Accepted: 15 March 2018
DOI: 10.1111/fwb.13106

ORIGINAL ARTICLE

WILEY Freshwater Biology

Spatial extent of analysis influences observed patterns of population genetic structure in a widespread darter species (Percidae)

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Funding information
Virginia Department of Game and Inland Fisheries; U.S. Department of Agriculture Hatch Program; Virginia Department of Mines, Minerals, and Energy; U.S. Department of Agriculture National Institute for Food and Agriculture; U.S. Geological Survey; Virginia Tech

Abstract

1. Connectivity among stream fish populations allows for exchange of genetic material and helps maintain genetic diversity, adaptive potential and population stability over time. Changes in species demographics and population connectivity have the potential to permanently alter the genetic patterns of stream fish, although these changes through space and time are variable and understudied in small-bodied freshwater fish.
2. As a spatially widespread, common species of benthic freshwater fish, the variegate darter (*Etheostoma variatum*) is a model species for documenting how patterns of genetic structure and diversity respond to increasing isolation due to large dams and how scale of study may shape our understanding of these patterns. We sampled variegate darters from 34 sites across their range in the North American Ohio River basin and examined how patterns of genetic structure and diversity within and between populations responded to historical population changes and dams within and between populations.
3. Spatial scale and configuration of genetic structure varied across the eight identified populations, from tributaries within a watershed, to a single watershed, to multiple watersheds that encompass Ohio River mainstem habitats. This multiwatershed pattern of population structuring suggests genetic dispersal across large distances was and may continue to be common, although some populations remain isolated despite no apparent structural dispersal barriers. Populations with low effective population sizes and evidence of past population bottlenecks showed low allelic richness, but diversity patterns were not related to watershed size, a surrogate for habitat availability. Pairwise genetic differentiation (F_{ST}) increased with fluvial distance and was related to both historic and contemporary processes. Genetic diversity changes were influenced by underlying population size and stability, and while instream barriers were not strong determinants of genetic structuring or loss of genetic diversity, they reduce population connectivity and may impact long-term population persistence.
4. The broad spatial scale of this study demonstrated the large spatial extent of some variegate darter populations and indicated that dispersal is more extensive than expected given the movement patterns typically observed for small-bodied, benthic fish. Dam impacts depended on underlying population size and stability,

with larger populations more resilient to genetic drift and allelic richness loss than smaller populations.

5. Other darters that inhabit large river habitats may show similar patterns in landscape-scale studies, and large river barriers may impact populations of small-bodied fish more than previously expected. Estimation of dispersal rates and behaviours is critical to conservation of imperilled riverine species such as darters.

KEYWORDS
large rivers, population connectivity, population resilience, stream fish, variegate darter

1 | INTRODUCTION

Disruptions of hydrologic connectivity affect river ecosystems and fish populations by changing species distributions and community dynamics, decreasing species movement and dispersal, and impeding nutrient and energy transfer (Fullerton et al., 2010), all of which can contribute to species imperilment and loss (Helfman, 2007; Jelks et al., 2008). The scope and nature of such effects on the conservation status of small-bodied and non-game species are not well understood, particularly in large rivers. Species responses to disrupted hydrologic connectivity would be better determined by understanding current and past population status, which is affected by factors including population size, growth potential, distribution, genetic structure and diversity, and dispersal rates.

Population parameter estimation has progressed using traditional survey data and techniques, including capture–recapture studies, species distribution mapping and occupancy modelling (e.g. Cao, Hinz, Metzke, Stein, & Holtop, 2016), but these are data-intensive and usually done at small extents. Further, these estimates typically are limited to current (not historical) population status. Hence, the demographic status of most species is unknown or only partially understood. Population genetics provides powerful, though underemployed tools (Bowman et al., 2016; Keller, Holderegger, van Strien, & Bolliger, 2015) for assessing population size, stability and genetic differentiation. The coupling of population and landscape genetics can clarify the impacts of landscape change on population structure, diversity, persistence and connectivity (Manel & Holderegger, 2013), thereby making species conservation planning and management more effective (Keller et al., 2015).

Fish population connectivity and genetic diversity and differentiation are driven by multiscale processes—both natural and anthropogenic. Key natural processes and factors include glaciation and deglaciation (Beneteau, Mandrak, & Heath, 2009; Boizard, Magnan, & Angers, 2009; Switzer, 2004; Turner & Trexler, 1998), drainage capture, river network structure and interconnectedness (Mullen, Woods, Schwartz, Sepulveda, & Lowe, 2010) and isolation by waterfalls or uninhabitable areas (Bessert & Orti, 2008; Boizard et al., 2009). Anthropogenic landscape changes such as habitat loss and barriers to movement may alter patterns of genetic structure and diversity (Blanchet, Rey, Etienne, Lek, & Loot, 2010; Johnson, Mitchell, & Harp, 2006), but such impacts on small-bodied fish are not well studied. Large population sizes and connectivity among populations help to mediate these genetic alterations, but genetic drift is common in populations with artificially limited dispersal, as caused by habitat fragmentation or loss (Bessert & Orti, 2008; Roberts, Angermeier, & Hallerman, 2013). Anthropogenic barriers (e.g. dams, reservoirs, culverts and degraded habitat) can impede fish dispersal and consequent isolation can cause the loss of rare alleles or random changes in allele frequencies, thereby increasing between-population genetic differentiation (Beneteau et al., 2009; Bessert & Orti, 2008; Haponski, Marth, & Stepien, 2007; Junker, Peter, Wagner, Mwaiko, & Germann, 2012; Roberts et al., 2013). Barriers also may alter the frequency and directionality of movement. For example, instream barriers eliminated upstream, but not downstream genetic dispersal in European bullhead (Cottidae: *Cottus gobio*), which suggests an important population-level role for passive dispersal of larvae across barriers (Junker et al., 2012).

In stream systems, the conceptual model of isolation by distance (IBD) (Rousset, 1997) is commonly used to estimate dispersal across sites from genetic differentiation (F_{ST}), as genetic differentiation is a function of dispersal of individuals across a certain distance. Observed versus expected patterns of differentiation can be compared to infer the relative importance of natural versus anthropogenic processes and factors (Crookes & Shaw, 2016), which then can inform conservation and management decisions. Determining patterns of genetic diversity and structure typical of stream fish populations can help distinguish natural versus human-induced changes in population genetic patterning and inform such decisions.

The spatial extent chosen for a population genetics study is crucial because it may impact study conclusions and management recommendations. For example, failing to sample enough individuals or populations can lead to a biased analysis or results that lack proper evolutionary context (Cushman & Landguth, 2010). However, studies of the landscape ecology of fish may be performed at an extent of specific management interest (e.g. within watershed or political boundaries) instead of the most relevant ecological extents, particularly given funding limitations or lack of ecological baseline information for understudied species, particularly dispersal and early life-history traits (Frimpong & Angermeier, 2009). Designing studies to match or exceed the dispersal ability of individuals (Anderson et al.,

2010; Keller et al., 2015) is especially problematic when dispersal distances are unknown, and arbitrary study extents may not capture underlying variability in genetic diversity, which limits clear assessment of anthropogenic impacts.

The literature on landscape-level genetics of freshwater fish focuses on three groups: game and commercial species, narrow-range and headwater species (e.g. Brogdon, Tabit, & Kral, 2003) and big-river migrants (Bessert & Orti, 2008). Many genetic studies of North American darters (Percidae) focus on rare species with small ranges, recent demographic bottlenecks or other features that diminish genetic diversity (e.g. Fluker, Kuhajda, Lang, & Harris, 2010). Collectively, these studies exclude most lotic fish and may bias current understanding of how their populations are structured. Study of genetic patterns in widely distributed and locally abundant species can elucidate typical genetic diversity patterns and the spatial scales of study appropriate for other species, particularly those that are understudied or imperilled (Galbraith, Zanatta, & Wilson, 2015).

Genetic diversity and differentiation of darter populations are linked to lifetime movement of individuals, which is difficult to measure with traditional methods (e.g. mark–recapture or radio telemetry) because of low recapture rates (Roberts & Angermeier, 2007) and small body size. Single-season mark–recapture studies indicate that individuals typically have small home ranges, although long-distance movements are extremely difficult to detect (Albanese, Angermeier, & Gowan, 2003; Roberts & Angermeier, 2007). The median (and maximum) extents of such documented movements vary among species, but range from 11 m (284 m) in fantail darter (Percidae: *Etheostoma flabellare*) (citations in Schwalb, Poos, & Ackerman, 2011) to 275 m (3,200 m) in Roanoke logperch (Percidae: *Percina rex*) (Roberts & Angermeier, 2007). However, these likely underestimate lifetime movement distances; for example, analyses of genetic markers show that juvenile Roanoke logperch can disperse at least 55 km/year (Roberts, Angermeier, & Hallerman, 2016). Movement studies typically focus on adults, whereas larval or juvenile dispersal could mediate dispersal between populations even if adults are sedentary (Fluker, Kuhajda, & Harris, 2014; Roberts et al., 2016). However, knowledge of larval and juvenile movement is lacking for most lotic fish species, including darters (Simon & Wallace, 2006).

The variegate darter (Percidae *Etheostoma variatum*) is a good model for examining landscape patterns of population genetics of widespread fish species. It is a small-bodied benthic riverine fish found in high-gradient riffles in medium and large streams and rivers throughout the upper Ohio River basin (Figure 1). Variegate darters spawn in riffles (May, 1969) and eggs are buried in sand pockets behind rocks and boulders. After hatching, larvae are pelagic and can be caught in surface plankton nets, indicating downstream drift. Young settle to the bottom within 6 weeks of hatch (18–26 mm) (J. E. Argentina, unpublished data; Simon & Wallace, 2006), although total duration of the drift period is unknown. Age 1+ darters are indistinguishable from older darters on the basis of standard length (Argentina, Angermeier, & Hallerman, 2013). Both sexes mature at age 2; maximum lifespan is 4 years (Lachner, Westlake, & Handwerk, 1950). Watersheds supporting variegate darters vary widely in size, connectivity to other watersheds and the extent of impacts including coal mining, agriculture, urbanisation and instream barriers over the past 100 years (Thomas, Emery, & McCormick, 2004). In small isolated watersheds, we expect small population sizes and low genetic diversity, although connectivity to other populations may mitigate these effects. In larger watersheds with greater habitat availability, we expect larger populations that can maintain genetic diversity, particularly if these watersheds are connected to others via fish dispersal. We expect decreasing connectivity and/or migration between sites to alter genetic diversity and structure, even where ample available habitat (and presumably large populations) exist.

Our objectives in this range-wide analysis of variegate darter population genetics were threefold: (1) to estimate and compare variation in genetic diversity and differentiation among populations, (2) to determine whether and how instream barriers (i.e. dams) between sites affect genetic structure and diversity through reduction in dispersal of breeders and (3) to examine how the spatial extent of analysis and population size and stability affect results and conclusions germane to the first two objectives. We use the broad spatial scale of this study to contextualise results because each watershed or population can be assessed separately.

2 | METHODS

2.1 | Field study design

We collected and analysed genetic material from tissue samples of adult variegate darters from 34 sites across its range (upper Ohio River basin) within most watersheds where they occur, from Indiana to New York (Figure 1, Table 1). We selected sampling sites to represent most major watersheds where variegate darters occur extensively, including the upper Allegheny River (sites 1–7), Monongahela River (site 9), Little Beaver Creek (site 10), Elk River (sites 11–12), Big Sandy River (sites 13–26), Tygarts Creek (site 27), Scioto River (sites 28–29), Little Miami River (site 30), Whitewater River (sites 31–32) and Kentucky River (sites 33–34). Site localities are described in Appendix S1. Samples were collected between 1987 and 2011, with most collected 2008–2011 (Appendix S1). Dams occur throughout the upper Ohio drainage, and lock-and-dams are particularly pervasive across the mainstem Ohio and Kentucky rivers. Chosen sample sites are isolated by these and other instream structures (Figure 1). Most barriers (mainstem Ohio, Lower Allegheny, Monongahela, Kanawha, Big Sandy and Kentucky rivers) are lock-and-dam structures, while upper Allegheny, lower Cheat (Monongahela drainage), Elk (Kanawha drainage) and Levisa (Big Sandy drainage) barriers are flood control or hydropower dams (Figure 1). The barrier on French Creek is a run-of-the-river dam that allows for continuous flow beneath the structure but creates a reservoir during high flows. While lock-and-dams are not complete barriers, we do not expect upstream passage and dispersal of the variegate darter given upstream habitats are maintained as deep, lotic environments to allow movement of large shipping vessels. Variegate darters are not commonly detected during fish surveys in large rivers with lock-and-

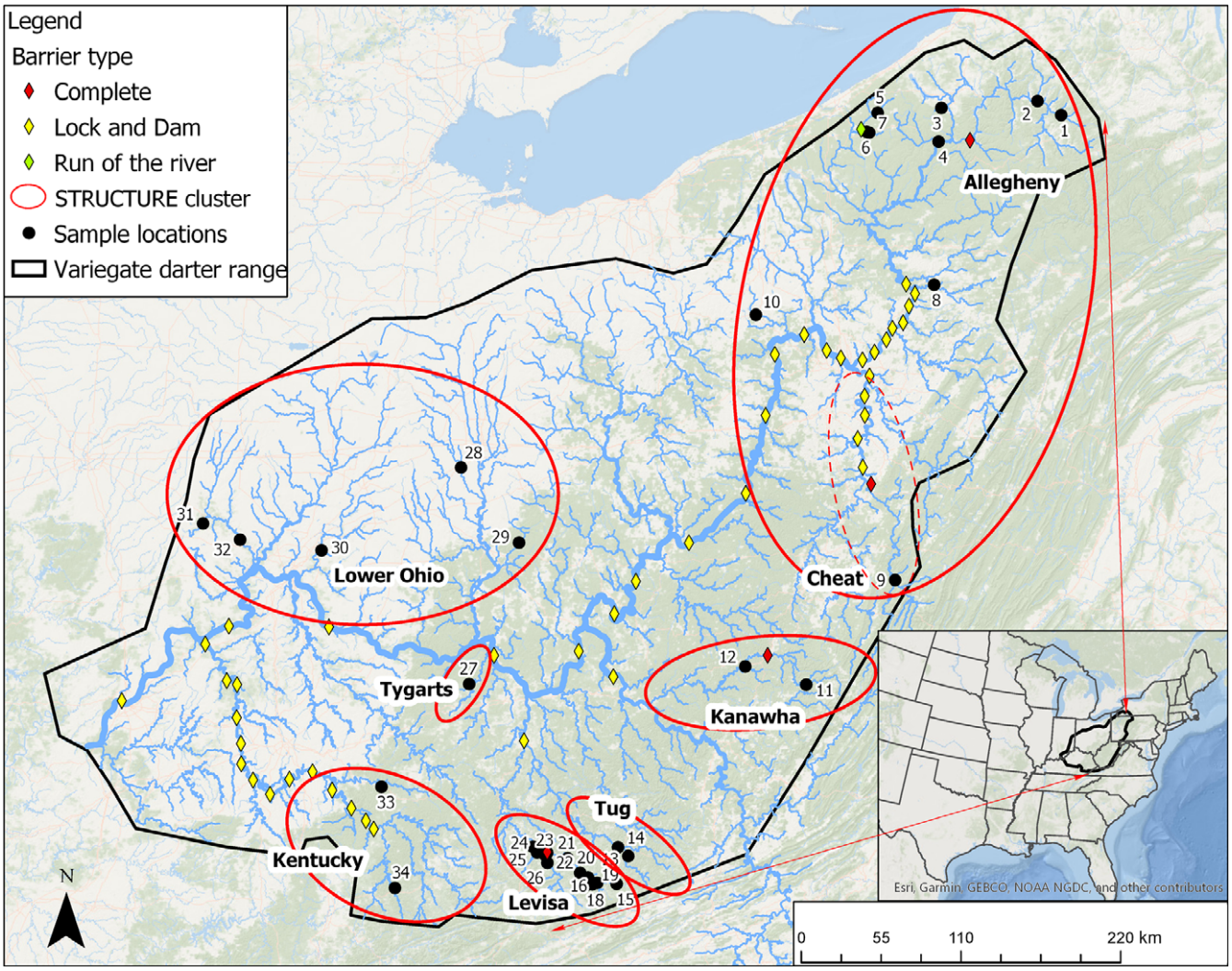

FIGURE 1 All major dams between sites and genetic sample locations across the upper Ohio River basin. Seven population clusters identified by STRUCTURE analysis (Pritchard et al., 2000) are shown as red ellipses; the dashed ellipse indicates potential sites of population structuring within the upper Ohio River. Sample locations are provided in Table 1

dams, and appear to be extremely rare in these habitats (Argent & Kimmel, 2014; Koryak, Bonislawsky, Locy, & Porter, 2008; Pearson & Krumholz, 1984).

Collection methods varied among sites. At sites in the upper Big Sandy and Elk River watersheds, adult fish were anaesthetised with MS-222, and a 5-mm × 5-mm section of tissue was removed from the caudal fin and stored in labelled envelopes. Collaborating researchers (see Acknowledgements) collected other samples and provided either small tissue or whole-fish samples stored in 95% ethanol. Samples were air-dried for 2–4 days, then stored at −20°C until DNA extraction. Sample sizes were 2–43 individuals per site, but were generally 20–30 per site (Appendix S1).

2.2 | Molecular markers

We used a Qiagen Gentra Puregene Tissue kit to extract DNA from preserved fin clips using the manufacturer's protocol. Genomic DNA was quantified and diluted to 50 ng/μl with biotechnology-grade water.

For all individuals processed at Virginia Tech, we selected DNA primers for 14 microsatellite loci (Switzer, Welsh, & King, 2008) and developed three multiplexes for cost-effectively scoring the collection of microsatellite loci of each individual. Forward primers were labelled with one of four fluorescent dyes: FAM, NED, PET or VIC (Applied Biosystems Inc., Foster City, CA, U.S.A.). Reverse primers were procured from Integrated DNA Technologies (Coralville, IA, U.S.A.). For multiplex one, markers were amplified in 10-μl reactions in the following reagent mix: 1 μl of 10 × ExTaq buffer (TaKaRa Bio, Inc. Otsu, Shiga, Japan); 0.8 μl of 2.5 mM each ExTaq pre-mixed dNTPs; 0.1 μl each of 20 μM *EosC6*, *EosC108*, *EosD10* and *EosC2* forward and reverse primers; 0.3 μl each of 20 μM *EosD107* forward and reverse primers; 0.1 μl of 5 units/μl Ex*Taq* polymerase (TaKaRa Bio, Inc., Otsu, Shiga, Japan); and 2 μl of 50 ng/μl template DNA. For multiplex two, markers were amplified in 10-μl reactions in the following reagent mix: 1 μl of 10× Ex*Taq* buffer; 0.8 μl of 2.5 mM each Ex*Taq* dNTPs (pre-mixed); 0.1 μl each of 20-μM *EosC112*, *EosC116*, *EosC208*, *EosD108* and *EosC207* forward and reverse

TABLE 1 Site-level values of rarefied allelic richness (A_r), observed heterozygosity (H_o), and expected heterozygosity (H_e) and corresponding site number, river names and population cluster (and subwatersheds) are shown. Standard deviations (*SD*) are shown in parentheses. Dash entries indicate Levisa sites 22–24 that were combined for analysis. Corresponding site locations and sampling dates are provided in Appendix S1. "S." = South

Site	River	Population cluster	Sample size	A_r	H_o (*SD*)	H_e (*SD*)
1	Bell Run	Allegheny	12	7.29	0.694 (0.27)	0.745 (0.24)
2	Allegheny	Allegheny	20	6.75	0.677 (0.25)	0.716 (0.26)
3	Stillwater	Allegheny	20	6.92	0.724 (0.23)	0.723 (0.24)
4	Brokenstraw	Allegheny	30	7.18	0.759 (0.24)	0.741 (0.24)
5	French	Allegheny	19	7.10	0.756 (0.22)	0.783 (0.19)
6	S. Branch French	Allegheny	33	6.96	0.725 (0.28)	0.726 (0.25)
7	S. Branch French	Allegheny	8	6.07	0.779 (.018)	0.790 (0.15)
8	Mahoning	Allegheny	8	5.79	0.788 (0.218)	0.782 (0.13)
9	Cheat	Monongahela	4	3.43	0.604 (0.368)	0.685 (0.19)
10	Middle Fork Beaver	Allegheny	4	4.64	0.768 (0.23)	0.773 (0.19)
11	Back Fork Elk	Kanawha	23	5.56	0.658 (0.25)	0.668 (0.25)
12	Birch	Kanawha	21	6.50	0.677 (0.26)	0.687 (0.25)
13	Dry Fork	Tug	31	6.27	0.743 (0.19)	0.734 (0.18)
14	Tug	Tug	17	6.03	0.710 (0.27)	0.735 (0.20)
15	Dismal	Levisa	2	2.43	0.875 (0.23)	0.792 (0.21)
16	Dismal	Levisa	2	2.36	0.750 (0.34)	0.778 (0.19)
17	Slate	Levisa	15	6.27	0.728 (0.21)	0.721 (0.22)
18	Levisa	Levisa	19	6.48	0.737 (0.26)	0.717 (0.24)
19	Levisa	Levisa	10	6.29	0.778 (0.14)	0.798 (0.12)
20	Levisa	Levisa	33	6.44	0.693 (0.26)	0.706 (0.25)
21	Levisa	Levisa	40	6.59	0.729 (0.22)	0.731 (0.21)
22	Levisa	Levisa	3	6.86	0.806 (.30)	0.844 (0.19)
23	Levisa	Levisa	36	–	0.736 (0.27)	0.731 (0.26)
24	Levisa	Levisa	18	–	0.794 (0.20)	0.783 (0.18)
25	Levisa	Levisa	10	6.93	0.777 (0.30)	0.740 (0.27)
26	Russell	Levisa	43	6.83	0.712 (0.26)	0.723 (0.26)
27	Tygarts	Tygarts	15	4.35	0.692 (0.20)	0.676 (0.17)
28	Big Darby	Lower Ohio (Scioto)	34	7.29	0.767 (0.19)	0.763 (0.21)
29	Salt	Lower Ohio (Scioto)	9	6.71	0.767 (0.21)	0.770 (0.21)
30	Little Miami	Lower Ohio	30	6.75	0.733 (0.20)	0.775 (0.20)
31	Salt	Lower Ohio (Whitewater)	5	5.36	0.787 (0.27)	0.757 (0.26)
32	Whitewater	Lower Ohio (Whitewater)	5	5.64	0.757 (0.25)	0.788 (0.24)
33	Middle Fork Red	Kentucky	7	6.21	0.747 (0.17)	0.830 (0.13)
34	Redbird	Kentucky	10	7.29	0.755 (0.20)	0.782 (0.20)

primers; 0.1 μl of 5 units/μl Ex*Taq* polymerase; and 2 μl of 50 ng/μl template DNA. For multiplex three, markers were amplified in 10-μl reactions in the following reagent mix: 1 μl of 10× Ex*Taq* buffer; 0.8 μl of 2.5 mM each Ex*Taq* dNTPs; 0.1 μl each of 20 μM *EosD11*, *EosC117*, *EosC3* and *EosC124* forward and reverse primers; 0.1 μl of 5 units/μl Ex*Taq* polymerase; and 2 μl of 50 ng/μl template DNA. Polymerase chain reactions were conducted in a MyCycler Thermal Cycler (Bio-Rad, Hercules, CA, U.S.A.) using an initial denaturation step (94°C, 90 s), followed by 30 cycles of denaturation (94°C, 30 s), annealing (55°C, 30 s) and extension (72°C, 45 s), with final extension (74°C, 10 min). Amplification products were separated in an ABI 3130 automated sequencer at the Virginia Biocomplexity Institute at Virginia Tech, which used a Genescan LIZ500 (Applied Biosystems Inc., Foster City, CA, U.S.A.) size standard. GeneMapper 3.5 (Applied Biosystems Inc., Foster City, CA, U.S.A.) was used to observe and score the amplification products. Raw fragment sizes were scored as allele sizes in base-pairs by eye.

We also included microsatellite data from 87 individuals from sites 1, 7–10, 27, 29 and 31–34 (Appendix S1), analysed for a separate study (Switzer, Welsh, & King, 2007). These data were corrected

for M13 primer addition and were cross-checked at each allele using individuals collected at the same site.

2.3 | Data analysis

We analysed data for 596 individuals from 34 sites (Figure 1). We evaluated rarefied allelic richness (A_r) over all loci standardised to 20 genes from each sample and one sample from each population (from genetic structure analysis), using HP-Rare 1.1 (Kalinowski, 2005) to account for sample size differences among sites, allowing more accurate comparison among sites and groups (Kalinowski, 2005).

We investigated genetic diversity within and genetic structure among populations using multiple methods. We used program ARLEQUIN 3.11 (Excoffier, Laval, & Schneider, 2006) to calculate mean expected (H_e) and observed (H_o) heterozygosities, test for Hardy–Weinberg equilibrium for each marker in each site group and to estimate between-site divergence (F_{ST}) between populations. The Hardy–Weinberg test used 10^5 burn-in steps followed by 10^6 steps in the Markov chain, and results were evaluated using a sequential Bonferroni adjusted $\alpha = 0.05$. Tests of observed genotype frequencies against Hardy–Weinberg expectations indicated no evidence of disequilibrium, so we included all loci in all analyses.

We used Pearson correlation coefficients between watershed area of the sample sites and genetic diversity metrics to test for effects of potential habitat availability or underlying spatial effects on population-level diversity. We investigated population genetic structure using program STRUCTURE (Pritchard, Stephens, & Donnelly, 2000) to assess multilocus genotypic data for evidence of distinct clusters within and across watersheds. STRUCTURE uses a Bayesian clustering algorithm to infer number of clusters (K) and to assign probabilities that individuals originate from particular population cluster within the K clusters available in a given iteration of the test. We analysed data for genetic structure at two spatial extents (watershed and range-wide) to examine interscale differences, both in general terms and in response to any effects of dams on population isolation and structuring in the two focal watersheds. Uneven sampling can affect the observed number of populations and the assignment of populations with smaller sample sizes (Puechmaille, 2016). To account for this effect, we randomly subsampled a maximum of 20 individuals from each sampled site. Once putative populations were assigned, we also subsampled from these to even the number of individuals from each population so that we could assess differences among assignments. We investigated models with $K = 1$–10 populations in the Levisa Fork watershed and upper Allegheny watershed, and $K = 1$–22 populations across the entire range of variegate darter. We plotted the largest log-likelihood values from each of the ten iterations of the model and selected the top model as that with the peak likelihood. The most likely number of populations (K) for range-wide and watershed analyses was selected using the maximum likelihood (STRUCTURE) and the change in likelihood over successive K values (Evanno, Regnaut, & Goudet, 2005); both methods returned the same result.

We assessed historical and contemporary changes to population sizes by estimating N_e, the current effective number of breeders, and by calculating the m-ratio (Garza & Williamson, 2001), the mean ratio of the number of alleles to the range in allele size. We estimated contemporary effective number of breeders (N_e) via program NeEstimator (Do et al., 2013) using the linkage disequilibrium model assuming random mating and an allelic cut-off of 0.05 to estimate N_e. Confidence intervals (95%) were estimated using the parametric method. N_e was estimated for clusters (as grouped by the STRUCTURE analysis results) to increase the number of individuals used for each estimate and to decrease confidence intervals around the estimates. The m-ratio (Garza & Williamson, 2001) can be used to detect historical bottlenecks in a population through a decrease in the ratio of average locus allelic diversity to allele size range; the ratio declines during bottlenecks because decreasing abundance leads to loss of rare alleles, while the range of allele sizes remains constant. We used program *M* Ratio (Garza & Williamson, 2001) to calculate the average m-ratio for each population, using the default values of pre-bottleneck theta (4), average size of mutations that are not one-step mutations (3.5) and proportion of time that mutations are not one-step (0.2), and the suggested cut-off value of 0.68 to assess evidence of historical bottlenecks in each population. We assumed m-ratios >0.68 indicate populations in a drift-to-mutation-rate equilibrium, while m-ratios <0.68 indicate that historic bottlenecks have occurred (Garza & Williamson, 2001).

2.4 | Genetic structure and analysis of spatial scale impacts

We used program TreeFit (Kalinowski, 2009) to estimate phylogenetic distances D_a (Nei et al., 1983) between population clusters. We visualised genetic relationships among population clusters using both the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and the neighbour-joining (NJ) methods for population tree construction, each bootstrapped 10,000 times. The trees were displayed using FigTree 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree). Outcomes of the two tree reconstruction algorithms (UPGMA and NJ) were examined for consistency and clarity between known a priori information about connectivity between sample locations and the results of STRUCTURE analysis to select the best tree for analysis.

We assessed intersite distances and barriers, so we could test the validity of our two conceptual models. Distances between sites were calculated using fluvial distance based on the NHDPlusV2 hydroline data set in ArcGIS 10.4 (ESRI, Redlands, CA). Large dams on all sampled streams were identified to determine location and type of physical barriers between sites (Figure 1). We conducted an analysis of IBD across all sample sites to investigate patterns of genetic differentiation among sites. F_{ST} was linearised as $F_{ST}/1 - F_{ST}$ (Rousset, 1997). We tested for correlations between fluvial distance and F_{ST} using Mantel tests in program PASSaGE (Rosenberg & Anderson, 2011). We used partial Mantel tests to test for correlations between numbers of dams between sites and F_{ST} given fluvial distance between sites.

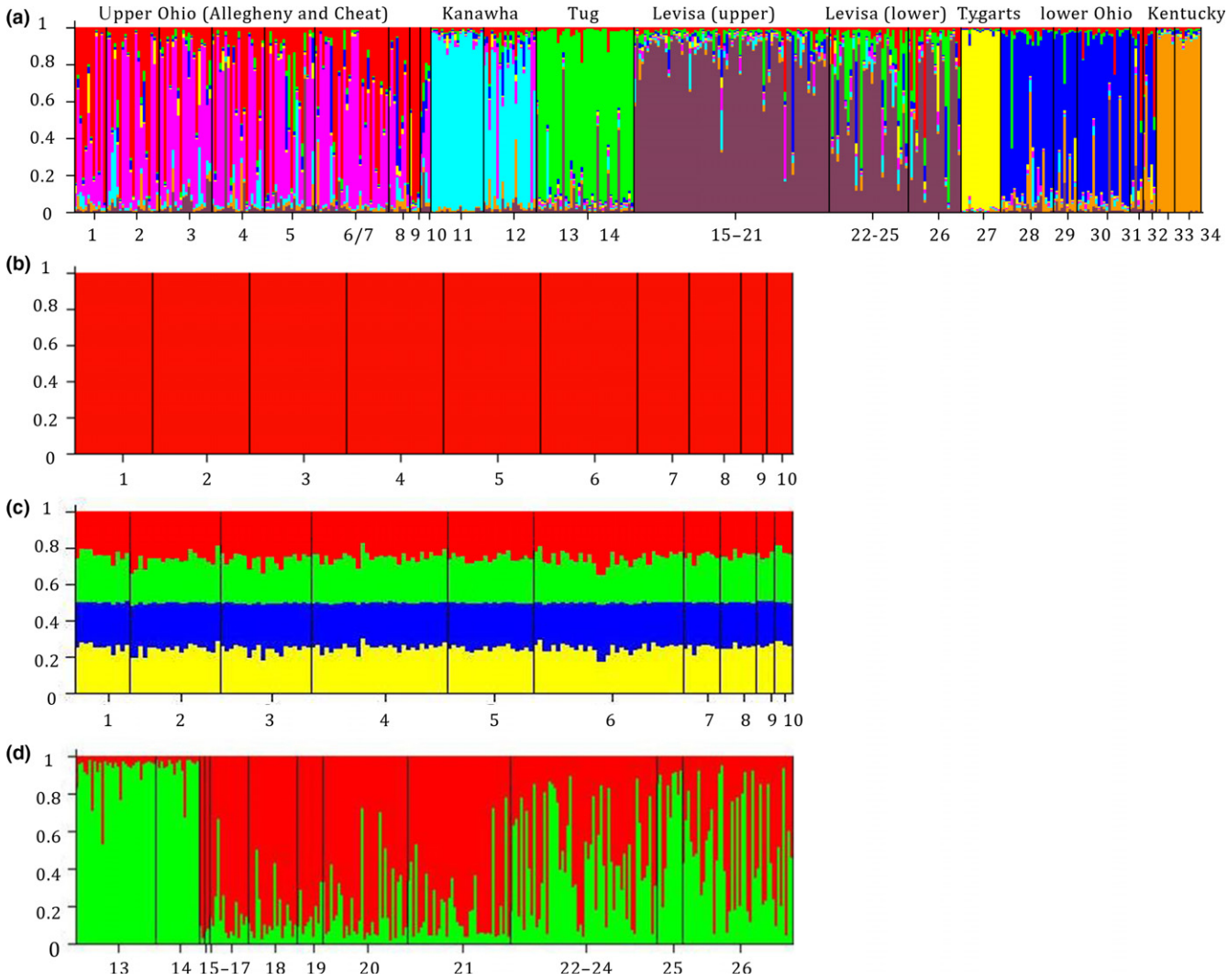

FIGURE 2 Results of genetic structure analysis of variegate darter populations: (a) across the species range, (b) most parsimonious (red) and (c) admixture (multicolour) models from the upper Allegheny River drainage, and (d) upper Big Sandy River drainage. Numbers along the *x*-axis indicate sample location as described in Table 1, and each colour bar indicates the genetic admixture of an individual. The *y*-axis shows the likelihood of each individual being assigned to a particular inferred cluster

3 | RESULTS

3.1 | Genetic diversity

Across all sampled sites, H_e ranged from 0.676 (SE = 0.166) in Tygarts Creek, KY to 0.844 (0.185) in Levisa Fork (tributary to Big Sandy River) downstream of Fishtrap Lake Dam, VA (Table 1). A_r at sites ranged from 2.36 to 7.29, although sites with very low A_r generally had low sample sizes (<10 individuals). The highest levels of A_r and H_e were observed in the upper Allegheny River (sites 1–8), central Ohio River (sites 28–31), upper Big Sandy River (Levisa, Tug and Russell forks, sites 15–26) and Kentucky River (sites 33 and 34) locations, and not confined to a particular portion of the species' range. All sites from the upper Allegheny River (sites 1–8) had similar levels of A_r, with the lowest at South Branch French Creek (site 7), although low sample size (N = 8) could have contributed to the low values. Elk River, WV (Kanawha River watershed, sites 11 and 12) and Tygarts Creek, KY (site 27) showed low levels of A_r (<6) and H_e compared to other watersheds. Cheat River (tributary to Monongahela River, site 9), Middle Fork Beaver Creek (upper Ohio River, site 10), Salt Creek (tributary to Whitewater River, site 31) and Whitewater River, IN (site 32) had low A_r, but the results may be biased by extremely low sample size. Watershed area, elevation and latitude were not significantly related to genetic diversity metrics across all sites. No significant correlations were found between A_r or H_e and watershed area ($p > .05$ for all correlations).

3.2 | Genetic structure within and among populations

Range-wide STRUCTURE analysis of the subsampled data assigned sites into one of eight multilocus genotypic clusters (Figure 2a), which correspond conceptually to biological populations. With the exception of the upper- and lower-most Ohio River sites, most

clusters were nested within watersheds, with the Ohio River mainstem representing a geographic breakpoint between clusters (Figure 1). Only Big Sandy River had tributaries split into two clusters: Tug Fork grouped separately from the Levisa Fork and Russell Fork sites.

Genetic structuring was unclear in the upper Ohio River watersheds (Figure 2a, sites 1–9), despite the presence of dams creating barriers among sites. Inferred group membership was approximately evenly split between two populations (indicating admixture) for all upper Allegheny River sites (1–8) with the exception of Cheat River, WV (site 9), which showed low admixture. Mahoning Creek in the Allegheny River watershed, PA (site 8), Cheat River, WV (site 9) and Middle Fork Beaver Creek, OH (site 10), a northern Ohio River tributary, were grouped with the upper Allegheny River sites. While there are no barriers between Mahoning Creek and Ohio River, both Middle Beaver Creek and Cheat River are highly isolated from other upstream sites by multiple lock-and-dam and hydropower dams (Figure 1).

In contrast to the range-wide STRUCTURE analysis showing Cheat River, WV (site 9) to be clearly assigned to a single cluster, STRUCTURE analysis confined to data from sites in the upper Ohio watershed did not reveal genetic structuring (i.e. $K = 1$ was the most parsimonious model), despite occurrences of dams that create putative barriers between sample sites (Figure 2b). Models with higher K values were not well supported and indicated admixture with no discernible patterns of genetic divergence (Figure 2c).

Genetic structuring was apparent in the lower Ohio River basin. Three tributaries to the Ohio River mainstem clustered together (Scioto River [sites 28–29], Little Miami River [site 30] and Whitewater rivers, sites 31–32), while the Kentucky River sites (33 and 34) clustered separately (Figure 2a). Sites in the Kentucky River watershed are isolated from the Ohio by 10 (site 33) and 14 (site 34) lock-and-dams, respectively, but other indications of genetic impacts, such as low A_r or low m-ratio values, were not apparent, and N_e was moderate.

Watershed-scale analyses of the Big Sandy River drainage performed in STRUCTURE indicated $K = 2$ clusters of variegate darters; fish from Levisa and Russell fork watersheds were assigned to one cluster, while fish from the Tug Fork watershed were assigned to a second (Figure 2d). The model of three populations, where sites were split into Tug Fork (sites 13–14), upper Levisa Fork (sites 15–21) and lower Levisa Fork (sites 22–26), had only slightly higher AIC values and indicated differentiation across the dam at Fishtrap Lake, likely from genetic drift.

3.3 | Effective population size

Analysis of the eight populations identified in STRUCTURE analysis showed effective population sizes (N_e) were variable, with four populations >500 (Table 2), a benchmark value set by the 50/500 rule for long-term conservation of evolutionary potential (Jamieson & Allendorf, 2012). Estimates of N_e for the Allegheny, Tug and Levisa (upper and lower groups split because of isolation and potential population structure) populations were above the $N_e > 500$ benchmark. Ohio and Kentucky river population estimates were between 50 and 500, indicating that these populations likely could avoid inbreeding depression but might not be large enough to maintain long-term evolutionary potential. Estimated N_e values in Tygarts Creek and Monongahela River populations are regarded as unresolved using the linkage disequilibrium approach due to small sample sizes.

The m-ratio values indicated past bottlenecks in five of the eight clusters (Table 2). Although the Allegheny and Big Sandy population clusters have large N_e, only the Kanawha River, lower Ohio River and Kentucky River population clusters showed evidence of long-term demographic stability.

3.4 | Phylogeographic analyses

Phylogenetic inferences largely supported the outcomes of STRUCTURE analysis. The range-wide unrooted neighbour-joining tree illustrates the genetic distances between samples, and branches roughly correspond to the respective Ohio River tributaries (Figure 3a). Branch length varies among watersheds, though, and Cheat River, WV (site 9), Tygarts Creek, KY (site 27) and Kentucky River (Redbird River and Middle Fork Red River, sites 33 and 34) sites have the longest branch lengths. Tygarts Creek, KY was considered a separate population in the STRUCTURE analysis, although it diverges from the base of the lower Ohio River branch.

Neither the range-wide nor the watershed-scale phylogenetic analysis of the upper Allegheny River sites showed apparent effects of dams on genetic distances among populations or between Bell Run (site 1) and the upper Allegheny River (site 2) sites and downstream sites (sites 3–7). Both analyses showed Cheat River, WV (site 9) to be genetically distant but within the grouping of the upper Ohio River sites. In the Big Sandy River basin, phylogenetic distances in the neighbour-joining tree (Figure 3b) showed a similar pattern as the STRUCTURE analysis, with the Levisa Fork upstream (sites 15–21) branching separately from Levisa Fork downstream (sites 22–25) and Russell Fork (site 26) populations, and all branching separately from the Tug Fork population (site 13–14). Neighbour-joining trees of the upper Ohio River population (Figure 3c) also showed a pattern of similar phylogenetic distances among sites except that Mahoning Creek, Cheat River and Middle Fork Beaver Creek (sites 8–10) had longer branch lengths than the other upper Allegheny sites.

3.5 | Effects of dams and distance on genetic distance

Pairwise F_{ST} between most populations was low (<0.06), slightly higher among pairwise groupings with Kentucky River (*c.* 0.1), and highest with pairwise groupings with Tygarts Creek (Table 3). Mantel test showed significant positive correlation ($r = .28$, $p < .02$) between pairwise F_{ST} and fluvial distance between sites (Figure 4), and only pairwise values with Tygarts Creek, KY (site 27) had F_{ST} values greater than 0.2. Dams are pervasive throughout the watershed, and maximum undammed fluvial distance between sites is 450 km. Numbers of dams and distance between sites were highly

TABLE 2 Population cluster-level values of effective population size (N_e) with upper and lower 95% confidence intervals, rarefied allelic richness (A_r) and *m*-ratios. Values in parentheses indicate number of individuals from each population analysed. N_e in Monongahela and Tygarts populations are unknown (–) due to low sample size

Population (*N*)	N_e	Lower 95%	Upper 95%	Allelic richness	*m*-Ratio
Allegheny (150)	752	403	3,774	6.90	0.551
Monongahela (8)	–	–	–	4.61	0.554
Kanawha (44)	170	87	1,040	5.10	0.708
Tug (48)	693	164	∞	6.27	0.583
Levisa (upper) (121)	8,020	575	∞	5.26	0.507
Levisa (lower) (110)	∞	11,682	∞	6.87	0.600
Tygarts (15)	–	–	–	4.35	0.640
Lower Ohio (83)	213	147	366	6.35	0.807
Kentucky (17)	215	56	∞	6.75	0.785

correlated; dams were not significantly correlated with F_{ST} after accounting for fluvial distance between sites. In the Big Sandy River drainage, Mantel tests revealed that F_{ST} significantly increased with geographic distance ($r = .496$, $p < .01$), but the correlation was not significant after accounting for the Fishtrap Lake Dam ($r = .079$, $p = .35$).

4 | DISCUSSION

4.1 | Phylogenetic patterns of genetic variation

We examined geographic patterns of population genetic variation in variegate darter in the light of the species' natural history and anthropogenic impacts. Natural historical events have their own genetic signature over which any anthropogenic impacts are superimposed. Additionally, there is a time-lag for populations to reach equilibrium between genetic drift and gene flow following habitat fragmentation. Such time lags may be hundreds or thousands of generations and are impacted by factors such as demographic expansion, bottlenecks and dispersal ability. Prior to the Pleistocene, flow paths of major river drainages differed from their paths today (reviewed by Mayden, 1988). Ancient drainage networks and patterns of post-glacial recolonisation have been invoked to explain patterns of genetic differentiation in several freshwater fish (Berendzen, Simons, & Wood, 2003; Berendzen, Simons, Wood, Dowling, & Secor, 2008; Near, Page, & Mayden, 2001), including variegate darters. Mitochondrial DNA analysis suggests variegate darter experienced several episodes of range expansion and fragmentation during the Pleistocene, with individuals dispersing from the ancient Teays River northward into the upper Ohio River system as drainage networks were reconfigured (Switzer, 2004). Our range-wide collections encompass potential glacial refugia such as the Kanawha, Big Sandy and Kentucky rivers, and more northerly areas that were recolonised after deglaciation. These historic drainage patterns and post-glacial expansion help to frame some of our results, such as lack of population structure in the upper Ohio watershed, the inference of genetic dispersal across large distances and the greater population genetic differentiation observed in the southern part of the species' range.

The combination of historical biogeographic processes and recent anthropogenic changes is reflected in current population genetic patterns in this wide-ranging species. Biogeographical processes are reflected in the observed phylogenetic relationships among populations (Figure 3a). Branch A includes Kanawha (11 and 12) and Allegheny (1–10) populations, branch B the Big Sandy and no derived populations, and branch C the Kentucky (33 and 34) and lower Ohio (27–32) refugia populations (Figure 3a). The nodes of the unrooted tree are near one another, and the respective branches are relatively long. The three phylogenetic groups, subject to natural and anthropogenic processes, became differentiated into the eight population clusters revealed by STRUCTURE analysis (Figure 2a). The Kanawha population cluster became differentiated from the upper Ohio cluster, and the Kentucky cluster from the lower Ohio cluster. The Big Sandy populations presumably were historically well connected, but have become differentiated due to anthropogenic changes into the Tug Fork and Levisa/Russell Fork population clusters, and the Tygarts Creek population became differentiated from the rest of the lower Ohio clade.

4.2 | Effects of scale on interpretation of genetic diversity patterns

Large sample sizes and spatial extent of variegate darter genetic analysis allowed us to quantify the high levels of genetic variability among watersheds and to identify populations and rivers with particularly low levels of genetic diversity. Historic bottlenecks were detected in most (66%) sampled population clusters, likely due to intense anthropogenic disturbances during the past 100 years, including coal mining, resource extraction, agriculture and urbanisation (Thomas et al., 2004). Low A_r was particularly evident in the Kanawha River, upper Levisa Fork and Tygarts Creek clusters (sites 10–11, 15–21 and 27); these population clusters also showed signs of low current (N_e) and past (*m*-ratio) population sizes. Differences in A_r and H_e among population clusters were not related to watershed size and are likely due to both biogeographic patterns and to the anthropogenic disturbances across the landscape. Distance was a significant factor in explaining pairwise genetic distances among sites across the range of variegate darters, although genetic differentiation was higher in pairwise values from Tygarts Creek (site 27) than even the most distant sites.

Comparison of local versus range-wide patterns of genetic variation reinforced the importance of considering large spatial extents for population genetic analyses, particularly when determining appropriate units for species management. Discrepancies between range-wide and watershed-scale genetic structure results were noted in the upper Ohio River, particularly in the Cheat River, WV (site 9), where a large data set showed evidence of genetic differentiation.

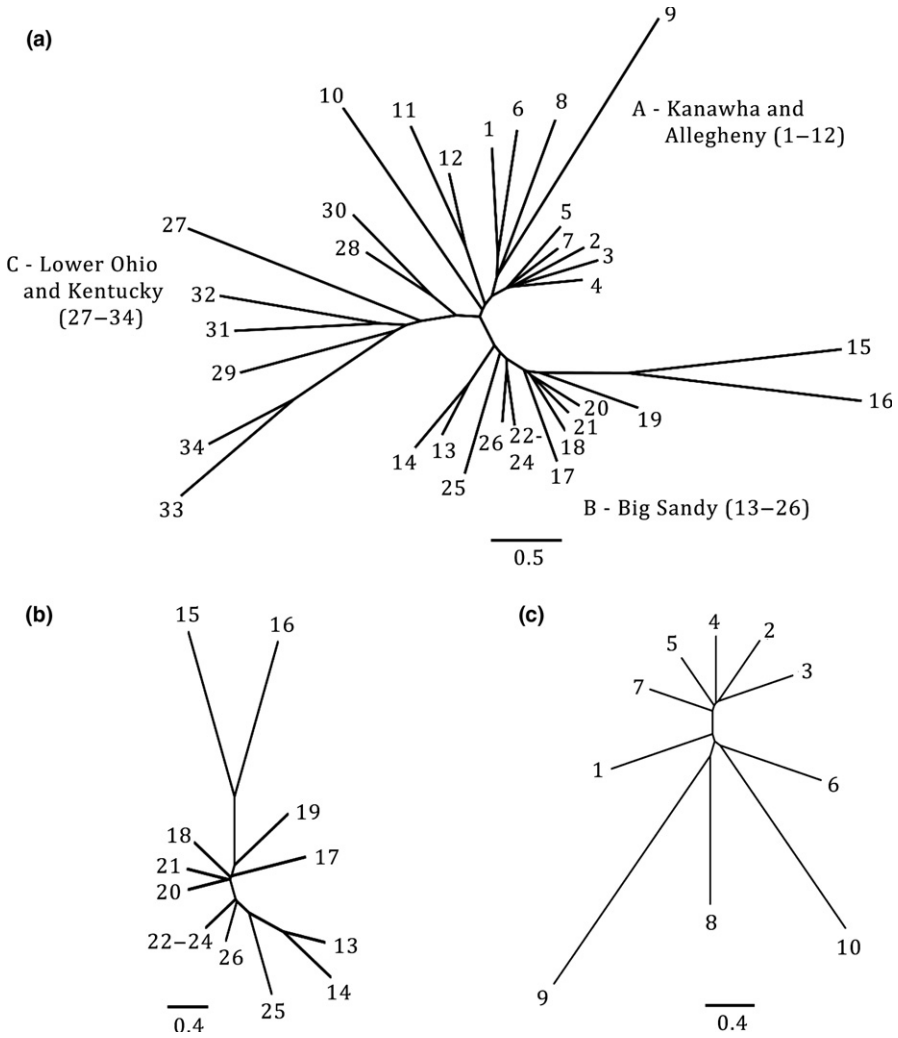

FIGURE 3 Unrooted neighbour-joining trees for populations of variegate darter from: (a) all sites across the species' range, with three clusters labelled according to drainage basin of sites; (b) upper Big Sandy River drainage; and (c) Allegheny River drainage. Site numbers for each branch and clusters are described in Appendix S1

TABLE 3 Pairwise F_{ST} values for variegate darter population clusters[a] excluding Monongahela cluster

	Allegheny	Kanawha	Tug	Levisa	Tygarts	Lower Ohio
Kanawha	0.041					
Tug	0.046	0.056				
Levisa	0.030	0.041	0.023			
Tygarts	0.091	0.121	0.114	0.101		
Lower Ohio	0.028	0.047	0.038	0.023	0.095	
Kentucky	0.067	0.058	0.065	0.047	0.139	0.042

[a]Population clusters are as described in Table 1.

The range-wide STRUCTURE results showed uncertainty about population assignments in the upper Ohio basin but showed evidence of two populations, although the signal was weak for some sites (i.e. there was equal likelihood for site membership in more than one cluster). Fine-scale STRUCTURE analysis from the upper Ohio sites indicated a single cluster, and further increases in the value of *K* showed genetic admixture. Furthermore, range-wide analysis showed that clusters can cross river mainstem and state lines (Monongahela River, Middle Fork Beaver Creek and surrounding watersheds grouped with the upper Ohio River sites).

The phylogenetic tree also showed genetic structuring within the upper Ohio tributaries, partially due to the effects of population declines and exacerbated by isolation by instream dams. The Cheat River, WV (site 9) clustered with the upper Ohio sites (sites 1–8), but had a longer branch than the other sites, and had higher-than-expected pairwise F_{ST} values. Interestingly, the two uppermost sites of the Allegheny River watershed, Bell Run and the Allegheny River, have been isolated by Allegheny Dam from all other downstream sites since the 1960s, but do not show the expected patterns of increased genetic structuring or a decline in genetic diversity metrics except for a slight decline in heterozygosity. We suspect that this muted effect of a large dam reflects the large and demographically stable populations upstream of the dam.

4.3 | Effects of dams on population genetic structure

Dams are widely known to affect ecological processes (Poff et al., 1997), including changes to fish populations, by acting as barriers to

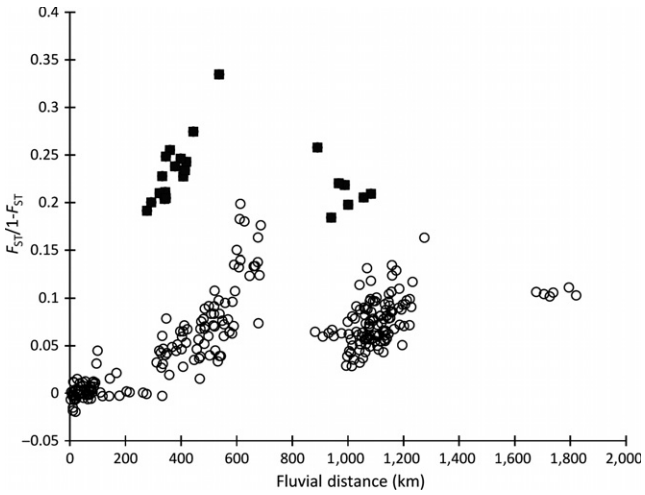

FIGURE 4 Plots of linearised F_{ST} ($F_{ST}/1 - F_{ST}$) versus pairwise fluvial distance (km) for all sites with sample sizes of at least 10 ($r = 0.28$, $p < 0.02$), with Tygarts Creek (Site 27) in closed black squares

migration and dispersal of large-bodied fish (Bessert & Orti, 2008), but the effect on non-migratory small-bodied stream fish is less thoroughly understood. Populations isolated by dams can show genetic changes, even in non-migratory fish such as darters, particularly for imperilled species with small populations (Beneteau et al., 2009; Roberts et al., 2013). However, effects can be mitigated by the amount of available habitat within each fragment, ecological traits of the study species and ability of species to cross barriers (Reid, Wilson, Mandrak, & Carl, 2008). We showed that dams can, but do not always, exacerbate genetic differentiation; evidence of impacts were not consistent among populations. Range-wide pairwise F_{ST} values of 0.1–0.2 were similar to those of other darter species (Roanoke logperch and Eastern sand darter [*Ammocrypta pellucia*]) in the few published studies that spanned multiple watersheds and long fluvial distances (more than 1,000 km) between sites (Ginson et al., 2015; Roberts et al., 2013), but much higher than those generally found in studies focused within watersheds (fluvial distances less than 500 km between size) (e.g. *Etheostoma blennioides*, Beneteau et al., 2009; *E. caeruleum*, Haponski et al., 2009), where values were often less than 0.1 and little genetic structure was evident. Genetic dissimilarity was primarily driven by increasing fluvial distance between sites, but both fluvial distance and anthropogenic impacts affected the diversity and population structure of variegate darter across its range. The magnitude of the effects of dams varied among watersheds because of variation in underlying population parameters such as breeding population size.

Lifetime movement and its importance to population structure warrant additional study in darters and other riverine fish. Although darters are often considered to have small home ranges based on instream movement studies (Schwalb et al., 2011), such studies typically focus on adult behaviour, and long-distance movements by individuals are difficult to detect in standard mark–recapture studies. In contrast to such expectations, juvenile and adult darters can disperse over large distances (at least 55 km in a single year for Roanoke logperch, Roberts & Angermeier, 2007; Roberts et al., 2016), although few other species have been studied. Our results provide evidence that variegate darters disperse across watershed and state boundaries, indicated by the lack of genetic structure within watersheds lacking physical barriers, even when sites are in different watersheds such as the respective lower Ohio River tributaries. Adult variegate darters are habitat specialists, preferring riffles with coarse bed substrate and swift waters in medium to large streams (Argentina et al., 2013; May, 1969), so typical daily movements by an adult are likely to be short distances. However, larval variegate darters display patterns of crepuscular drift (J. E. Argentina, unpublished data; Simon & Wallace, 2006), which may play a significant role in long-distance dispersal. Larvae in many darter species, particularly within the genus *Percina*, are pelagic and enter drift after hatching (Simon & Wallace, 2006), but drift timing, duration and distance are largely unknown. Adult and larval dispersal may be reduced by increased mortality in lentic habitats created by reservoirs and by the physical barrier of the dam itself. Although we could not estimate rates of dispersal across watersheds, lack of population structure in rivers in the lower Ohio River indicates genetic dispersal occurs across at least 400 km and through very large river habitats. Many other large river ecologically similar species of small-bodied Percids occur throughout the Ohio River basin and may be similarly impacted by anthropogenic changes and isolation due to barriers (e.g. river darter [*Percina shumardi*], slenderhead darter [*P. phoxocephala*] or spotted darter [*Etheostoma maculatum*]).

Underlying population size and management activities (e.g. stocking) can affect genetic diversity and population structure (Crookes & Shaw, 2016), and patterns of genetic diversity among populations are expected to change in response to anthropogenic impacts. For example, large, connected fish populations from high-quality habitats show higher levels of genetic variability and reduced population genetic differentiation in cutthroat trout *Oncorhynchus clarkii*, a large-bodied Salmonid species (Neville, Dunham, & Peacock, 2006), and we found similar patterns in a small-bodied benthic stream species. Diversity metrics showed large variation across populations, but were generally high in the upper Allegheny and French Creek watersheds, which have large N_e. These large, demographically stable variegate darter populations have high genetic diversity and show resilience to some of the negative impacts of isolation by dams. Alternatively, small, isolated populations with historic population bottlenecks, such as those in the Levisa Fork (sites 15–21), can show the signature of genetic drift over 60 years (up to 30 generations), a process that will probably continue to occur unless barriers to dispersal are mitigated. Dam removal could facilitate dispersal among stream reaches and increase genetic connectivity across the population. Kentucky River cluster (sites 33 and 34) also shows genetic effects of isolation by dams, including population genetic structuring and low N_e, although genetic diversity remains high and we saw no evidence of past bottlenecks.

4.4 | Conservation implications

Demographic parameters such as population size and dispersal rate can interact with landscape changes to determine the strength of anthropogenic impacts on population differentiation. Instream barriers were associated with increased differentiation in some watersheds, but not in others, demonstrating the importance of spatial replicates and large data sets in understanding the strength and direction of anthropogenic impacts on darter populations. Spatially extensive sampling can help prioritise species conservation by contextualising typical diversity and effective population sizes. We suspect that further spatially extensive studies would show that other large river species exhibit similar spatial variability in genetic diversity and response to population isolation. Overall, our results reinforce the importance of large spatial scales of study and management when linking landscape and population effects to stream fish genetic structure and diversity.

Genetic pattern differences among populations suggest that effective variegate darter management should address population-specific strengths and vulnerabilities. In the upper Ohio River watershed where populations are resilient to the influence of dams because of robust breeding populations, maintenance of habitat quality might be a management priority. By contrast, habitat restoration or population augmentation might be critical for long-term population stability in watersheds where populations show decreased genetic diversity, low N_e and considerable population structuring. When population decline is due to habitat fragmentation or destruction, management might focus on increasing connectivity between small, isolated watersheds such as the upper Cheat River, WV or upper Levisa Fork, VA. On the other hand, high pairwise F_{ST} values and low genetic diversity, despite the lack of physical isolation from other populations, indicate small population size. A combination of habitat improvement and population augmentation could increase genetic diversity and stability.

In the United States, 46% of all freshwater fish species and 44% of darters are imperilled, and the numbers of imperilled species are increasing (Jelks et al., 2008). Habitat destruction and range restriction are the most frequent causes of fish imperilment, and habitat fragmentation is a component of both these causes. Fragmentation by dams in large rivers clearly causes genetic isolation and differentiation in large-bodied and migratory fish and has been a focus of salmon restoration in the American West, but the potential impacts of fragmentation in large rivers on small-bodied fish (e.g. darters) have been historically overlooked. Further clarity on movement and dispersal ages, rates and distances would be especially helpful in prioritisation of specific actions aimed at reducing imperilment due to population isolation, including increasing connectivity via instream barrier removal (e.g. New York DEC, 2015), improving habitat quality and translocating breeding individuals. Our research supports previous findings that fragmentation in rivers increases genetic differentiation in riverine darter species (Roberts et al., 2013), especially in small populations. Our results affirm the need for more studies with large spatial extents and replicated study sites (Keller et al., 2015) to clarify how population size, species traits and underlying genetic variability interact to determine impacts of instream barriers in rivers, and provide useful comparisons for contextualising genetic diversity of imperilled species (e.g. Fluker et al., 2010).

ACKNOWLEDGMENTS

Research support was provided by the Virginia Department of Game and Inland Fisheries (VDGIF) and the Virginia Department of Mines, Minerals, and Energy. Genetic samples from Ohio, Pennsylvania and New York were graciously collected and donated by Todd Crail at University of Toledo and Dr. Robert Daniels at the New York State Museum. Joanne Davis Printz completed all Virginia Tech laboratory analyses. We thank Jamie Roberts for analytical suggestions and helpful feedback. Many technicians were critical in collection of field data. Field collections were made under the auspices of IACUC protocol 08-133-FIW. The participation of author EH was supported in part by the Virginia Agricultural Experiment Station through the U.S. Department of Agriculture National Institute for Food and Agriculture. We mourn the passing of co-author Tim King, valued collaborator, colleague and friend. The Virginia Cooperative Fish and Wildlife Research Unit is jointly sponsored by the U.S. Geological Survey, Virginia Tech, VDGIF, and Wildlife Management Institute. Use of trade names or commercial products does not imply endorsement by the U.S. government.

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REFERENCES

Albanese, B., Angermeier, P. L., & Gowan, C. (2003). Designing mark–recapture studies to reduce effects of distance weighting on movement distance distributions of stream fishes. *Transactions of the American Fisheries Society*, *132*, 925–939. https://doi.org/10.1577/T03-019

Anderson, C. D., Epperson, B. K., Fortin, M., Holderegger, R., James, P. M. A., Rosenberg, M. S., … Spear, S. (2010). Considering spatial and temporal scale in landscape-genetic studies of gene flow. *Molecular Ecology*, *19*, 3565–3575. https://doi.org/10.1111/j.1365-294X.2010.04757.x

Argent, D. G., & Kimmel, W. G. (2014). Patterns of ichthyofaunal distribution and connectivity in navigable and free-flowing reaches of a major river system: The Allegheny River in Pennsylvania. *River Research and Applications*, *30*, 631–638. https://doi.org/10.1002/rra.2668

Argentina, J. E., Angermeier, P. L., & Hallerman, E. M. (2013). *Population ecology of Variegate Darter* (Etheostoma variatum) *in Virginia*. Final report to the Virginia Department of Mines, Minerals, and Energy, Richmond, VA. Retrieved from https://vtechworks.lib.vt.edu/handle/10919/23699

Beneteau, C. L., Mandrak, N. E., & Heath, D. D. (2009). The effects of river barriers and range expansion of the population genetic structure and stability in Greenside Darter (*Etheostoma blennioides*) populations. *Conservation Genetics*, *10*, 477–487. https://doi.org/10.1007/s10592-008-9627-9

Berendzen, P. B., Simons, A. M., & Wood, R. M. (2003). Phylogeography of the Northern Hogsucker, *Hypentelium nigricans* (Teleostei:Cypriniformes): Genetic evidence for the existence of the ancient Teays River. *Journal of Biogeography*, *30*, 1139–1152. https://doi.org/10.1046/j.1365-2699.2003.00888.x

Berendzen, P. B., Simons, A. M., Wood, R. M., Dowling, T. E., & Secor, C. L. (2008). Recovering cryptic diversity and ancient drainage patterns in eastern North America: Historical biogeography of the *Notropis rubellus* species group (Teleostei: Cypriniformes). *Molecular Phylogenetics and Evolution*, *46*, 721–737. https://doi.org/10.1016/j.ympev.2007.07.008

Bessert, M. L., & Orti, G. (2008). Genetic effects of habitat fragmentation on blue sucker populations in the upper Missouri River (*Cycleptus elongatus* Lesueur, 1918). *Conservation Genetics*, *9*, 821–832. https://doi.org/10.1007/s10592-007-9401-4

Blanchet, S., Rey, O., Etienne, R., Lek, S., & Loot, G. (2010). Species-specific responses to landscape fragmentation: Implications for management strategies. *Evolutionary Applications*, *3*, 291–304. https://doi.org/10.1111/j.1752-4571.2009.00110.x

Boizard, J., Magnan, P., & Angers, B. (2009). Effects of dynamic landscape elements on fish dispersal: The example of creek chub (*Semotilus atromaculatus*). *Molecular Ecology*, *18*, 430–441. https://doi.org/10.1111/j.1365-294X.2008.04044.x

Bowman, J., Greenhorn, J. E., Marrotte, R. R., McKay, M. M., Morris, K. Y., Prentice, M. B., & Wehtje, M. (2016). On applications of landscape genetics. *Conservation Genetics*, *17*, 753–760. https://doi.org/10.1007/s10592-016-0834-5

Brogdon, S. M., Tabit, C. R., & Kral, L. G. (2003). Population structure of the Tallapoosa darter (*Etheostoma tallapoosae*). *Southeastern Naturalist*, *2*, 487–498. https://doi.org/10.1656/1528-7092(2003)002[0487:PSOTTD]2.0.CO;2

Cao, Y., Hinz, L., Metzke, B., Stein, J., & Holtop, A. (2016). Modeling and mapping fish abundance across wadeable streams of Illinois, USA, based on landscape-level environmental variables. *Canadian Journal of Fisheries and Aquatic Sciences*, *73*, 1031–1046. https://doi.org/10.1139/cjfas-2015-0343

Crookes, S., & Shaw, P. W. (2016). Isolation by distance and non-identical patterns of gene flow within two river populations of the freshwater fish *Rutilus rutilus* (L. 1758). *Conservation Genetics*, *17*, 861–874. https://doi.org/10.1007/s10592-016-0828-3

Cushman, S. A., & Landguth, E. L. (2010). Scale dependent inference in landscape genetics. *Landscape Ecology*, *25*, 967–979. https://doi.org/10.1007/s10980-010-9467-0

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillet, B. K., & Ovenden, J. R. (2013). NeEstimator V2: Reimplementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Molecular Ecology Resources*, *14*, 209–214.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*(2611), 2620.

Excoffier, L. G., Laval, G., & Schneider, S. (2006). *Arlequin, version 3.01: An integrated software package for population genetics data analysis*. Retrieved from http://cmpg.unibe.ch/software/arlequin3

Fluker, B. L., Kuhajda, B. R., & Harris, P. M. (2014). 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. https://doi.org/10.1111/fwb.12283

Fluker, B. L., Kuhajda, B. R., Lang, N. J., & Harris, P. M. (2010). Low genetic diversity and small long-term population size in the spring endemic watercress darter, *Etheostoma nuchale*. *Conservation Genetics*, *11*, 2267–2279. https://doi.org/10.1007/s10592-010-0111-y

Frimpong, E., & Angermeier, P. L. (2009). Fish Traits: A database of ecological and life-history traits of freshwater fishes of the United States. *Fisheries*, *34*, 487–495. https://doi.org/10.1577/1548-8446-34.10.487

Fullerton, A. H., Burnett, M., Steel, E. A., Flintcroft, R. L., Pess, G. R., Feist, B. E., … Sanderson, B. L. (2010). Hydrological connectivity for riverine fish: Measurement challenges and research opportunities. *Freshwater Biology*, *55*, 2215–2237.

Galbraith, H. S., Zanatta, D. T., & Wilson, C. C. (2015). Comparative analysis of riverscape genetic structure in rare, threatened and common freshwater mussels. *Conservation Genetics*, *16*, 845–857. https://doi.org/10.1007/s10592-015-0705-5

Garza, J. C., & Williamson, E. G. (2001). Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology*, *10* (305), 318.

Ginson, R., Walter, R. P., Mandrak, N. E., Beneteau, C. L., & Heath, D. D. (2015). Hierarchical analysis of genetic structure in the habitat-specialist Eastern Sand Darter (*Ammocrypta pellucida*). *Ecology and Evolution*, *5*, 695–708. https://doi.org/10.1002/ece3.1392

Haponski, A. E., Bollin, T. L., Jedlicka, M. A., & Stepien, C. A. (2009). Landscape genetic patterns of the rainbow darter *Etheostoma caeruleum*: A catchment analysis of mitochondrial DNA sequences and nuclear microsatellites. *Journal of Fish Biology*, *5*, 2244–2268. https://doi.org/10.1111/j.1095-8649.2009.02414.x

Haponski, A. E., Marth, T. A., & Stepien, C. A. (2007). Genetic divergence across a low-head dam: A preliminary analysis using logperch and greenside darters. *Journal of Great Lakes Research*, *33*, 117–126. https://doi.org/10.3394/0380-1330(2007)33[117:GDAALD]2.0.CO;2

Helfman, G. S. (2007). *Fish conservation: A guide to understanding and restoring global aquatic biodiversity and fishery resources*. Washington, DC: Island Press.

Jamieson, I. G., & Allendorf, F. W. (2012). How does the 50/500 rule apply to MVPs? *Trends in Ecology & Evolution*, *27*, 578–584. https://doi.org/10.1016/j.tree.2012.07.001

Jelks, H. L., Walsh, S. J., Burkhead, N. M., Contreras-Balderas, S., Diaz-Pardo, E., Hendrickson, D. A., … Warren, Jr, M. L. (2008). Conservation status of imperiled North American and diadromous fishes. *Fisheries*, *33*, 372–407. https://doi.org/10.1577/1548-8446-33.8.372

Johnson, R. L., Mitchell, R. M., & Harp, G. L. (2006). Genetic variation and genetic structuring of a numerically declining species of darter, *Etheostoma moorei* Raney & Suttkus, endemic to the upper Little Red River, Arkansas. *American Midland Naturalist*, *156*, 37–44. https://doi.org/10.1674/0003-0031(2006)156[37:GVAGSO]2.0.CO;2

Junker, J., Peter, A., Wagner, C. E., Mwaiko, S., & Germann, B. (2012). River fragmentation increases localized population genetic structure and enhances asymmetry of dispersal in bullhead (*Cottus gobio*). *Conservation Genetics*, *13*, 545–556. https://doi.org/10.1007/s10592-011-0306-x

Kalinowski, S. T. (2005). HP-Rare: A computer program for performing rarefication on measures of allelic diversity. *Molecular Ecology Notes*, *5*, 187–189. https://doi.org/10.1111/j.1471-8286.2004.00845.x

Kalinowski, S. T. (2009). *TREEFIT: A computer program for evaluating how well evolutionary trees fit genetic distance data*. Retrieved from http://www.montana.edu/kalinowski

Keller, D., Holderegger, R., van Strien, M. J., & Bolliger, J. (2015). How to make landscape genetics beneficial for conservation management? *Conservation Genetics*, *16*, 503–512. https://doi.org/10.1007/s10592-014-0684-y

Koryak, M., Bonislawsky, P., Locy, D., & Porter, B. A. (2008). Use of benthic trawling to supplement electrofishing in characterizing the fish community of the Allegheny River navigation channel in Pennsylvania, USA. *Journal of Freshwater Ecology*, *23*, 491–494. https://doi.org/10.1080/02705060.2008.9664232

Lachner, E. A., Westlake, E. F., & Handwerk, P. S. (1950). Studies on the biology of some percid fishes from western Pennsylvania. *American Midland Naturalist*, *43*, 92–111. https://doi.org/10.2307/2421881

Manel, S., & Holderegger, R. (2013). Ten years of landscape genetics. *Trends in Ecology & Evolution*, *28*, 614–621. https://doi.org/10.1016/j.tree.2013.05.012

May, B. (1969). Observations on the biology of the variegated darter, *Ethestoma variatum* (Kirtland). *Ohio Journal of Science*, *69*, 85–92.

Mayden, R. L. (1988). Vicariance biogeography, parsimony, and evolution in North American freshwater fishes. *Systematic Zoology*, *37*, 329–355. https://doi.org/10.2307/2992197

Mullen, L. B., Woods, H. A., Schwartz, M. K., Sepulveda, A. J., & Lowe, W. H. (2010). Scale-dependent genetic structure of the Idaho giant salamander (*Dicamptodon aterrimus*) in stream networks. *Molecular Ecology*, *19*, 898–909. https://doi.org/10.1111/j.1365-294X.2010.04541.x

Near, T. J., Page, L. M., & Mayden, R. L. (2001). Intraspecific phylogeography of *Percina evides* (Percidae: Etheostomatinae): An additional test of the Central Highlands pre-Pleistocene vicariance hypothesis. *Molecular Ecology*, *10*, 2235–2240. https://doi.org/10.1046/j.1365-294X.2001.01362.x

Nei, M., Tajima, F., & Tateno, Y. (1983) Accuracy of estimated phylogenetic trees from molecular data. II: gene frequency data. *Journal of Molecular Evolution*, *19*, 153–170.

Neville, H. M., Dunham, J. B., & Peacock, M. M. (2006). Landscape attributes and life history variability shape genetic structure of trout populations in a stream network. *Landscape Ecology*, *21*, 901–916. https://doi.org/10.1007/s10980-005-5221-4

New York DEC. (2015). *New York State wildlife action plan*. Albany, NY: New York State Department of Environmental Conservation.

Pearson, W. D., & Krumholz, L. A. (1984). *Distribution and status of Ohio River fishes (No. ORNL/Sub-79-7831/1)*. Louisville, KY: Water Resources Laboratory, Louisville University.

Poff, N. L., Allan, N. L., Bain, M. B., Karr, J. R., Prestagaard, K. L., Richter, B. D., … Stromberg, J. C. (1997). The natural flow regime: A paradigm for river conservation. *BioScience*, *47*, 769–784. https://doi.org/10.2307/1313099

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959.

Puechmaille, S. J. (2016). The program STRUCTURE does not reliably recover the correct population structure when sampling is uneven: Subsampling and new estimators alleviate the problem. *Molecular Ecology Resources*, *16*, 608–627. https://doi.org/10.1111/1755-0998.12512

Reid, S. M., Wilson, C. C., Mandrak, N. E., & Carl, L. M. (2008). Population structure and genetic diversity of black redhorse (*Moxostoma duquesnei*) in a highly fragmented watershed. *Conservation Genetics*, *9*, 531–546. https://doi.org/10.1007/s10592-007-9367-2

Roberts, J. H., & Angermeier, P. L. (2007). Spatiotemporal variability of stream habitat and movement of three species of fish. *Oecologia*, *151*, 417–430. https://doi.org/10.1007/s00442-006-0598-6

Roberts, J. H., Angermeier, P. L., & Hallerman, E. M. (2013). Distance, dams and drift: What structures populations of an endangered, benthic stream fish? *Freshwater Biology*, *58*, 2050–2064. https://doi.org/10.1111/fwb.12190

Roberts, J. H., Angermeier, P. L., & Hallerman, E. M. (2016). Extensive dispersal of Roanoke logperch (*Percina rex*) inferred from genetic marker data. *Ecology of Freshwater Fish*, *25*, 1–16. https://doi.org/10.1111/eff.12177

Rosenberg, M. S., & Anderson, C. D. (2011). PASSaGE: Pattern analysis, spatial statistics and geographic exegesis. Version 2. *Methods in Ecology and Evolution*, *2*(3), 229–232. https://doi.org/10.1111/j.2041-210X.2010.00081.x

Rousset, F. (1997). Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics*, *145*, 1219–1228.

Schwalb, A. N., Poos, M. S., & Ackerman, J. D. (2011). Movement of logperch—The obligate host fish for endangered snuffbox mussels: Implications for mussel dispersal. *Aquatic Sciences*, *73*, 223–231. https://doi.org/10.1007/s00027-010-0171-6

Simon, T. P., & Wallace, R. (2006). *Reproductive and early life history of fishes in the Ohio River drainage, Volume 4: Percidae – perch, pikeperch, and darters*. Boca Raton, FL: CRC Press.

Switzer, J. F. (2004). *Molecular systematics and phylogeography of the* Etheostoma variatum *species group (Actinopterygii: Percidae)*. Ph.D. dissertation, Saint Louis University, St. Louis, MO.

Switzer, J. F., Welsh, S. A., & King, T. L. (2007). *A molecular genetic investigation of hybridization between* Etheostoma osburni and Etheostoma variatum *in the New River Drainage, West Virginia*. Final Report to West Virginia Division of Natural Resources, Elkins, WV. 54 pp.

Switzer, J. F., Welsh, S. A., & King, T. L. (2008). Microsatellite DNA primers for the candy darter, *Etheostoma osburni* and variegate darter, *Etheostoma variatum*, and cross-species amplification in other darters (Percidae). *Molecular Ecology Resources*, *8*, 335–338. https://doi.org/10.1111/j.1471-8286.2007.01946.x

Thomas, J. A., Emery, E. B., & McCormick, F. H. (2004). Detection of temporal trends in Ohio River fish assemblages based on lockchamber surveys (1957–2001). *American Fisheries Society Symposium*, *2004*, 1–19.

Turner, T. F., & Trexler, J. C. (1998). Ecological and historical associations of gene flow in darters (Teleostei: Percidae). *Evolution*, *52*, 1781–1801. https://doi.org/10.1111/j.1558-5646.1998.tb02256.x

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How to cite this article: Argentina JE, Angermeier PL, Hallerman EM, Welsh SA. Spatial extent of analysis influences observed patterns of population genetic structure in a widespread darter species (Percidae). *Freshwater Biol*. 2018;63:1185–1198. https://doi.org/10.1111/fwb.13106