminated between impounded and unimpounded streams in phase 2 comparisons were also correlated with crayfish assemblage structure. Minimum and maximum discharge, D50, and LWD were correlated with assemblage structure, explaining 27% of assemblage variation (Figure 3.3B). Turbidity, D50, maximum discharge, and top predator biomass were also correlated with crayfish CPUEs and sizes (Table 3.4).

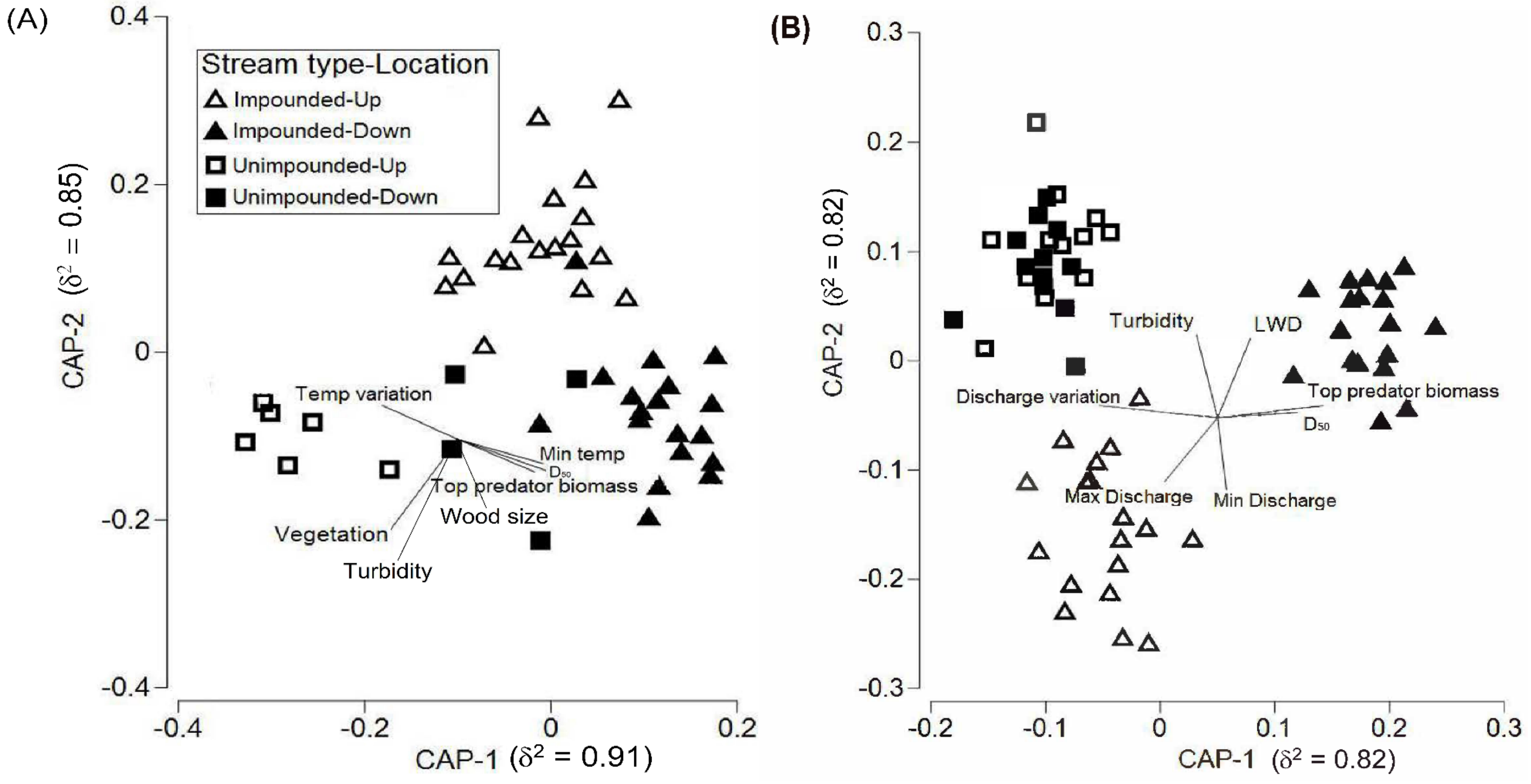


Figure 3.6. Canonical analysis of principal coordinates (CAP) of stream characteristic data (resemblance measure: Euclidean distance) from sites up- and downstream in impounded and unimpounded streams in phase 1 (A) and 2 (B) comparisons. Note that some symbols overlap. Black vectors represent raw Pearson correlations of stream characteristic that contributed > 30% to the dissimilarity between stream types. The length and direction of the vector show the magnitude and correlation, respectively, for each variable. Temp variation = temperature variation (°C); all other abbreviations as in Figure 3.3.

Table 3.3. Mean (SD) stream characteristic values from up and downstream sections of impounded and unimpounded streams. ** = impounded versus unimpounded stream discriminate variables. Abbreviations follow Table 3.1.

	Impounded		Unimpounded	
Stream characteristics	Upstream	Downstream	Upstream	Downstream
Phase 1 comparisons				
Max water temperature (°C)	23.63 (2.46)	24.43 (3.72)	23.62 (2.60)	22.44 (1.65)
**Min water temperature	7.28 (1.36)	11.42 (1.57)	5.95 (2.07)	8.86 (4.27)
**Temperature variation	21.3 (4.10)	14.95 (3.69)	22.95 (5.06)	24.83 (6.67)
Width to depth ratio	0.53 (0.21)	0.61 (0.38)	0.88 (0.85)	0.25 (0.08)
Canopy cover (%)	56.4 (19.7)	48.8 (22.4)	68.7 (28.1)	44.8 (5.9)
D50	36.4 (23.1)	350.3 (679.8)	66.5 (35.9)	18.3 (5.1)
D16	8.1 (10.7)	13.1 (25.2)	12.4 (10.3)	0.1 (0.1)
D84	152.6 (104.9)	514.5 (829.3)	497.1 (894.8)	37.7 (2.7)
**Aquatic vegetation (%)	9.3 (5.7)	15.4 (9.7)	15.7 (13.0)	32.0 (5.0)
SWD	6.6 (3.5)	5.6 (1.8)	5.8 (1.0)	6.9 (3.4)
LWD	6 (4)	12 (8)	4 (1)	8 (3)
Wood size (mm)	33.2 (21.5)	49.8 (25.3)	28.4 (27.1)	86.8 (37.3)
Riffle (%)	20.8 (7.7)	24.3 (11.2)	26.7 (7.5)	20.8 (10.2)
Conductivity (µS/cm)	0.21 (0.18)	0.19 (0.06)	0.22 (0.05)	0.09 (0.01)
DO	6.95 (1.05)	7.15 (0.66)	6.73 (3.26)	6.22 (1.05)
pH	7.19 (0.30)	7.53 (0.28)	7.39 (0.35)	7.57 (0.15)
Turbidity (NTU)	10.0 (3.0)	16.1 (7.4)	16.6 (7.2)	14.1 (6.3)
Fish abundance (N/100 m^2)	2.3 (1.2)	1.4 (0.7)	1.7 (1.3)	3.0 (2.0)
Predator fish abundance	0.2 (0.2)	0.3 (0.2)	0.2 (0.2)	0.1 (0.1)
Predator fish biomass (g)	505.8 (342.8)	513.1 (361.4)	115.8 (60.6)	167.6 (171.3)
Mean predator fish biomass	12.3 (4.9)	15.1 (8.9)	5.4 (2.6)	4.1 (1.6)
**Top predator fish biomass	158.3 (207.5)	247.7 (221.4)	4.9 (8.0)	21.2 (25.3)
Mean top predator fish biomass	34.3 (41.0)	28.1 (18.2)	1.9 (2.7)	7.4 (8.8)
Phase 2 comparisons				
Max water temperature	22.39 (2.52)	25.15 (2.82)	26.58 (3.92)	25.68 (2.90)
Min water temperature	9.01 (4.17)	10.43 (2.59)	7.75 (4.72)	8.87 (4.48)
Temperature variation	21.68 (10.56)	18.14 (6.48)	25.30 (12.73)	24.53 (12.48)
Width to depth ratio	0.76 (0.73)	0.82 (0.49)	0.50 (0.27)	0.34 (0.16)
Canopy cover	50.6 (21.93)	42.5 (17.2)	48.9 (19.46)	49.5 (13.8)
**D50	42.3 (59.4)	363.5 (664.3)	51.2 (49.4)	20.9 (12.3)
D16	3.8 (3.9)	32.1 (51.8)	18.3 (24.8)	1.8 (1.2)
D84	264.7 (411.9)	623.9 (882.7)	882.1 (945.7)	364.0 (712.2)
Aquatic vegetation	18.9 (17.1)	14.7 (9.5)	18.8 (9.4)	21.4 (12.1)
SWD	7.5 (4.3)	8.3 (3.3)	10.0 (2.9)	10.1 (4.2)
**LWD	5 (6)	8 (7)	6 (7)	12 (6)
Wood size	46.3 (72.2)	36.6 (20.8)	30.0 (17.9)	73.6 (42.7)
Riffle	21.5 (11.2)	31.9 (10.1)	19.5 (13.2)	13.2 (7.5)
Conductivity	0.20 (0.16)	0.19 (0.04)	0.24 (0.06)	0.22 (0.12)
DO	7.12 (0.82)	7.05 (1.07)	6.76 (2.34)	5.51 (1.06)
pH	7.25 (0.50)	7.54 (0.34)	7.53 (0.34)	7.21 (0.32)
**Turbidity	7.5 (6.4)	7.8 (3.0)	13.6 (9.17)	12.4 (7.8)
**Max discharge (m^3/s)	391.91 (499.73)	21.17 (18.83)	62.14 (131.48)	356.00 (421.13)
**Min discharge	1.04 (1.52)	0.22 (0.09)	0.04 (0.05)	0.16 (0.20)
**Discharge variation	198.81 (33.78)	130.00 (59.45)	229.32 (55.74)	223.22 (58.42)
Flashiness	0.59 (0.21)	0.22 (0.15)	0.65 (0.13)	0.63 (0.14)
Peak flow day	210 (127)	189 (116)	216 (133)	216 (133)
Days zero flow	2 (3)	0 (0)	15 (20)	4 (7)

	Impounded		Unimpounded	
Stream characteristics	Upstream	Downstream	Upstream	Downstream
Phase 2 comparisons				
Fish abundance	8.0 (7.9)	17.4 (19.0)	7.2 (10.3)	8.4 (6.0)
Predator fish abundance	3.8 (4.4)	2.81 (3.26)	4.37 (4.63)	4.44 (4.64)
Predator fish biomass	550.5 (387.8)	846.6 (674.0)	409.2 (570.3)	392.4 (220.3)
Mean predator fish biomass	8.4 (5.1)	15.1 (9.3)	8.0 (6.3)	10.9 (8.4)
**Top predator fish biomass	77.2 (103.5)	309.4 (326.8)	28.2 (43.9)	32.2 (78.5)
Mean top predator fish biomass	22.7 (29.1)	50.5 (32.9)	8.0 (12.0)	27.6 (79.2)

Table 3.4. Linear models for crayfish assemblages' relationships with stream characteristics that discriminate between impounded and unimpounded streams. CPUE = catch per unit effort (*N*/100 m^2); Small crayfish size = the 25th percentiles of adult postorbital carapace lengths; all other abbreviations are as in Table 3.1.

Comparison	Dependent variables	Explanatory variables	Estimate	*F*	*P*	R^2
Phase 1	Juvenile CPUE	Min. temperature	-1.243	2.76	0.10	0.39
		Temperature variation	-1.000	1.54	0.22	
		D50	0.065	0.33	0.57	
		Aquatic vegetation	**0.528**	**9.07**	**0.05**	
		Top Predator Biomass	**-0.104**	**8.03**	**< 0.01**	
		Wood size	-0.138	0.57	0.46	
		Turbidity	0.622	2.42	0.13	
	Adult CPUE	**Min. temperature**	**-0.990**	**5.44**	**0.02**	0.55
		Temperature variation	-0.138	0.09	0.76	
		D50	0.084	1.90	0.18	
		Aquatic vegetation	**0.248**	**5.50**	**0.02**	
		Top Predator Biomass	**-0.083**	**16.30**	**< 0.01**	
		Wood size	0.097	1.02	0.32	
		Turbidity	-0.429	3.69	0.06	
	Small crayfish size	Min. temperature	-0.166	2.49	0.13	0.20
		Temperature variation	-0.065	1.80	0.20	
		D50	0.003	0.14	0.72	
		Aquatic vegetation	**-0.060**	**4.39**	**0.05**	
		Top Predator Biomass	0.006	0.78	0.39	
		Wood size	-0.043	2.00	0.18	
		Turbidity	0.039	1.48	0.24	
Phase 2	Juvenile CPUE	**Turbidity**	**0.873**	**11.42**	**< 0.01**	0.53
		LWD	-0.226	1.32	0.26	
		D50	-0.191	2.06	0.16	
		Discharge variation	1.016	3.69	0.06	
		Min discharge	-0.107	0.07	0.80	
		Max discharge	-0.039	0.11	0.74	
		Top predator biomass	-0.072	3.07	0.09	
	Adult CPUE	Turbidity	-0.120	0.71	0.40	0.24
		LWD	-0.015	0.02	0.89	
		D50	-0.022	0.11	0.75	
		Discharge variation	-0.269	1.38	0.24	
		Min discharge	-0.090	0.08	0.78	
		Max discharge	**0.146**	**3.96**	**0.05**	
		Top predator biomass	**-0.056**	**4.31**	**0.04**	
	Small crayfish size	Turbidity	-0.146	1.83	0.19	0.34
		LWD	-0.065	1.07	0.31	
		D50	**0.058**	**8.99**	**< 0.01**	
		Discharge variation	0.227	0.36	0.55	
		Min discharge	**-0.090**	**4.18**	**0.05**	
		Max discharge	-0.040	1.96	0.18	
		Top predator biomass	0.272	3.14	0.09	

3.3.3 Assessment of land use differences among drainages and years

Forest and agriculture were the dominant land uses (mean: 77% and 16%, respectively) in Bear Creek drainage watersheds (Table 3.5; Appendix H). The largest changes between historical and recent land uses were agricultural lands converted to forest (mean = 8.0% change) and water (mean = 3.2% change). Water land use increased due to stream impoundments. Recent (2014 vs. 2015) and historical (1970/71 vs 2015) land use did not differ statistically in the Bear Creek drainage (PERMANOVA $F_{1,5} = 0.44$, $P = 0.71$; PERMANOVA $F_{1,5} = 3.40$, $P = 0.16$, respectively). Nonetheless, forest increased in all watersheds and water increased in only watersheds with impounded streams, indicating that impoundments (i.e., water land use differences) may discriminate between assemblage structures in Bear Creek drainage watersheds.

In the Cahaba River drainage, forest and developed lands were dominant during the study (2015 mean = 69% and 21%, respectively), and forest and agriculture were dominant historically (1960 mean = 72% and 16%, respectively) (Table 3.5; Appendix H). Nonetheless, recent and historical land use (1960 vs. 2015) did not differ statistically in the drainage (PERMANOVA $F_{1,3} = 5.62$, $P = 0.26$). Although not statistically different, the increase in developed lands may be biologically significant. The largest changes between historical and recent land uses were forest and agriculture converted to developed lands (mean = 15% change), which could negatively impact stream complexity and water quality. Because aerial photographs pre-dating the Little Cahaba River dam were not available, we could not assess land use changes since the pre-dam era.

Crayfish assemblage differences between impounded and unimpounded streams were inconsistent between drainages. Assemblage structure and juvenile CPUE differed between impounded and unimpounded streams in only the Bear Creek drainage, indicating that factors other than the impoundment and its environmental changes may impact crayfish assemblages in the Cahaba River drainage. Likewise, land use differed between Bear Creek and Cahaba River drainages (PERMANOVA $F_{1,9} = 26.20$, $P < 0.01$), with agricultural lands highly correlated with Bear Creek drainage streams ($r = 0.92$) and developed lands highly correlated with Cahaba River drainage streams ($r = 0.95$).

Table 3.5. Recent (2014 and 2015) and historical (1960, 1970 or 1971) land use percentages (and % difference from 2015) for each watershed in Bear Creek and Cahaba River drainages.

		Little Bear	Cedar	Rock
Bear Creek drainage				
Current land use				
2015	Agriculture	12.8	14.5	8.2
	Water	3.0	3.8	1.0
	Barren	2.3	2.4	2.6
	Forest	79.9	74.3	86.2
	Developed	2.0	5.0	1.9
2014	Agriculture	17.1 (-4.3)	16.3 (-1.8)	12.1 (-3.9)
	Water	3.2 (-0.2)	4.2 (-0.4)	0.7 (0.3)
	Barren	3.2 (-0.9)	4.5 (-2.1)	3.2 (-0.6)
	Forest	74.5 (5.4)	70.4 (3.9)	82.3 (3.9)
	Developed	2.0 (0.0)	4.6 (0.4)	1.7 (0.2)
Historical land use				
1970-71	Agriculture	23.0 (-10.2)	23.1 (-8.6)	13.4 (-5.2)
	Water	0.1 (2.9)	0.4 (3.4)	0.8 (0.2)
	Barren	0.8 (1.5)	1.6 (0.8)	6.6 (-4.0)
	Forest	75.1 (4.8)	71.4 (2.9)	77.6 (8.6)
	Developed	1.1 (0.9)	3.6 (1.4)	1.7 (0.2)
Cahaba River drainage				
Current land use		Little Cahaba	Shades	
2015	Agriculture	1.7	4.2	
	Water	2.5	0.6	
	Barren	5.2	4.3	
	Forest	76.0	62.6	
	Developed	14.6	28.3	
Historical land use				
1960	Agriculture	19.2 (-17.5)	12.1 (-7.9)	
	Water	2.4 (-0.1)	0.1 (0.5)	
	Barren	3.2 (-2.0)	7.3 (-3.0)	
	Forest	69.1 (6.9)	74.1 (-11.5)	
	Developed	6.0 (8.6)	6.3 (22.0)	

3.3.4 Assemblage changes with distance from impoundments

Assemblage structure changed with distance up- and downstream of impoundments. However, in both phases, the effect of distance on assemblage structure depended on stream section (Phase 1: PERMANOVA $F_{10,35} = 2.21$, $P = 0.01$; Phase 2: $F_{10,35} = 2.38$, $P = 0.01$). In phase 1, assemblage structure changed with distance upstream of impoundment only in Cedar Creek (sites C4 and C3 compared to sites C2 and C1, $P < 0.05$; all other comparisons, $P > 0.05$). Sites near the impoundment were dominated by *F. erichsonianus*, but *F. validus* became dominant with distance upstream. In phase 2, assemblage structure changed with distance downstream from impoundments in Little Bear Creek (site LB6 compared to sites LB9 and LB10 $P < 0.05$; all other comparisons, $P > 0.05$) and Little Cahaba River (site LC5 compared to sites LC7 and LC8, $P < 0.05$; all other comparisons, $P > 0.05$). In Little Bear Creek, *F. erichsonianus* and *F. validus* were the only species collected directly downstream of impoundments, but *F. compressus* became abundant with distance downstream. In Little Cahaba River, *F. erichsonianus* and *F. virilis* were the only species collected directly downstream of impoundments, but *C. striatus* and *C. coosae* became abundant with distance downstream.

3.4 Discussion

Impoundments altered the structure of both up- and downstream crayfish assemblages. We detected greater assemblage differences down- than upstream of impoundments and greater assemblage differences in the Bear Creek than Cahaba River drainage. The most consistent results across drainages were changes in the dominant species in up- and downstream sections of unimpounded streams and greater CPUEs and smaller adults collected downstream in unimpounded than impounded streams. Similarly, CPUE was lower downstream of small impoundments in Alabama and Mississippi in previous studies (Hartfield 2010, Adams 2013). Greater predator abundance and reduced variation in minimum and maximum flows and temperatures were correlated with lower CPUEs downstream of impoundments in the current study, while differences in channel morphology contributed to CPUE differences in Mississippi (Adams 2013). Lower CPUEs for crayfishes in impounded streams differed from the higher standing crops documented for other macroinvertebrates (Williams and Winget 1979, Bunn and Arthington 2002). Additionally, this is the first study to assess effects of relatively large impoundments on crayfish assemblages and the first to assess impoundment effects on adult crayfish sizes.

Physical variables within a stream system gradually change with distance from headwaters to mouth, causing a subsequent change in biota (Vannote et al. 1980, Matthews 2012). However, these natural changes are interrupted when streams are impounded, often resetting the natural continuum for physical and biotic variables (Ward and Stanford 1983). For instance, bedrock and large boulders, resembling our furthest upstream sites, were present at sites right below impoundments. Additionally, substrate sizes gradually decreased with distance away from impoundments, just as documented with movement up-to downstream in unimpounded streams (Ellis and Jones 2013). Similarly, for crayfishes in impounded streams, sampling up to 55 km along stream lengths, assemblage structure did not differ between up- and downstream sections. In impounded streams, *F. validus* and *F. erichsonianus* dominated both stream sections in the Bear Creek drainage and *F. virilis* dominated both stream sections in the Cahaba River drainage. Conversely, in unimpounded streams, sampling up to 30 km along stream lengths, assemblage structure and adult sizes differed between up- and downstream sections. The dominant crayfish species gradually shifted with distance downstream in unimpounded streams.

Faxonius validus and *F. virilis* dominated the uppermost sites in Rock and Shades creeks, respectively, and *F. erichsonianus* dominated the lowermost sites in both streams. The dominant species in upper and lower most sites were abundant at sites in middle sections.

In the Bear Creek drainage, the most notable differences in crayfish assemblages up- and downstream of impoundments were the dominance of two species (*F. validus* and *F. erichsonianus,* the most dominant species in the drainage) at all sites in both impounded streams. Conversely, in unimpounded streams, while *F. validus* and *F. erichsonianus* were abundant, *C. striatus* and *F. compressus* were also dominant at up- and downstream sites, respectively. Similarly, in the Sipsey Fork drainage where both *F. validus* and *C. striatus* were present, *F. validus* was abundant in both free-flowing and impounded stream sections, while *C. striatus* was abundant in unimpounded tributaries (Adams et al. 2015). *Faxonius validus* and *F. erichsonianus* prefer run habitats (73% and 78% of individuals, respectively, collected in runs) with vegetation and woody debris, burrowing only during dry conditions (Bouchard and Bouchard 1976, Cooper and Hobbs 1980). Conversely, *F. compressus* prefers riffles (81% of individuals were collected in riffles) with gravel and cobble substrate, digging shallow burrows under substrate (Bouchard 1972, Jones and Eversole 2015). Additionally, *C. striatus* seasonally occupies streams (found equally in riffles and runs), burrowing in stream banks and floodplains during portions of the year (Bouchard 1978, Hobbs 1989, Stoeckel et al. 2011). Larson et al. (2017) and Stites et al. (2017) findings, suggest that *F. compressus* and *C. striatus* may provide different functions and occupy different trophic levels than *F. validus* and *F. erichsonianus* due to their differences in preferred habitats and burrowing behaviors. These assemblage changes may alter stream ecosystem processes (e.g., processing of substrate and macrophytes) in impounded streams.

Abiotic and biotic factors impacted by impoundments (e.g., flow and temperature regime, aquatic vegetation, interstitial space, and predators) can drive changes in crayfish assemblages (Wang et al. 2011, Chu et al. 2015). Less variable temperature and flow regimes was correlated with less abundant and diverse crayfish assemblages. Aquatic species have evolved life history strategies that are synchronized with long-term flow patterns and receive life history cues from temperature regimes (Bunn and Arthington 2002). Crayfish mating, spawning, foraging, and growth are all linked to water temperatures or flows (Lowery 1988, Carral et al. 1994, Mead 2008, Barnett et al. 2017). Creating more stable (i.e., flood control impoundments) or more erratic (i.e., hydroelectric impoundments) flow and temperature regimes can drastically impact key life history events, with the timing of changes (i.e., during mating season, during the release of juveniles) playing a key role in the level of impact (Gore and Bryant 1990, Bunn and Arthington 2002, Karplus et al. 2003). Not only did flow and temperature regimes impact crayfish assemblages, but habitat availability also impacted crayfish abundance. Small crayfishes (juveniles and adults) were less abundant in sites with less vegetation and smaller substrates, which occurred more frequently in impounded streams. Aquatic vegetation, woody debris, and interstitial spaces are important for juvenile crayfish recruitment (i.e., egg and age-0 crayfish survival) and protection from predators (Stein 1977, Englund and Krupa 2000, Olsson and Nyström 2009). With less habitat and more crayfish predators in impounded streams (Ward and Stanford 1979, Phillips and Johnston 2004) due to fish stocking and more favorable conditions, crayfish CPUEs were lower in impounded streams.

Differences in stream sizes and flow regimes may impact stream crayfish assemblages (Flinders and Magoulick 2003). In this study, maximum and minimum flows discriminated between upstream sections of impounded and unimpounded streams, with higher maximum and minimum flows in impounded streams. Because flow regimes of sections upstream of impoundments are not greatly impacted, flow regimes in these sections should resemble unimpounded streams. Thus, in this study, flow differences in upstream sections indicate that unimpounded streams were not perfect controls for impounded streams. Nonetheless, impounded and unimpounded upstream sections had similar flow variatons. For example, impounded (Little Bear Creek) and unimpounded (Rock Creek) streams in the Bear Creek drainage both had isolated pools, with reaches completely dry by the end of each summer. Although stream size does impact crayfish species presence, similarities in discharge variation between upstream sections of impounded and unimpounded streams indicated that species diversity within each drainage was likely similar before impoundments.

Impoundments typically have their greatest effects at locations closest to the impoundment with a gradual recovery of abiotic factors to more natural conditions with distance away from impoundments (Camargo and Voelz 1998, Voelz and Ward 1991). In this study, lentic conditions and sediment deposition decreased with distance upstream of impoundments, while hypoxia and substrate sizes decreased and stream temperatures increased with distance downstream. Like the gradual recovery of abiotic factors, fish, mussels, and aquatic insects also show a gradual change in assemblage structure with distance downstream from impoundments (Voelz and Ward 1991, Kinsolving and Bain 1993, McGregor and Garner 2003). Similarly, gradual changes in crayfish assemblage structure occurred with distance downstream of impoundments in Little Bear Creek and Little Cahaba River, while upstream changes with distance occurred in Cedar Creek. Downstream assemblage structure changes in Little Bear Creek and Little Cahaba River included increases in the CPUE and richness of less dominant species within the drainage, indicating species recovery with distances downstream of impoundments. Conversely, less dominant species were never abundant upstream of impoundments. Thus, upstream recovery did not occur in any impounded stream. Likewise, fish assemblages upstream of impoundments were dominated by generalists and did not change with distance upstream (Herbert and Gelwick 2003, Falke and Gido 2006, Guenther and Spacie 2006).

Habitat complexity is one of the most important factors influencing the diversity and health of stream communities, and a key driver of this complexity is the land use in a stream's watershed (Angermeier and Karr 1984, Wang et al. 2001). Numerous anthropogenic disturbances, including urbanization and deforestation, often degrade streams, homogenizing stream habitats and assemblages (Scott and Helfman 2001, Rahel 2002, Allan 2004). The Cahaba River drainage was more urbanized than the Bear Creek drainage, which had more agriculture. In the Cahaba River drainage, only adult CPUE and adult crayfish sizes varied between impounded and unimpounded streams, while in the Bear Creek drainage, assemblage structure, adult and juvenile CPUEs, and adult crayfish sizes differed. These differences indicate that additional factors beyond impoundments and their subsequent environmental changes may impact crayfish assemblage structure and juvenile CPUE in the Cahaba River drainage. Watersheds such as those in the Cahaba River drainage, with high amounts of developed land, contain impervious surfaces that alter water delivery to streams, ultimately creating flashier hydrographs (Hollis 1975, Booth 1991, Wheeler et al. 2005). Like changes caused by impoundments (Ward 1976, Baxter 1977, Wood and Petts 1994, Thomaz et al. 2007), streams in urbanized watersheds undergo numerous changes including: increased peak flows, bank erosion, fine sediments, and abundances of large piscivorous fishes; decreased aquatic vegetation and interstitial space; and changes in water chemistry (Wolman 1967, Limburg and Schmidt 1990, Gregory et al. 1992, Wheeler et al. 2005, Slawski et al. 2008). These changes affect stream biota in both impounded and unimpounded streams within the watershed. Furthermore, similar stream biota changes may occur in impounded streams without urbanization and unimpounded streams with urbanization. Future research assessing the effects of impoundments in highly urbanized watersheds is needed.

As with urbanization, introduced species can also change stream ecosystems. Invasive species are the leading contributor to population declines of native crayfishes globally (Lodge et al. 2000, Richman et al. 2015) and are likely negatively impacting Cahaba River drainage crayfish populations. *Faxonius virilis*, an introduced species, was present throughout impounded and unimpounded streams in the Cahaba River drainage, while only native crayfishes were present in Bear Creek drainage streams. Invasive species often displace native species due to their high densities and ability to outcompete natives for food and habitat (Light et al. 1995, Hill and Lodge 1999, Twardochleb et al. 2013). The roles that crayfish play in trophic processing (i.e., processing detritus, altering the composition of macrophytes and substrate) often increases in streams with invasive species, altering stream habitats by reducing macrophytes and aquatic vegetation, changing substrate compositions, and creating more turbid waters (Chambers et al. 1990, Twardochleb et al. 2013). *Faxonius virilis* is one of the most widespread invasive crayfishes in the USA (Larson and Olden 2011), present in 34 states outside of its natural range (Donahou 2019), yet its invasive effects are understudied (Larson et al. 2018). Future research assessing the effects of impoundments in watersheds with invasive crayfishes is needed.

Water management objectives differ between drainages, with Bear Creek drainage impoundments managed for flood control and the Cahaba River drainage impoundment managed for water use. Nonetheless, all were storage impoundments that stabilized and increased predictability of downstream flows (Poff et al. 1997). Increased flow predictability may allow a habitat generalist to outcompete a fluvial specialist, causing an overall decline in species diversity (Ward and Stanford 1979; Haxton and Findlay 2008). Flow stabilization also reduces stream-floodplain interactions (Baxter 1977), which is an important factor for crayfishes (e.g., secondary burrowers) using stream habitats seasonally (Helms et al. 2013). Both impoundments also released water from the hypolimnion, which can lead to cold, hypoxic, high nutrient conditions downstream (Petts 1984, Marshall et al. 2006, Haxton and Findlay 2008).

Many crayfishes are highly sensitive to anthropogenic habitat modifications (Richman et al. 2015), in part due to the small natural ranges of many species (Taylor et al. 2007). Over 30 USA and Canadian crayfish species are known from five or fewer locations and numerous others are restricted to a single drainage (Taylor et al. 1996, Lodge et al. 2000). Thus, even small, isolated anthropogenic habitat modifications can reduce crayfish abundance or even extirpate crayfishes. Understanding these effects is often difficult due to lack of historical samples and data deficiencies (Richman et al. 2015). Nonetheless, negative impacts to crayfish assemblages can have ripple effects throughout stream systems (Nyström 2002). Crayfishes can alter both instream and floodplain habitats through processing of leaf litter and macrophytes, as well as the mobilization of substrate by burrowing (Statzner et al. 2000, Dorn and Wojdak 2004, Johnson et al. 2011). Crayfishes also play important trophic role by releasing nutrients and breaking down detritus for use by other invertebrates, preying on other invertebrates, fishes, and amphibians, and serving as prey for over 200 aquatic and terrestrial species (Hobbs 1993, Parkyn et al. 1997, Englund and Krupa 2000, Usio 2000, Holdich 2002).

There are over 20,000 large impoundments in the southeastern USA (NID 2013) and over 270 crayfish species endemic to the region (Taylor et al. 2007). If small impoundments are also considered, it is estimated that most southeastern states have 0.3 to 1 impoundment/km^2 (Smith et al. 2002, Adams 2013), affecting hundreds of crayfish species in thousands of stream kilometers. Although this study focused on stream crayfishes, primary burrowing crayfish species that utilize floodplains are also likely negatively impacted due to the decrease in flood events in impounded streams (Baxter 1977). In addition, tributaries of impounded streams also experience changes in flow and temperature regimes, habitat complexity, and water chemistry, which impact tributary stream biota (Penczak et al. 2009, Roghair et al. 2016). Studies are needed to assess the impact of impoundments on floodplain connectivity and burrowing crayfishes as well as crayfishes in tributaries of impounded streams. Impoundment management type (e.g., storage vs. run-of-river impoundment) will influence impoundment effects, but only impacts of storage impoundments have been assessed for crayfishes (Adams 2013).

Dams and their impoundments altered crayfish assemblages up- and downstream of impoundments relative to unimpounded streams. Impoundments created conditions less favorable for all species except the two dominant species within each drainage, with similar assemblages up- and downstream of impoundments. Nonetheless, assemblage structure began to recover with distance downstream of impoundments. More stable temperature and flow regimes, as well as less aquatic vegetation and larger substrates in impounded streams were correlated with assemblage differences between impounded and unimpounded streams. Additionally, other anthropogenic changes (i.e., land use changes, introduction of invasive species) also played a role in shaping crayfish assemblages in both impounded and unimpounded streams. Greater differences between impounded and unimpounded streams were detected in the drainage with less anthropogenic change and no introduced crayfish species. Impoundments impact crayfish assemblages in thousands of stream kilometers, possibly altering stream systems by decreasing the numerous ecosystem services that crayfishes provide.

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APPENDIX D

SITE LOCATIONS

Appendix D. Site locations and distance (km) from midpoint (i.e., midpoint dam in impounded streams) for sampling sites in Bear Creek and Cahaba River drainages. Dam sizes (dam height, m [area impounded, ha]), and year dam completed listed, as well as USGS site numbers and TVA discharge locations. See Appendix G for further explanation of Rock Creek discharge location. Site latitudes and longitudes are in decimal degrees in map datum WGS 84. Site labels correspond to those in Figure 1. U = unimpounded; I = impounded; Up = upstream; Dn = downstream; Lat. = latitude; Long. = longitude; CC = Cedar Creek; LB = Little Bear Creek.

Drainage	Stream	Stream type	Site label	Stream Section	County	Lat.	Long.	Distance	Dam size	Year	USGS/TVA site
Bear	Rock	U	R1	Up	Colbert	34.6047	-87.9064	13.56			CC TVA inflow
			R2	Up	Colbert	34.5969	-87.9223	11.50			
			R3	Up	Colbert	34.6008	-87.9794	4.02			
			R4	Dn	Colbert	34.5999	-88.0360	1.87			
			R5	Dn	Colbert	34.6236	-88.0760	10.49			
			R6	Dn	Colbert	34.6328	-88.0925	12.63			
	Cedar	I	C1	Up	Franklin	34.4412	-87.7196	26.71			
			C2	Up	Franklin	34.4639	-87.7513	19.29	29.3 (1700)	1979	
			C3	Up	Franklin	34.4711	-87.8023	11.12			
			C4	Up	Franklin	34.4840	-87.8259	5.34			CC TVA inflow
			C5	Dn	Franklin	34.5471	-87.9780	0.30			CC TVA discharge
			C6	Dn	Franklin	34.5528	-87.9846	1.69			
			C7	Dn	Franklin	34.5599	-87.9980	2.79			
			C8	Dn	Franklin	34.5481	-88.0179	6.63			
	Little Bear	I	LB1	Up	Franklin	34.3640	-87.7330	24.64	25.6 (631.3)	1975	
			LB2	Up	Franklin	34.3775	-87.7760	20.07			
			LB3	Up	Franklin	34.3808	-87.8064	15.81			
			LB4	Up	Franklin	34.3819	-87.8350	11.60			
			LB5	Up	Franklin	34.4026	-87.8742	6.22			LB TVA inflow
			LB6	Dn	Franklin	34.4549	-87.9846	0.10			LB TVA discharge
			LB7	Dn	Franklin	34.4560	-87.9830	1.25			
			LB8	Dn	Franklin	34.4609	-88.0042	4.68			
			LB9	Dn	Franklin	34.4880	-88.0350	11.84			
			LB10	Dn	Franklin	34.5011	-88.0492	14.91			

Drainage	Stream	Stream type	Site label	Stream Section	County	Lat.	Long.	Distance	Dam size	Year	USGS/TVA site
Cahaba	Shades	U	S1	Up	Jefferson	33.3755	-86.8611	16.85			
			S2	Up	Jefferson	33.3549	-86.8781	14.07			
			S3	Up	Jefferson	33.3544	-86.9391	3.84			
			S4	Dn	Jefferson	33.3263	-86.9490	2.69			2423630
			S5	Dn	Jefferson	33.3074	-86.9623	5.60			
			S6	Dn	Jefferson	33.2945	-86.9831	9.08			
	Little Cahaba	I	LC1	Up	St. Clair	33.5721	-86.5201	17.56	16.8 (425)	1911	
			LC2	Up	Jefferson	33.5461	-86.5490	12.82			
			LC3	Up	Jefferson	33.5181	-86.5830	6.80			2423397
			LC4	Up	Jefferson	33.4999	-86.6132	1.28			
			LC5	Dn	Shelby	33.4605	-86.6692	0.05			
			LC6	Dn	Shelby	33.4584	-86.6733	0.92			
			LC7	Dn	Jefferson	33.4395	-86.6974	5.60			
			LC8	Dn	Jefferson	33.4373	-86.7017	6.80			2423414

APPENDIX E

FISH SPECIES

Appendix E. Total numbers of each fish species collected up and downstream of impounded and unimpounded streams by drainage in descending order of abundance. Up = upstream; down = downstream; * = crayfish predator; ** = top crayfish predator (see text for explanation); Bear = Bear Creek drainage; Cahaba = Cahaba River drainage.

		Impounded		Unimpounded		
Drainage	Fish species	Up	Down	Up	Down	Total
Bear	Largescale Stoneroller *Campostoma oligolepis*	2,242	1,282	62	31	3,617
	Redline Darter *Etheostoma rufilineatum*	1,316	1,287	0	115	2,718
	Snubnose Darter *Etheostoma simoterum*	1,204	461	2	1	1,668
	*Striped Shiner *Luxilus chrysocephalus*	356	260	39	186	841
	Blackside Snubnose Darter *Etheostoma duryi*	307	316	80	91	794
	*Banded Sculpin *Cottus carolinae*	364	223	0	93	680
	Rainbow Darter *Etheostoma caeruleum*	332	185	47	13	577
	*Longear Sunfish *Lepomis megalotis*	195	117	30	164	506
	Greenside Darter *Etheostoma blennioides*	362	64	26	9	461
	Northern Hogsucker *Hypentelium nigricans*	312	100	18	12	442
	Stripetail Darter *Etheostoma kennicotti*	19	105	224	56	404
	*Creek Chub *Semotilus atromaculatus*	228	37	71	45	381
	Bigeye Chub *Hybopsis amblops*	275	0	0	0	275
	Scarlet Shiner *Lythrurus fasciolaris*	67	46	52	106	271
	*Bluegill *Lepomis macrochirus*	166	74	5	11	256
	Blackspotted Topminnow *Fundulus olivaceus*	78	52	40	45	215
	Bluntnose Minnow *Pimephales notatus*	62	47	39	23	171
	**Rock Bass *Ambloplites rupestris*	54	108	1	5	168
	Common Logperch *Percina caprodes*	106	56	0	5	167
	*Green Sunfish *Lepomis cyanellus*	49	48	45	19	161
	*Whitetail Shiner *Cyprinella galactura*	88	65	1	0	154
	Brook Silverside *Labidesthes sicculus*	6	144	0	1	151
	Black Redhorse *Moxostoma duquesnei*	108	21	0	2	131
	Northern Studfish *Fundulus catenatus*	48	52	0	0	100
	Western Mosquitofish *Gambusia affinis*	34	29	4	22	89
	**Largemouth Bass *Micropterus salmoides*	45	25	12	1	83
	**Spotted Bass *Micropterus punctulatus*	11	62	1	6	80
	Mimic Shiner *Notropis volucellus*	2	61	3	14	80
	Blueside Darter *Etheostoma jessiae*	0	56	8	12	76
	*Spotfin Shiner *Cyprinella spiloptera*	42	31	0	1	74
	Rosyside Dace *Clinostomus funduloides*	43	0	5	2	50
	Weed Shiner *Notropis texanus*	2	2	1	42	47
	Dusky Darter *Percina sciera*	1	37	0	7	45
	Golden Redhorse *Moxostoma erythrurum*	28	9	3	1	41
	Bigeye Shiner *Notropis boops*	2	2	0	35	39
	*Slender Madtom *Noturus exilis*	32	2	0	2	36

		Impounded		Unimpounded		
Drainage	Fish species	Up	Down	Up	Down	Total
Bear	*Black Madtom *Noturus funebris*	28	0	0	1	29
	*Brindled Madtom *Noturus miurus*	4	25	0	0	29
	Bullhead Minnow *Pimephales vigilax*	19	4	1	5	29
	Johnny Darter *Etheostoma nigrum*	9	0	3	9	21
	**Yellow Bullhead *Ameiurus natalis*	9	3	0	1	13
	**Channel Catfish *Ictalurus punctatus*	1	12	0	0	13
	Dollar Sunfish *Lepomis marginatus*	2	1	5	3	11
	*Warmouth *Lepomis gulosus*	0	2	3	6	11
	Western Creek Chubsucker *Erimyzon claviformis*	0	1	5	4	10
	Redfin Pickerel *Esox americanus*	0	0	0	8	8
	Silver Redhorse *Moxostoma anisurum*	3	0	2	0	5
	Bluehead Chub *Nocomis leptocephalus*	5	0	1	0	6
	*Pirate Perch *Aphredoderus sayanus*	0	0	0	5	5
	Spotted Sucker *Minytrema melanops*	1	3	0	0	4
	Least Brook Lamprey *Lampetra aepyptera*	4	0	0	0	4
	*White Crappie *Pomoxis annularis*	3	1	0	0	4
	*Longnose Gar *Lepisosteus osseus*	0	4	0	0	4
	**Flathead Catfish *Polydictis olivaris*	3	0	0	0	3
	Threadfin Shad *Dorosoma petenense*	0	3	0	0	3
	Telescope Shiner *Notropis telescopus*	1	2	0	0	3
	Common Carp *Cyprinus carpio*	0	3	0	0	3
	Golden Shiner *Notemigonus crysoleucas*	0	2	0	0	2
	Redear Sunfish *Lepomis microlophus*	0	2	0	0	2
	Gilt Darter *Percina evides*	0	2	0	0	2
	Smallmouth Buffalo *Ictiobus bubalus*	0	1	0	0	1
	Gizzard Shad *Dorosoma cepedianum*	0	1	0	0	1
	Mobile Logperch *Percina kathae*	0	1	0	0	1
	Highland Shiner *Notropis micropteryx*	0	1	0	0	1
	*Redspotted Sunfish *Lepomis miniatus*	0	0	0	1	1
	*Freckled Madtom *Noturus nocturnus*	1	0	0	0	1
Cahaba	Largescale Stoneroller *Campostoma oligolepis*	285	281	168	13	747
	Longear Sunfish *Lepomis megalotis*	178	227	188	106	699
	Banded Sculpin *Cottus carolinae*	184	253	0	0	437
	Tricolor Shiner *Cyprinella trichroistia*	203	156	44	4	407
	Bluegill *Lepomis macrochirus*	97	115	102	79	393
	Blackbanded Darter *Percina nigrofasciata*	59	101	92	55	307
	Alabama Shiner *Cyprinella callistia*	76	57	43	34	210
	Alabama Hogsucker *Hypentelium etowanum*	44	85	61	17	207
	Redspotted Sunfish *Lepomis miniatus*	49	113	7	9	178

Drainage	Fish	Impounded		Unimpounded		
		Up	Down	Up	Down	Total
Cahaba	Green Sunfish *Lepomis cyanellus*	114	1	28	20	163
	Western Mosquitofish *Gambusia affinis*	43	5	66	27	141
	Alabama Darter *Etheostoma ramseyi*	0	141	0	0	141
	Silverstripe Shiner *Notropis stilbius*	78	11	29	19	137
	Alabama Bass *Micropterus henshalli*	33	24	24	18	99
	Blackspotted Topminnow *Fundulus olivaceus*	9	17	36	10	72
	Cahaba Bass *Micropterus cahabae*	8	44	12	0	64
	Mobile Logperch *Percina kathae*	21	38	0	3	62
	Speckled Darter *Etheostoma stigmaeum*	2	16	14	28	60
	Creek Chub *Semotilus atromaculatus*	2	39	0	0	41
	Blacktail Shiner *Cyprinella venusta*	0	2	31	6	39
	Warmouth *Lepomis gulosus*	2	10	9	11	33
	Blackside Darter *Percina maculata*	0	22	6	0	28
	Redspot Darter *Etheostoma artesiae*	0	15	7	0	22
	Rock Darter *Etheostoma rupestre*	0	0	2	19	21
	Clear chub *Hybopsis winchelli*	0	8	10	0	18
	Emerald Shiner *Notropis atherinoides*	0	1	16	0	17
	Largemouth Bass *Micropterus salmoides*	7	2	4	1	14
	Blacktail Redhorse *Moxostoma poecilurum*	0	0	9	2	11
	Redear Sunfish *Lepomis microlophus*	3	4	0	2	9
	Mimic Shiner *Notropis volucellus*	4	0	0	3	7
	Yellow Bullhead *Ameiurus natalis*	4	1	1	0	6
	Dollar Sunfish *Lepomis marginatus*	0	0	0	6	6
	Striped Shiner *Luxilus chrysocephalus*	5	0	0	1	6
	Greenbreast Darter *Etheostoma jordani*	0	1	0	5	6
	Riffle Minnow *Phenocobius catostomus*	0	0	0	4	4
	Mountain Shiner *Lythrurus lirus*	0	2	0	2	4
	Black Redhorse *Moxostoma duquesnii*	0	3	0	0	3
	Flathead Catfish *Pylodictis olivaris*	0	0	1	1	2
	Spotted Sucker *Minytrema melanops*	0	0	2	0	2
	Golden Shiner *Notemigonus crysoleucas*	0	1	0	0	1
	Golden Redhorse *Moxostoma erythrurum*	0	0	0	1	1
	Chain Pickerel *Esox niger*	0	0	0	1	1
	Blackstripe Topminnow *Fundulus notatus*	0	0	1	0	1
	Dusky Darter *Percina sciera*	0	0	1	0	1

APPENDIX F

LAND USE ACCURACY ASSESSMENT

Appendix F. Accuracy assessment of Landsat 8 image land use classifications in Bear Creek and Cahaba River drainages.

Drainage Bear Creek 2015	ag	forest	barren	water	developed	total	omission	Overall accuracy	Kappa statistic
ag	**19**	0	0	0	0	19	0.00	92.50	0.86
forest	5	**75**	1	1	0	82	0.09		
barren	1	0	**7**	0	0	8	0.13		
water	0	1	0	**5**	0	6	0.17		
developed	0	0	0	0	**5**	5	0.00		
total	25	76	8	6	5	120			
commission	0.24	0.01	0.13	0.17	0.00				
2014									
ag	**25**	0	0	0	0	25	0.00	98.33	0.98
forest	0	**55**	0	0	0	55	0.00		
barren	2	0	**6**	0	0	7	0.29		
water	0	0	0	**17**	0	17	0.00		
developed	0	0	0	0	**15**	15	0.00		
total	27	55	6	17	15	120			
commission	0.07	0.00	0.00	0.00	0.00				
Drainage Cahaba River 2015								87.50	0.76
ag	**8**	0	0	0	0	8	0.0		
forest	0	**72**	0	0	5	77	0.06		
barren	0	3	**5**	0	0	8	0.38		
water	0	0	0	**5**	1	6	0.17		
developed	0	6	0	0	**15**	21	0.29		
total	8	81	5	5	21	120			
commission	0.00	0.11	0	0	0.29				

APPENDIX G

DATA CREATION

Phase 1 Data Creation

We calculated spring (March 20 – June 21) stream temperature characteristics from NOAA air temperature data (Muscle Shoals, AL, KMSL, NOAA weather station). For each site, we regressed our hourly water temperatures on NOAA air temperatures from the same timeframe and used these equations to estimate spring water temperatures. We calculated fall (September 22–December 21) 2015 stream temperature characteristics from our hourly water temperatures.

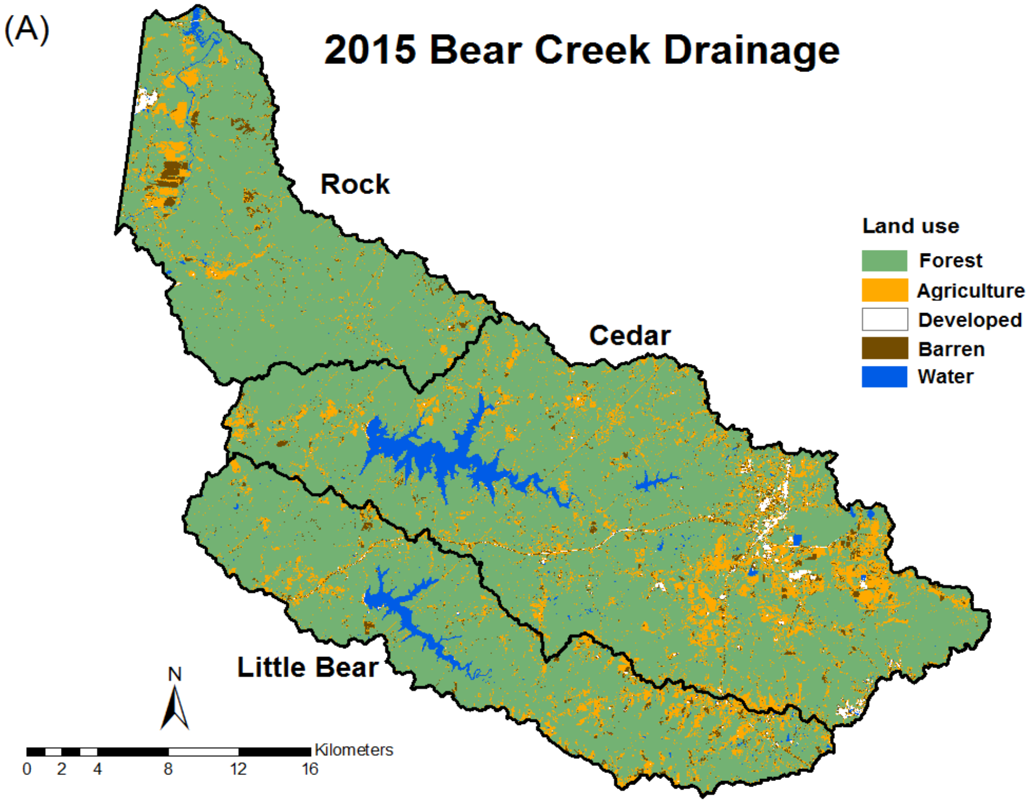
Predatory fish length-weight polynomial relationships (weight = $[a * \text{length}^2] - [b * \text{length}] + c$) were calculated for each species from multi-pass electrofishing surveys, and constants a, b, and c were estimated. We used these relationships to estimate the biomass of predator fish collected and calculated total and average predator and top predator fish biomass.

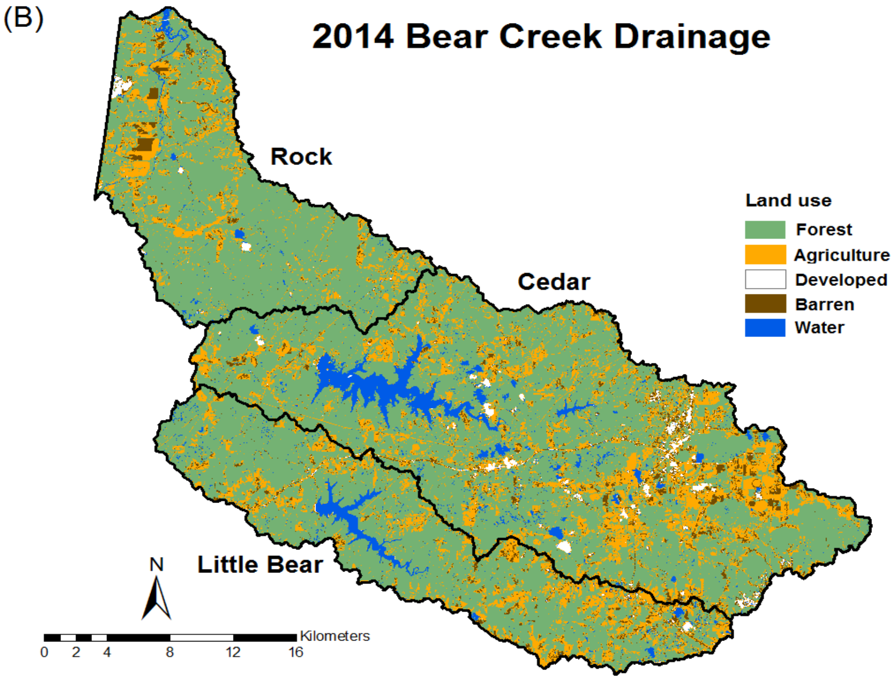
Phase 2 Data Creation

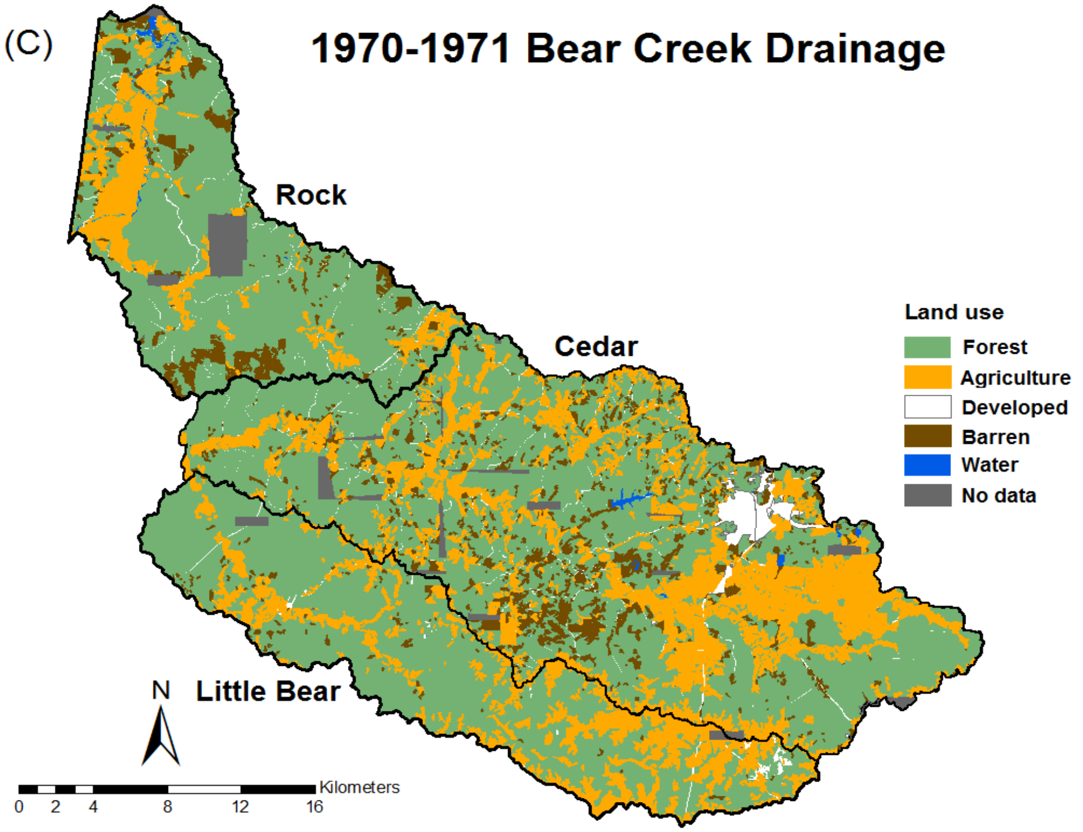
We calculated spring and fall discharge characteristics (Table 3.2) from one site up- and downstream (Appendix D) on Bear Creek and Cahaba River drainage impounded streams using TVA and USGS discharge data, respectively. We calculated discharge characteristics from one site on unimpounded streams. Because there was no discharge gage on Rock Creek, we regressed discharge measured during sampling at site R1 (furthest upstream Rock Creek site) on Cedar Creek impoundment inflow data due to their close proximity. For each stream, we used the discharge measured during sampling to assess the linear relationship of discharge with movement downstream (discharge = $[a * \text{distance}] + b$). Linear relationships were calculated separately for up- and downstream sections of impounded streams. We used the constants estimated in these relationships to calculate discharge characteristics from USGS and TVA discharge data. Discharges were likely underestimated because we did not take into account changes in tributary discharge.

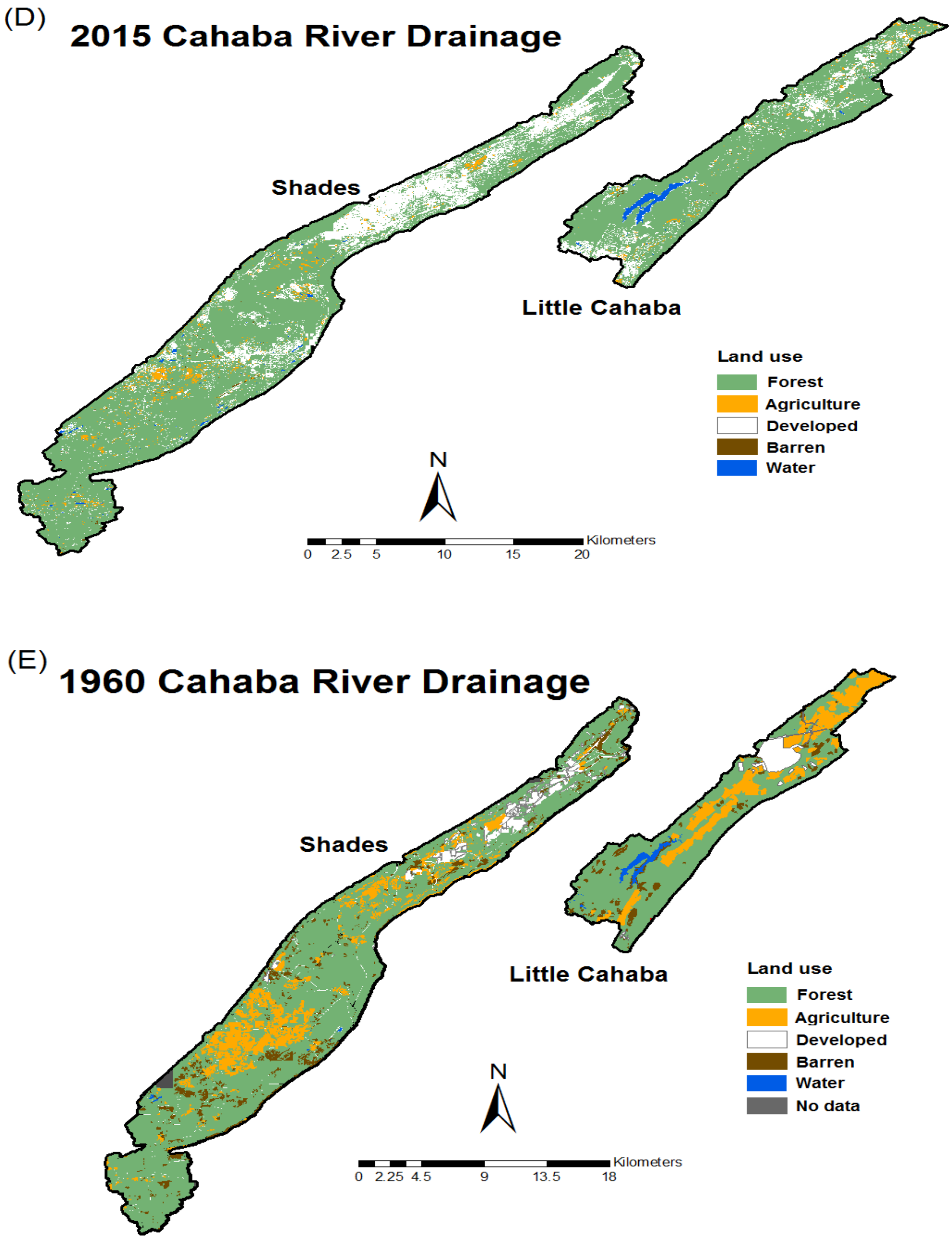
APPENDIX H

LAND USE CLASSIFICATIONS OF SATELLITE AND AERIAL IMAGES


(A)
2015 Bear Creek Drainage
Rock
Cedar
Little Bear
Land use
Forest
Agriculture
Developed
Barren
Water
N
Kilometers
0
2
4
8
12
16


(B)
2014 Bear Creek Drainage
Rock
Cedar
Little Bear
Land use
Forest
Agriculture
Developed
Barren
Water
N
Kilometers
0
2
4
8
12
16


(C)
1970-1971 Bear Creek Drainage
Rock
Cedar
Little Bear
Land use
🟩 Forest
🟧 Agriculture
☐ Developed
🟫 Barren
🟦 Water
⬛ No data
N
Kilometers
0
2
4
8
12
16



Appendix H. Bear Creek (A-C) and Cahaba River (D-E) drainage recent (A, B, D) and historical (C, E) land cover classification results of aerial photography (C, E) and Landsat 8 (A, B, D) data using visual and supervised maximum likelihood classifications. Outlined polygons represent each streams' watershed, with stream watersheds labeled outside of polygons. Historical aerial photography was taken in 1960 for all watersheds in the Cahaba River drainage, in 1970 for the Rock Creek watershed, and 1971 for the Cedar and Little Bear creek watersheds.

CHAPTER IV:

CRAYFISH POPULATIONS GENETICALLY FRAGMENTED IN STREAMS IMPOUNDED FOR 36–104 YEARS

Abstract

Dams and their associated impoundments may restrict gene flow among populations of numerous freshwater species, leading to genetic isolation. This process can reduce genetic diversity and effective population size, increasing risk of local extinction. Here we present the first assessment of the impacts of dams and impoundments on stream crayfish populations, using genetic tools. We studied crayfishes from multiple up- and downstream sites in three impounded and two unimpounded southeastern USA streams in Bear Creek and Cahaba River drainages, Alabama. Using mitochondrial DNA (COI gene) sequence data generated from population-level sampling of two abundant crayfishes, *Faxonius validus* and *F. erichsonianus* (Decapoda: Cambaridae), we assessed species' spatial genetic structure and genetic diversity. We also compared results between the species, which had different stream preferences and ranges (*F. validus* prefers smaller streams and has a smaller range). For both species, levels of genetic diversity (number of haplotypes, and haplotypic and nucleotide diversity) were the same or higher in impounded than unimpounded streams. However, unimpounded stream local populations (populations in up- and downstream sections) displayed high genetic similarity and bidirectional gene flow, whereas impounded stream crayfishes typically displayed genetic differentiation and unidirectional, downstream gene flow. Little to no gene flow occurred among local populations in the stream impounded for the greatest duration (104 years). Additionally, within the Bear Creek drainage, less gene flow occurred among local populations in the stream with the largest impoundment. Although impoundments decreased connectivity among local populations of both *F. validus* and *F. erichsonianus*, the magnitude of genetic effects was species-specific, with greater differentiation between *F. validus* populations up- and downstream of impoundments. In an ecologically short timeframe, impoundments have fragmented stream

crayfish populations, and even species with relatively high abundances and large ranges suffered negative genetic effects of fragmentation. Our findings suggest that size of impoundments and time since dam closure also impacted crayfish genetic structures. Ultimately, the feedbacks between genetic and demographic effects on fragmented populations may decrease the probability of long-term persistence.

4.1 Introduction

Over 20,000 large dams (> 15 m high) impound streams in the southeastern United States (NID 2013). Dams fragment populations of stream fauna by physically blocking dispersal and migration, reducing floodplain connectivity, and creating unfavorable conditions for pre-disturbance fauna (Baxter 1977, Watters 1996). Habitat fragmentation can cause genetic isolation of local populations, with the degree of observed isolation dependent on the spatial and temporal scales analyzed. Reduction or prevention of gene flow due to habitat fragmentation can increase genetic divergence among isolated populations, largely owing to the effects of genetic drift or selection (Lande 1976, Vandergast et al. 2007, Bessert and Ortí 2008). Isolated populations may also experience decreased recruitment, adaptive potential, and persistence due to reduced genetic diversity and effective population sizes (N_e). In small populations, these threats may be compounded by inbreeding depression (i.e., the phenotypic expression of deleterious recessive alleles that usually reside in gene pools at low frequency; Crnokrak and Roff 1999, Dixo et al. 2009), further increasing extinction risk (MacArthur and Wilson 1967, Lande 1988, Pringle 1997).

Decreased connectivity has led to increased genetic subdivision between up- and downstream populations isolated by impoundments in numerous aquatic organisms including fishes, mussels, and insects (Yamamoto et al. 2004, Kelly and Rhymer 2005, Alp et al. 2012). Consistent with expectations for the effects of genetic drift in small isolated populations (Charlesworth and Charlesworth 1987, Crnokrak and Roff 1999, Hedrick 2005), reduced genetic diversity in aquatic insect populations separated by impoundments has been reported, particularly for species with limited dispersal (Monaghan et al. 2002, Watanabe and Omura 2007, Watanabe et al. 2010). In fishes, impoundments have impacted populations in numerous ways, including loss of genetic variation within isolated populations, genetic discontinuities across formerly connected populations (Faulks et al. 2011, Fluker et al. 2014), and phenotypic deformities and local extinctions, especially in upstream populations (Morita and Suzuki 1999, Morita and Yamamoto 2002).

Crayfishes are vulnerable to anthropogenic habitat modifications (Richman et al. 2015), including damming, water management, and urban development. For many species, this vulnerability is exacerbated by their small natural ranges (Taylor et al. 2007). Consequently, crayfish populations are declining worldwide, with 48% of North American crayfish species threatened (Taylor et al. 2007) and extinction rates thought to be rapidly increasing (Richman et al. 2015). Crayfishes play an important role in stream ecosystem trophic processes by altering the composition of macrophytes and substrates, processing detritus, and transferring energy to predators including fishes, birds, and other crayfishes (Chambers et al. 1990, Hanson et al. 1990, Momot 1995, Rabeni et al. 1995, Statzner et al. 2003). Despite their functional importance, to our knowledge, no previously published study has examined the impacts of impoundments on crayfish genetic structures. Although numerous studies have examined the effects of impoundments on other stream organisms (e.g., mussels [Abernethy et al. 2013, Galbraith et al. 2015], aquatic insects [Monaghan et al. 2002, Alp et al. 2012], and fishes [Yamamoto et al.

2004, Neville et al. 2009]), crayfishes ability to move across land makes it difficult to predict the impacts of instream barriers from existing studies.

In the present study, we assessed the impacts of dams and impoundments on the population genetics of crayfishes in the southern Appalachian region of Alabama, USA (ARC 2009). Alabama has the most diverse freshwater fauna in North America (Lydeard and Mayden 1995, Duncan 2013) but also has over 2,000 large dams (NID 2013). Similarly, the southern Appalachian region is the global center of crayfish diversity (Crandall and Buhay 2008) and a region where numerous impoundments were built during the last 115 years (Morse et al. 1993, NID 2013). We focused on two crayfish species, *Faxonius validus* and *F. erichsonianus* (Decapoda: Cambaridae), that were abundant in the region. *Faxonius erichsonianus* and *F. validus* share many ecological traits typical of stream crayfishes but differ in stream size preferences and ranges. Like many stream crayfishes, both species live 3–4 years, have a September–November mating season (Holdich 2002), and are tertiary burrowers typically found under rocks in shallow mud burrows and in leaf litter and aquatic plant habitats (Bouchard 1972, Williams and Bivens 2001, Hopper et al. 2012). *Faxonius erichsonianus* occurs in medium to large streams with moderate currents and rocky substrates in six southeastern states from western Tennessee down to northern Mississippi and Alabama and east to northwestern Georgia, western North Carolina, and southwestern Virginia (Hobbs 1981). In contrast, *F. validus* occurs in small intermittent to medium-sized perennial streams and springs in the Tennessee and Black Warrior river basins in northern Alabama and southern Tennessee (Cooper and Hobbs 1980, Hobbs 1989). From a conservation perspective, both are considered stable species (Adams et al. 2010a, b); nonetheless, 20% of currently imperiled crayfishes in the United States and Canada are *Faxonius* spp. (Taylor *et al.,* 2007).

The goal of this study was to compare the population genetic structures of *F. validus* and *F. erichsonianus* between unimpounded and impounded streams. We addressed three questions (Table 4.1): 1) Is genetic diversity reduced in crayfish populations in impounded streams compared to unimpounded streams? 2) Do dams and impoundments inhibit crayfish dispersal, resulting in geographically structured populations? and 3) Do the two focal crayfish species show concordant responses to impoundments?

Table 4.1. Research questions, statistical analyses, and expected results. Local population = up- or downstream crayfish population.

Research Question	Statistical analyses	Expected result if impoundments impacted crayfish population genetics
Question 1: Is genetic diversity reduced in impounded streams?	ANOVA	Less genetic diversity in local populations of impounded than unimpounded streams
		Less genetic diversity in up- than downstream local populations in impounded streams
Question 2: Do impoundments limit dispersal and enhance genetic population structure?	TCS haplotype networks	Geographically structured networks in impounded streams only
	SAMOVA	Distinct genetic populations identified for up- versus downstream sites in impounded streams only
	AMOVA	Significant genetic differentiation between local populations in impounded streams only
	Isolation by distance (IBD)	IBD within local populations of impounded streams when analyzed separately, but not when analyzed together
	Migrate-n	Unidirectional downstream or no gene flow between impounded stream local populations, but bidirectional gene flow in unimpounded streams
		Smaller effective populations sizes upstream of impoundments, but no differences between effective population sizes of local populations in unimpounded streams
Question 3: Do species show concordant responses to impoundments?	Qualitatively examined	Similar patterns in genetic diversity, genetic structure, and gene flow matrices for both species in impounded versus unimpounded streams

4.2 Methods

4.2.1 Study Areas

We sampled crayfishes from five streams in the Bear Creek and Cahaba River drainages, Alabama, USA (Figure 4.1). In the Bear Creek drainage (Tennessee River Basin), we sampled two impounded (Little Bear and Cedar creeks) and one unimpounded (Rock Creek) stream. In the Cahaba River drainage (Mobile River Basin), we sampled one impounded (Little Cahaba River) and one unimpounded (Shades Creek) stream. Both drainages are valuable ecological resources due to diverse aquatic faunal communities and numerous imperiled species contained within them (Allen 2001, McGregor and Garner 2003, Phillips and Johnston 2004). The Bear Creek drainage has four flood control impoundments, and the Cahaba River drainage has one major impoundment. Importantly, both drainages had long segments of impounded and unimpounded streams with similar habitats (e.g., distinct riffle-run complexes) and species assemblages that were accessible to sample.

Impounded streams each had one earthen storage dam. Little Bear Creek dam was completed in 1975, and is 25.6 m high and 739.1 m long, creating a 631 ha reservoir. Cedar Creek dam, completed in 1979, is 29.3 m high and 963.2 m long, forming a 1700 ha reservoir. Little Cahaba River dam is considerably older than the others, originally constructed in 1911 and later expanded to its current size, 16.8 m high and 64.9 m long, in 1929, resulting in a 425 ha reservoir (Purdy Lake). Little Bear and Cedar creek impoundments were used for flood control, and Little Cahaba River impoundment was used for water storage. Each year from November until February and during heavy rain events hypolimnetic water is released in Little Bear and Cedar creeks. In Little Cahaba River, hypolimnetic water is released when water flow in the river is too low to meet water usage demands.

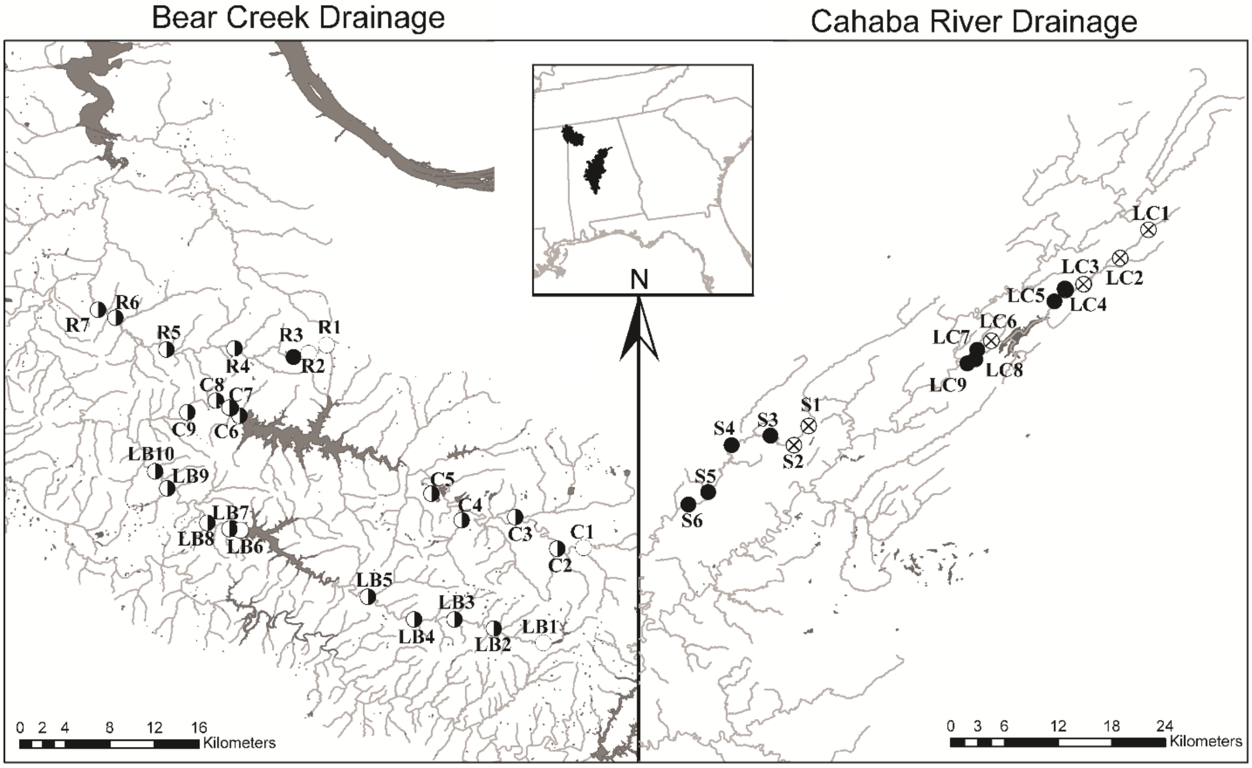


Figure 4.1. Map of Bear Creek and Cahaba River drainages, Alabama, with collection sites represented by labeled circles. Sites are labeled in increasing order from up- to downstream, with letters representing stream names (R = Rock Creek, C = Cedar Creek, LB = Little Bear Creek, S = Shades Creek, and LC = Little Cahaba River). Filled circles = *Faxonius erichsonianus* collection sites; unfilled circles = *F. validus* collections sites; half-filled circles = *F. erichsonianus* and *F. validus* collection sites; encircled X = sample sites from which neither of the two target species were collected. Inset shows drainage locations within the southeastern United States, with the Bear Creek Drainage in the northwest corner and the Cahaba River Drainage in the center of Alabama.

4.2.2 Population sampling

Faxonius erichsonianus and *F. validus* individuals were collected in the Bear Creek drainage, and *F. erichsonianus* individuals were collected in the Cahaba River drainage. We sampled 6–10 sites along each of the five streams, resulting in 41 sites from which one or both species were sampled (Figure 4.1). In each stream, for both species, we sampled three to five sites in up- and downstream sections (hereafter referred to as "local populations"). In impounded streams, these two sections were separated by the impoundment. We sampled 26 sites in the Bear Creek drainage (10 in Little Bear Creek, 9 in Cedar Creek, and 7 in Rock Creek) and 15 sites in the Cahaba River drainage (9 in Little Cahaba River and 6 in Shades Creek). We selected sites at set intervals up- and downstream of impoundments and mimicked the pattern in unimpounded streams. If a predetermined sampling location was inaccessible, we instead sampled the closest accessible site. Sites ranged from 0–31 km away from impoundments and up to 30 km along the length of unimpounded streams. Sampling with a variety of methods (i.e., kick seining, electroshocking, nest trapping, dip netting) from 2015 to 2017, we collected 143 *F. validus* and 179 *F. erichsonianus* individuals (i.e., an average of six individuals per site). Immediately after sampling, we recorded crayfish species and preserved them in 95% ethanol.

4.2.3 Genetic data collection

We extracted genomic DNA from crayfish leg tissue using a DNeasy blood and tissue kit (Qiagen, Valencia, CA), following the manufacturer's recommendations. For all individuals, a portion of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified via polymerase chain reaction (PCR), using primers LCO1490 and HCO2198 (Folmer et al. 1994). We performed PCR amplifications in a final volume of 15 µL containing 1.5 µL genomic DNA, 3 μL of 5x buffer (Promega, Madison, WI), 1.1 μL of $MgCl_2$ (25mM, Promega), 2.5 μL of deoxynucleotides (1.25 μM, Promega), 0.75 μL of Bovine Serum Albumin (10 mg/ μL, New England Biolabs, Ipswich, MA), 4.5 μL of dH_2O, 0.15 μL of Go-*Taq* (5U/ μL, Promega), and 0.75 μL of each primer (10 μM). Thermocycling conditions for PCR amplifications were: 95 ˚C for 2 min (1 cycle), 95 ˚C for 30 sec, 50 ˚C for 30 sec, 72 ˚C for 1 min (35 cycles), and a final extension at 72 ˚C for 2 min (1 cycle). We used agarose gel electrophoresis to assess the quality and estimate the size (in base pairs [bp]) of amplified products via comparison to a 100-bp ladder. Amplified products were purified using ExoSAP-IT® (Affymetrix, Santa Clara, CA) and sequenced on an Applied Biosystems 3730x Genetic Analyzer at Yale University's DNA Analysis Facility on Science Hill. Sequence chromatograms were manually edited, aligned, and assessed for quality via translating into amino acids in order to confirm the absence of premature stop codons, using MEGA v. 7 (Kumar et al. 2016). We further assessed data quality by comparing our sequences to those in the NCBI's GenBank database. All sequences generated in the present study are available from GenBank under accession numbers #####-####.

4.2.4 Genetic analyses

We investigated the effects of impoundments on genetic diversity, spatial genetic structure, and connectivity mediated by dispersal and gene flow. Table 4.1 summarizes the suite of complementary analytical approaches used to address each research question and the associated expected outcomes if impoundments affected crayfish populations. Below, we provide a detailed description of each analysis.

4.2.4.1 Genetic diversity comparisons

To examine if impoundments affected genetic diversity within crayfish populations, we assessed the relationships between measures of genetic diversity and stream types (i.e., impounded/unimpounded). For COI sequence data from each species, we used DNAsp v. 5.10.01 (Librado and Rozas 2009) to calculate three genetic diversity indices (i.e., sample size-scaled number of haplotypes, haplotypic diversity, and nucleotide diversity) at each site. Briefly, the sample size-scaled number of haplotypes (h/N) is the number of different haplotypes (h) at each site scaled by the number of individuals sampled (N). Haplotypic diversity (hd) is the probability that a randomly chosen pair of haplotypes are different from one another. Nucleotide diversity (π) is the average proportion of nucleotides that differ between pair of sequences. To test whether genetic diversity was lower in impounded streams relative to unimpounded streams, we compared genetic diversity indices between stream types, site locations (up/downstream), and stream identity using separate analysis of variance (ANOVA) models for each species. Interactions among stream type, site location, and stream identity were included. Analyses were performed with the *car* package (Fox and Weisberg 2011) in R v. 3.4.4 (R Core Team 2018), using Tukey's HSD post hoc tests to further analyze significant results. Histograms and scatterplots of model residuals did not exhibit departure from normality or heterogeneity, respectively.

4.2.4.2 Spatial distribution of genetic variation and gene flow analyses

We used five approaches to characterize gene flow among local populations, and the spatial distributions of genetic variation within and among populations of each crayfish species. First, for each species, we estimated evolutionary relationships among haplotypes using statistical parsimony networks (Clement et al. 2000) calculated using PopART v. 1.2.1 (Leigh and Bryant 2015). We used this approach because haplotype networks often better illustrate genetic divergence at the intraspecific level than do strictly bifurcating phylogenetic trees, especially in cases with multiple haplotypes derived from a single ancestral sequence, and where ancestral sequences are still extant (Templeton et al. 1992). Following network construction, we color-coded the local population of each sampled haplotype to facilitate visual assessment of spatial structure.

Second, to define genetic populations (i.e., natural partitions of genetic data identified *a posteriori* on the basis of haplotype frequencies and mutational differences) that are maximally differentiated from each other, we used spatial analysis of molecular variance (SAMOVA) implemented in SAMOVA v. 2.0 (Dupanloup et al. 2002). This method is based on a simulated annealing procedure that maximizes the proportion of genetic variance explained by differences among groups of individuals sampled from one or more geographic locations (F_{CT}). We selected the best-fit DNA sequence evolution model identified by the corrected Akaike information criterion (AIC_c), using jModeltest v. 2.1.10 (Darriba et al. 2012). SAMOVA analyses were based on 100 simulated annealing steps and *a priori* definition of the number of groups (K), with a maximum of two groups estimated per stream (i.e., 2 to 6 for *F. validus* and 2 to 10 for *F. erichsonianus*). For each analysis, we identified the optimal value of K by maximizing F_{CT}.

Third, for each species, we assessed haplotype frequency-based genetic differentiation (F_{ST}) between each stream's local populations (i.e., geographically delineated up- vs. downstream groups) using analysis of molecular variance (AMOVA) calculated in Arlequin v. 3.5.2.2 (Excoffier and Lischer 2010), with a null distribution generated via 10,000 permutations.

Fourth, we evaluated whether genetic differentiation was a product of isolation by distance (IBD). We examined evidence for IBD within each stream, separately, by correlating matrices of genetic distances (i.e., proportion of nucleotides that differ between each pair of sequences) among individuals with their corresponding geographic distances (i.e., the shortest waterway route between each site from which individuals were sampled) using Mantel tests. To assess IBD, we performed independent analyses of pairwise comparisons for each local population and for all conspecific samples collected from a given stream. All geographic distances were determined using ArcGIS (ESRI, Redlands, CA). We performed IBD tests with the *ade4* package (Chessel et al. 2004) in R, and used 10,000 randomizations to measure the significance of each test (Bohonak 2002).

In a fifth analysis, we estimated parameter values for N_e of local populations, and migration (m) between each stream's local populations, using Migrate-n v. 3.6.11 (Beerli and Felsenstein 2001). To determine N_e and m, we used a mutation rate (μ) of 2.2×10^{-8} substitutions per site per generation based on Cunningham et al. (1992) estimates for crabs, assuming a generation time of 1 year and equal sex ratios (Cooper 1975, Holdich 2002). Notably, Migrate-n estimates of the mutation-scaled effective population size ($\theta = N_e\mu$ for mitochondrial DNA) and mutation-scaled immigration rates ($M = m/\mu$) does not assume symmetrical bi-directional gene flow between a pair of populations, but instead partitions immigration from emigration, enabling inferences about directionality of gene flow. For these analyses, we used a static heating scheme with four parallel chains, temperature values of 1, 1.5, 3, and 1×10^6, and a swapping interval of one. In all analyses, we ran five long Markov chain Monte Carlo (MCMC) simulations with 1×10^4 genealogies discarded as burn-in and recorded 1×10^6 steps every 20 generations, resulting in 2×10^6 sampled genealogies averaged over five independent replicates. We assessed convergence of MCMC simulations by evaluating the consistency of estimates across replicate runs. Using likelihood ratio tests, for each stream's local crayfish populations, we assessed: (1) differences between estimated N_e, (2) significance of departure from the null hypothesis of symmetric gene flow as estimated via migration rates, and (3) significance of departure from the null hypothesis of complete genetic isolation (i.e., zero gene flow).

4.3 Results

We obtained mitochondrial COI sequences from 143 *F. validus* and 179 *F. erichsonianus* individuals, with final alignments of 618-bp and 640-bp, respectively. For *F. validus,* the alignment contained 25 polymorphic sites and 28 unique haplotypes. For each stream, sample size-scaled number of haplotypes (*h*/*N*) ranged from 0.17–0.31 (7–16 haplotypes/stream). Within local populations, haplotypic diversity (*h*d) ranged from 0.23–0.76 and nucleotide diversity (π) ranged from 0.001–0.004 (Table 4.2). Notably, all *F. validus* haplotypes sampled from Rock Creek (7 haplotypes) were unique to that stream, a result not found elsewhere. For *F. erichsonianus,* the mitochondrial COI alignment contained 68 polymorphic sites and 42 haplotypes. For each stream, *h*/*N* ranged from 0.11–0.32 (5–14 haplotypes/stream). Within local populations, *h*d ranged from 0.23–0.91 and π ranged from 0.0004–0.007 (Table 4.2).

Table 4.2. Mean genetic diversity (± SD) of up- and downstream local crayfish populations in each stream. *N* = number of sites where target species collected; Up = upstream; Dn = downstream; I = impounded; U = unimpounded; Dist. = stream distance between the most up- and downstream sites containing the species within the local population; *h* = number of haplotypes; *h*d = haplotype diversity; *π* = nucleotide diversity.

Local population (*N*)	Site codes	Stream type	Dist. (km)	No. crayfish	*h*	*h*d	π
Faxonius validus							
Up Little Bear (5)	LB1–5	I	18.42	28	5	0.47 (0.20)	0.002 (0.001)
Dn Little Bear (5)	LB6–10	I	14.83	30	7	0.71 (0.06)	0.003 (0.001)
Up Cedar (5)	C1–5	I	25.23	31	8	0.70 (0.10)	0.004 (0.003)
Dn Cedar (4)	C6–9	I	6.32	21	9	0.76 (0.10)	0.003 (0.001)
Up Rock (3)	RC1–2,4	U	10.33	19	4	0.23 (0.29)	0.001 (0.001)
Dn Rock (3)	RC5–7	U	11.51	14	4	0.44 (0.50)	0.002 (0.003)
F. erichsonianus							
Up Little Bear (4)	LB2–5	I	18.42	21	5	0.79 (0.20)	0.006 (0.010)
Dn Little Bear (4)	LB7–10	I	14.83	23	2	0.23 (0.30)	< 0.001 (0.001)
Up Cedar (4)	C2–5	I	21.37	20	9	0.91 (0.06)	0.005 (0.004)
Dn Cedar (4)	C6–9	I	6.32	24	7	0.77 (0.04)	0.002 (0.001)
Up Rock (2)	RC3–4	U	7.87	12	6	0.70 (0.10)	0.005 (< 0.001)
Dn Rock (3)	RC5–7	U	11.51	18	4	0.36 (0.40)	0.002 (0.002)
Up Little Cahaba (2)	LC4–5	I	6.51	13	6	0.88 (0.03)	0.006 (0.005)
Dn Little Cahaba (3)	LC7–9	I	5.88	19	4	0.45 (0.40)	0.001 (0.001)
Up Shades (2)	S3–4	U	6.52	14	5	0.83 (0.03)	0.007 (0.007)
Dn Shades (2)	S5–6	U	5.52	15	4	0.64 (0.15)	0.001 (0.001)

4.3.1 Genetic diversity comparisons

Haplotypic diversity differed between impounded and unimpounded streams for *F. validus,* but not *F. erichsonianus*. For *Faxonius validus*, *h*d was higher in impounded than unimpounded streams ($F_{1,19}$ = 8.69, $P < 0.01$); however, *π* and *h*/*N* did not differ between streams with impoundments versus without impoundments, or between sites up- and downstream of impoundments (all *P* values > 0.05). For all streams, *F. erichsonianus π*, *h*d, and *h*/*N* were higher in up- than downstream sites, irrespective of impoundments ($F_{1,20}$ = 16.67, $P < 0.001$; $F_{1,20}$ = 13.09, $P < 0.01$; $F_{1,20}$ = 5.36, $P = 0.03$, respectively).

4.3.2 Spatial distribution of genetic variation and gene flow analyses

4.3.2.1 Statistical parsimony haplotype networks

Faxonius validus haplotype networks displayed strong geographic structure only for impounded streams' local populations (Appendix I-A), indicating dispersal limitations. The most common haplotype was shared by 82% of individuals in Rock Creek (84% and 79% of individuals in the up- and downstream local populations, respectively). Conversely, the most common haplotypes were shared by only 55% of individuals in Little Bear Creek (62% and 48% of individuals up- and downstream, respectively) and 23% of individuals in Cedar Creek (41% and 5% of individuals up- and downstream, respectively). The *F. erichsonianus* haplotype network for samples from Little Cahaba River showed indications of geographic structure between local populations (Appendix I-B). An absence of shared haplotypes up- and downstream of the impoundment indicated little to no gene flow between local populations. The two most common haplotypes in all unimpounded streams (Rock and Shades creeks) and impounded streams in the Bear Creek drainage (Cedar and Little Bear creeks) were shared by 58–86% and 55–100% of the up- and downstream local populations, respectively (Appendix I-B).

4.3.2.2 Spatial analysis of molecular variance (SAMOVA)

For *F. validus*, we identified six genetic populations within the Bear Creek drainage. All SAMOVA analyses, which collectively assessed the fit of 2–6 groups, were significant ($P < 0.05$; Figure 4.2A). Nonetheless, F_{CT} was maximized when assuming six groups, which explained 44% of variation among groups. Each SAMOVA analysis grouped all Rock Creek (unimpounded stream) sites together. Five groups were identified for sites within impounded streams (Little Bear and Cedar creeks; Figure 4.2A). All sites downstream of Cedar Creek impoundment, as well as two sites downstream of Little Bear Creek impoundment, grouped together. The remaining sites downstream of Little Bear Creek impoundment grouped with sites upstream in Little Bear Creek. Two and four groups were identified upstream of Little Bear and Cedar creek impoundments, respectively. When we analyzed two groups, all Rock Creek sites grouped together, and all Little Bear and Cedar Creek sites grouped together, indicating one genetic population in the unimpounded stream and one to five genetic populations in impounded streams (Figure 4.2A).

For *F. erichsonianus*, we identified 10 genetic populations using SAMOVA. All analyses, estimating 2–10 groups, were significant ($P < 0.05$; Figure 4.2B). However, F_{CT} maximized at nine groups, explaining 81% of variation among them. Six genetic populations were identified in the Bear Creek drainage and three in the Cahaba River drainage (Figure 4.2B). Each stream in the Bear Creek drainage grouped separately. In addition, for each stream, one upstream site formed a separate group, indicating two genetic populations per stream. Each SAMOVA analysis grouped all Shades Creek sites with all sites downstream of Little Cahaba River impoundment. Each site upstream of Little Cahaba River impoundment formed its own group. When two groups were analyzed, all sites in the Bear Creek Drainage grouped together, and all sites in the Cahaba River Drainage grouped together (Figure 4.2B).

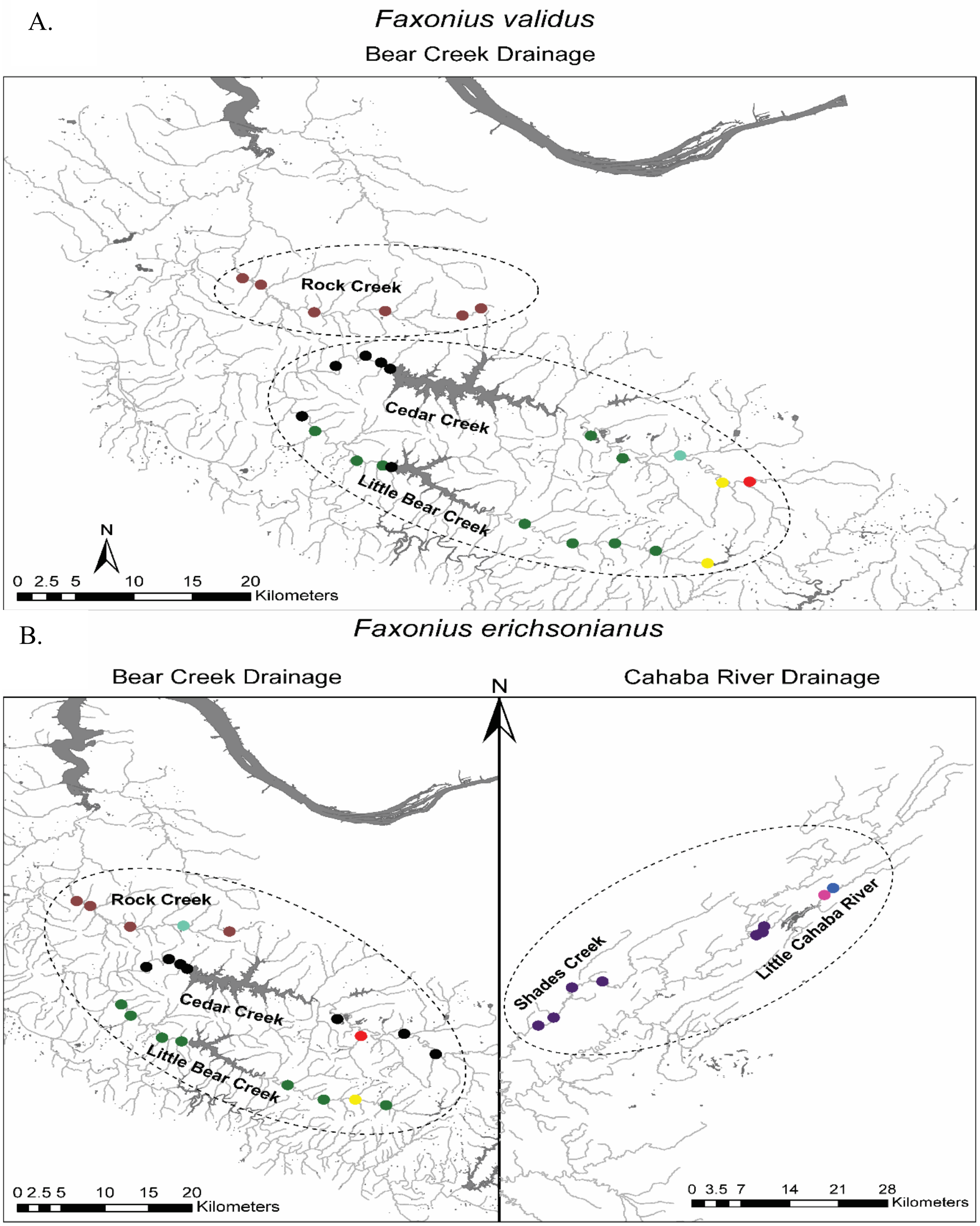


Figure 4.2. Map of Bear Creek (A–B) and Cahaba River (B) drainages, with SAMOVA groupings of populations that maximized F_{CT} for *Faxonius validus* (A; 6 groups) and *F. erichsonianus* (B; 9 groups) represented by colored circles, and the groupings for the lowest number of groups analyzed (2) represented by dashed lined circles. All sites with the same colored circle were grouped together by SAMOVA analyses.

4.3.2.3 Analysis of molecular variance (AMOVA)

For both species, F_{ST} (a measure of genetic differentiation between each stream's local populations) was highest between those populations in impounded streams (Table 4.3). For *F. validus,* differentiation between local populations was present only in impounded streams (Table 4.3). Similarly, for *F. erichsonianus,* local populations were differentiated in two impounded streams (Little Bear Creek and Little Cahaba River) but not in unimpounded streams. However, local populations in Cedar Creek (impounded) were not differentiated.

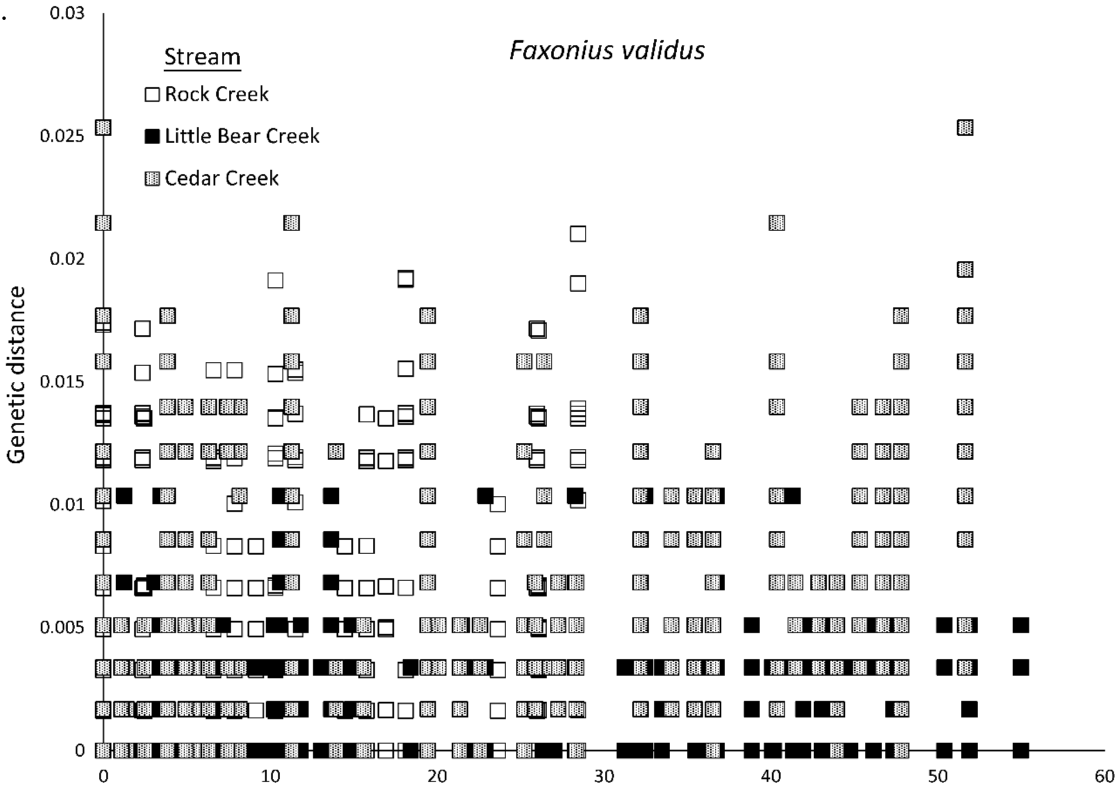
Table 4.3. Pairwise F_{ST} values (P-values) between each stream's local populations of *Faxonius validus* and *F. erichsonianus*. I = impounded; U = unimpounded.

Stream	*Faxonius validus*	*Faxonius erichsonianus*
Little Bear (I)	**0.129 (< 0.01)**	**0.058 (0.02)**
Cedar (I)	**0.127 (< 0.01)**	0.011 (0.22)
Rock (U)	0.000 (0.46)	0.033 (0.08)
Little Cahaba (I)		**0.331 (< 0.01)**
Shades (U)		0.022 (0.16)

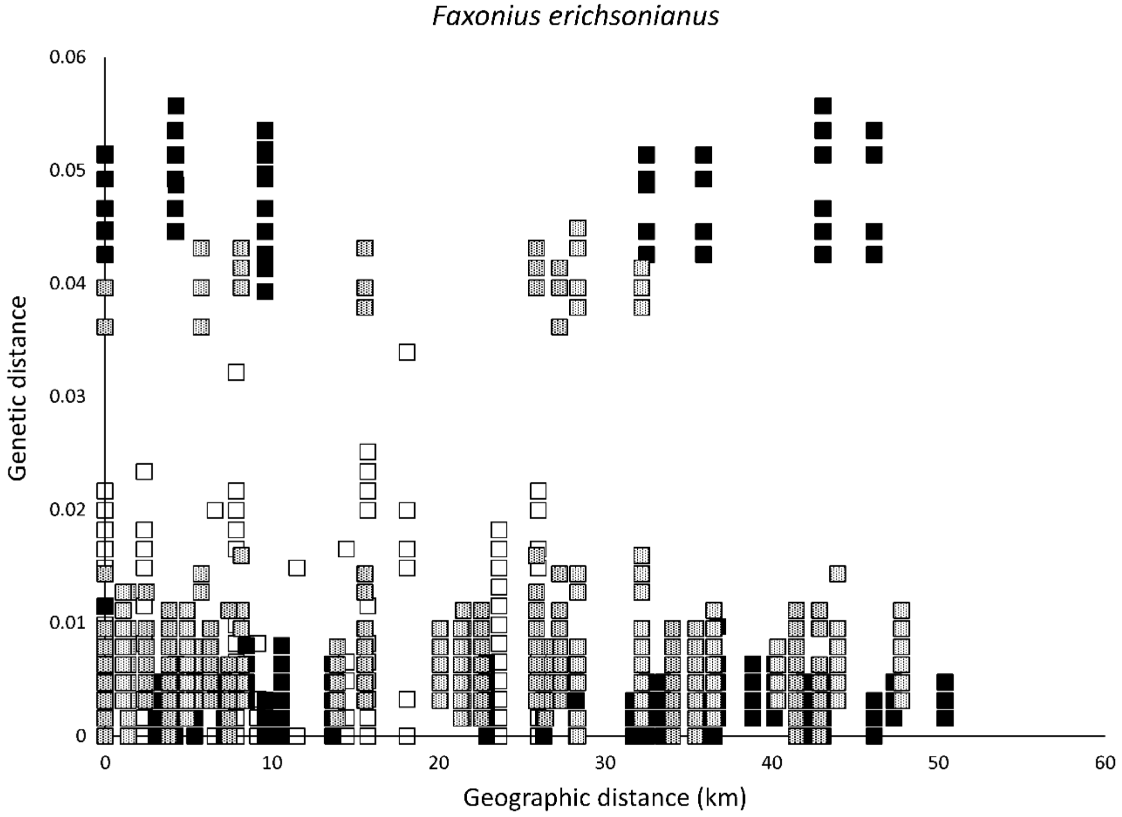
4.3.2.4 Isolation by distance (IBD)

Isolation by distance was detected for *F. erichsonianus* individuals in one impounded stream (Little Cahaba River; Figure 4.3C) but not for *F. validus* individuals within any stream (all P values > 0.05; Figure 4.3A). For *F. erichsonianus* individuals in Little Cahaba River, genetic and geographic distance were positively associated ($r = 0.18$, $P < 0.001$; Figure 4.3C).

A.


Faxonius validus
Stream
☐ Rock Creek
■ Little Bear Creek
▨ Cedar Creek
Genetic distance
0.03
0.025
0.02
0.015
0.01
0.005
0
0
10
20
30
40
50
60

B.


Faxonius erichsonianus
Genetic distance
0.06
0.05
0.04
0.03
0.02
0.01
0
0
10
20
30
40
50
60
Geographic distance (km)

C.

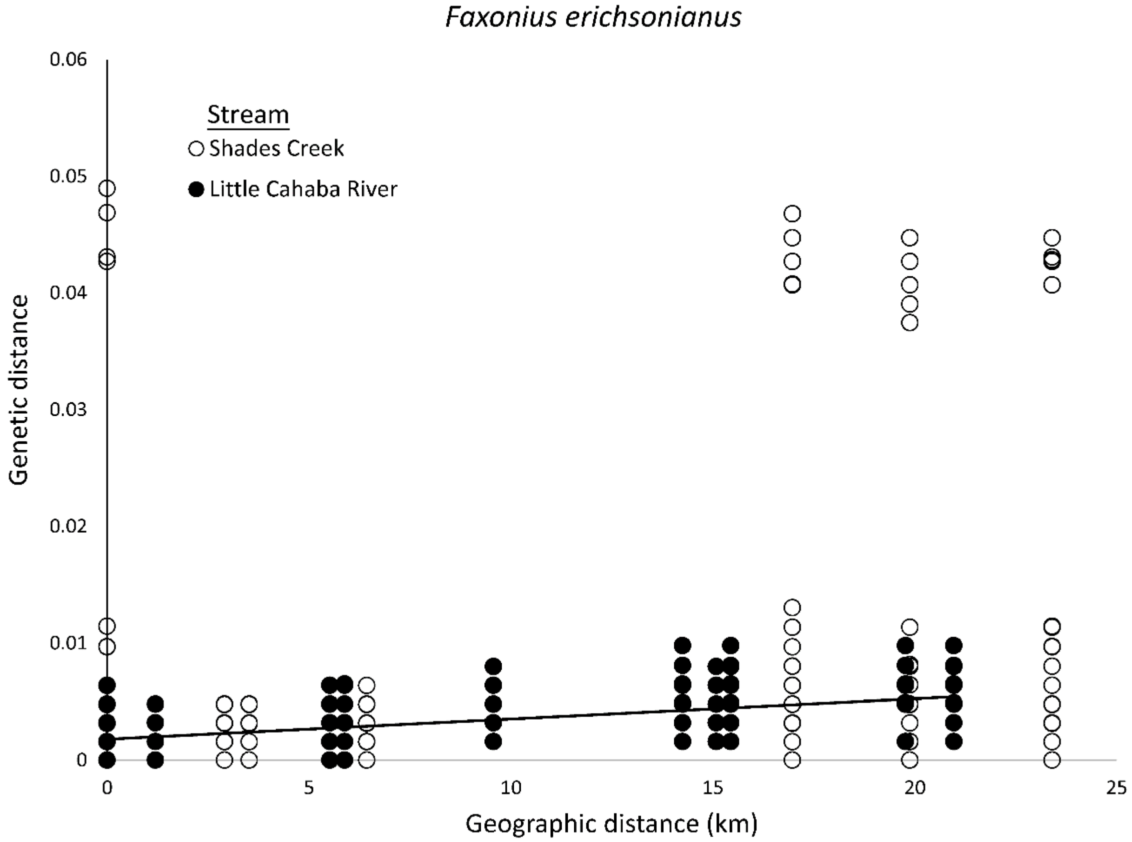


Figure 4.3. Scatter plot of pairwise genetic distances (proportion of nucleotides that differ between each pair of sequences) and geographic distances for all *Faxonius validus* (A) and *F. erichsonianus* (B–C) individuals collected at sites within the Bear Creek (A–B) and Cahaba River (C) drainages. Trend line represents a significant correlation between genetic and geographic distance of *F. erichsonianus* individuals within the Little Cahaba River population. Unfilled squares and circles represent unimpounded streams. Filled squares and circles represent impounded streams.

4.3.2.5 Migrate-n estimates of gene flow directionality and effective population sizes

Our data indicated bidirectional gene flow between local populations of *F. validus* within Little Bear and Rock creeks and unidirectional, downstream gene flow between local populations in Cedar Creek. In Rock Creek (unimpounded), more gene flow occurred down- than upstream, but up- and downstream gene flow did not differ between local populations within Little Bear Creek (impounded) (Table 4.4). In addition, gene flow was higher in the unimpounded stream than impounded streams. No differences were detected between local populations' N_e for any stream (all P values > 0.05).

Local populations of *F. erichsonianus* exhibited bidirectional gene flow in unimpounded streams and unidirectional, downstream or no gene flow in impounded streams. In unimpounded streams, downstream gene flow exceeded upstream gene flow (Table 4.4). No gene flow occurred between local populations in Little Cahaba River. In the Bear Creek drainage, unidirectional, downstream gene flow occurred between local populations in both impounded streams (Little Bear and Cedar creeks). Local populations' N_e did not differ for any stream (all P values > 0.05).

Table 4.4. Migrate-n estimates (P-values) of mean up- and downstream migration rates (m = number of migrant individuals/generation) and log likelihood-ratio tests results (only P-values displayed) of differences between up- and downstream m and between effective population sizes (N_e) in up- versus downstream local populations for *Faxonius validus* and *F. erichsonianus*. Significance indicates migration rates greater than zero, or differences between up- and downstream m and N_e. I = impounded; U = unimpounded.

	Upstream m (P-value, null: $m = 0$)	Downstream m (P-value, null: $m = 0$)	m differences (P-value, null: $m_{up} = m_{down}$)	N_e differences (P-value, null: $Ne_{up} = Ne_{down}$)
Faxonius validus				
Little Bear Creek (I)	**5.3 (< 0.01)**	**1.7 (< 0.01)**	(0.48)	(0.92)
Cedar Creek (I)	< 0.1 (0.98)	**1.4 (< 0.01)**	**(< 0.01)**	(0.98)
Rock Creek (U)	**7.4 (< 0.01)**	**19.8 (< 0.01)**	(0.66)	(0.96)
Faxonius erichsonianus				
Little Bear Creek (I)	< 0.1 (0.97)	**173.0 (< 0.01)**	**(< 0.01)**	(0.97)
Cedar Creek (I)	0.2 (0.93)	**30.9 (< 0.01)**	**(< 0.01)**	(0.98)
Rock Creek (U)	**1.2 (0.01)**	**20.3 (< 0.01)**	**(< 0.01)**	(0.98)
Little Cahaba River (I)	< 0.1 (0.98)	< 0.1 (0.08)	(0.16)	(0.95)
Shades Creek (U)	**0.8 (0.02)**	**28.1 (< 0.01)**	**(< 0.01)**	(0.99)

4.4 Discussion

For both *F. validus* and *F. erichsonianus*, gene flow between local populations was reduced in impounded streams, which is consistent with the expected response to habitat fragmentation (Meffe and Vrijenhoek 1988, Frankham 1996, Dixo et al. 2009). In contrast, local populations in unimpounded streams displayed high genetic connectivity and bidirectional dispersal and gene flow. Although genetic isolation among local populations in impounded streams is common for fishes (Nielsen et al. 1999, Yamamoto et al. 2004) and aquatic insects (Monaghan et al. 2002, Watanabe and Omura 2007), most studies examining other stream organisms found no evidence for prevention of gene flow (e.g., mussels; Abernethy et al. 2013, Fuller 2017: snails; Liu and Hershler 2009: amphipods; Berettoni et al. 1998).

Detecting genetic signatures of recent fragmentation using molecular data has been difficult (Sumner et al. 2004, Richmond et al. 2009), particularly when using markers that do not mutate at exceptionally fast rates. For example, genetic differences among fragmented local populations of Alabama stream fishes were detected using hypervariable nuclear microsatellite loci but not using mitochondrial DNA sequence data (Fluker et al. 2014). However, here we detected differences among local populations of crayfishes in streams that were impounded for only 36 (Cedar Creek), 40 (Little Bear), and 104 (Little Cahaba River) years on the basis of mitochondrial DNA sequences. Even if we assume a relatively short (1 year) generation time, this outcome suggests that restrictions to gene flow among local populations, and subsequent genetic drift within them, were substantial in impounded streams (Lacy 1987, Dixo et al. 2009). These findings are of particular interest in biodiversity hotspots, such as the southeastern USA (Lydeard and Mayden 1995, Noss et al. 2015), where almost all aquatic systems are fragmented by impoundments.

Dispersal among local populations within impounded streams depends on reservoir size (Ward and Stanford 1979, Petts 1989). The dams in the Bear Creek drainage were similar ages and dimensions, but the impoundment on Cedar Creek was three times larger than that on Little Bear Creek. The larger impoundment presumably constituted a larger area to which lotic crayfishes were poorly adapted, and thus created a less permeable barrier to dispersal. For both species, less gene flow occurred in Cedar than Little Bear creek. Although gene flow among local populations differed between impounded streams in this study, all impoundments negatively impacted dispersal, with little to no gene flow also occurring in Little Cahaba River. Conversely, in an unpublished study in Alabama, small (dam height < 10 m; average reservoir size 20 ha), low-head mill dam impoundments did not negatively impact movement in all crayfishes studied (Hartfield 2010), indicating that larger impoundments can exacerbate fragmentation effects.

Longer periods of isolation decrease population sizes, reproductive success, and within population genetic diversity, consequently decreasing the likelihood of a populations persisting (Zwick 1992, Lowe and Allendorf 2010, Mims et al., 2016). We detected little to no gene flow between local populations of *F. erichsonianus* in Little Cahaba River, the stream with the smallest reservoir in this study but the longest time impounded (more than two times longer than Little Bear and Cedar creeks). Conversely, we detected unidirectional, downstream gene flow between local populations of *F. erichsonianus* in Little Bear and Cedar creeks. These findings for crayfishes are consistent with those for fishes, where genetic diversity was reduced in impounded populations isolated for longer periods, leading to the extirpation of small populations (Morita and Yamamoto 2002, Yamamoto et al. 2004). Furthermore, our findings suggest that impounded streams' genetic fragmentation increased in concert with impoundment size and time since dam closure. Nonetheless, because we did not have replicates of small and large impoundments or young and old dams, our study design did not allow us to test these hypotheses. Future research with replicates of various sized and aged impoundments is needed to investigate how impoundment size and time since dam closure interacts with crayfishes' genetic fragmentation.

Although gene flow differed between impounded and unimpounded streams for both crayfishes, differences were not consistent across species. Intrinsic biological and life history characteristics, such as dispersal ability, ecological specialization, and physiological tolerance often determine the degree of impact that habitat fragmentation has on natural populations (Luoy et al. 2007, Reid et al. 2008, Alp et al. 2012). In Little Bear Creek, we detected bidirectional gene flow between local populations of *F. validus* and unidirectional, downstream gene flow between local populations of *F. erichsonianus*. *Faxonius validus* preference for smaller streams (Cooper and Hobbs 1980, Hobbs 1989) may cause members of this species to naturally disperse upstream at higher rates than members of *F. erichsonianus* (Hobbs 1981). Steeper slopes and faster water velocities usually decrease upstream dispersal, and crayfishes' abilities to navigate these conditions will influence upstream dispersal rates (Bernardo et al. 2011). Additionally, downstream gene flow was higher between local populations of *F. erichsonianus* than *F. validus* in all Bear Creek drainage streams. Gut contents of fishes from impoundments in the Bear Creek drainage indicated that *F. erichsonianus* was the dominant crayfish prey, comprising 88% (37 of 42) of identified crayfishes (Barnett unpublished data). *Faxonius erichsonianus* has a larger geographic range than *F. validus* and consequently may have a broader niche (Brown 1984, Slatyer et al. 2013). With a larger niche breadth, *F. erichsonianus* may be able to tolerate greater environmental changes caused by impoundments and successfully disperse downstream within impounded systems. Overall, impacts of impoundments vary, at least in part, according to dispersal tendency and species habitat preferences.

Levels of genetic diversity and estimated N_e were not lower in impounded stream populations, with *h*d in impounded streams the same or higher than *h*d within unimpounded streams. Although initially counter-intuitive, this result is not entirely unexpected. Fragmentation increases the probability of differentiation due to genetic drift or selection within local populations (Kimura and Crow 1963, Templeton et al. 1990, Heggenes and Røed 2006), resulting in fewer shared haplotypes between local populations. These differentiating processes, along with retentions of pre-existing genetic diversity, often increase overall genetic diversity.

Upstream local populations tended to have higher genetic diversity than downstream populations, which is not indicative of isolated upstream populations that have experienced subsequent size reduction. Similarly, π was higher in upstream local populations for crayfishes in other impounded (mill dams) and unimpounded (breached or relict mill dams) Alabama streams (Hartfield, 2010). Ecological factors, such as decreased habitat quality (e.g., reduced interstitial spaces, fewer riffle-pool complexes) and increased predation, may have reduced genetic variation in downstream local populations. These factors, along with downstream environmental changes (e.g., flow alteration, decreased dissolved oxygen, and decreased temperatures) due to impoundments, can exacerbate the loss of genetic diversity in impounded streams (Ward 1976, Watters 1996, Bunn and Arthington 2002, Cumming 2004). The location of impoundments within drainages can also influence genetic diversity (Stanford and Ward, 2001). Impoundments closer to headwaters, with fewer tributaries upstream, have larger impacts on upstream populations due to greater isolation. Impoundments in the current study were 30–57 km ($\bar{x}$ = 43 km) downstream of headwaters. Due to the distance of impoundments from headwaters in the current study it is unlikely that impoundment location impacted our results. Furthermore, fragmentation and reduction of gene flow by impoundments may have increased genetic diversity among local populations, but within local populations, other factors (e.g., gene flow from tributaries, environmental and habitat changes caused by impoundments, stochastic environmental events, time after fragmentation) may have enhanced or diminished genetic diversity.

Faxonius validus and *F. erichsonianus* had high levels of π in impounded and unimpounded streams when compared to other crayfish populations (Brown 1981, Grandjean and Souty-Grosset 2000, Fetzner and Crandall 2001). Nonetheless, few studies have assessed *Faxonius* spp. at the population level, and like Australian crayfishes (*Cherax* spp. and *Geocherax* spp.) that are also found in biodiversity hotspots, crayfishes in this study showed high π and *h*d (Munasinghe et al. 2004, Bentley et al. 2010). High levels of genetic diversity in the genus *Faxonius* may result from past fragmentation (i.e., changes conditions associated Pleistocene glacial cycles) which altered pre-Pleistocene river drainage patterns in the region (Crandall and Templeton 1999, Fetzner and Crandall 2003). The high diversity levels may also reflect the presence of multiple refugia during the Pleistocene (Fetzner and Crandall, 2003), as well as low levels of gene flow among stream populations.

One potential bias in our study design was that in the Bear Creek drainage, our sample sites encompassed a shorter overall stream length in the unimpounded stream than in the impounded streams. Unfortunately, no comparable unimpounded streams as long as the impounded streams exist in the drainage. Nonetheless, the impounded and unimpounded streams sampled in the Cahaba River drainage were of similar lengths, and genetic differences between all impounded and unimpounded streams were documented.

Our findings have important implications for crayfishes in impounded streams. First, *F. validus* and *F. erichsonianus* were the most abundant and widespread crayfish species within sampled streams, and evidence of population fragmentation for these species was detectable within the relatively short time since impoundment (36–104 years). This implies that many crayfish populations in impounded streams (i.e., with relatively large impoundments built before 1980) may be genetically isolated. Presumably, ecologically specialized species and those with smaller N_e, lower genetic variation, and higher sensitivity to stochastic environmental events (Franzén and Nilsson 2010, Li et al. 2014) may suffer more severe effects of stream fragmentation by impoundments. Second, local populations upstream of impoundments are at risk of local extinction due to the lack of upstream dispersal in most impounded streams. This risk is greatest in drainages with impoundments near headwaters causing greater isolation of upstream local populations. Conservation strategies focused on enhancing connectivity may be beneficial in impounded streams by preventing the loss of genetic diversity and extinction within isolated local populations, especially in streams like those in the current study with high levels of genetic diversity. Furthermore, mechanisms like fish ladders may be beneficial to enhance movement across dams (Welsh and Loughman, 2018), facilitating gene flow among populations of species that can survive in impoundments. Dam removal is also likely to benefit stream crayfishes by increasing connectivity among local populations (Reid et al. In Press).

This study presents the first examination and comparison of changes to crayfish population genetic structure in impounded streams. Habitat fragmentation due to impoundments has reduced gene flow, potentially leading to decreased persistence of isolated local populations. Decreases in crayfishes' genetic diversity and population sizes can cause cascading effects through stream ecosystems, affecting trophic dynamics and organic matter processing in streams

(Chambers et al. 1990, Momot 1995, Rabeni et al. 1995). Our results suggest that negative genetic effects of fragmentation may be detectable relatively soon after dam closure, and even crayfishes with high abundances, large ranges, and high levels of genetic diversity are impacted.

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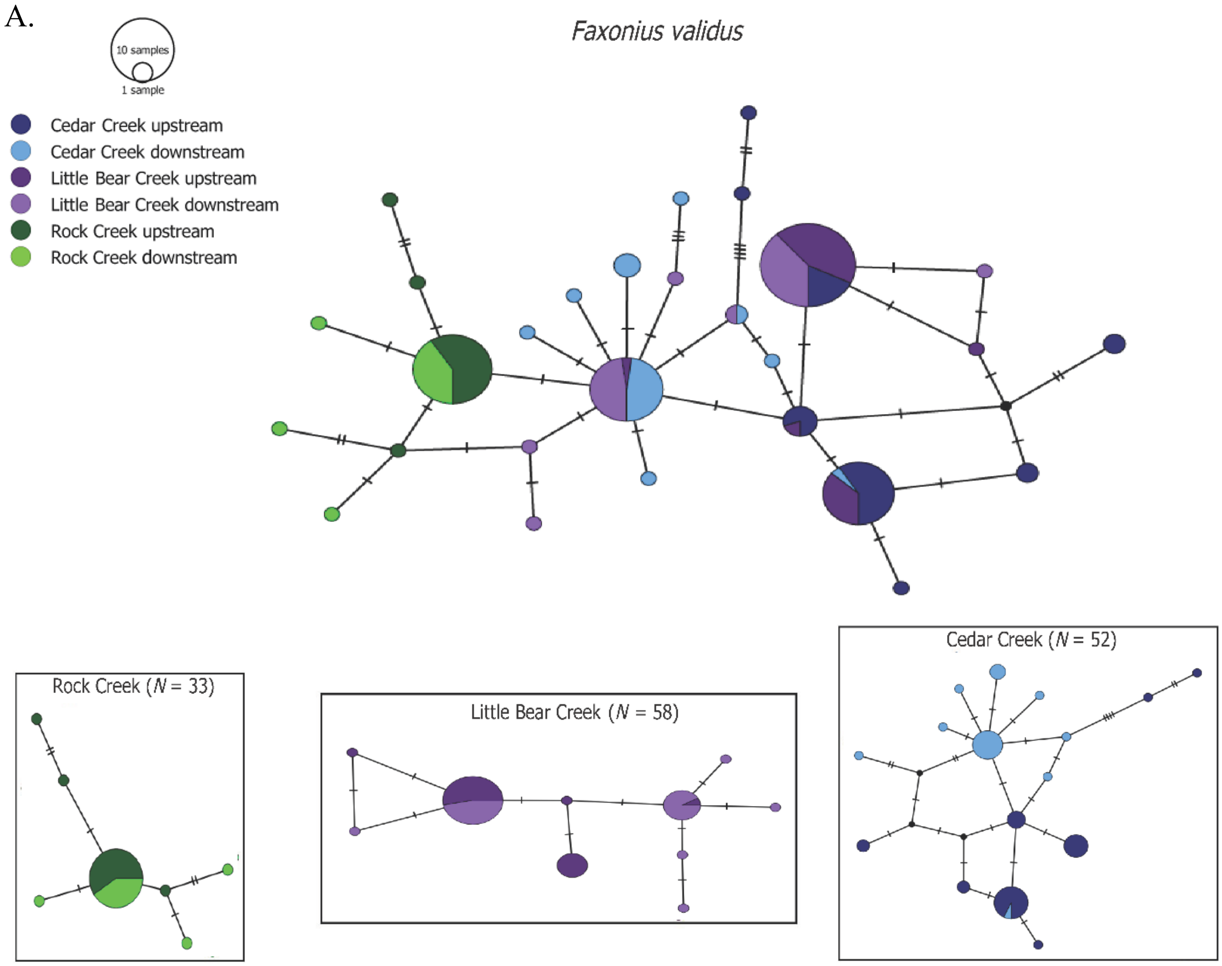
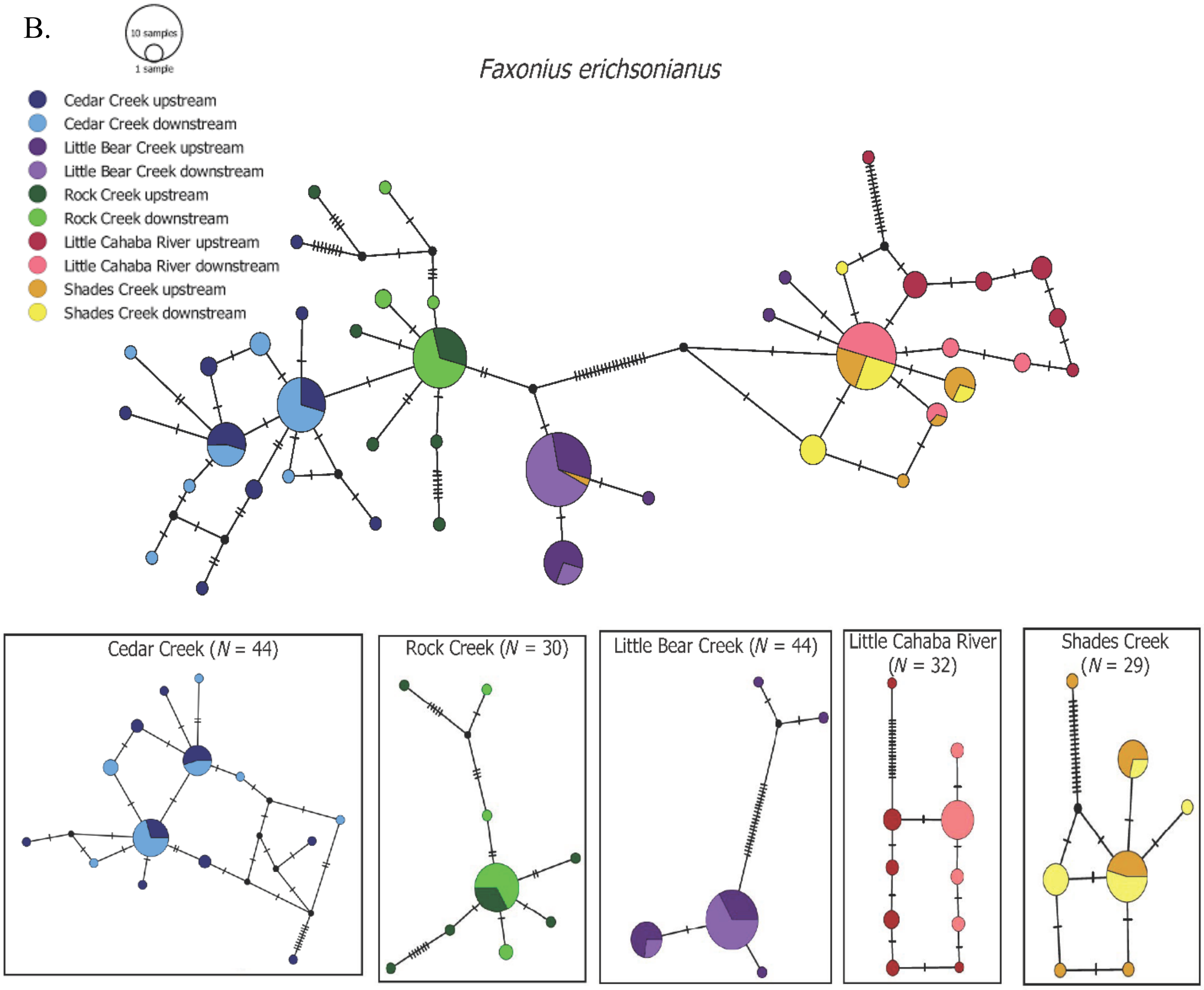
APPENDIX I

HAPLOTYPE NETWORKS


A.
10 samples
1 sample
Cedar Creek upstream
Cedar Creek downstream
Little Bear Creek upstream
Little Bear Creek downstream
Rock Creek upstream
Rock Creek downstream
Faxonius validus
Rock Creek (*N* = 33)
Little Bear Creek (*N* = 58)
Cedar Creek (*N* = 52)



Appendix I. *Faxonius validus* (A) and *F. erichsonianus* (B) haplotype networks based on 618-bp and 640-bp mitochondrial COI sequence alignments, respectively. Each circle represents a unique haplotype. Lines connecting haplotypes indicate a single mutational difference between haplotypes, whereas dashes along lines represent multiple additional mutations, with each dash corresponding to one mutation. The size of each circle is proportional to the frequency with which that haplotype was sampled. *N* = number of individuals collected.

CHAPTER V: CONCLUSION

Freshwater ecosystems are highly diverse in species and habitats, but they are considered our most imperiled ecosystems (Chaplin et al. 2000). About 60% of the world's rivers are being regulated (McAllister et al. 2001), impacting streams' physiochemical properties including modifications to temperature regimes, water chemistry, channel geomorphology, and floodplain connectivity (Ward 1976, Baxter 1977, Voelz and Ward 1990, Rahel 2002). One in three freshwater species are threatened with extinction world-wide (Collen et al. 2014), and crayfishes are among the most threatened taxa (Chaplin et al. 2000, Collen et al. 2014), with habitat loss and degradation, in part due to stream regulation by impoundments, one of the top threats (Wilcove et al. 2000). Although threats to crayfishes have been identified, only in recent years have studies, such as this one, attempted to quantify the relationship between crayfish communities and stream regulation (Hartfield 2010, Adams 2013).

Because of the ecological importance (Momot 1995, Rabeni et al. 1995, Statzner et al. 2003) and population declines (Taylor et al. 2007) of crayfishes, interest in understanding and conserving crayfishes and their habitats has increased. Despite this, effective sampling methods that quantify crayfishes' distributions and assemblage structures have not been thoroughly assessed (Barnett and Adams 2018, Budnick et al. 2018). Selecting effective sampling methods is vital for every study design of empirical studies, but can be challenging when sampling in lotic environments with variable habitat parameters (i.e., depth, flow, and substrate). Thus, our first objective was to assess the effectiveness of kick seining, electrofishing, and nest trapping for sampling stream crayfishes and fishes in southern Appalachian streams. In the current study, kick seining and electrofishing were the most effective methods to assess crayfish and fish richness and abundance, respectively. Sampling accuracy increased when both methods were combined. The effectiveness of each method depended on stream characteristics, and using both methods offset biases. Furthermore, using methods that decrease sampling biases and simultaneously sampling more than one focal group can cut down on sampling effort and cost, and contribute to a better understanding of stream community composition.

Once quantitative methods have been established, scientists can properly assess anthropogenic impacts to crayfish assemblages. Disconnecting habitats by impounding streams altered species' distributions and food availability, leading to dramatic shifts in ecosystem and community structures (Watters 1996, Cumming 2004, McLaughlin et al. 2006). Similarly, in the current study, impoundments altered the structure of both up- and downstream crayfish assemblages, decreasing the abundance and sizes of crayfishes downstream of impoundments and creating similar assemblages up- and downstream of impoundments. These changes were associated with stream physiochemical alterations. Physical variables within a stream system gradually changed with movement from headwaters to mouth, causing a subsequent change in biota (Vannote et al. 1980, Matthews 2012). However, these natural changes are interrupted when dams are built on streams, often resetting the natural continuum for physical and biotic variables (Ward and Stanford 1983). In addition, a gradual recovery of abiotic factors to more natural conditions is common with distance away from impoundments (Voelz and Ward 1991, Camargo and Voelz 1998). Like physical variables, the dominant crayfish species in the current study gradually shifted in unimpounded streams with distance downstream. Conversely, crayfish assemblages in impounded streams did not differ between up- and downstream sections. Nonetheless, with movement downstream of impoundments crayfish species richness and abundance increased, indicating species recovery with distances downstream of impoundments. Decreases in species abundance and diversity in impounded streams, as well as shifts in dominant species, are consistent findings across taxa (Mackay and Waters 1986, Voelz and Ward

1990, Gherke et al. 2002, McGregor and Garner 2003, Gangloff et al. 2011). While specific physiochemical changes (i.e., timing of high flow events) may impact taxa differently depending on life histories and adaptive capabilities (Bunn and Arthington 2002, Carlisle et al. 2010), overall impoundment effects negatively impact numerous taxa, and managing streams to mimic natural flow regimes is necessary to maintain diverse stream communities (Propst and Gido 2004, Kiernan et al. 2012).

Although longitudinal crayfish assemblage changes were documented in both study drainages, changes differed between the highly developed drainage with an invasive crayfish species and the less developed drainage with only native crayfishes, indicating that other confounding factors may have impacted our ability to assess impoundment effects on crayfish assemblages. Numerous anthropogenic disturbances including land development, deforestation, and introduction of invasive species often degrade stream habitats and create physiochemical changes that can impact stream assemblages similar to impoundment effects (Scott and Helfman 2001, Rahel 2002, Allan 2004). These changes negatively impact impounded and unimpounded streams, decreasing the differences between stream types. For instance, in the current study, more urbanization and an invasive crayfish species were present in the Cahaba River drainage, whereas more agriculture and only native crayfishes were present in the Bear Creek drainage. Greater crayfish assemblage differences were detected between impounded and unimpounded streams in the Bear Creek than Cahaba River drainage. Thus, changes to crayfish assemblages are likely determined by numerous interacting anthropogenic disturbances, making it difficult to isolate anthropogenic impacts within a drainage affected by numerous anthropogenic disturbances.

Impoundments not only impact stream species assemblages, they also impact gene flow and genetic structure of stream populations. Dams and their associated impoundments restrict gene flow among populations, increasing the risk of local extinction (Baxter 1977, Watters 1996). The degree of gene flow restriction is taxon dependent. Genetic isolation among local populations in impounded streams is common for fishes (Nielsen et al. 1999, Alp et al. 2004) and aquatic insects (Monaghan et al. 2002, Watanabe and Omura 2007), but based on (albeit it limited) available data, this is not necessarily the case for mussels (Abernethy et al. 2013, Fuller, 2017), snails (Liu and Hershler, 2009), and amphipods (Berettoni et al. 1998). Intrinsic biological and life history characteristics, such as dispersal ability and physiological tolerance, often determine the degree of impact that impoundments have on populations (Luoy et al. 2007, Reid et al. 2008, Alp et al. 2012). In this study, I detected genetic isolation or unidirectional, downstream gene flow among local populations of crayfishes in impounded streams. Decreases in genetic diversity and population sizes can lead to local extinction, causing cascading effects through stream ecosystems (Momot 1995, Rabeni et al. 1995). With already declining crayfish populations, conservation efforts are needed to maintain genetically diverse crayfish populations by increasing population connectivity, subsequently, decreasing chances of local extinction.

Scientists have found it difficult to detect genetic signatures of recent fragmentation using molecular data for numerous organisms (Sumner et al. 2004, Richmond et al. 2009). Nonetheless, I detected differences between crayfish populations using mitochondria DNA. Genetic effects of fragmentation were also detectable relatively soon after dam closure (36–104 years). These findings indicate that crayfishes may be a useful indicator taxon to assess impoundment effects on stream population genetic structures.

There is still much to learn about impoundment effects on crayfishes. More research is necessary to fully understand effects to stream crayfishes and conserve their populations and essential habitats. For instance, research is needed to understand the effects of other impoundment types (i.e., run-of-river impoundments) on crayfish assemblages and assess the interactions between impoundment effects and other anthropogenic disturbances. Additionally, because numerous crayfish species have small natural ranges, assessing the genetic impacts of both small and large impoundments on less abundant crayfishes with more restricted ranges is also necessary (Taylor et al. 2007). Although much work is needed, it is clear that impoundments and its subsequent physiochemical changes and barrier effects have major consequences on crayfishes and other stream organisms, which can cause detrimental changes to both aquatic and terrestrial ecosystem structure and function.

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- Phylogenetic illustrations
    - PopART

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- Aquatic organism identifications

Statistical techniques and data management

- ArcGIS
- Statistical analyses
    - R, SPSS, PC-Ord, PRIMER

- Database management

Laboratory techniques

- Mussel aging
- Water clarity analyses with spectrometer

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Barnett, Z. C. 2018. Defeating the odds: trailblazing women. Women in STEM annual dinner, University of Mississippi. 25 Oct. Oral Presentation. Invited.

Barnett, Z. C., R. C. Garrick, C. A. Ochs, and S. B. Adams. 2018. Detectable effects of impoundments on the genetic structure of crayfish (*Faxonius* spp.) in Alabama 43 years after dam closure. International Association of Astacology. 12 July. Oral Presentation. Volunteer.

Pârvulescu, L., L. Neculae, E. Kaslik, C. Zaharia. Z. Barnett, M. M. Dalosto, J. M. Furse, T. Kawai, S. Santos, and O. I. Sîrbu. 2018. International Association of Astacology. 11 July. Poster Presentation. Volunteer.

Barnett, Z. C., S. B. Adams, and C. A. Ochs. 2018. Environmental and biological impacts to crayfish populations in impounded and unimpounded streams in Alabama. Society of Freshwater Science. 23 May. Oral Presentation. Volunteer.

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Barnett, Z. C. 2017. Crayfish ecology and importance in Mississippi. Strawberry Plains Audubon. 18 March. Oral Presentation. Invited.

Barnett, Z.C., S. Adams, C. Ochs. 2017. Effects of flood-control impoundments on the community assemblage of stream crayfish and their fish predators. Alabama Fisheries Society. Tuscaloosa, AL. 28 February. Oral Presentation. Invited.

---- Southern Hardwood Forest Research Group. Stoneville, MS. 23 February. Oral Presentation. Invited.

Barnett, Z.C., S. Adams, C. Ochs. 2017. Effects of flood-control impoundments on the community assemblage of stream crayfish. Alabama Crayfish and Mollusk Meeting. 18 January. Oral Presentation. Volunteer.

---- International Association of Astacology. Madrid, Spain. 8 September 2016. Oral Presentation. Volunteer.

---- USDA FS Stream Ecology Lab. Oxford, MS. 25 August 2016. Oral Presentation. Invited.

Sims, B. and Z. Barnett. 2016. Habitat use of three crayfish genera in the Cahaba River Drainage, Alabama, USA. USDA FS Stream Ecology Lab. Oxford, MS. 27 July. Oral Presentation. Invited.

Barnett, Z.C. 2016. Effects of impoundments on community assemblage of crayfish in the Cahaba River Drainage. Birmingham Water Works Board. 20 July. Oral Presentation. Invited.

S. Adams, C. Roghair, J.A. Cochran, J. Moran, A. Dolloff, M.L. Warren, Jr., W.R. Haag, C. Krause, S. McGregor, C. Johnson, Z. C. Barnett. 2016. Aquatic community changes along riverine lacustrine transitions of Lewis Smith Reservoir, Bankhead National Forest, Alabama: crayfish surprises. Alabama Crayfish ad Mollusk Meeting. 19 – 20 January. Oral Presentation. Invited.

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---- University of Mississippi. Biology Department. Oxford, MS. 22 April 2015. Oral Presentation. Invited.

----USDA Forest Service, Center for Bottomland Hardwood Research, Stream Ecology lab. Oxford, MS. 16 April 2015. Oral Presentation. Invited.

Ochs, C. and Z. Barnett. 2015. Research Methods in Biology. University of Mississippi, BISC 300. Oxford, MS. 24 August. Oral Presentation. Invited.

Choice, Z.D. S. Adams, and B. Rosamond. 2014. Habitat use and life history of secondary burrower, *Procambarus sp. cf. viaeviridis*, in Mississippi, USA. International Association of Astacology. Sapporo, Japan. 20 – 26 September. Oral Presentation. Volunteer.

Choice, Z.D., Frazer, T.K., and Jacoby, C.A. 2014. Light requirements of seagrasses determined from historical records of light attenuation. Benthic Ecology Meeting. University of North Florida, Jacksonville, FL, 21 March. Oral Presentation. Volunteer.

Choice, Z.D., Frazer, T.K., and Jacoby, C.A. 2012. Seagrass light requirements in the spring-fed estuaries of northwestern Florida. Minorities in Agriculture, Natural Resources, and other Related Sciences National Meeting. Atlanta, GA. 29 March – 1 April. Oral Presentation. Volunteer.

Choice, Z.D., Detenbeck, N., and Rego, S. 2011. Wind energy effect on seagrass estuaries. Minorities in Agriculture, Natural Resources, and other Related Sciences National Meeting. Overland Park, KS. 31 March – 2 April. Poster. Volunteer.

Choice, Z.D., Ollinger, M., Muth, M.K., and Karns, S.A. 2010. Food Safety Audits, Plant Characteristics, and Food Safety Technology Use in Meat and Poultry Plants. Minorities in Agriculture, Natural Resources, and other Related Sciences National Meeting. Orlando, FL. 24 – 27 March. Oral. Volunteer.

Research Highlights

Shaull-Thompson, R. Women in science, Zanethia Barnett. United States Department of Agriculture, Southern Research Station. https://srs.fs.usda.gov/women-in-science/zanethia-barnett/

Myers, J. M. 2018. Vernal crayfish life history and habitat use. CompassLive, United States Department of Agriculture, Southern Research Station. https://www.srs.fs.usda.gov/compass/2018/03/29/vernal-crayfish-life-history-and-habitat-use/.

Farmer, S. 2015. Sunlight to seagrasses: U.S. Forest Service research shines light on threatened coastal plant. CompassLive, United States Department of Agriculture, Southern Research Station. http://blogs.usda.gov/2015/02/11/sunlight-to-the-seagrasses-u-s-forest-service-research-shines-light-on-threatened-coastal-plant/.

Buck, B. 2014. UF/IFAS research findings shed light on seagrass needs. University of Florida. https://news.ifas.ufl.edu/2014/04/ufifas-research-findings-shed-light-on-seagrass-needs/.

Funding

Cost share agreement between USDA Forest Service Southern Research Station, South Carolina National Forest and University of Mississippi, 2018 ($12,501)

Birmingham Audubon Society Walter F. Coxe Research Grant, 2015 ($1,500)

USDA Forest Service Washington Office Professional Development Funds, 2015–present ($150,000)

Honors, Awards, Fellowships

Society of Freshwater Science, Runner-Up Award for Best Oral Presentation in Applied Research, 2018

International Association of Astacology, Outstanding student presentation award, 2018

USDA Forest Service, SPOT Award, Outstanding presence of mind and quick action to a field emergency situation, 2018.

Alabama Fisheries Association Scholarship Recipient, 2017

USDA Forest Service Inspiring Woman Award, Outstanding Mentor/Coach, 2016

American Fisheries Society Hutton Scholar’s Mentor, 2016

University of Mississippi Minority Fellowship, 2014–present

USDA Forest Service Chief Scholars Fellowship, 2012

Best thesis in the Interdisciplinary Ecology program of the School of Natural Resources and Environment at the University of Florida, 2012

Minorities in Agriculture, Natural Resources and Related Sciences Oral Research Contest Winner, 2010

USDA 1890 Scholar, 2006–2010

Scientific Contributions

Journal of Fish and Wildlife Management, Peer Reviewer

Marine Pollution Bulletin, Peer Reviewer

North American Journal of Fisheries Management, Peer Reviewer

US Fish and Wildlife Service, Peer Reviewer

Mississippi River Basin Regional Panel of the Aquatic Nuisance Species Task Force, Member

International Association of Astacology, Member, Outreach Award and Social Media Committees

Society Freshwater Science, Member

Crustacean Society, Member

American Fisheries Society, Member

Alabama Fisheries Association, Member

Invasive Crayfish Collaborative Team, Member

Outreach

Recruitment of high schoolers to aquatic biology, St. Paul Missionary Baptist Church College Preparatory Workshop, Olive Branch, MS, 12 January 2019

The importance of crayfish. Lafayette Upper Elementary School, 4th grade QUEST Gifted and Talented Class. 12 October 2018.

Aquatic invertebrates of the Lower Mississippi River: Life in the River. Smithsonian Institution Traveling Water/Ways Exhibit. 29 August 2018.

Oxford Intermediate School Science Fair Judge, Oxford, MS, 2018.

Mississippi Region VII Science Fair Judge, University of Mississippi, Oxford, MS, 2017–2018

Demonstrated field gear use and gave tours to students for aquatic ecology summer camps, 2016 and 2018.

Crayfish ecology and importance in Mississippi—discussion and field demonstration. Strawberry Plains Audubon. 18 March 2017.

Oxford Elementary School Science Fair Judge, Oxford, MS, 2015.

Mentoring and Management

Coordinated volunteer workers training and field assignments for stream research

Coordinated projects using resources and personnel across multiple organizations

Developed and organized annual college preparatory workshop for high school students at St. Paul Missionary Baptist Church and surrounding area, Olive Branch, MS, 2019-present

Organize annual 5k to raise money for school supplies for underprivileged kids, St. Paul Missionary Baptist Church, 2017–present

USDA Dive Safety Officer, 2013-present

Coordinated monthly brown bag lunch seminars for the Forest Service, Center for Bottomland Hardwoods Research, Stream Ecology Lab, 2015-present

Recruited and mentored American Fisheries Society Hutton Scholar, 2016

Tutored students from elementary to college in English, math, and science, 2013–2014

Developed summer literacy program for Clear Creek Baptist Church, Oxford, MS, 2013

Educated and informed students about government job opportunities, 2009–2013