

Crayfish populations genetically fragmented in streams impounded for 36–104 years

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Abstract

1. Dams and their associated impoundments may restrict dispersal and gene flow among populations of numerous freshwater species within stream networks, leading to genetic isolation. This can reduce effective population sizes and genetic diversity, increasing the risk of local extinction.
2. We studied crayfishes from multiple up- and downstream sites in three impounded and two unimpounded streams in the Bear Creek and Cahaba River drainages, Alabama, U.S.A. Using mitochondrial DNA (cytochrome oxidase subunit I gene) sequence data generated from population-level sampling of two abundant native crayfishes, *Faxonius validus* and *Faxonius erichsonianus* (Decapoda: Cambaridae), we assessed species' spatial genetic structure and genetic diversity, estimated the magnitude and directionality of gene flow, and compared results between the species.
3. For both species, levels of genetic diversity (number of haplotypes, and haplotypic and nucleotide diversity) were the same or higher in impounded compared to unimpounded streams. Conversely, crayfish populations in up- and downstream sections of unimpounded streams displayed high genetic similarity and bidirectional gene flow, whereas in impounded streams, crayfish populations typically had greater up- and downstream genetic differentiation and predominantly unidirectional, downstream gene flow.
4. Although impoundments were associated with lower connectivity between up- and downstream sections for *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.
5. In an ecologically short timeframe, impoundments appear to have fragmented stream crayfish populations, and even species with relatively high abundances and large ranges had lower gene flow among populations in impounded streams compared to unimpounded streams. In addition, feedbacks between genetic and demographic effects on fragmented populations may decrease the probability of long-term persistence.

KEYWORDS

connectivity, dam, habitat fragmentation, impoundment, mitochondrial DNA

1 | INTRODUCTION

At present, over 20,000 large dams (>15 m high) impound streams in the south-eastern U.S.A. (NID, 2013). Dams fragment populations of stream fauna by physically blocking dispersal and gene flow, reducing floodplain connectivity, and creating unfavourable conditions for pre-disturbance fauna (Baxter, 1977; Watters, 1996). With the installation of dams, parts of rivers are often converted from lotic to lentic habitats and natural flow variability is greatly reduced in other portions, causing changes in biotic and abiotic patterns as well as ecological processes (Baxter, 1977; Cumming, 2004; Ward & Stanford, 1983; Watters, 1996). Thus, dams dramatically alter stream physiochemical properties including flow and temperature regimes, channel geomorphology, and water chemistry (Baxter, 1977). The impacts of these changes can depend on dam height, impoundment size, physiographic setting, location within the drainage, and location along the stream. In addition, the cumulative effects of these hydrological alterations may cause fragmentation within stream biological assemblages (Carlisle, Falcone, Wolock, Meador, & Norris, 2010; Ward & Stanford, 1982).

Habitat fragmentation can cause genetic isolation of stream populations in up- and downstream sections of impounded streams, with the degree of isolation dependent on the spatial and temporal scales analysed, as well as the life history characteristics of the species and structure of the habitat (Hughes, 2007; Hughes, Huey, & Schmidt, 2013). Habitat fragmentation can also reduce or prevent dispersal among populations and subsequent mating (i.e. gene flow), increasing genetic divergence, largely owing to the effects of genetic drift, or in some cases, selection favouring local adaptation (Bessert & Ortí, 2008; Lande, 1976; Vandergast, Bohonak, Weissman, & Fisher, 2007). Isolated populations may be subject to decreased recruitment, reduced adaptive potential, and lower probability of persistence due to loss of genetic diversity and reduced 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 & Roff, 1999; Dixo, Metzger, Morgante, & Zamudio, 2009), further increasing risk of local extinction (Lande, 1988; MacArthur & Wilson, 1967; Pringle, 1997). As these changes are most pronounced in small populations, evidence of population fragmentation can become harder to distinguish in large populations, such that there may be considerable *lag times* before impacts become detectable. Also, past environmental processes (e.g. Pleistocene glacial–interglacial cycles) can decrease our ability to distinguish between recent and historical fragmentation.

Increased genetic subdivision between up- and downstream populations isolated by impoundments has been reported for numerous aquatic organisms including fishes, mussels, and insects (Kelly & Rhymer, 2005; Yamamoto, Morita, Koizumi, & Maekawa, 2004). Consistent with expectations for the effects of genetic drift in small isolated populations (Charlesworth & Charlesworth, 1987; Crnokrak & Roff, 1999; Hedrick, 2005), reduced genetic diversity in aquatic insect

populations separated by impoundments has been documented, particularly for species with limited dispersal (Hughes, Schmidt, & Finn, 2009; Monaghan, Spaak, Robinson, & Ward, 2002; Watanabe, Monaghan, Takemon, & Omura, 2010; Watanabe & Omura, 2007). In fishes, impoundments have impacted populations in numerous ways, including loss of genetic diversity within populations, genetic discontinuities across formerly connected populations (Faulks, Gilligan, & Beheregaray, 2011; Fluker, Kuhajda, & Harris, 2014), and phenotypic deformities and local extinction, especially in upstream segments (Morita & Suzuki, 1999; Morita & 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, and extinction rates thought to be rapidly increasing (Richman et al., 2015; Taylor et al., 2007). 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, Hanson, Burke, & Prepas, 1990; Hanson, Chambers, & Prepas, 1990; Momot, 1995; Rabeni, Gossett, & McClendon, 1995; Stutzner, Peltret, & Tomanova, 2003). Despite their functional importance, to our knowledge no previously published study has examined the impacts of impoundments on crayfishes' population genetic structures. Although numerous studies have investigated the effects of impoundments on other stream organisms (e.g. mussels [Abernethy, McCombs, Siefferman, & Gangloff, 2013; Galbraith, Zanatta, & Wilson, 2015], aquatic insects [Monaghan et al., 2002], and fishes [Neville, Dunham, Rosenberger, Umek, & Nelson, 2009; Yamamoto et al., 2004]), the ability of crayfishes to walk across land complicates extrapolating from results of existing studies and predicting the impacts of instream barriers on crayfish populations (Hughes, 2007; Hughes et al., 2013).

We assessed the impacts of dams and impoundments on the population genetics of crayfishes in northern Alabama, U.S.A. Alabama has the most diverse freshwater fauna in North America (Duncan & Wilson, 2013; Lydeard & Mayden, 1995), and northern Alabama is in the southern Appalachian region (ARC, 2009), which is the global centre of crayfish diversity (Crandall & Buhay, 2008). High levels of endemism and morphologically cryptic diversity within crayfishes have been documented in this region (Helms, Vaught, Suci, & Santos, 2015), as well as elsewhere in the south-eastern U.S.A. (Fetzner & DiStefano, 2008; Figiel, 2016). Intraspecific genetic variation often exists at the stream or basin level (Bentley, Schmidt, & Hughes, 2010; Mathews et al., 2008) with historical vicariance events implicated as potential drivers of high local endemism (Loughman, Henkanathgedara, Fetzner, & Thoma, 2017). In the last 115 years, numerous impoundments were built in this region (Morse, Stark, & Patrick McCafferty, 1993; NID, 2013), and these may have further isolated populations in up- and downstream sections. In this study, we focused on

two crayfish species, *Faxonius validus* and *Faxonius erichsonianus* (Decapoda: Cambaridae), which are abundant in large impounded streams of the southern Appalachian region of Alabama. The dam heights of these large impoundments are considerable and are, therefore, likely to prevent upstream dispersal by crayfishes unless phoresy (e.g. via humans or birds) facilitates such countercurrent movements.

Faxonius erichsonianus and *F. validus* share many ecological traits typical of stream crayfishes but differ in stream size preferences and geographic ranges. Like many stream crayfishes, both species live 3–4 years, have a September–November mating season (via sexual reproduction; Holdich, 2002), and are tertiary burrowers, retreating to shallow burrows when waterbodies dry or during egg laying and brooding. Both species are typically found under rocks in shallow mud burrows and in leaf litter, or among aquatic plants (Bouchard, 1972; Hopper, Huryn, & Schuster, 2012; Williams & Bivens, 2001). We collected *F. erichsonianus* and *F. validus* in streams with 11 other crayfish species (Barnett, 2019). *Faxonius erichsonianus* occurs in medium to large streams with moderate currents and rocky substrates in six south-eastern states from western Tennessee south to northern Mississippi and Alabama and east to north-western 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 & Hobbs, 1980; Hobbs, 1989). From a conservation perspective, both species are considered stable (Adams, Schuster, & Taylor, 2010a, 2010b); nonetheless, 20% of currently imperiled crayfishes

in the U.S.A. and Canada are members of the genus *Faxonius* (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 1): (1) Is genetic diversity lower in crayfish populations in impounded streams compared to unimpounded streams? (2) Do dams and impoundments inhibit crayfish dispersal and gene flow, resulting in geographically structured populations? and (3) Do the two focal crayfish species show concordant differences between impounded and unimpounded streams?

2 | METHODS

2.1 | Study areas

We sampled crayfishes from five perennial streams in the Bear Creek and Cahaba River drainages, Alabama, U.S.A. (Figure 1; Table 2). In the Bear Creek drainage (Tennessee River Basin; drainage area size: 2,450 km²), we sampled two impounded (Little Bear and Cedar creeks) and one unimpounded (Rock Creek) stream (mean stream length: 61.5 km). In the Cahaba River drainage (Mobile River Basin; drainage area size: 4,800 km²), we sampled one impounded stream (Little Cahaba River, located in the upper Cahaba River drainage in St. Clair, Jefferson, and Shelby counties) and one unimpounded stream (Shades Creek; mean stream length: 41.6 km). Both drainages are valuable ecological resources due to diverse aquatic faunal communities and numerous imperiled

TABLE 1 Research questions, statistical analyses, and expected results (numbered sequentially with superscripts)

Research question	Statistical analyses	Expected result if impoundments impacted crayfish population genetics
Question 1: Is genetic diversity lower in crayfish populations in impounded streams compared to unimpounded streams?	ANOVA	¹ Less genetic diversity in up- and downstream sections of impounded than unimpounded streams ² Genetic diversity differences (less genetic diversity in up- than downstream sections) more pronounced in impounded streams
Question 2: Do dams and impoundments inhibit crayfish dispersal and gene flow, resulting in geographically structured populations?	TCS haplotype networks	³ Geographically structured networks in impounded streams only
	SAMOVA	⁴ Distinct genetic populations identified for up- versus downstream sites in impounded streams only
	AMOVA	⁵ Significant genetic differentiation between crayfish in up- and downstream sections in impounded streams only
	Isolation by distance (IBD)	⁶ IBD within up- and downstream sections of impounded streams when analysed separately, but not when analysed together
Question 3: Do the two focal crayfish species show concordant differences between impounded and unimpounded streams?	Migrate-n	⁷ Unidirectional downstream or no gene flow between up- and downstream sections of impounded streams, but bidirectional gene flow in unimpounded streams ⁸ Smaller effective population sizes upstream of impoundments, but no differences between effective population sizes in up- and downstream sections of unimpounded streams
	Qualitatively examined	⁹ Similar patterns in genetic diversity, genetic structure, and gene flow matrices for both species in impounded versus unimpounded streams

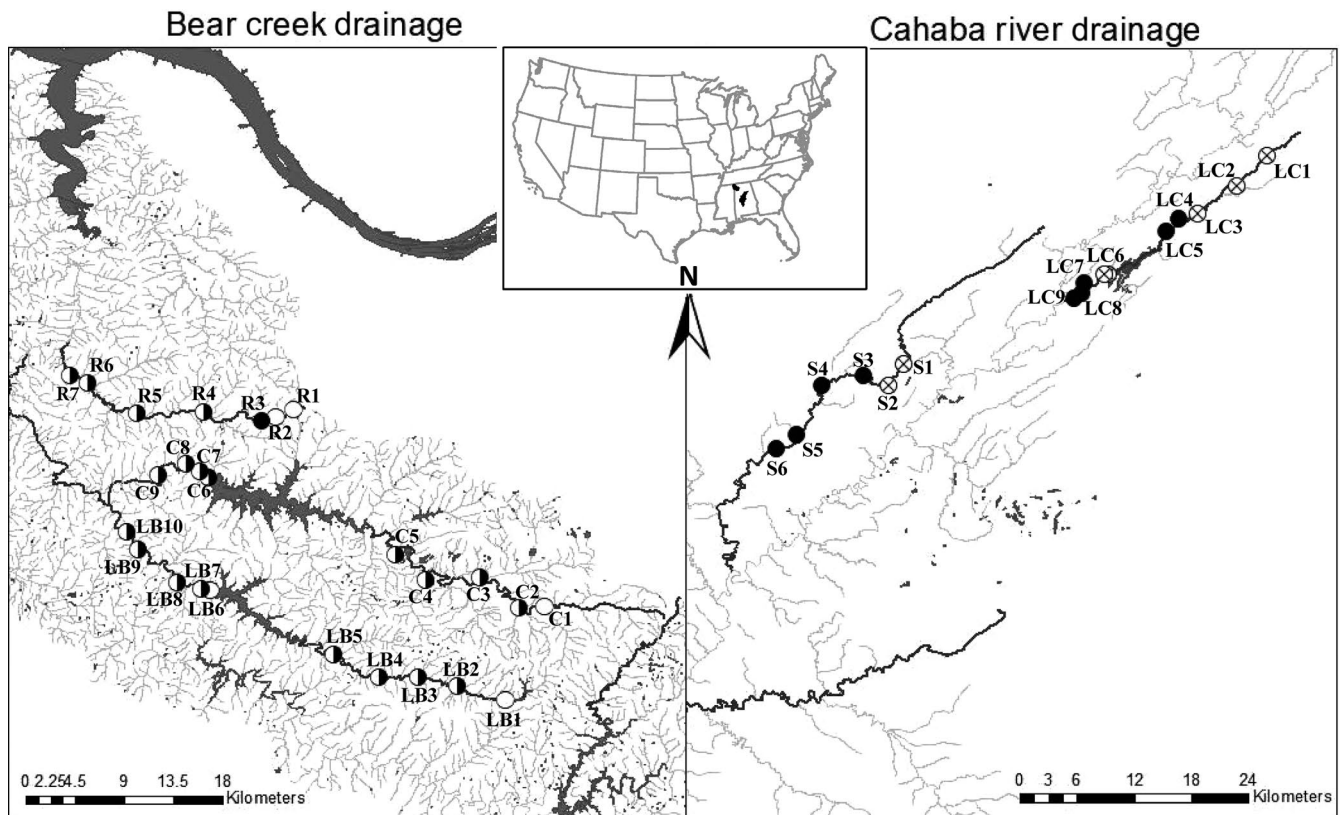


FIGURE 1 Map of Bear Creek and Cahaba River drainages, Alabama, U.S.A., with collection sites represented by labelled circles. Sites are labelled in increasing order from up- to downstream, with letters representing stream names (R = Rock Creek, C = Cedar Creek, LB = Little Bear Creek, S = Shades Creek, and LC = Little Cahaba River). Filled circles = *Faxonius erichsonianus* collection sites; unfilled circles = *Faxonius 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 south-eastern U.S.A., with the Bear Creek Drainage in the northwest corner and the Cahaba River Drainage in the centre of Alabama

species contained within them (Allen, 2001; McGregor & Garner, 2003; Phillips & Johnston, 2004). The Bear Creek drainage had four flood control impoundments, and the Cahaba River drainage had 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. The Little Bear Creek dam was completed in 1975, and was 25.6 m high and 739.1 m long, creating a 631-ha reservoir (reservoir length: 15 km). The Cedar Creek dam, completed in 1979, is 29.3 m high and 963.2 m long, forming a 1,700 ha reservoir (reservoir length: 15 km). The Little Cahaba River dam was considerably older than the others, originally constructed in 1911 and later expanded in 1929 to its current size, 16.8 m high and 64.9 m long, resulting in a 425 ha reservoir (Purdy Lake; reservoir length: 7 km). The Little Bear and Cedar creek impoundments were used for flood control, and the Little Cahaba River impoundment was used for water storage. Each year from November until February and during heavy rain events, hypolimnetic water was released in Little Bear and Cedar creeks. In the Little Cahaba River, hypolimnetic water was released when water flow in the river was too low to meet urban water usage demands.

2.2 | Population sampling

Faxonius erichsonianus and *F. validus* individuals were collected from the Bear Creek drainage, whereas only *F. erichsonianus* was collected from the Cahaba River drainage. In each stream, we sampled three to five sites in both up- and downstream sections. We selected sites at set intervals up- and downstream of impoundments and then mimicked the same pattern in unimpounded streams, with similar distances between up- and downstream sections of impounded (\bar{X} = 16 km; range = 8–20 km) and unimpounded streams (\bar{X} = 10 km; range = 8–12 km). Although no physical barrier was present in unimpounded streams, we divided each of these into up- and downstream sections to facilitate comparisons with impounded streams for genetic diversity metrics that are sensitive to sample size asymmetry. This approach of demarcating two groups in each of the unimpounded streams also attempted to make use of natural geographic clusters of sampling sites, such that the largest spatial separation of sites was commonly associated with a between-group (cf. within-group) partition. Although our approach also ensured that none of the groups within unimpounded streams were represented by an unusually small geographic spread of sites, we do recognise that it was not inherently based on biological criteria, other than the

TABLE 2 Mean genetic diversity (\pm SD) of crayfishes in up- and downstream sections of each stream

Stream section (N)	Site codes	Stream type	Distance (km)	Annual Q	No. crayfish	<i>h</i>	<i>hd</i>	π
<i>Faxonius validus</i>								
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	2.38	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	7.58	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)
<i>Faxonius erichsonianus</i>								
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	2.38	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	7.58	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	0.69	13	6	0.88 (0.03)	0.006 (0.005)
Dn Little Cahaba (3)	LC7–9	I	5.88	2.26	19	4	0.45 (0.40)	0.001 (0.001)
Up Shades (2)	S3–4	U	6.52	3.07	14	5	0.83 (0.03)	0.007 (0.007)
Dn Shades (2)	S5–6	U	5.52	3.07	15	4	0.64 (0.15)	0.001 (0.001)

Abbreviations: Annual Q, mean annual discharge (m^3/s); Distance, stream distance between the most up- and downstream sites containing the species within the stream section; Dn, downstream; *h*, number of haplotypes; *hd*, haplotype diversity; I, impounded; N, number of sites where target species collected; U, unimpounded; Up, upstream; π , nucleotide diversity.

general expectation of dispersal limitation. Sites ranged from <1 to 31 km away from impoundments and spanned up to 30 km along the stream length of unimpounded streams. Along each of the five streams, we sampled 6–10 sites, resulting in 41 sites from which one or both species were collected (Figure 1). 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 (nine in the Little Cahaba River and six in Shades Creek).

From 2015 to 2017, sampling was conducted using a variety of field techniques (i.e. kick seining, electroshocking, nest trapping, dip netting), resulting in the collection of 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 whole specimens in 95% ethanol, so that they would be available for future morphological and genomic analyses. *Faxonius validus* and *F. erichsonianus* were the most abundant (66 and 23% of individuals collected, respectively) and widespread species (collected within 96 and 72% of sites, respectively) within sampled streams (Barnett, 2019). Due to their high abundance, our sampling was unlikely to have negatively impacted local population persistence.

2.3 | Genetic data collection

We extracted genomic DNA from crayfish leg tissue using a DNeasy blood and tissue kit (Qiagen), 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, using primers LCO1490 and HCO2198 (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994). We performed polymerase chain reaction amplifications in a final volume of 15 μl containing 1.5 μl genomic DNA, 3.0 μl 5 \times buffer (Promega), 1.1 μl MgCl_2 (25 mM, Promega), 2.5 μl deoxy-nucleotides (1.25 μM , Promega), 0.75 μl bovine serum albumin (10 mg/ μl ; New England Biolabs), 4.5 μl dH_2O , 0.15 μl Go-Taq DNA polymerase (5U/ μl , Promega), and 0.75 μl of each primer (10 μM). Thermocycling conditions were: 95°C for 2 min (1 cycle), 95°C for 30 s, 50°C for 30 s, 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) 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 DNA to amino acids in order to confirm the absence of premature stop codons, using MEGA v.7 (Kumar, Stecher, & Tamura, 2016). We further assessed data quality by using BLAST searches (Altschul, Gish, Miller, Myers, & Lipman, 1990) to compare our sequences to those in the National Center for Biotechnology Information's nucleotide database. All sequences generated in this study are

available from GenBank under accession numbers MN053979–MN054048 (Table S1).

2.4 | Statistical analyses

We investigated the effects of impoundments on genetic diversity, spatial genetic structure, and connectivity mediated by dispersal and gene flow. Table 1 summarises the suite of complementary analytical approaches used to address each research question, and the associated outcomes expected under a scenario in which impoundments did affect crayfish populations. Below, we provide a detailed description of each analysis.

2.4.1 | Genetic diversity comparisons

To examine if impoundments affected genetic diversity within crayfish populations, we assessed 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 & Rozas, 2009) to calculate three genetic diversity indices (i.e. sample size-scaled number of haplotypes, haplotypic diversity, and nucleotide diversity) at each collection site. Briefly, the sample size-scaled number of haplotypes (h/N) is the number of different haplotypes (h) at each site divided by the number of individuals sampled and sequenced (N). Haplotypic diversity (hd) is the probability that a randomly chosen pair of haplotypes is different from one another. Nucleotide diversity (π) is the average number of nucleotide differences between a pair of randomly selected haplotypes, per nucleotide position. To test whether genetic diversity was different in impounded streams relative to unimpounded streams, we compared genetic diversity indices among 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. Due to the limited number of stream replicates within each watershed, watershed was not included as a variable. Analyses were performed with the *car* package (Fox & Weisberg, 2011) in R v.3.4.4 (R Core Team, 2018), using Tukey's post hoc tests to further analyse significant results. Histograms and scatterplots of model residuals did not exhibit departure from normality or heterogeneity, respectively.

2.4.2 | Spatial distribution of genetic variation, and gene flow analyses

We used five approaches to characterise dispersal and gene flow between crayfish populations in up- and downstream sections, and the spatial distributions of genetic variation within and among populations. First, for each species, we estimated phylogenetic relationships among haplotypes using statistical parsimony networks (Clement, Posada, & Crandall, 2000) calculated using PopART v.1.2.1

(Leigh & Bryant, 2015). We used this approach because haplotype networks can better illustrate genetic divergence at the intraspecific level than do strictly bifurcating phylogenetic trees, especially in cases where multiple haplotypes are derived from a single ancestral sequence, and/or where ancestral sequences are still extant (Templeton, Crandall, & Sing, 1992).

Second, to define genetic populations (i.e. natural partitions of genetic data identified a posteriori based on haplotype sequences and their frequencies) that are maximally differentiated from each other, we used spatial analysis of molecular variance implemented in SAMOVA v.2.0 (Dupanloup, Schneider, & Excoffier, 2002). This method is based on a simulated annealing procedure that maximises 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 model of evolution, as identified by the corrected Akaike information criterion (AIC_c), using jModeltest v.2.1.10 (Darriba, Taboada, Doallo, & Posada, 2012). Spatial analysis of molecular variances (SAMOVAs) were based on 100 simulated annealing steps and several different a priori delineations of the number of groups (K), with a minimum of two groups estimated per stream (i.e. a total of 2–6 groups for *F. validus*, and 2–10 for *F. erichsonianus*). For each analysis, we identified the optimal value of K by maximising F_{CT} .

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

Fourth, we evaluated whether genetic differentiation was consistent with isolation by distance (IBD), whereby a positive correlation exists between geographic and genetic distance, owing to dispersal limitation over the spatial scale that was sampled. We examined evidence for IBD within each stream, separately, by assessing correlation between 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 pair of sites from which individuals were sampled) using Mantel tests. To determine whether a pattern of IBD existed, we performed independent analyses of pairwise comparisons for each stream's up- and downstream section and for all conspecific samples collected from a given stream. All geographic distances were determined using ArcGIS (ESRI), and IBD tests were performed with the *ade4* package (Chessel, Dufour, & Thioulouse, 2004) in R, using 10,000 randomisations to measure the significance of each test (Bohonak, 2002).

In the fifth analysis, we estimated values for N_e and migration (m) of crayfish in up- and downstream sections of each stream using Migrate-n v.3.6.11 (Beerli & Felsenstein, 2001). Briefly, Migrate-n estimates of the mutation-scaled effective population size ($\theta = N_e\mu$ for mitochondrial DNA), and mutation-scaled immigration rates ($M = m/\mu$) that do not assume symmetrical bi-directional gene flow between a pair of populations, but instead partition immigration

from emigration, enabling inferences about directionality of gene flow to be made. 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 replicate runs. We assessed convergence of MCMC simulations by evaluating the consistency of estimates across replicates. To convert mutation-scaled parameter estimates to raw values of N_e and m , we used a mutation rate (μ) of 2.2×10^{-8} substitutions per site per generation based on Cunningham's, Blackstone, and Buss (1992) estimates for crabs, and assuming a 1-year generation time and equal sex ratios for each of the focal crayfish species (Cooper, 1975; Holdich, 2002). Using likelihood ratio tests for crayfish in up- and downstream sections of each stream, we assessed: (1) differences between estimated N_e against the null hypothesis of equivalency (i.e. $N_{e1} = N_{e2}$); (2) significance of departure from the null hypothesis of symmetric gene flow (i.e. $m_1 = m_2$); and (3) significance of departure from the null hypothesis of complete genetic isolation (i.e. $m_1 = m_2 = 0$).

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, h/N ranged from 0.17 to 0.31 ($h = 7$ –16). Within up- and downstream sections of streams, hd ranged from 0.23 to 0.76 and π ranged from 0.001 to 0.004 (Table 2). Notably, all *F. validus* haplotypes sampled from Rock Creek ($h = 7$ 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 to 0.32 ($h = 5$ –14). Within up- and downstream sections of streams, hd ranged from 0.23 to 0.91 and π ranged from <0.001 to 0.007 (Table 2).

3.1 | Genetic diversity comparisons

Haplotype diversity differed between impounded and unimpounded streams for *F. validus*, but not *F. erichsonianus* (Figure 2a). For *F. validus*, hd was significantly higher in impounded than unimpounded streams ($F_{1,19} = 8.69$, $p < 0.01$); however, π and h/N did not meaningfully differ between streams with versus without impoundments. None of the *F. validus* genetic diversity metrics differed between up- and downstream sections of impounded or unimpounded streams, individually (p value range = 0.14–0.93; Figure 2b,c). For *F. erichsonianus*, π , hd , and h/N were significantly higher in up- than downstream sections of streams, individually (p value range = 0.001–0.007; Figure 2b,c). For *F. erichsonianus*, π , hd , and h/N were significantly higher in up- than downstream sections of streams, individually (p value range = 0.001–0.007; Figure 2b,c). For *F. erichsonianus*, π , hd , and h/N were significantly higher in up- than downstream sections of streams, individually (p value range = 0.001–0.007; Figure 2b,c).

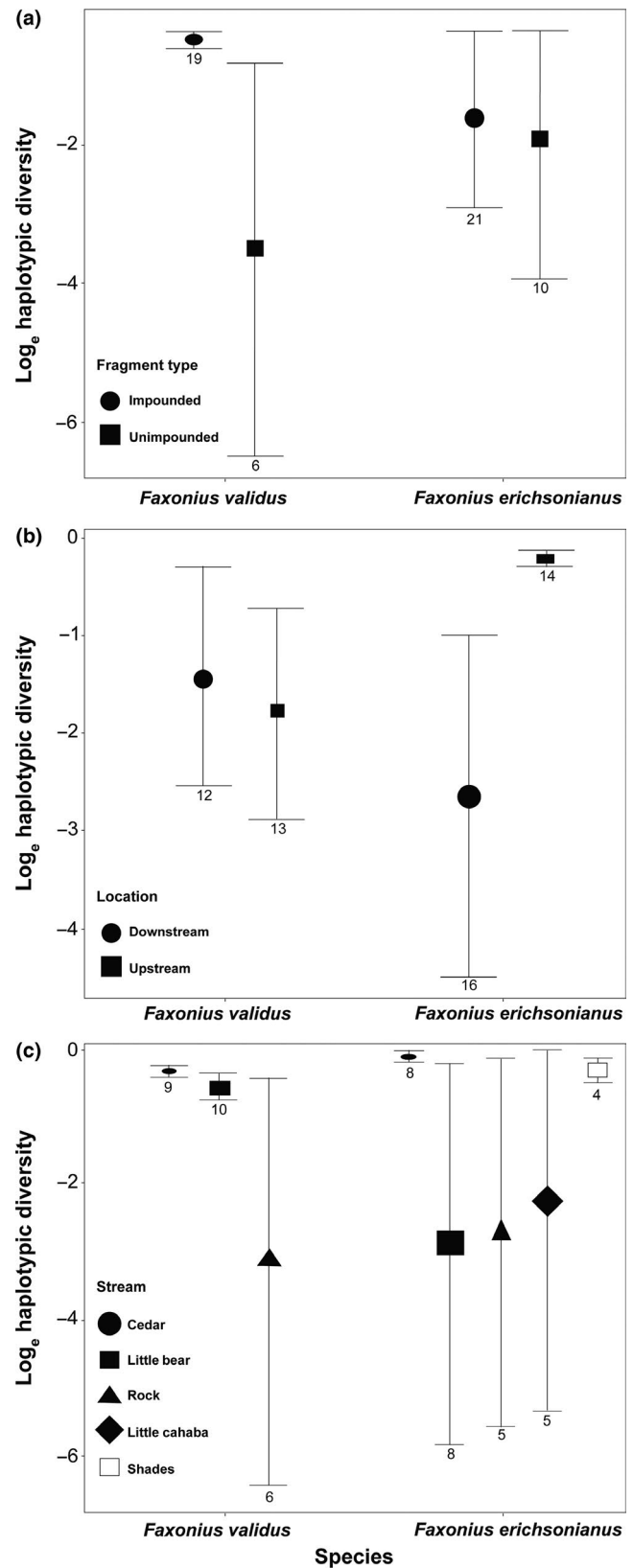


FIGURE 2 *Faxonius validus* and *Faxonius erichsonianus* mean \log_e haplotype diversity \pm 95% confidence interval (CI) for fragment types (impounded versus unimpounded; a), locations (up- versus downstream; b), and streams (c). Numbers below CIs represent the number of sites sampled within each category

$p < 0.001$; $F_{1,20} = 13.09$, $p < 0.01$ [Figure 2b]; $F_{1,20} = 5.36$, $p = 0.03$, respectively).

3.2 | Spatial distribution of genetic variation, and gene flow analyses

3.2.1 | Statistical parsimony haplotype networks

Faxonius validus haplotype networks displayed strong geographic structure only for up- and downstream sections of impounded streams (Figure 3a), indicating that dispersal and gene flow were limited in impounded streams. The most common haplotype in Rock Creek was shared by 82% of individuals (84 and 79% of individuals in the up- and downstream sections, respectively). Conversely, the most common haplotype was 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 based on samples from the Little Cahaba River showed indications of geographic structure between up- and downstream sections (Figure 3b). An absence of shared haplotypes up- and downstream of the impoundment is consistent with little to no dispersal or gene flow between populations in each stream section. The two most common haplotypes in both 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 crayfishes in the up- and downstream sections, respectively (Figure 3b).

3.2.2 | Spatial analysis of molecular variance

For *F. validus*, we identified six genetic clusters within the Bear Creek drainage. All SAMOVA analyses, which collectively assessed the fit of $K = 2$ –6 groups, were significant ($p < 0.05$; Figure 4a). Nonetheless, F_{CT} was maximised 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 4a). All sites downstream of the Cedar Creek impoundment, as well as two sites downstream of the Little Bear Creek impoundment, grouped together. The remaining sites downstream of the 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 analysed $K = 2$ groups, all Rock Creek sites grouped together, and all Little Bear and Cedar Creek sites grouped together (Figure 4a).

For *F. erichsonianus*, we identified 10 genetic clusters using SAMOVA. All analyses estimating $K = 2$ –10 groups were significant ($p < 0.05$; Figure 4b). However, F_{CT} was maximised at nine groups, which explained 81% of variation. Six groups were identified in the Bear Creek drainage, and three in the Cahaba River drainage (Figure 4b). Each stream in the Bear Creek drainage grouped separately. In addition, for each stream, one upstream site formed a separate group, indicating two genetic clusters per stream. Each SAMOVA analysis grouped all Shades Creek sites with all sites downstream of the Little Cahaba River impoundment. Each site upstream of the Little Cahaba River impoundment formed its own group. When $K = 2$ groups were analysed, all sites in the Bear Creek Drainage grouped together, as did all sites in the Cahaba River Drainage (Figure 4b).

3.2.3 | Analysis of molecular variance

For both species, F_{ST} was highest between populations in impounded streams (Table 3). For *F. validus*, we detected differentiation between crayfishes in up- and downstream sections only in impounded streams (Table 3). Similarly, for *F. erichsonianus*, up- and downstream sections were differentiated in two of the impounded streams (Little Bear Creek and Little Cahaba River) but not in the unimpounded streams. However, crayfish in up- and downstream sections of Cedar Creek (impounded) were not differentiated.

3.2.4 | Isolation by distance


Isolation by distance was detected for *F. erichsonianus* individuals in one impounded stream (Little Cahaba River; $r = 0.18$, $p < 0.001$; Figure 5b,c) but not for *F. validus* individuals within any stream (p -value range: 0.09–0.62; Figure 5a). There was also no IBD detected for *F. erichsonianus* or *F. validus* individuals in up- and downstream sections, independently, in any stream (p -value range: 0.07–0.96).

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

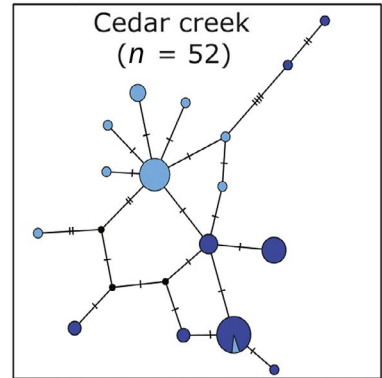
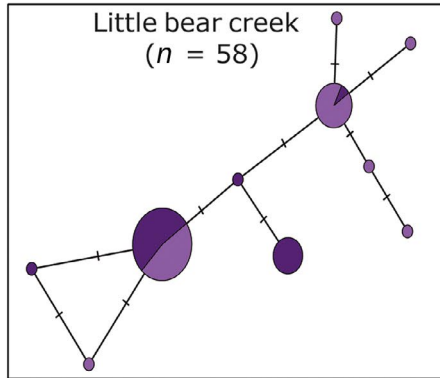
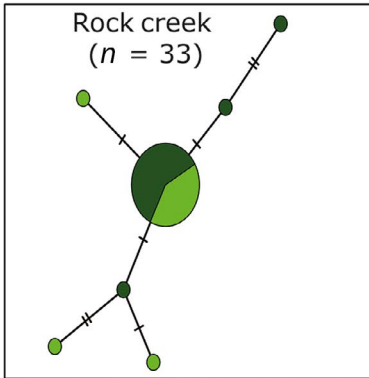
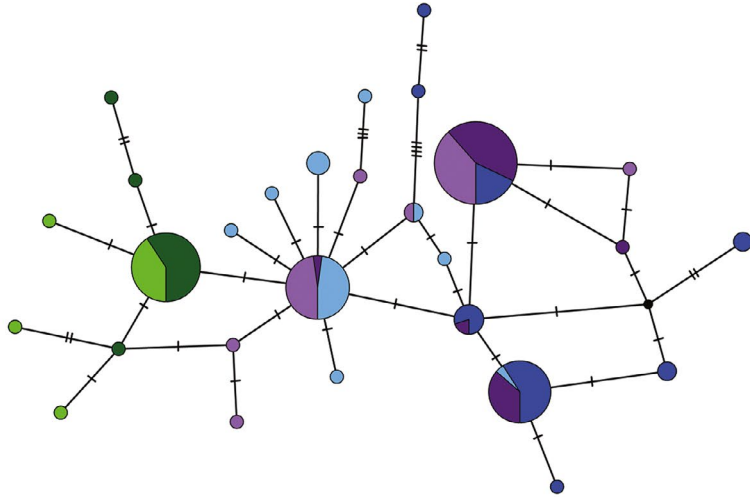
Our data indicated bidirectional gene flow between *F. validus* populations in the up- and downstream sections of Little Bear and Rock

FIGURE 3 *Faxonius validus* (a) and *Faxonius erichsonianus* (b) haplotype networks based on 618-bp and 640-bp mitochondrial COI sequence alignments, respectively. Each full haplotype network includes all samples of a species, while separate boxes represent each creek's haplotype network separately. 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


(a) *Faxonius validus*

10 samples

 1 sample

- Cedar creek upstream
- Cedar creek downstream
- Little bear creek upstream
- Little bear downstream
- Rock creek upstream
- Rock creek downstream



(b) *Faxonius erichsonianus*

10 samples

 1 sample

- Cedar creek upstream
- Cedar creek downstream
- Little bear creek upstream
- Little bear downstream
- Rock creek upstream
- Rock creek downstream
- Little cahaba river upstream
- Little cahaba river downstream
- Shades creek upstream
- Shades creek downstream

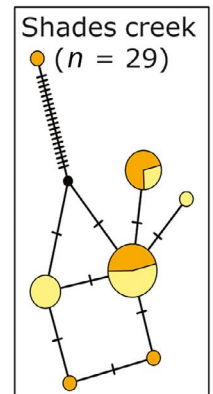
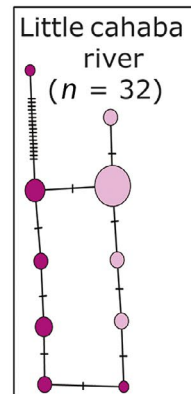
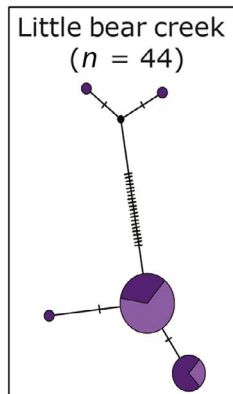
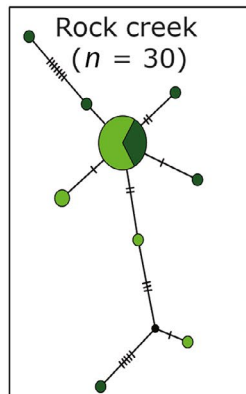
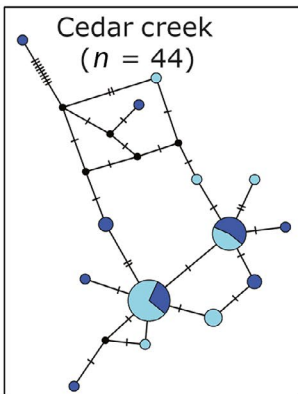
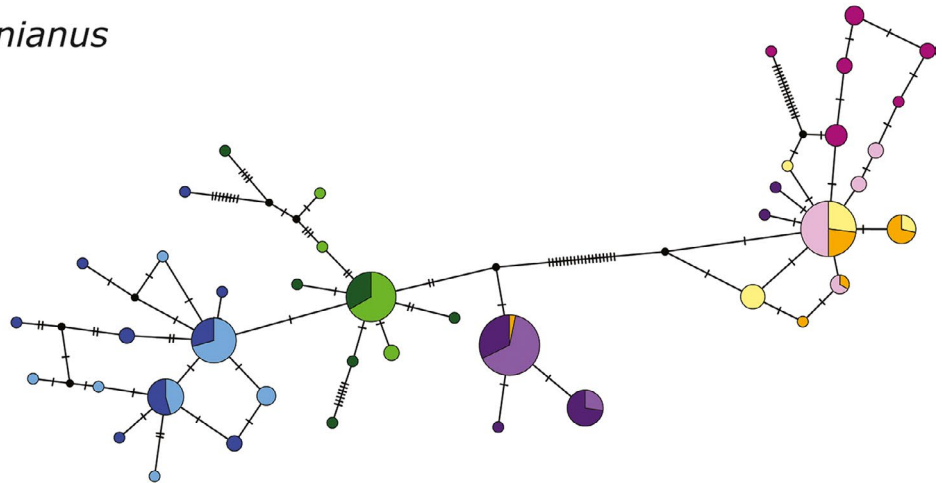
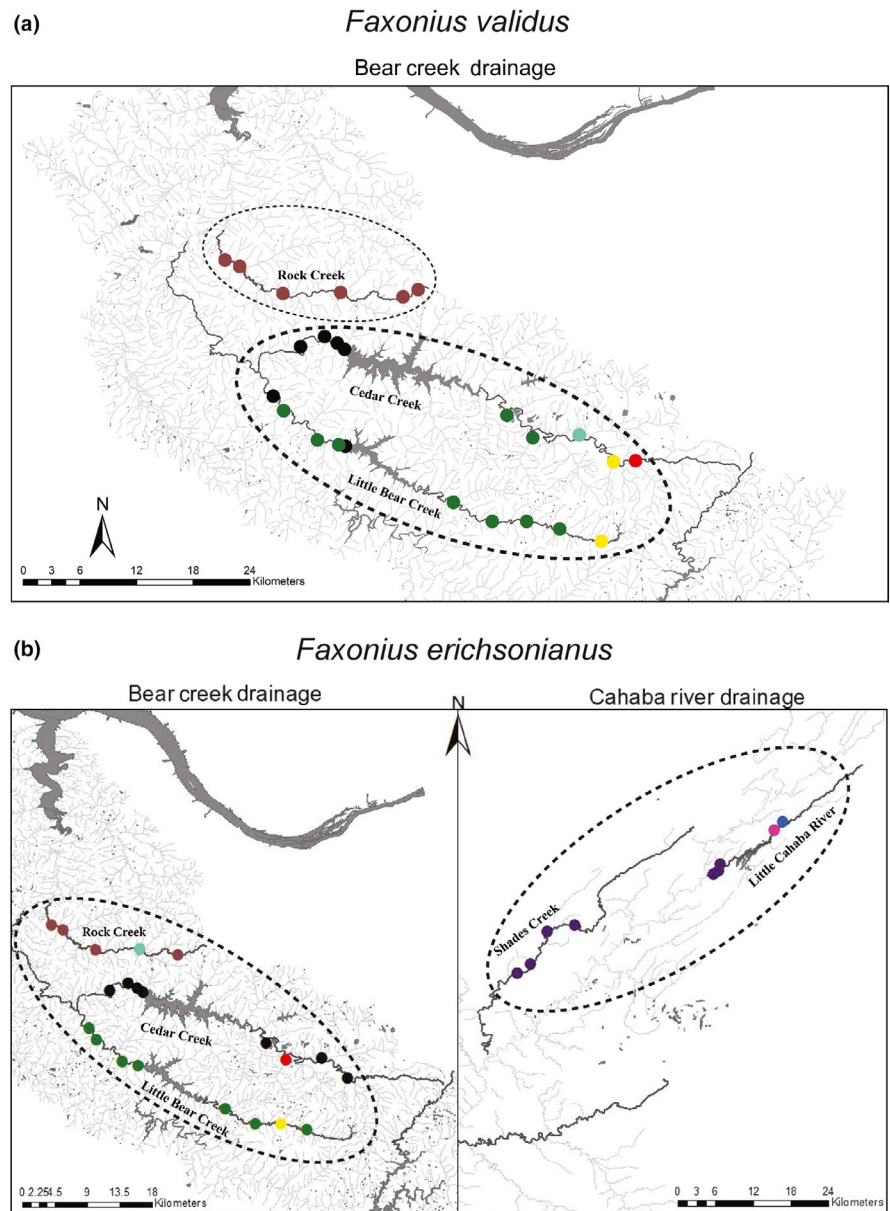


FIGURE 4 Map of Bear Creek (a–b) and Cahaba River (b) drainages, with SAMOVA groupings of populations that maximised F_{CT} for *Faxonius validus* (a; six groups) and *Faxonius erichsonianus* (b; nine groups) represented by coloured circles, and the groupings for the lowest number of groups analysed (2) represented by dashed lined circles. All sites with the same coloured circle were grouped together by SAMOVA



streams, and unidirectional, downstream gene flow between sections of Cedar Creek. In Rock Creek, more gene flow occurred down- than upstream, but up- and downstream gene flow did not differ within

TABLE 3 *Faxonius validus* and *Faxonius erichsonianus* pairwise F_{ST} values (p -values) between up- and downstream sections of each stream

Stream	<i>F. validus</i>	<i>F. erichsonianus</i>
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)

Note: Significant F_{ST} values indicate genetic differentiation between stream sections.

Bold values represent significant (P -values ≤ 0.05) F_{ST} values.

Abbreviations: I, impounded; U, unimpounded.

Little Bear Creek (Table 4). In addition, gene flow between populations was higher in the unimpounded stream than in impounded streams. Differences in N_e between up- and downstream sections were not statistically significant (p -value range: 0.90–0.98) in any stream.

Faxonius erichsonianus in up- and downstream sections exhibited bidirectional gene flow between populations in unimpounded streams and unidirectional, downstream or no gene flow in impounded streams. In unimpounded streams, downstream gene flow was greater than upstream gene flow (Table 4). No gene flow occurred between crayfish populations in up- and downstream sections of the Little Cahaba River. In the Bear Creek drainage, unidirectional downstream gene flow occurred between crayfish populations in up- and downstream sections in both impounded streams (Little Bear and Cedar creeks). Differences in N_e between up- and downstream sections of any stream were not statistically significant (p -value range: 0.89–0.97).

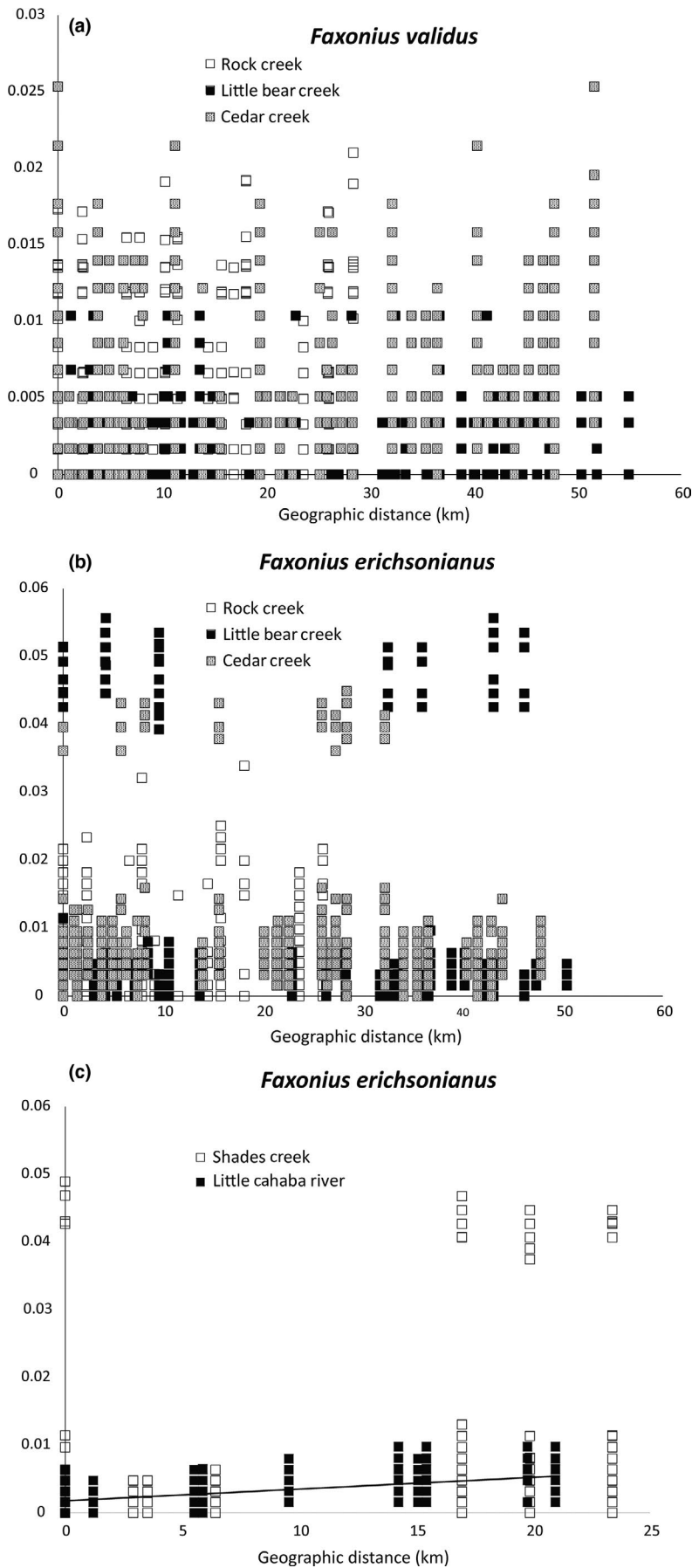


FIGURE 5 Scatter plot of pairwise genetic distances (i.e. proportion of nucleotides that differ between each pair of sequences) and waterway distances for all *Faxonius validus* (a) and *Faxonius erichsonianus* (b–c) individuals collected at sites within the Bear Creek (a–b) and Cahaba River (c) drainages. Trend lines represent a significant correlation between genetic and geographic distance of *F. erichsonianus* individuals within the Little Cahaba River population. Unfilled squares represent unimpounded streams. Filled squares represent impounded streams

TABLE 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) of *Faxonius validus* and *Faxonius erichsonianus* in up- versus downstream sections of streams

	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: $N_{e_{up}} = N_{e_{down}}$)
<i>Faxonius validus</i>				
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)
<i>Faxonius erichsonianus</i>				
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)

Note: Significance indicates migration rates >0 , or differences between up- and downstream m and N_e .

Bold values are significant (P -values ≤ 0.05).

Abbreviations: I, impounded; U, unimpounded.

4 | DISCUSSION

Freshwater ecosystems are highly diverse in species and habitats, but they are considered one of the most imperiled ecosystem types (Chaplin, Gerrard, Watson, Master, & Flack, 2000). One in three freshwater species is threatened with extinction worldwide, and crayfishes are among the most threatened taxonomic groups (Chaplin et al., 2000; Collen et al., 2014). Notably, habitat loss and restricted gene flow among populations, in part due to stream regulation by impoundments, is one of the top threats (Wilcove, Rothstein, Dubow, Phillips, & Losos, 2000). Although threats to crayfishes have been identified, the relationship between stream regulation and crayfish genetic structure and dispersal has not been extensively studied (Hartfield, 2010). In the present study, we provide evidence that dams and impoundments are associated with lower dispersal and gene flow between up- and downstream crayfish populations, indicating that these structures lead to habitat fragmentation. Not all of our results were as anticipated (i.e. expected results 1, 2, 6, 8); however, our data showed that crayfish populations were more geographically structured (low, one-way, or no gene flow between up- and downstream sections) in impounded than unimpounded streams, indicating that dams were decreasing connectivity between populations. Gene flow limitation may lead to lower genetic diversity, reduced population sizes, and local extinction, causing cascading effects through stream ecosystems (Momot, 1995; Rabeni et al., 1995). Because there is a strong correlation between changes in gene flow/genetic structure and damming of rivers (Monaghan et al., 2002; Nielsen, Hansen, & Loeschcke, 1999; Watanabe & Omura, 2007; Yamamoto et al., 2004), we inferred a cause and effect relationship. Nonetheless, a shortcoming of this and many other studies examining the effects of dams and impoundments on stream communities is the lack of

data before dam installation, such that a *space for time* approach becomes necessary, albeit with the potential for confounding factors to affect inferences (Damgaard, 2019).

For both *F. validus* and *F. erichsonianus*, gene flow from down- to upstream populations was lower in impounded streams compared to unimpounded streams, which is consistent with the expected response to stream habitat fragmentation (Fluker et al., 2014; Hudman & Gido, 2013; Meffe & Vrijenhoek, 1988). In contrast, crayfish populations in up- and downstream sections of unimpounded streams displayed high genetic connectivity and bidirectional dispersal and gene flow. Differences between the magnitude of gene flow between crayfish populations in up and downstream sections were qualitatively compared due to statistical limitations. While the number of migrants needed to maintain genetic connectivity differs by taxa and population, studies show that a minimum of one migrant per generation is needed to minimise loss of genetic diversity owing to drift (Mills & Allendorf, 1996; Nathan, Kanno, & Vokoun, 2017; Wright, 1931). All unimpounded stream populations had around one or more migrants per generation, indicating that genetic diversity may be maintained through this gene flow. Although genetic isolation between populations in up- and downstream sections of impounded streams is common for fishes (Nielsen et al., 1999; Yamamoto et al., 2004) and aquatic insects (Monaghan et al., 2002; Watanabe & Omura, 2007), most studies examining other stream organisms have not found clear evidence of gene flow limitation due to impoundments (e.g. mussels: Abernethy et al., 2013; Fuller, 2017; snails: Liu & Hershler, 2009; amphipods: Berettoni & Hervant, 1998). Gene flow differences across studies may result from contrasting taxon-specific dispersal ability, generation times, and population sizes, as well as differences in the genetic markers analysed, or magnitude of gene flow prior to dam installation (Abernethy et al., 2013; Berettoni & Hervant, 1998; Fuller, 2017; Liu & Hershler, 2009).

Detecting genetic signatures of recent fragmentation using molecular data has been difficult (Richmond, Reid, Ashton, & Zamudio, 2009; Sumner, Jessop, Paetkau, & Moritz, 2004), particularly when using markers that do not mutate at exceptionally fast rates. For example, genetic differences between fragmented populations of Alabama stream fishes were detected using hypervariable nuclear microsatellite loci but not using mitochondrial DNA sequences (Fluker et al., 2014). However, in the present study using mitochondrial COI sequences, we detected differences between crayfishes in up- and downstream sections of streams that were impounded for only 36 years (Cedar Creek), 40 years (Little Bear), and 104 years (Little Cahaba River). Even if we assume a relatively short (1 year) generation time, this outcome suggests that restrictions to dispersal and gene flow between crayfish populations in up- and downstream sections of streams, and subsequent genetic drift within these locations, were substantial in impounded streams (Dixo et al., 2009; Lacy, 1987). Although we detected IBD in the Little Cahaba River, there was no clear signature of IBD and no differences among sections in unimpounded streams. Accordingly, we inferred that genetic differences between populations in the Little Cahaba River were caused by impoundments. These findings are of particular interest in biodiversity hotspots, such as the south-eastern U.S.A. (Lydeard & Mayden, 1995; Noss et al., 2015), where almost all aquatic systems are fragmented by impoundments.

Gene flow between populations in up- and downstream sections of impounded streams can depend on reservoir size (Petts, 2018; Ward & Stanford, 1979), with larger reservoirs usually causing greater shifts in biophysical and biotic patterns, potentially creating less favourable stream habitats than smaller reservoirs (Ward & Stanford, 1983). The dams in the Bear Creek drainage were of 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 less permeable barrier to dispersal. For both species, lower upstream gene flow occurred in Cedar than Little Bear creek. Conversely, small (dam height <10 m; average reservoir size 20 ha), low-head mill dam impoundments in Alabama did not negatively impact movement in all crayfish species studied (Hartfield, 2010), indicating that larger impoundments can exacerbate fragmentation effects.

Longer periods of isolation can lead to reduced population size, reproductive success, and genetic diversity, consequently decreasing the likelihood of population persistence (Lowe & Allendorf, 2010; Mims, Hauser, Goldberg, & Olden, 2016; Zwick, 1992). For *F. erichsonianus* populations, we detected little to no dispersal and gene flow between up- and downstream sections in the Little Cahaba River. Conversely, in Little Bear and Cedar creeks, we detected unidirectional, downstream dispersal and gene flow between up- and downstream sections. The Little Cahaba River had the smallest reservoir in this study but was impounded for the longest time (more than two times longer than Little Bear and Cedar creeks) and had the least amount of gene flow between populations. These findings are consistent with those for fishes, where genetic diversity was lower among impounded populations isolated for longer periods (Morita & Yamamoto, 2002; Yamamoto et al., 2004). However, with only one

stream impounded for a long period, our study design did not allow us to rigorously test the hypothesis that longer periods of isolation lead to reduced genetic diversity and gene flow. Further research is needed to investigate how size and duration of impoundments interact to affect levels of genetic isolation.

Although gene flow differed between populations in impounded and unimpounded streams for both crayfishes, the nature of these differences was not consistent across species. Life history characteristics such as dispersal ability, ecological specialisation, and physiological tolerance often determine the degree of impact that habitat fragmentation has on populations (Alp, Keller, Westram, & Robinson, 2012; Luoy et al., 2007; Reid, Wilson, Mandrak, & Carl, 2008). In Little Bear Creek, we detected bidirectional dispersal and gene flow between up- and downstream *F. validus* populations, but unidirectional, downstream gene flow between *F. erichsonianus* populations. *Faxonius validus*' preference for smaller streams (Cooper & Hobbs, 1980; Hobbs, 1989) may cause members of this species to naturally disperse upstream at higher rates than members of *F. erichsonianus* (Hobbs, 1981). Steep slopes and fast water velocities usually decrease upstream versus downstream dispersal, and crayfishes' abilities to navigate these conditions can influence upstream dispersal rates (Bernardo, Costa, Bruxelas, & Teixeira, 2011). Additionally, downstream dispersal and gene flow was higher for *F. erichsonianus* than *F. validus* populations in all Bear Creek drainage impounded streams. Gut contents of fishes from impoundments in the Bear Creek drainage indicate that *F. erichsonianus* was the dominant crayfish prey of predatory fishes, comprising 88% (37 of 42) of identified crayfishes (Z. Barnett, unpublished data). Our results suggest that *F. erichsonianus* may be better able than *F. validus* to tolerate impounded habitats. In addition, *F. erichsonianus* also has a larger geographic range than *F. validus* and, consequently, may have a broader niche (Brown, 1984; Slatyer, Hirst, & Sexton, 2013). With a broader niche breadth, *F. erichsonianus* may be more tolerant of, and better able to disperse through, altered habitats created by dams. Overall, impacts of impoundments vary, at least in part, according to dispersal ability and species' habitat preferences (Hughes, 2007; Hughes et al., 2013).

Genetic diversity and estimated N_e were not lower in impounded than unimpounded stream populations, with h_d in impounded streams the same or higher than h_d in unimpounded streams. Given that fragmentation increases the probability of differentiation due to genetic drift or selection within isolated populations (Heggenes & Røed, 2006; Kimura & Crow, 1963; Templeton, Shaw, Routman, & Davis, 1990) resulting in fewer shared haplotypes among populations, it is possible that these differentiating processes inflated measures of overall genetic diversity in cases where substructure existed. For *F. erichsonianus* in both impounded and unimpounded streams, upstream populations tended to have higher genetic diversity than downstream populations, which is not indicative of isolated upstream populations that have experienced recent size reductions. Similarly, π was higher in upstream populations for crayfishes in other impounded (mill dams) and unimpounded (breached or relict mill dams) Alabama streams (Hartfield, 2010). The location of dams within drainages

can influence genetic diversity (Stanford & Ward, 2001), whereby dams closer to headwaters, with fewer tributaries upstream, have larger impacts on upstream populations by isolating smaller populations. Dams in this study were at least 29 km downstream of headwaters and so they probably isolated potentially large upstream populations of the focal species. Although we assumed that we sampled all genetically relevant populations present within the study streams, the maximum likelihood approach used to estimate migration rates is relatively insensitive to ghost populations (i.e. those that were not sampled, but did exchange, or continue to exchange, alleles with sampled populations; Beerli, 2004). In our study, fragmentation and gene flow limitation associated with impoundments appear to have increased genetic differentiation of crayfish populations between each stream's up- and downstream sections. However, within up- and downstream sections, factors other than impoundments (e.g. gene flow from tributaries, stochastic environmental events, competition from invasive species) may have also impacted levels of genetic diversity.

Faxonius validus and *F. erichsonianus* had high levels of π and hd in impounded and unimpounded streams when compared to values reported for populations of other crayfish species (Brown, 1981; Fetzner & Crandall, 2001; Grandjean & Souty-Grosset, 2000). Nonetheless, few studies have assessed *Faxonius* spp. at the population level, and our results concur with studies of Australian crayfishes (*Cherax* spp. and *Geocheerax* spp.) in biodiversity hotspots (Bentley et al., 2010; Munasinghe, Burrige, & Austin, 2004). High levels of genetic diversity in *Faxonius* species may result from historical factors (e.g. Pleistocene glacial-interglacial cycles) which altered river drainage patterns in the region (Crandall & Templeton, 1999; Fetzner & Crandall, 2003), as well as low contemporary levels of gene flow among stream populations.

One potential bias in our study design was that in the Bear Creek drainage, our sampling sites spanned a shorter overall length in the unimpounded stream than in the corresponding impounded streams. Unfortunately, unimpounded streams with lengths comparable to the impounded streams do not exist in this drainage. However, our results for the Bear Creek drainage are supported by results for the Cahaba River drainage where sampling sites in impounded and unimpounded streams covered similar stream lengths, and genetic differences between these streams were apparent from our data.

Our findings have important implications for understanding crayfish population dynamics in impounded streams. First, *F. validus* and *F. erichsonianus* were the most abundant and widespread crayfish species within the sampled streams, and evidence of population fragmentation for these species was detectable within a relatively short time since impoundment (i.e. only 36–104 years). This indicates that many crayfish populations in impounded streams (i.e. those with relatively large impoundments built before 1980) may be genetically isolated to some extent. Presumably, ecologically specialised species and those with small N_e , low genetic variation, and high sensitivity to stochastic environmental events (Franzén & Nilsson, 2010; Li, Jovelín, Yoshiga, Tanaka, & Cutter, 2014) may suffer more severe effects of stream fragmentation by impoundments.

Second, crayfish populations upstream of impoundments are at risk of local extinction due to the lack of detectable upstream dispersal and gene flow in most impounded streams. This risk is greatest in drainages with impoundments near headwaters isolating smaller upstream populations. Conservation strategies focused on enhancing connectivity may be beneficial in impounded streams, especially in streams like those in the current study with high levels of genetic diversity. For example, assisted translocation of individuals from down- to upstream populations could facilitate gene flow. Dam removal is also likely to benefit stream crayfishes by increasing connectivity between up- and downstream populations (Reid et al., 2019). Mechanisms such as fish ladders may also enhance crayfish movement across dams (Welsh & Loughman, 2018); however, studies that assess the use of fish ladders by crayfishes are needed.

This study presents one of the first comparisons of differences in crayfish population genetic structure in impounded streams compared to spatially proximate unimpounded reference streams. Bidirectional gene flow was lower between impounded than unimpounded stream populations, potentially leading to decreased persistence of isolated populations. Decreases in crayfishes' genetic diversity and population sizes can cause cascading effects through stream ecosystems due to their keystone role in stream environments, with their omnivory and mobility affecting trophic dynamics and organic matter processing (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 may be negatively impacted.

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CONFLICT OF INTEREST

Authors have no conflicts of interest to declare.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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