Dissimilar effects of low-head dams on the genetic structure of riverine fishes

Shannon C. F. Smith[1,4], Robert E. Colombo[1,5], Trent Thomas[2,6], and Devon B. Keeney[3,7]

[1]Eastern Illinois University, 600 Lincoln Avenue, Charleston, Illinois 61920 USA
[2]Department of Natural Resources, 301 South Date Street, Gibson City, Illinois 60936 USA
[3]Le Moyne College, 1419 Salt Springs Road, Syracuse, New York 13214 USA

Abstract: The impacts of low-head dams on the genetic structure of small-bodied riverine fishes have not been fully explored. We used both novel and existing microsatellites to assess how 2 low-head dams affect genetic diversity and differentiation in Longear Sunfish (*Lepomis megalotis*) and Bluntnose Minnow (*Pimephales notatus*) in an Illinois river system. We expected reduced genetic diversity above dams in both species and greater genetic differentiation in Longear Sunfish, given the species' more sedentary life history. No detectable patterns of genetic diversity were observed for *L. megalotis*, whereas a decrease in allelic richness and gene diversity occurred in *P. notatus* above 1 of the impoundments. The genetic differentiation analyses F_{ST}, principal components analysis, and Bayesian clustering revealed that slight genetic differences occurred among some *L. megalotis* throughout the study area, but these differences were not associated with either dam. In contrast, the same methods showed there were 2 genetically distinct populations of *P. notatus* separated by 1 of the 2 low-head dams. Our results show that dams do not necessarily impede gene flow or movement of *L. megalotis* in this region and that small genetic differences are likely the result of the species' limited home range. However, the strong genetic differentiation we found in *P. notatus* is probably a result of poor-quality habitat created by a dam that restricts *P. notatus* movement. Our data show that even small dams can influence genetic structure in river systems but that they affect species in different ways that are not necessarily intuitive based on the life history of a species.

Key words: freshwater fishes, low-head dams, *Lepomis megalotis*, *Pimephales notatus*, genetic structure, genetic diversity, microsatellites, Illinois, river

Construction of impoundments for industrial, agricultural, and domestic purposes has been altering river hydrology for more than a century (Jansson et al. 2007). Dams, and the impoundments they create, can negatively alter rivers and streams by reducing connectivity, influencing flow regime, and changing physical habitat (Ward and Stanford 1995, Pringle 2003, Jansson et al. 2007). These changes can severely alter fish populations. For example, in systems with impassable barriers, reduced connectivity and habitat alterations hinder fish passage and can result in population collapse (Bunn and Arthington 2002, Pess et al. 2008, Burroughs et al. 2010, Nislow et al. 2011). Reduced fish passage across dams can also influence genetic diversity within a population and genetic differentiation among populations (Meldgaard et al. 2003, Stamford and Taylor 2005, Bessert and Ortí 2008, Faulks et al. 2011). Fish populations above dams can lose genetic diversity or become genetically distinct because of increased genetic drift when they are reduced in size and isolated from other populations (Meldgaard et al. 2003, Yamamoto et al. 2004, Faulks et al. 2011, Junker et al. 2012).

Few studies have investigated the effects of small, low-head (run-of-river) dams on the genetic structure of riverine fishes, although multiple studies have assessed the genetic effects large dams have on salmonids and other large-bodied fishes (Neraas and Spruell 2001, Meldgaard et al. 2003, Yamamoto et al. 2004, Whiteley et al. 2006, Bessert and Ortí 2008, Peterson and Ardren 2009). The studies that have evaluated the effects of smaller dams have found mixed results. For example, weirs did not appear to influence Black Redhorse (*Moxostoma duquesnei*) genetic diversity or differentiation (Reid et al. 2008b), and dams had no negative

effect on the genetic structure of the Rio Grande Silvery Minnow (*Hybognathus amarus*; Alò and Turner 2005). Conversely, low-head dams caused interpopulational genetic structuring in Dorado (*Salminus hilarii*; Esguícero and Arcifa 2010), and impounds contributed to genetic differentiation and loss of genetic variation in the Macquarie Perch (*Macquaria australasica*; Faulks et al. 2011). Other studies have highlighted the negative impacts of reservoir impoundments on riverine fishes (Franssen 2012, Fluker et al. 2013, Hudman and Gido 2013). Variability in observed impacts may depend on the river system, physical characteristics of the impounds, and life-history characteristics of focal species. However, it remains infeasible to generalize about the impacts of low-head dams on fish genetic structure, especially nongame and small-bodied species, because of the inherent variability within and among aquatic systems and fish species and the lack of data on the genetic structure of many riverine fishes.

We therefore examined how low-head dams influenced genetic structure in 2 riverine fish species, Longear Sunfish (*Lepomis megalotis*, family Centrarchidae) and Bluntnose Minnow (*Pimephales notatus*, family Cyprinidae). We studied these 2 species in 2 rivers impounded by low-head dams in east-central Illinois. We chose these species because they are common, warm-water species that have widespread ranges and similar life spans but different life histories (Smith 1979, Wells and Haynes 2007, Jacquemin et al. 2013). *Lepomis megalotis* are eurytopic but prefer relatively shallow lotic habitats (<90 cm) away from silt and strong current (Smith 1979, Schaefer et al. 1999). *Pimephales notatus* are somewhat more tolerant of silt and strong currents and are widespread and abundant in Illinois. *Pimephales notatus* prefer hard-bottomed pools in lotic systems but occur everywhere except in swamps and heavily silted waterbodies (Smith 1979, Jacquemin et al. 2013). *Lepomis megalotis* are relatively sedentary and have home ranges of 30–61 m (mean = 37 m; Berra and Gunning 1972). In contrast, *P. notatus* exhibit shoaling behaviors, have seasonal population fluctuations in Illinois streams, and have life-history characteristics typical of *r*-strategists (Smith 1963, Dewey 1981, Jacquemin et al. 2013). In addition, *P. notatus* are relatively mobile within stream networks and have low recapture rates and movements >1 km (Smith 1963, Dewey 1981).

Our goals were to provide baseline data on the genetic diversity and structure of *L. megalotis* and *P. notatus* river populations and to determine whether 2 low-head dams in these rivers influence the population genetics of these 2 species. Our study allowed for a direct comparison of how the life-history traits of *L. megalotis* and *P. notatus* influence how their genetic structure is influenced by dams because we compare these species over the same region. We expected that we would find direct evidence of dams creating genetic structure, especially in the more sedentary

L. megalotis, because both dams have been in place for approximately a century. We also expected to find reduced genetic diversity upstream of the dams. Our findings provide important insights into the basic biology of these fishes and the anthropogenic impacts on them because both species are widely distributed and low-head dams are common in the region and elsewhere. There is an urgent need for additional research assessing the impacts of impounds on the genetic structure of fishes because of the ubiquity of impounds on rivers (Doyle et al. 2000) and the need to justify their removal (Bednarek 2001). As baseline data on the impacts of low-head dams accumulate, the ability of biologists to assess the effects of dams and predict the consequences of their removal should improve.

METHODS
Study area

The impounded Vermilion and North Fork Vermilion rivers are located in Danville, Illinois. The Danville Dam on the Vermilion River was built in 1914 and is a barrier between the lower 35 km of the Vermilion River mainstem and the 3341-km^2 drainage network upstream of the dam (Fig. 1; IDNR 2013). The Ellsworth Park Dam was built in the 1920s on the North Fork Vermilion River ~0.85 km upstream from the confluence of the Vermilion and North Fork Vermilion rivers (Fig. 1). Both dams are classified as low-head dams (structures <4.6 m in height; IDNR 2013, USACE 2013). Water flow over these structures is intermittent and varies by season. During late summer, there can be little to no spillover, whereas in early spring, the structures can be nearly submerged because of high discharge.

We sampled fish at twelve 100-m sites on the Vermilion River and the North Fork Vermilion River. We sampled 6 sites on each river: 2 sites below each dam, 2 in the pool above the dam, and 2 upriver of the pool above the dam (Fig. 1). We named sites as follows: Site names begin with either V (Vermillion River) or NF (North Fork Vermillion River). Sites were then labeled based on their location relative to the dam with either B (below-dam), P (above-dam pool), or R (upriver). We ended site names with either 1 (closer to the dam) or 2 (farther from the dam), because there were two of each site type.

Genetic analyses

We collected *L. megalotis* and *P. notatus* in both autumn and spring from 2012 to 2015. We captured individuals with boat- or barge-mounted pulsed DC electrofishing (both seasons; 60 Hz, 25% duty cycle), beach seines (autumn), and mini-fyke nets (spring) at each site. We identified all fish >100 mm to species, measured them to the nearest millimeter, and weighed them to the nearest gram in the field. We euthanized individuals <100 mm and preserved them in 95% ethanol for later identification and
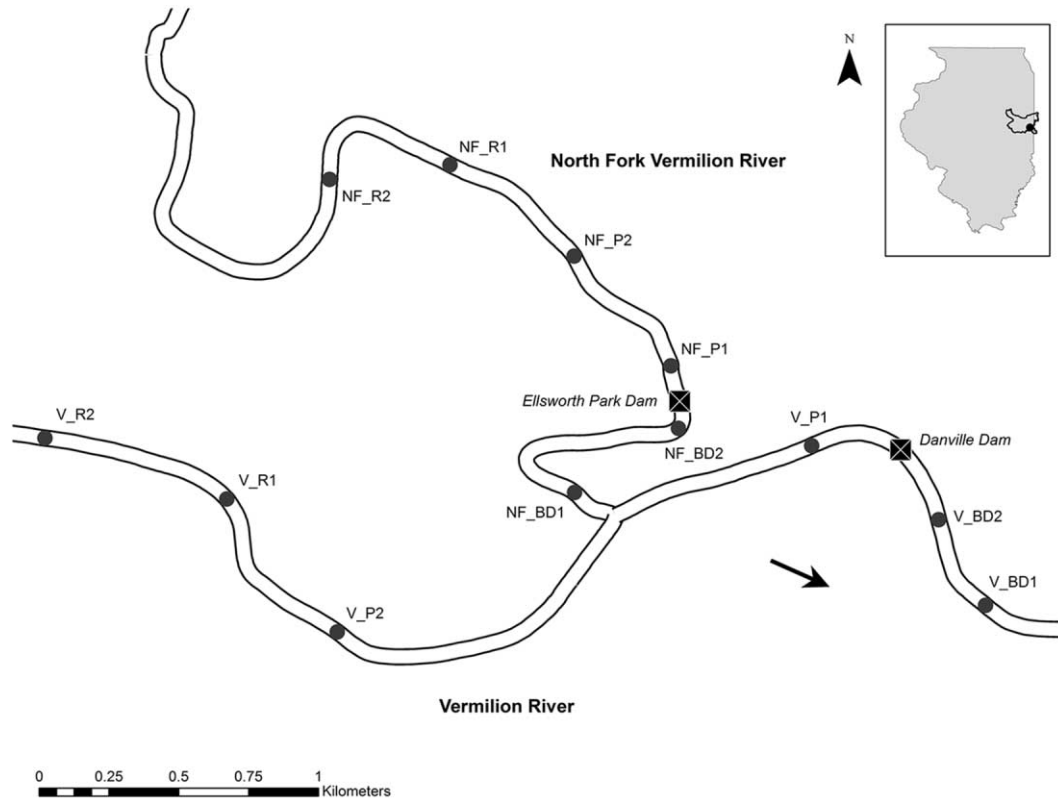
Figure 1. Map of Vermilion River (V) and North Fork Vermilion River (NF) sampling sites near Danville, Illinois. Below dam sites (BD1, BD2), pool sites (P1, P2), and upriver sites (R1, R2). The Danville Dam is located at lat 40°07′20.23″N, long 87°37′53.97″W. The Ellsworth Park Dam is located at lat 40°07′26.0″N, long 87°38′19.65″W. Arrow indicates flow direction. Figure created with ArcMap (ESRI, Redlands, California).

measurement. We preserved a portion of the caudal fin of each fish in 95% ethanol for DNA analysis.

We conducted genetic analyses on 12 microsatellite loci for 426 *L. megalotis* and 363 *P. notatus* individuals. Sample sizes for each site ranged from 27 to 48 for *L. megalotis* and 2 to 52 for *P. notatus* (Tables 1, 2). We extracted DNA by placing ~0.5 cm^2 of fin tissue in 400 µL of 5% Chelex containing 0.1 mg/mL proteinase K, incubating this mixture at 60°C for 2–12 h, and then heating it to 100°C for 8 min (modified from Walsh et al. 1991). We then used 2 preexisting primer pairs (Gotoh et al. 2013) and 10 novel primer pairs we developed for this study (GenBank accession numbers MH521016–MH521025) for *L. megalotis* and 12 preexisting primer pairs for *P. notatus* (Landis et al. 2009; Table S2) to quantify genetic variation. We developed the novel primers following the methods outlined for the freshwater snails *Valvata tricarinata* and *Promenetus exacuous* (Yurco and Keeney 2018). Following this protocol, we used ~3 µg of DNA from the fin of a single V_P2 *L. megalotis* for ION Torrent PGM sequencing at the University of Wisconsin-Madison Biotechnology Center.

We amplified the microsatellite loci with 3 primer polymerase chain reactions (PCR) (Schuelke 2000) and used the fluorescent-labeled microsatellite primer tag (CAGTCGGGCGTCATCA) as a 3rd primer. One primer from each locus-specific pair also contained this 5′ primer tag sequence (Table S2). The PCR reactions included 1X Type-it® Multiplex PCR Master Mix (QIAGEN), 0.2 µm standard locus primer, 0.02 µm locus primer with tag sequence, and 0.2 µm fluorescent-labeled tag in a total of 10 µL. The thermal cycling conditions included an initial heat activation for 5 min at 95°C, 30 cycles of 30 s at 95°C, 90 s at 56°C or 60°C (Table S2), 30 s at 72°C, and a final extension of 30 min at 60°C. We pooled the individual locus PCRs (*Leme279* and *Leme246* as well as *Leme454* and *Leme239* were multiplexed) post-PCR to create sets of 4 loci labeled with the dyes NED, 6FAM, PET, and VIC. We genotyped these sets on an ABI 3730xl 96-Capillary Genetic Analyzer at the DNA Analysis Facility at Yale University. We scored and binned allele peaks with the software Geneious (version 8.1; Kearse et al. 2012). We then used MicroChecker (version 2.2; Van Oosterhout et al. 2004) to examine loci for scoring errors from stuttering, large allele dropout, and the presence of null alleles. We also used GENEPOP (version 4.2; Raymond and Rousset 1995) to check for deviations from Hardy-Weinberg expectations

Table 1. *Lepomis megalotis* genetic diversity, bottleneck inferences (*p*-values), and effective population size estimates per region and site. n = number of fish, A = mean number of alleles, AR = allelic richness, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = inbreeding coefficient, IAM = infinite alleles model, SMM = stepwise mutation model, and N_e = effective population size.

Region/Site	n	A	AR	H_O	H_E	F_{IS}	IAM	SMM	N_e (95% CI)
Below Danville Dam	57	7.05	4.13	0.66	0.67	0.02	0.001	0.979	348 (154–∞)
V_BD1	30	7.18	4.17	0.68	0.68	0.01	0.001	0.924	84 (43–495)
V_BD2	27	6.92	4.08	0.64	0.66	0.02	0.026	0.924	∞ (114–∞)
Above Danville Dam	230	7.42	4.22	0.67	0.69	0.03	0.001	0.983	5286 (1298–∞)
V_P1	27	6.92	4.24	0.68	0.69	0.02	0.007	0.765	76 (41–275)
V_P2	32	7.00	4.24	0.66	0.68	0.03	0.017	0.810	123 (62–951)
V_R1	37	7.67	4.26	0.69	0.69	0.00	0.001	0.961	∞ (205–∞)
V_R2	41	7.58	4.12	0.63	0.67	0.06	0.004	0.935	∞ (358–∞)
NF_BD1	48	7.92	4.30	0.66	0.70	0.06	0.004	0.924	3076 (219–∞)
NF_BD2	45	7.42	4.12	0.67	0.68	0.02	0.001	0.945	314 (121–∞)
Above Ellsworth Dam	139	7.34	4.14	0.66	0.67	0.01	0.005	0.993	1969 (625–∞)
NF_P1	39	7.50	4.18	0.68	0.67	−0.01	0.005	0.945	152 (80–790)
NF_P2	41	7.42	4.10	0.65	0.67	0.02	0.017	0.995	226 (101–∞)
NF_R1	30	7.67	4.20	0.68	0.68	0.00	0.032	0.912	∞ (917–∞)
NF_R2	29	6.75	4.08	0.64	0.67	0.05	0.017	0.924	200 (68–∞)

within sites and linkage disequilibrium between loci pairs. For all significance tests with multiple comparisons, we used an adjusted critical value based on the BY false discovery rate (BY-FDR) method (Narum 2006).

We pooled genetic data from fish from the same sample site collected in different seasons and years after pairwise

F_{ST} analyses, clustering analyses, and Hardy-Weinberg equilibrium analyses did not detect significant differences or deviations (please see below for details of each method). We calculated the number of alleles, expected heterozygosity (gene diversity), observed heterozygosity, and F_{IS} with GENEPOP (version 4.2; Raymond and Rousset 1995).

Table 2. *Pimephales notatus* genetic diversity, bottleneck inferences (*p*-values), and effective population size estimates per region and site. n = number of fish, A = mean number of alleles, AR = allelic richness, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = inbreeding coefficient, IAM = infinite alleles model, SMM = stepwise mutation model, and N_e = effective population size.

Region/Site	n	A	AR	H_O	H_E	F_{IS}	IAM	SMM	N_e (95% CI)
Below Danville Dam	88	14.46	5.35	0.77	0.78	0.01	0.207	0.997	1904 (686–∞)
V_BD1	39	14.25	5.45	0.79	0.78	−0.01	0.103	0.973	285 (141–5740)
V_BD2	49	14.67	5.25	0.75	0.77	0.03	0.207	0.998	30,579 (434–∞)
Above Danville Dam	179	11.74	5.40	0.78	0.79	0.01	0.120	0.998	2216 (1200–12,199)
V_P1	7	6.50	5.33	0.76	0.76	0.00			
V_P2	47	14.00	5.34	0.75	0.78	0.04	0.139	0.994	808 (271–∞)
V_R1	20	11.17	5.44	0.81	0.79	−0.02	0.232	0.768	∞ (134–∞)
V_R2	52	14.83	5.48	0.77	0.80	0.03	0.139	0.997	533 (256–∞)
NF_BD1	22	11.25	5.43	0.81	0.79	−0.03	0.260	0.992	113 (53–∞)
NF_BD2	31	12.67	5.38	0.79	0.78	−0.01	0.062	0.926	∞ (247–∞)
Above Ellsworth Dam	96	8.13	4.93	0.75	0.76	0.01	0.027	0.999	2888 (698–∞)
NF_P1	6	5.67	5.16	0.76	0.80	0.04			
NF_P2	2	2.67		0.73	0.70	−0.03			
NF_R1	36	11.42	5.00	0.76	0.76	0.00	0.074	0.997	225 (111–4185)
NF_R2	52	12.75	4.86	0.75	0.75	0.00	0.062	0.999	809 (265–∞)

To correct for differences in sample sizes, we used the program HP-RARE (version 1.0; Kalinowski 2005), which calculates rarefied allelic richness within sites and within regions separated by dams. To compare the 2 species, we set the number of genes to 10 for both species. *Pimephales notatus* population NF_P2 had <10 genes, so we did not calculate allelic richness for this population. To test for the influence of dams on genetic diversity, we grouped sample sites into 3 regions: below Danville Dam (V_BD1 and V_BD2), above Danville Dam (V_P1, V_P2, V_R1, V_R2, NF_BD1, and NF_BD2), and above Ellsworth Park Dam (NF_P1, NF_P2, NF_R1, and NF_R2). We compared allelic richness, gene diversity, and F_{IS} among these 3 regions with 5000 permutations in FSTAT (version 2.9.3; Goudet 1995, 2001). After we analyzed *P. notatus* genetic structure, we also compared population metrics above and below the Ellsworth Park Dam for both species. We did this comparison by combining the Danville Dam regions into a single region and comparing it to the above Ellsworth Park Dam region.

We tested whether there was evidence of recent genetic bottlenecks within sample sites and regions with the infinite allele (IAM) and stepwise mutation (SMM) models with the Wilcoxon signed-rank test as implemented in BOTTLENECK (version 1.2.02; Cornuet and Luikart 1996, Piry et al. 1999). We also conducted separate analyses for the 2 largest seasonal samples for each species (*P. notatus* in autumn 2014 and autumn 2015; *L. megalotis* in autumn 2013 and spring 2014). We examined genetic bottlenecks for all sites that had ≥10 fish, although results for samples with <30 fish should be interpreted with caution (Piry et al. 1999). We estimated effective population size (N_e) for each sample site with the linkage disequilibrium method (Waples and Do 2010) as implemented in NeEstimator (version 2.1; Do et al. 2014) after removing singleton alleles. To decrease potential biases from genetic structure and any slight temporal variation (Wang et al. 2016), we also calculated N_e estimates separately for single seasons within sites when we sampled ≥10 fish. However, results for samples with <25 fish must be interpreted with caution (Waples and Do 2010).

We used FSTAT (version 2.9.3; Goudet 1995, 2001) to calculate F_{ST}, a measure of genetic differentiation. We quantified the overall significant genetic differentiation with the θ estimator of F_{ST} and 95% confidence intervals (CI) that we obtained by bootstrapping across loci (Weir and Cockerham 1984). We also calculated pairwise F_{ST} estimates between all sample site pairs. We determined significance based on 5,000 replicates. To determine whether dams influenced variation in genetic structure, we pooled sample sites into the 3 regions identified previously (below Danville Dam, above Danville Dam, and above Ellsworth Park Dam) and then used analyses of molecular variance (AMOVA; GenAlEx, version 6.503; Peakall and Smouse 2006, 2012) to test for differences among the 3 regions.

This analysis partitioned genetic variation among the 3 regions (F_{RT}), among sites within regions (F_{SR}), and among sites overall (F_{ST}). We conducted all analyses with missing data interpolated and 9999 permutations. We also analyzed patterns of genetic differentiation with principal components analysis (PCoA) of allele frequencies with the covariance matrix as implemented in GenAlEx (version 6.503; Peakall and Smouse 2006, 2012). We did distance-based RDA (dbRDA) with the *capscale* function in the R package *vegan* to examine isolation-by-distance between linearized F_{ST} ($F_{ST}/1$-F_{ST}) and river distance (R version 3.5; R Project for Statistical Computing, Vienna, Austria; R Development Core Team 2017, Oksanen et al. 2018). This method ordinates given dissimilarity matrices and then analyzes the results with redundancy analysis. We evaluated the significance of the constraining variables on linearized F_{ST} using the *anova.cca* function, an ANOVA-like permutation test (Oksanen et al. 2018).

We used STRUCTURE (version 2.4.3; Pritchard et al. 2000) to infer population differentiation by assigning individual genotypes to populations and probabilistically estimating the number of genetic populations via Bayesian clustering. We ran STRUCTURE with an admixture model with 10 iterations, a burn-in length of 100,000, and 100,000 steps in the Markov chain Monte Carlo (MCMC). We conducted separate runs for each species with both the mean estimated log probability of data ($\ln(P(D))$; Pritchard et al. 2000) and ΔK (Evanno et al. 2005) as the selection criteria and K values ranging from 1 to 12. We explored all potential clustering arrangements for biological implications and interpreted additional patterns that were biologically meaningful (Meirmans 2015).

RESULTS
Genetic diversity
Lepomis megalotis All 12 *L. megalotis* loci were polymorphic. The total number of alleles per locus ranged from 3 to 34 (Table S3), and the mean number of alleles per locus within sites ranged from 6.75 to 7.92 (Table S3). Individual locus observed heterozygosity (H_O) within sites ranged from 0.15 to 1.00 (Table S3). Over all loci, H_O was similar among sampling sites and ranged from 0.63 to 0.69. These values were consistent with the expected heterozygosity (H_E) that ranged from 0.66 to 0.70 (Table 1). We detected significant deviations from Hardy-Weinberg equilibrium in 3 loci, each in only a single sample site (*Leme373* in V_BD1, possible null alleles; *Leme372* in V_R1, possible null alleles; and *Leme279* in NF_R1, heterozygote excess; Table S3). All sample sites conformed to Hardy-Weinberg expectations as estimated by 12 loci. Loci pairs from our entire sample set displayed no significant departures from linkage equilibrium. We found no clear patterns of allelic richness among samples sites or regions separated by dams, and average values ranged from 4.08 to 4.26 among sites

and from 4.13 to 4.22 among regions separated by dams (Tables 1, S3). Neither allelic richness ($p = 0.983$), gene diversity ($p = 0.242$), nor F_{IS} ($p = 0.610$) varied significantly among the 3 regions separated by dams or between sites above and below the Ellsworth Park Dam (allelic richness: $p = 0.972$; gene diversity: $p = 0.117$; F_{IS}: $p = 0.344$).

We did detect evidence of genetic bottlenecks for all 3 regions and all 12 sample sites with the IAM, but not with the SMM (Table 1). Effective population size estimates were typically high, with wide confidence intervals and upper 95% CI approaching infinity, indicating that there is no evidence for variation in genetic characteristics caused by genetic drift (Do et al. 2014; Table 1). We found smaller estimates for V_BD1, V_P1, V_P2, and NF_P1. However, separate N_e seasonal comparisons were not always consistent, and most estimates had large confidence intervals (Table S1). Overall, we did not detect an effect of dams on N_e.

Pimephales notatus   All 12 *P. notatus* loci were polymorphic. The number of alleles at each site ranged from 7 to 51 per locus, and the mean number of alleles per locus within sites ranged from 2.67 to 14.83 (11.17–14.83 in sites with >10 fish sampled; Table S4). Individual locus H_O within sites ranged from 0.00 to 1.00 (0.45–1.00 in sites with >10 fish sampled; Table 2). Over all loci, H_O within sites ranged from 0.73 to 0.81, whereas H_E varied from 0.70 to 0.80 (Table S4). We detected significant deviations from Hardy-Weinberg equilibrium in 3 loci, each in only a single sample site (*Pino234* in V_R1, heterozygote excess; *Pino209* in NF_BD2, potential null alleles; and *Pino222* in NF_R2, potential null alleles; Table S4). All sites conformed to Hardy–Weinberg expectations as estimated by 12 loci. Loci pairs from our entire sample set displayed

no significant departures from linkage equilibrium. Average allelic richness values ranged from 4.86 to 5.48 among sites (with the smallest averages all above the Ellsworth Park Dam) and from 4.93 to 5.40 among regions separated by dams (Table 2). Neither allelic richness ($p = 0.219$), gene diversity ($p = 0.070$), nor F_{IS} ($p = 0.954$) varied significantly among the 3 regions separated by dams. Allelic richness ($p = 0.030$) and gene diversity ($p = 0.006$) were significantly lower above versus below the Ellsworth Park Dam, but F_{IS} did not differ ($p = 0.696$).

We detected a genetic bottleneck above Ellsworth Park Dam with the IAM but not with the SMM. None of the 10 sample sites with the ≥10 individuals necessary to test for bottlenecks displayed significant signals of genetic bottlenecks. However, 2 of the sites above the Ellsworth Park Dam had too few individuals to test and the other 2 had relatively low *p*-values (Table 2). Ne estimates were similar for the 3 regions, and individual sites were characterized by wide confidence intervals that typically included infinity (Tables 2, S1).

Population differentiation

Lepomis megalotis   Global F_{ST} for *L. megalotis* was very low and was significant ($F_{ST} = 0.003$, 95% CI = 0.000–0.006, $p = 0.0174$), indicating that there is genetic heterogeneity within the study area. Four out of 66 pairwise F_{ST} comparisons between sites were significant (Table 3; FDR $\alpha = 0.017$ for 11 comparisons of each population). The AMOVA supported our global F_{ST} findings and indicated that more variation was partitioned within regions than among regions separated by dams ($F_{ST} = 0.003$, $p = 0.014$; $F_{SR} = 0.002$, $p = 0.053$; $F_{RT} = 0.001$, $p = 0.104$). For the PCoA, PC1 and PC2 explained 39% and 27% of the variance in allele frequencies, respectively. Site pairs

Table 3. Bluntnose minnow (above dashes) and *Lepomis megalotis* (below dashes) pairwise F_{ST} comparisons. (*) indicates nominal significance ($p < 0.05$), (**) indicates significance with false discovery rate ($p < 0.017$ for 11 multiple comparisons), NA = not able to determine significance because of small sample size.

Site	V_BD1	V_BD2	V_P1	V_P2	V_R1	V_R2	NF_BD1	NF_BD2	NF_P1	NF_P2	NF_R1	NF_R2
V_BD1	–	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.019	0.010[NA]	0.016**	0.019**
V_BD2	0.001	–	0.007	0.002	0.000	0.000	0.000	0.000	0.016	0.000[NA]	0.010**	0.020**
V_P1	0.002	0.005	–	0.008	0.019	0.004	0.004	0.006	0.034	0.020[NA]	0.018**	0.031**
V_P2	0.000	0.000	0.007*	–	0.000	0.000	0.000	0.000	0.016	0.007[NA]	0.013**	0.014**
V_R1	0.000	0.000	0.007	0.000	–	0.000	0.000	0.001	0.022	0.000[NA]	0.008**	0.016**
V_R2	0.001	0.001	0.006	0.001	0.000	–	0.000	0.001	0.015	0.001[NA]	0.010**	0.015**
NF_BD1	0.000	0.010**	0.006*	0.002	0.002	0.004	–	0.000	0.011	0.010[NA]	0.007**	0.018**
NF_BD2	0.000	0.001	0.000	0.002	0.000	0.000	0.000	–	0.011	0.000[NA]	0.012**	0.015**
NF_P1	0.002	0.007	0.015**	0.004	0.007	0.006**	0.007	0.002	–	0.000[NA]	0.006	0.008
NF_P2	0.001	0.000	0.005	0.001	0.005*	0.006**	0.007	0.003	0.000	–	0.000[NA]	0.000[NA]
NF_R1	0.003	0.008	0.000	0.007	0.006	0.003	0.000	0.003	0.009	0.004	–	0.002
NF_R2	0.005	0.006**	0.011	0.002	0.001	0.005*	0.001	0.001	0.000	0.000	0.009	–

V_P1, NF_R1 and NF_P1, NF_R2 were the most divergent from the remaining sites along the PC1 axis, whereas V_BD2 and NF_BD1 were most divergent along the PC2 axis (Fig. 2A). There was no effect of distance on genetic differentiation revealed by dbRDA ($F_{1,10}$ = 1.335, p = 0.351). STRUCTURE analysis with $\ln(P(i))$ supported the F_{ST} analyses and indicated that only 1 genetic population ($K = 1$) was present throughout the study area. The highest ΔK value was $K = 2$ (Fig. 3A), and we observed a relatively elevated value for $K = 5$ (Fig. 3B). ΔK cannot find the optimum K when $K = 1$ (Evanno et al. 2005), but these scenarios suggest very slight allele frequency differences in several sites, such as V_P1, NF_BD1, and NF_R1.

Pimephales notatus   The global F_{ST} for *P. notatus* was slightly higher than for *L. megalotis* (F_{ST} = 0.006, 95% CI = 0.003–0.010, $p < 0.001$). Sixteen out of 54 possible
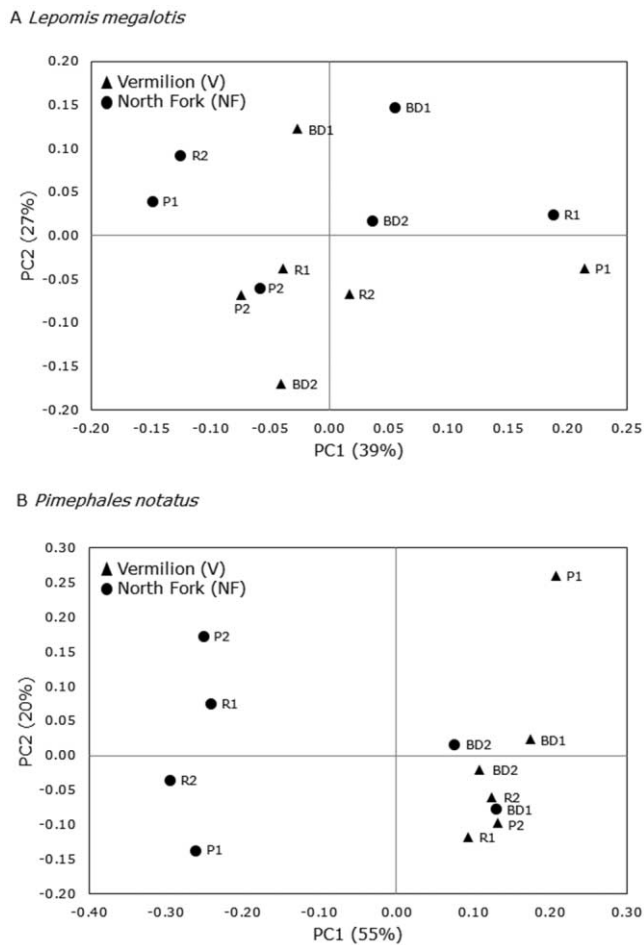

Figure 2. Principal components ordination of allele frequency variation across 12 sample sites for *Lepomis megalotis* (A) and *Pimephales notatus* (B). BD1 and BD2 = below dam sites, P1 and P2 = pool sites, and R1 and R2 = upriver sites (R1, R2) for the Vermilion River (triangles) and North Fork Vermilion River (circles).

pairwise F_{ST} comparisons between sites were significant, though significance for 12 comparisons could not be determined because of small sample sizes within sites (Table 3; FDR α = 0.017). F_{ST} comparisons between sites showed consistent and distinct patterns that indicated fish from sites NF_R1 and NF_R2 were genetically distinct from all sites in the Vermilion River and NF_BD1 and NF_BD2. The sample size of 6 for NF_P1 prohibited pairwise F_{ST} values comparing NF_P1 to other sites from being statistically significant, even though they were some of the highest F_{ST} values obtained (Table 2). Estimates of F_{ST} for NF_P2 were based on only 2 fish, which prevented us from accurately assessing whether this population differed genetically from others. The AMOVA supported our global F_{ST} findings and indicated that more variation was partitioned among regions separated by dams than among sites within those regions (F_{ST} = 0.009, $p < 0.001$; F_{SR} = 0.001, p = 0.222; F_{RT} = 0.008, $p < 0.001$). PC1 and PC2 explained 55% and 20% of the variance in allele frequencies in the PCoA, respectively. Sites NF_P1, NF_P2, NF_R1, and NF_R2 were most divergent along the PC1 axis, whereas V_P1 and NF_P2 were most divergent along the PC2 axis (Fig. 2B). The dbRDA analysis showed that distance had a significant effect on F_{ST} ($F_{1,10}$ = 12.27; p = 0.020). However, this result was driven by comparisons with the 4 divergent sites above the Ellsworth Park Dam, and distance did not significantly influence F_{ST} throughout the rest of the study after we removed those sites ($F_{1,6}$ = 8.04; p = 0.124). STRUCTURE $\ln(P(D))$ and ΔK values both corroborated the pairwise F_{ST} comparisons because these analyses suggested that there are 2 genetic populations in the study area ($K = 2$). Thus, NF_P1, NF_P2, NF_R1, and NF_R2 were genetically distinct from sites downstream of the Ellsworth Park Dam and all sites in the Vermilion River (Fig. 3C). Alternative values of K did not suggest additional genetic structuring throughout the region.

DISCUSSION
Genetic diversity

Fragmentation of river systems by dams can reduce genetic diversity in fish populations above dams (Meldgaard et al. 2003, Yamamoto et al. 2004, Skalski et al. 2008, Junker et al. 2012). However, dams do not always influence genetic diversity, and whether they do depends on multiple factors, including dam permeability, size of the river fragments above and between barriers, and the life span of study species (Reid et al. 2008a, b). In this study, *L. megalotis* and *P. notatus* showed different spatial patterns of genetic diversity (allelic richness and gene diversity). *Lepomis megalotis* genetic diversity was similar throughout the study region and did not indicate an influence of dams, whereas *P. notatus* had lower genetic diversity above than below the Ellsworth Park Dam. Our focal species have relatively similar, short life spans (typical maximum ages of 6 y for
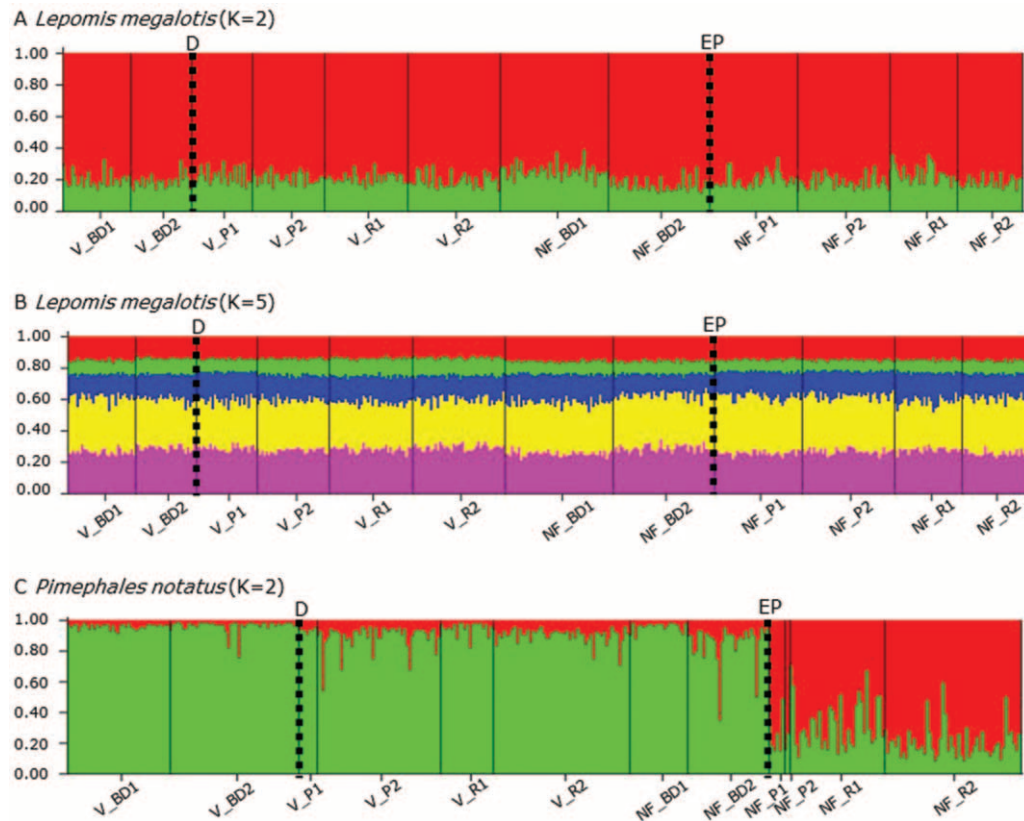
Figure 3. Results of STRUCTURE analyses for *Lepomis megalotis* $K = 2$ (A), *L. megalotis* $K = 5$ (B), and *Pimephales notatus* $K = 2$ (C). Thin lines separate sites, and thick dotted lines indicate locations of dams. D = Danville Dam, EP = Ellsworth Park Dam, BD = below dam, P = pool, and R = upriver. Vertical bars represent the proportion of assignment of individual fish to each cluster represented by a different color.

L. megalotis and 5 y for *P. notatus*; Smith 1979). The absence of genetic diversity patterns among sites regardless of dam presence in *L. megalotis* is therefore probably a result of dam permeability, the size of populations inhabiting the rivers, or both of these factors.

We found that only 1 of the 2 low-head dams influenced the genetic diversity of *P. notatus*. A previous study found that more available habitat increased allelic diversity in a small-bodied cyprinid and a demersal percid, possibly by causing genetic bottlenecks, and that population size and home range influenced genetic diversity (Fluker et al. 2013). Another study found that the genetic diversity of above-barrier Brook Trout (*Salvelinus fontinalis*) was positively related to the size of discrete "patches" of suitable habitat areas, potentially resulting from decreased spawning habitat (Whiteley et al. 2013). In our study area, another low-head dam impounds the river ~2.2 km upstream of the upstream-most North Fork Vermilion River site (NF_R2), and a larger dam impounding Lake Vermilion is located 5.42 km upstream of NF_R2. These additional dams may result in a relatively small area of suitable habitat for *P. notatus* above the Ellsworth Park Dam, which, coupled

with isolation from populations below the dam, may cause the marked decrease in genetic diversity that we observed. Our results may therefore be a result of a relatively general pattern whereby small areas of available habitat in combination with habitat isolation cause allelic diversity to decrease.

We only found evidence for a regional genetic bottleneck in *P. notatus* above the Ellsworth Park Dam with the IAM analysis. In contrast, there was evidence of genetic bottlenecks for *L. megalotis* at all sample sites with the IAM. The ability to detect genetic bottlenecks is highly dependent on the mutational process, and the SMM analysis is more conservative and has lower power to detect bottlenecks than the IAM with highly variable microsatellite loci (Cornuet and Luikart 1996). However, the SMM may be more appropriate for tetra-nucleotide repeats characterized by fewer alleles (Cornuet and Luikart 1996). The intermediate 2-phase model (TPM) produced intermediate results that varied based on the proportion of mutation allocated to each of the IAM and SMM models (data not shown). Given the relatively large number of alleles for most of the loci used in our study, we think that the IAM results are

more accurate. We were unable to determine whether current N_e estimates coincided with genetic bottleneck results because the 95% CIs were too large to provide accurate N_e inferences. We sampled during multiple seasons to increase the robustness of our genetic differentiation analyses, but our results suggest more intensive within-season sampling is necessary to accurately estimate N_e. *Lepomis megalotis* has a limited home range (Berra and Gunning 1972), so it is likely that seasonal fluctuations in water quality or quantity force fish out of the region and cause periodic fluctuations in effective population sizes. These seasonal events could include algal and diatom blooms or the high-water events that occur above the dams. *Pimephales notatus* has higher mobility and habitat tolerance than *L. megalotis*, which may make many of their populations less susceptible to these fluctuations (Smith 1963, Dewey 1981, Jacquemin et al. 2013).

Population differentiation

We did not detect strong genetic differentiation among *L. megalotis* populations in the study area. Low-head dams do not, therefore, appear to isolate *L. megalotis* populations or act as a barrier to their gene flow. Some populations were weakly differentiated, such as V_BD2 and V_P1, but this differentiation is probably caused by *L. megalotis* having a small home range and moving out of its home range seasonally to find shallower waters in warmer months (Berra and Gunning 1972). The seasonal movements of these fish, coupled with the intermittent submergence of these low-head dams, probably prevent genetic differentiation from occurring in the study area.

We observed distinct genetic structuring in *P. notatus*, such that all Vermilion River sites and sites below the Ellsworth Park Dam were genetically distinct from sites above the Ellsworth Park Dam. However, the Danville Dam did not lead to distinct populations, which indicates that some low-head dams are traversable. The genetic structure we observed is therefore probably not because the Ellsworth Park Dam is completely impassable but is instead an indirect result of the effects of the dam on the river habitat. The upstream portion of the Ellsworth Dam has a 0.4-km stretch of poor quality habitat that is characterized by deep water, silty substrate, and little to no surface water velocity above this dam (Smith et al. 2017). The habitat in this reach was the poorest-quality habitat in the study area as classified by the Ohio Qualitative Evaluation Index (Smith et al. 2017), which takes into account multiple habitat metrics (Rankin 2006). This habitat is unlike other reaches of these rivers and is an artifact of the Ellsworth Park Dam.

The small-bodied *P. notatus* may exhibit behavioral avoidance of the poor-quality lentic-type habitat found above the Ellsworth Park Dam, despite their tolerance of a variety of habitats, and avoid passing through such unsuitable habitat in the river. This behavior would isolate them from the *P. notatus* downstream of the Ellsworth Park Dam and in the Vermilion River mainstem. Our sampling results support this hypothesis. We caught very few *P. notatus* in these 2 sites, despite targeted collecting efforts over multiple seasons and years (S. Smith, personal observation). We also observed low abundances of *P. notatus* in the pool site above the Danville Dam (V_P1, $n = 7$), which is also a poor-quality habitat but is shorter than the pool above Ellsworth Park Dam. The population above Danville Dam showed the second-highest differentiation from other *P. notatus* populations, after the isolated population above Ellsworth Park Dam sites (Table 3, Fig. 2B). Previous studies have also found that poor quality habitat can impede fish movement. One of these studies, on Creek Chub (*Semotilus atromaculatus*), found that lentic habitats inhibited gene flow (Hudman and Gido 2013). Additionally, the gene flow of 2 shiner species was impeded by reservoir habitats that are poor corridors for the movement of small-bodied fish (Franssen 2012, Fluker et al. 2013).

Dam- and species-dependent impacts on genetic characteristics

The ability to detect genetic differentiation among fragmented populations is influenced by the effectiveness of the barrier to dispersal, time since population isolation, and species-specific characteristics such as effective population size, generation time, and life span. Species with large population sizes, long life spans, or both may not display detectable genetic differences among populations separated by dams as quickly as shorter-lived species with smaller populations (Hoffman et al. 2017). However, any effects of the dams should be detectable because both of these dams were built in the early 1900s and both focal species have relatively short life spans and generation times.

A key finding of this study is that low-head dams affect the genetic structure of 2 species differently and that different movement abilities and life-history traits influence fish sensitivity to barriers. However, the variation in the sensitivity of a species to dams may not be obvious based on its life history or movement patterns alone. This lack of predictability is underscored by our finding that there is stronger differentiation in *P. notatus*, even though we expected stronger genetic structure in *L. megalotis* because it is a more sedentary species and individuals have smaller home ranges. Another key finding was that 2 similar dams had different effects on genetic structure in *P. notatus*. Thus, our results suggest that fish have a wide range of sensitivity to dams and that small impoundments like low-head dams might not influence genetic diversity or differentiation in some species while leading to differentiation in others. The degree of influence appears to differ based on the ability of a fish species to traverse the structures or the habitat that has been altered by the structures. Therefore, our results suggest that it is essential to include species with different movement behaviors and life-history characteristics in studies that aim to holistically evaluate the effects of instream structures on genetic diversity and differentiation.

ACKNOWLEDGEMENTS

LITERATURE CITED

Alò, D., and T. E. Turner. 2005. Effects of habitat fragmentation on effective population size in the endangered Rio Grande Silvery Minnow. Conservation Biology 19:1138–1148.

Bednarek, A. 2001. Undamming rivers: a review of the ecological impacts of dam removal. Environmental Management 27:803–814.

Berra, T. M., and G. E. Gunning. 1972. Seasonal movement and home range of the Longear Sunfish, *Lepomis megalotis* (Rafinesque), in Louisiana. American Midland Naturalist 88:368–375.

Bessert, M. L., and G. Ortí. 2008. Genetic effects of habitat fragmentation on blue sucker populations in the upper Missouri River (*Cycleptus elongatus* Lesueur, 1918). Conservation Genetics 9:821–832.

Bunn, S. E., and A. H. Arthington. 2002. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Journal of Environmental Management 30:492–507.

Burroughs, B. A., D. B. Hayes, K. D. Klomp, J. F. Hansen, and J. Mistak. 2010. The effects of the Stronach Dam removal on fish in the Pine River, Manistee County, Michigan. Transactions of the American Fisheries Society 139:1595–1613.

Cornuet, J. M., and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014.

Dewey, M. R. 1981. Seasonal abundance, movement, and diversity of fishes in an Ozark stream. Journal of the Arkansas Academy of Science 35:33–39.

Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillett, and J. R. Ovenden. 2014. NeEstimator V2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Molecular Ecology Resources 14: 209–214.

Doyle, M. W., E. H. Stanley, M. A. Luebke, and J. M. Harbor. 2000. Dam removal: physical, biological, and societal considerations. Joint Conference on Water Resources Engineering and Water Resources Planning and Management 2000: Building Partnerships 104:1–10.

Esguícero, A. L. H., and M. S. Arcifa. 2010. Fragmentation of a Neotropical migratory fish population by a century-old dam. Hydrobiologia 638:41–53.

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

Faulks, L. K., D. M. Gilligan, and L. B. Beheregaray. 2011. The role of anthropogenic vs. natural in-stream structures in determining connectivity and genetic diversity in an endangered freshwater fish, Macquarie perch (*Macquaria australasica*). Evolutionary Applications 4:589–601.

Fluker, B. L., B. R. Kuhajda, and P. M. Harris. 2013. The effects of riverine impoundment on genetic structure and gene flow in two stream fishes in the Mobile river basin. Freshwater Biology 59:526–543.

Franssen, N. R. 2012. Genetic structure of a native cyprinid in a reservoir-altered stream network. Freshwater Biology 57:155–165.

Gotoh, R. O., S. Tamate, J. Yokoyama, H. B. Tamate, and N. Hanzawa. 2013. Characterization of comparative genome-derived simple sequence repeats for acanthopterygian fishes. Molecular Ecology Resources 13:461–472.

Goudet, J. 1995. FSTAT (version 1.2): a computer program to calculate *F*-statistics. Journal of Heredity 86:485–486.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). (Available from: http://www2.unil.ch/popgen/softwares/fstat.htm)

Hoffman, J. R., J. R. Willoughby, B. J. Swanson, K. L. Pangle, and D. T. Zanatta. 2017. Detection of barriers to dispersal is masked by long lifespans and large population sizes. Ecology and Evolution 7:9613–9623.

Hudman, S. P., and K. B. Gido. 2013. Multi-scale effects of impoundments on genetic structure of creek chub (*Semotilus atromaculatus*) in the Kansas River basin. Freshwater Biology 58:441–453.

IDNR (Illinois Department of Natural Resources). 2013. Danville Dam and Ellsworth Park Dam modifications. Strategic Planning Study, Vermilion County, Illinois.

Jacquemin, S. J., E. Martin, and M. Pyron. 2013. Morphology of Bluntnose Minnow *Pimephales Notatus* (Cyprinidae) covaries with habitat in a central Indiana watershed. American Midland Naturalist 169:137–146.

Jansson, R., C. Nilsson, and B. Malmqvist. 2007. Restoring freshwater ecosystems in riverine landscapes: the roles of connectivity and recovery processes. Freshwater Biology 52:589–596.

Junker, J., A. Peter, C. E. Wagner, S. Mwaiko, B. Germann, O. Seehausen, and I. Keller. 2012. River fragmentation increases localized population genetic structure and enhances asymmetry of dispersal in bullhead (*Cottus gobio*). Conservation Genetics 13:545–556.

Kalinowski, S. T. 2005. HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Molecular Ecology Notes 5:187–189.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, and A. Drummond. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649.

Landis, J. B., M. J. Grose, E. O. Wiley, and S. P. Hudman. 2009. Characterization of 35 novel microsatellite loci for ecological and evolutionary studies of the Bluntnose Minnow (*Pimephales notatus*). Molecular Ecology Resources 9:864–867.

Meirmans, P. A. 2015. Seven common mistakes in population genetics and how to avoid them. Molecular Ecology 24:3223–3231.

Meldgaard, T., E. E. Nielsen, and V. Loeschcke. 2003. Fragmentation by weirs in a riverine system: a study of genetic variation in time and space among populations of European grayling (*Thymallus thymallus*) in a Danish river system. Conservation Genetics 4:735–747.

Narum, S. R. 2006. Beyond Bonferroni: less conservative analyses for conservation genetics. Conservation Genetics 7:783–787.

Neraas, L. P., and P. Spruell. 2001. Fragmentations of riverine systems: the genetic effects of dams on bull trout (*Salvelinus confluentus*) in the Clark Fork River system. Molecular Ecology 10:1153–1164.

Nislow, K. H., M. Hudy, B. H. Letcher, and E. P. Smith. 2011. Variation in local abundance and species richness of stream fishes in relation to dispersal barriers: implications for management and conservation. Freshwater Biology 56:2135–2144.

Oksanen, J., G. F. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2018. vegan: community ecology package. R package version 2.4-6. (Available from: https://CRAN.R-project.org/package=vegan)

Peakall, R., and P. E. Smouse. 2006. GenALEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295.

Peakall, R., and P. E. Smouse. 2012. GenALEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539.

Pess, G. R., M. L. McHenry, T. J. Beechie, and J. Davies. 2008. Biological impacts of the Elwha River dams and potential salmonid responses to dam removal. Northwest Science 82:72–90.

Peterson, D. P., and W. R. Ardren. 2009. Ancestry, population structure, and conservation genetics of Arctic grayling (*Thymallus arcticus*) in the upper Missouri River, USA. Canadian Journal of Fisheries and Aquatic Sciences 66:1758–1774.

Piry, S., G. Luikart, and J.-M. Cornuet. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity 90:502–503.

Pringle, C. 2003. What is hydrologic connectivity and why is it ecologically important? Hydrological Processes 17:2685–2689.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959. http://pritchardlab.stanford.edu/publications/pdfs/PritchardEtAl00.pdf

Rankin, E. T. 2006. Methods for assessing habitat in flowing waters: using the qualitative habitat evaluation index (QHEI). State of Ohio Environmental Protection Agency, Groveport, Ohio.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249.

R Development Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org

Reid, S. M., C. C. Wilson, L. M. Carl, and T. G. Zorn. 2008a. Species traits influence the genetic consequences of river fragmentation on two co-occurring redhorse (Moxostoma) species. Canadian Journal of Fisheries and Aquatic Sciences 65:1892–1904.

Reid, S. M., Wilson, C. C., Mandrak, N. E. and L. M. Carl. 2008b. Population structure and genetic diversity of black redhorse (*Moxostoma duquesnei*) in a highly fragmented watershed. Conservation Genetics 9:531–546.

Schaefer, J. F., W. I. Lutterschmidt, and L. G. Hill. 1999. Physiological performance and stream microhabitat use by the centrarchids *Lepomis megalotis* and *Lepomis macrochirus*. Environmental Biology of Fishes 54:303–312.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature Biotechnology 18:233–234.

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

Smith, P. W. 1963. A study of seasonal distribution of fishes in the Kaskaskia River ditch, a highly modified stream in eastern Illinois. Copeia 1963:251–259.

Smith, P. W. 1979. The fishes of Illinois. University of Illinois Press, Chicago.

Smith, S. C. F., T. Thomas, R. Hastings, S. J. Meiners, and R. E. Colombo. 2017. Low-head dam impacts on habitat and the functional composition of fish communities. River Research and Applications 33:680–689.

Stamford, M. D., and E. B. Taylor. 2005. Population subdivision and genetic signatures of demographic changes in Arctic grayling (*Thymallus arcticus*) from an impounded watershed. Canadian Journal of Fisheries and Aquatic Sciences 62:2548–2559.

USACE (United States Army Corps of Engineers). 2013. National inventory of dams database. (Available from: http://nid.usace.army.mil/cm_apex/f?p=838:3:0::NO)

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535–538.

Walsh, P. S., D. A. Metzger, and R. Higuchi. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506–513.

Wang, J., E. Santiago, and A. Caballero. 2016. Prediction and estimation of effective population size. Heredity 117:193–206.

Waples, R. S., and C. Do. 2010. Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Evolutionary Applications 3:244–262.

Ward, J. V., and J. D. Stanford. 1995. Ecological connectivity in alluvial river systems and its disruption by flow regulation. River Research and Applications 11:105–119.

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

Wells, S. M., and J. M. Haynes. 2007. Status of the Longear Sunfish, *Lepomis megalotis*, in Western New York, USA. Final Report for State Wildlife Grant (SWG) T-5, Project 2, MOU-5292, New York State Department of Environmental Conservation.

Whiteley, A. R., J. A. Coombs, M. Hudy, Z. Robinson, A. R. Colton, K. H. Nislow, and B. H. Letcher. 2013. Fragmentation and patch size shape genetic structure of brook trout populations. Canadian Journal of Fisheries and Aquatic Sciences 70:678–688.

Whiteley, A. R., P. Spruell, B. E. Rieman, and F. W. Allendorf. 2006. Fine-scale genetic structure of bull trout at the southern limit of their distribution. Transactions of the American Fisheries Society 135:1238–1253.

Yamamoto, S., K. Morita, I. Koizumi, and K. Maekawa. 2004. Genetic differentiation of white-spotted char (*Salvelinus leucomaenis*) populations after habitat fragmentation: spatial-temporal changes in gene frequencies. Conservation Genetics 5:529–538.

Yurco, P., and D. B. Keeney. 2018. Characterization of tri and tetra-nucleotide microsatellite loci for the freshwater snails *Promenetus exacuous* (Planorbidae) and *Valvata tricarinata* (Valvatidae) and their utility in population genetic studies. BMC Research Notes 11:204.