Conservation Genetics (2018) 19:1379–1392
https://doi.org/10.1007/s10592-018-1107-2

RESEARCH ARTICLE

Role of drainage and barriers in the genetic structuring of a tessellated darter population

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Received: 5 April 2018 / Accepted: 7 September 2018 / Published online: 12 September 2018

Abstract

While population genetic structuring is easily identified, the causes of the structure can be difficult to determine. Habitat fragmentation in aquatic systems has often been identified as a major source of increased population structure and decreased genetic diversity in fish, including benthic resident species such as darters. However, these findings are often not replicated across natural and manmade barriers and come from endangered or threatened populations where the genetic structure is likely already compromised due to small population size. To evaluate the factors involved in structuring a healthy darter population, we genotyped 506 tessellated darters from 18 sites in three different river drainages and one large lake. Sites were all in the same watershed but separated from one another by one or more of three different types of barriers: dams, natural fall lines and causeways. We found that while diversity and allele frequency varied largely by drainage, within drainage variation was minimal even across multiple barriers. No single barrier type appeared to be more formidable than any other. Our results indicate that healthy populations of darters may naturally be structured by drainage, but likely disperse across barriers enough to retain drainage-wide homogeneity.

Keywords Habitat fragmentation · Microsatellite · Genetic diversity · Dams · Streams

Introduction

Issues associated with habitat fragmentation are at the forefront of modern conservation planning in both terrestrial and aquatic systems (Haddad et al. 2015). Aquatic systems are particularly vulnerable to the loss of connectivity as a consequence of habitat fragmentation. The construction of dams and culverts in riverine systems often interrupts hydrology (Ligon et al. 1995; Shaw et al. 2016) and blocks fish migrations. Loss of connectivity in rivers can have negative effects on both migratory and resident fish populations (Peacock et al. 2016), leading to population declines and loss of genetic diversity (Winston et al. 1991; Meldgaard et al. 2003).

Barriers in aquatic systems range from large hydroelectric dams and waterfalls to smaller low-head dams, weirs, culverts and natural cascades. In the United States of America, large dams often receive the most public attention as a source of fragmentation, but small dams less than 15 m high outnumber large dams almost 18–1 and impound three to four times more water in aggregate than large dams (Rosenberg et al. 2000). Because even a 1-m barrier is impassible to many fish, the relative impact of small dams on stream connectivity is high.

Though anthropogenic alterations such as dams can negatively influence species that inhabit rivers by decreasing connectivity and increasing genetic distance among populations (Helfman 2007), most lotic systems are naturally fragmented by waterfalls that may have isolated populations for thousands of years. For example, populations of cutthroat trout (*Oncorhynchus clarkii clarkia*) in rivers along the coast of Alaska fragmented by natural waterfalls show clear signs of asymmetric gene flow and high population structure above and below waterfalls (Whiteley et al. 2010). Determining the impact of anthropogenic habitat fragmentation relative to

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10592-018-1107-2) contains supplementary material, which is available to authorized users.

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natural fragmentation may help predict the future influence of dams and identify natural levels of population structure across barriers.

Much of what is known about river fragmentation comes from research focused on migratory and/or adfluvial fish such as salmonids (e.g. Clemento et al. 2009). However, fragmentation also impacts stream residents such as perch, darters, and catfish (Leclerc et al. 2008; Beneteau et al. 2009; Sotola et al. 2017). Species respond to fragmentation differently; for example, upstream gene flow for bullhead (*Cottus gobio*) was completely blocked by small dams in the Sense River in Switzerland, causing substantial genetic structure (Junker et al. 2012), whereas, populations of blue sucker (*Cycleptus elongates*) in the Missouri River experienced only minimal changes in genetic diversity and showed no strong genetic structure across 3000 km of river fragmented by six dams (Bessert and Orti 2008). Therefore, studying how barriers influence population structure in multiple species continues to be important to understand the consequences of habitat fragmentation.

Darters (Percidae) are a particularly good species group for examining effects of barriers, as they have life history traits that make them sensitive to habitat fragmentation. Over 140 species of darter are present in North America and are common residents in most freshwater environments (Kuehne and Barbour 2015). Darters prefer benthic habitats and tend to have relatively limited dispersal ability, so they are vulnerable to issues commonly associated with dams, including pollution, habitat loss, and reduced population connectivity (e.g. Juracek et al. 2017; Magoulick and Lynch 2015). Consequently, darters are a disproportionately endangered group, with 44% of darters listed as vulnerable, threatened or endangered (Helfman 2007; Jelks et al. 2008).

Decreased connectivity due to dams has had genetic consequences for many threatened or endangered species of darters and is believed to contribute to population declines (Beneteau et al. 2009; George et al. 2010; Sterling et al. 2012). However, most species of darters evolved in naturally fragmented environments and disperse only short distances even in connected regions of streams (Dammeyer et al. 2013). Case studies that evaluate the genetic structure of darter populations that span both natural and manmade barriers, but remain relatively healthy (e.g. contain high genetic diversity and population size), may help to identify the range of genetic sub-structuring that occurs naturally in darter populations (Richardson et al. 2016). Tessellated darters (*Etheostoma olmstedi*) are found in Lake Champlain and its tributaries, and are considered to be "abundant" in Vermont (Vermont National Heritage Inventory 2017). Tessellated darters are not exploited, and the only anthropogenic activity that may have affected stream populations was an increase in sedimentation during a period of deforestation in the 1800s (Marsden and Langdon 2012); populations are likely to have recovered from any effects during this period, as streams have steadily increased in substrate quality during subsequent reforestation (Wang et al. 1997; McBride et al. 2008).

Our objectives were to describe the level of genetic structure in a healthy population of darters and identify the relative influence of natural versus manmade fragmentation on the genetic structure and diversity of darter populations. We analyzed genetic data collected from tessellated darters sampled across three types of barriers (lake causeways, dams and natural fall lines) throughout the Lake Champlain watershed in Vermont. We structured our analysis to evaluate five hypotheses: (1) tessellated darter populations are genetically structured among Lake Champlain drainages by distance and by barriers; (2) genetic diversity decreases with distance from Lake Champlain which is presumed to have the highest genetic diversity; (3) both natural and manmade barriers increase population structure and decrease genetic diversity; (4) movement across instream barriers is primarily downstream, while movement across lake barriers is similar in both directions; (5) the magnitude of a barrier's effect on diversity and structure is related to barrier age and type.

Methods

Study location

The study was conducted in the Lake Champlain watershed, which spans 21,326 km^2. Lake Champlain is long (193 km) and narrow (20 km at the widest point), spans the border between New York and Vermont, USA, and Vermont and Quebec, CA and drains north into Quebec. Three large islands naturally divide the northern portion of Lake Champlain into eastern and western arms (Fig. 1). Seven causeways built between 1800 and 1900 link the islands to the mainland and isolate the lake further into four major basins: the Main Lake and a northeastern arm which is subdivided into Missisquoi Bay at the north end, the Inland Sea in the center, and Malletts Bay at the south end. All the causeways have one or two shallow openings (1–9 m deep) that allow some flow of water and passage of boats and fish. The three tributaries to Lake Champlain sampled in this study drain into three lake basins: Lewis Creek (southern Main Lake), Indian Brook (Malletts Bay), and the Missisquoi River (Missisquoi Bay). These tributaries all contain populations of tessellated darters and have one dam and a natural waterfall within the study area (Table 1). Indian Brook is the smallest stream, with a drainage area of 16.8 km^2 and mean discharge of 0.5 $m^3\ s^{-1}$. Lewis Creek has a moderate size drainage of 200 km^2 and a mean discharge of 3.1 $m^3\ s^{-1}$. The Missisquoi River is one of the largest tributaries to Lake Champlain with a drainage area of 2201.5 km^2 and mean discharge of

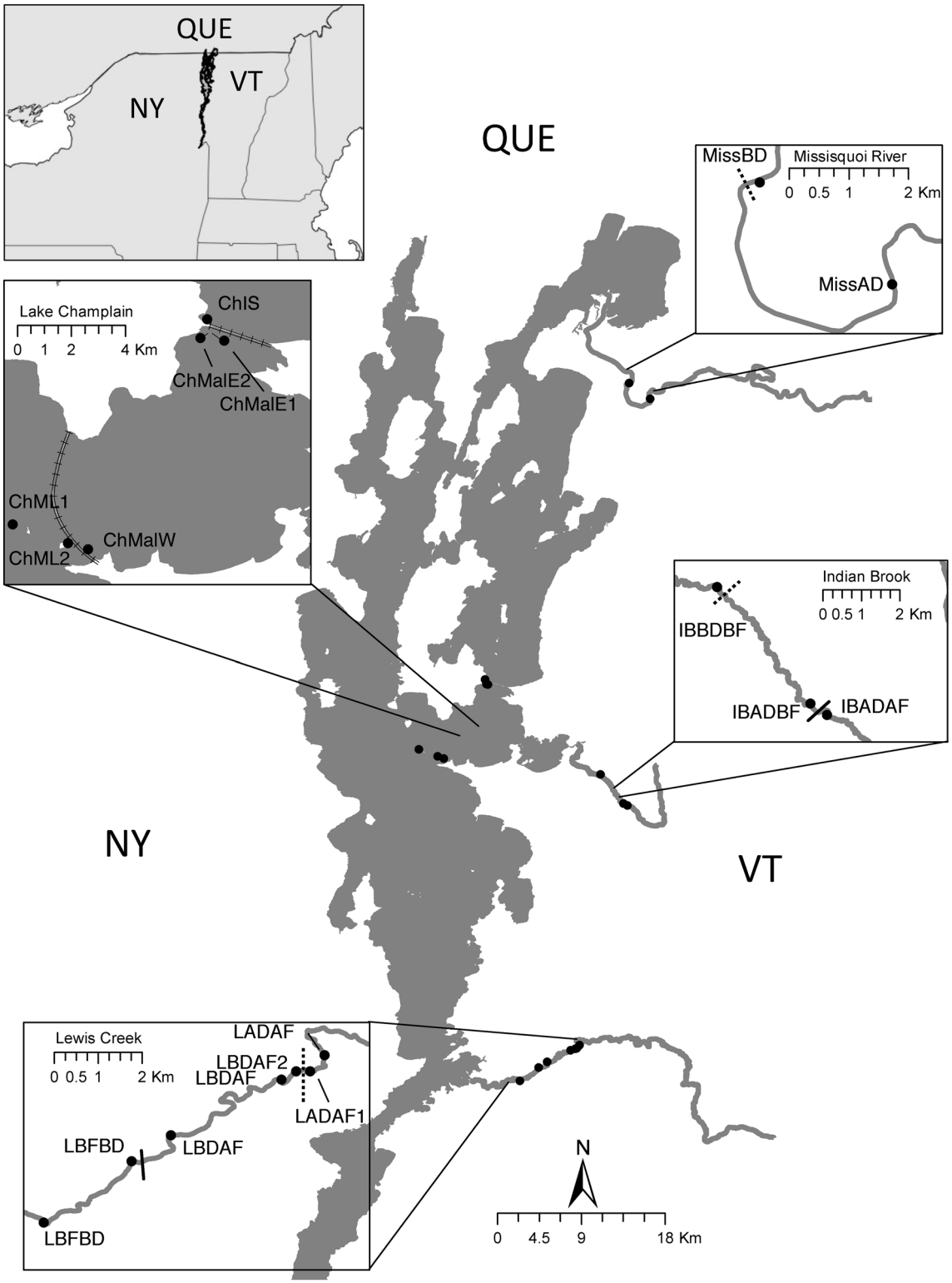

Fig. 1 Sampling sites (black dots) for tessellated darters collected from Lake Champlain and three Lake Champlain tributaries (Missisquoi River, Indian Brook, and Lewis Creek). Three types of potential barriers to darter dispersal are indicated in inset maps: fall lines (solid lines), dams (broken lines) and causeways (double line with hash marks). Site codes indicate location (e.g. Ch = Champlain) followed by location relative to barriers for rivers (e.g. AD = Above Dam, BF = Below Fall line) or basin for Lake Champlain sites (e.g. Mal = Malletts Bay). Full site names and codes can be found in Table S4

Table 1 Basic characteristics of the seven barriers in the Lake Champlain basin evaluated in this study

Barrier name	Latitude	Longitude	Type	Year built	YBP isolation	Height (m)	River drainage area (km^2)	Mean discharge ($m^3\ s^{-1}$)
Lewis Creek FL	44.2600	−73.2126	Fall line	NA	12,000	3.8	200	3.1
Lewis Creek Dam	44.2787	−73.1772	Dam	1980	37	4.0	200	3.1
Indian Brook FL	44.5148	−73.1277	Fall line	NA	12,000	5.5	17	0.5
Indian Brook Dam	44.5418	−73.1526	Dam	1900	117	3.7	17	0.5
Missisquoi Dam	44.9206	−73.1279	Dam	1920	97	5.8	2202	35.0
Outer Malletts CW	44.5648	−73.3112	Causeway	1899	98	0.0	21,326	NA
Sandbar CW	44.6312	−73.2561	Causeway	1850	167	0.0	21,326	NA

FL natural fall line, *CW* causeway, *YBP* years before present

35 $m^3\ s^{-1}$. The height of dams was taken from the height reported in the Vermont Dam Inventory managed by the Vermont Department of Environmental Conservation. No dam examined in this study contained a fish passage system and therefore each dam should be a complete upstream barrier to fish. Because the fall lines are partially eroded and form multiple cascades, a single height measurement would not be descriptive of the barrier. However, elevation drops of 0.5–1.0 m were observed at each fall line, which should be sufficient to prevent upstream movement of tessellated darters. To quantify the heights of fall lines consistently, the height of each fall line was defined by first creating a path of the entire cascade region of the fall line as determined visually in the field and then confirmed using topography in Google Earth. Next, the elevation profile of the entire path was used to identify the 20-m section with the steepest slope and defined the height of the fall line as the change in elevation across the steepest 20 m section of total path because this section was most likely to be the greatest barrier to migration. These measurements confirm that all fall line heights were equivalent to dam heights and therefore reasonable barriers to tessellated darters.

Fish sampling and genetic analysis

Fish were captured using a combination of beach seines, dip nets, and benthic trawls at 18 sites throughout Lake Champlain and the three tributaries (Fig. 1). Specifically, we targeted populations separated by two causeways in the lake, and by a natural fall line and dam in each of the three tributaries, allowing comparison between populations separated by a causeway, dam, fall line, dam and fall line, or no barrier (i.e., distance alone). While sampling was conducted at all sites indicated, no tessellated darters were found above or directly below the fall line in the Missisquoi River and therefore only samples from above and below the dam were analyzed in the study (see paragraph one of results for details). The sampling strategy also allowed comparisons between tributaries relative to lake populations, and downstream relative to upstream populations. Individuals were killed in the field and preserved in 95% ethanol. In the laboratory, fish were placed in 2-ml centrifuge tubes filled with fresh 95% ethanol for storage, generally within 24 h of sampling.

DNA was extracted from samples using a 5% Chelex-100 suspension. For each sample, approximately 1 mm^3 of muscle tissue was placed in 200 µl PCR tube with 150 µl of 5% Chelex-100 solution and 5 µl proteinase-K (Qiagen). Samples were incubated at 55 °C for 8 h followed by 99 °C for 10 min, 37 °C for 1 min, and 99 °C for 10 min and held at 4 °C or frozen at − 20 °C for polymerase chain reactions (PCRs). PCR was conducted for 12 microsatellite loci previously identified for the *Etheostoma* genus (Table S1); D1, Eo4, Eo6, Eo7 (DeWoody et al. 2000), Eca46EPA, Eca49EPA (Tonnis 2006), C2, C6, D116 (Switzer et al. 2008), Ebl3, Ebl6 (Beneteau et al. 2007) and Esc26b (Gabel et al. 2008). Loci C2, EO7, and Eca46EPA were found to be monomorphic after genotyping 99 individuals and were removed from future analysis, leaving a total of nine microsatellite loci. Loci were amplified in multiplex reactions when possible in 12.5 µl reactions containing 6.25 µl 2X Taq DNA Polymerase Master Mix (New England BioLabs Inc.), 0.8 µM μl^{-1} fluorescently labeled forward and unlabeled reverse primer, and DNA template. The general PCR program used was 95 °C for 2 min, 30 cycles at 95 °C for 30 s, 20 s at marker-specific annealing temperature (Table S1), 68 °C for 30 s followed by a final extension of 68 °C for 10 min. Fragment analysis of PCR products were analyzed in the University of Vermont Advanced Genome Technologies Core using an Applied Biosystems 3130 Genetic Analyzer and a ROX 500 size standard and scored using GENEMAPPER software (Applied Biosystems).

Statistical analysis

All loci were assessed for the presence of null alleles with MICRO-CHECKER version 2.2.3 (Van Oosterhout et al.

2004). Conformance to Hardy–Weinberg equilibrium (HWE) expectations at each locus within each population using exact testing, observed (H_O) and expected (H_E) heterozygosity, F_{IS} and allelic richness scaled to the smallest population and using rarefaction to account for differences in sample size were estimated using the basicStats() function of the diveRsity package v.1.9.90 in R version 3.3.3 (Keenan et al. 2013; R Core Team 2015). Any deviations from HWE following Bonferroni correction for multiple comparisons were assessed for heterozygote excess or deficiency reported in the basicStats() function output. Effective population size of each sampled location was calculated using a linkage disequilibrium method in N_eESTIMATOR (Do et al. 2014) with minimum acceptable allele frequencies of 0.02 which was used for microsatellite loci by Do et al. (2014) to avoid upward bias of effective population size estimates and all other parameters set to default conditions.

Asymmetrical upstream and downstream migration across barriers was evaluated for all drainages separately using the experimental divMigrate() function using default parameters in the diveRsity package v.1.9.90 in R which uses the method described in Sundqvist et al. (2016), Keenan et al. (2013). In brief, this method works by generating a hypothetical pool of migrants for a given pair of populations and then estimates a measure of genetic differentiation between each population and the hypothetical pool. The estimated directional genetic differentiation can then be used to estimate relative levels of migration. After migration was estimated among all sites, a two-way analysis of variance (ANOVA) was used to determine if migration differed across different barrier types and upstream versus downstream.

To evaluate genetic clustering without a priori assumptions of population, two different approaches were used. First, variation among and within each drainage was assessed using STRUCTURE (Pritchard et al. 2000) deployed through the ParallelStructure package for R (Besnier and Glover 2013). STRUCTURE was run hierarchically, first on the complete dataset and then on sites within each drainage. Each dataset was examined separately through five replicate runs of 100,000 replicates and a 10,000 cycles burn-in at k = 1–5. Discriminate analysis of principal components (DAPC) implemented in ADEGENET package v.2.0.1 was used as a second clustering estimator and run hierarchically like STRUCTURE (Jombart 2008; Jombart et al. 2010). Clusters were identified as overlapping groups in DAPC bi-plots. Possible genetic structure among sample sites was evaluated further using pairwise comparisons of F_{ST} and G'_{ST}, and 95% confidence intervals calculated using the diveRsity R package. Because G'_{ST} is standardized and therefore performs better for loci with multiple alleles and is not an estimate that is dependent on single-step mutation model which are sensitive to issues of homoplasy common in microsatellite loci, we chose G'_{ST} as the estimate of genetic distance (Hedrick 1999, 2005a; Sefc et al. 2007). However, standardized estimates of genetic distance can bias migration estimates by inflating distance estimates and therefore should not be used as an estimate of gene flow (Hedrick 2005a). Therefore, estimates of F_{ST} are also reported in supplementary materials and were used to compare against power estimates.

We tested for the statistical power to detect genetic differentiation at five different expected levels of F_{ST} (0.001, 0.0025, 0.005, 0.01, and 0.05), given the sample sizes, number of loci and allele frequencies used in this study, using POWSIM (Ryman and Palm 2006; Ryman et al. 2006). POWSIM simulates the sampling of genes from a specified number of population with a set effective population size (2000 for this study) that have diverged by drift for t number of generations. Samples from the simulated populations are then used to test for genetic homogeneity using Fisher's exact test and χ^2-tests. Power is then defined as the proportion of significant results obtained over multiple replicate simulations (2000 for this study).

To evaluate how drainage, upstream-distance, number of barriers and barrier type impacted genetic diversity (H_E, H_O, and allelic richness), we used a series of variance and covariance analyses (ANOVA and ANCOVA). Differences in genetic diversity among basins and upstream distance were evaluated using a two-way ANOVA with the diversity estimate as the response variable and drainage and upstream distance as the predictor variables. To test if barrier type influenced the change in genetic diversity from downstream to upstream populations, the change in diversity was calculated between every two pairs of sites within the same drainage as the difference between the downstream diversity estimate and the upstream diversity estimate for a given pair of sites. Next, differences in the change in diversity (H_E, H_O, and allelic richness) across five barrier types (no barrier, causeway, dam, fall line, dam and fall line) were assessed using an ANOVA with pairwise change in diversity as the response variable and barrier type as the predictor variable. The pairwise change in diversity was used as the response variable rather than point estimates of diversity themselves to directly assess the influence of barriers on diversity while partially controlling for effects of upstream distance and variation in diversity among drainages. Any significant effects were investigated using Tukey's honestly significant tests (Tukey 1949). For all statistical tests, significance was determined based on an alpha level of 0.05.

Generalized linear models

To determine how drainage, distance, number of barriers, barrier type and barrier age impacted genetic distance (G'_{ST}), we used a generalized linear models (GLM) approach. Unlike more traditional approaches such as partial Mantel

tests to a single predictive variable, GLM can combine multiple predictors and likelihood statistics can be employed to compare among models (Storfer et al. 2007). Landscape features were chosen to limit collinearity and models were purposefully kept simplistic, comparing only a single feature in addition to a null model of isolation by distance (IBD) at a time. Models were fit using the *glm()* function in the stats package in R with pairwise G'_{ST} as the response variable and one or more landscape features as the predictor variable and assuming a Gaussian distribution. Because G'_{ST} is standardized, it cannot be used as an estimate of gene flow. However, our goal was to identify the relative influence of landscape features on genetic distance, not estimate migration among sites. Therefore, using a standardized method such as G'_{ST} allows for comparison of genetic distance while controlling for differences in genetic diversity throughout the study system that would influence non-standardized estimates of genetic distance (Hedrick 1999, 2005a).

Eight total models in two broad categories were run to describe genetic distance of tessellated darters. Category 1 included three null models of genetic distance across the total study area (global models hereafter; Table 3). Model 1 was our null global model and evaluated the influence of isolation by waterway distance (IBD) on genetic distance among all sampled sites. Geographic distance was measured in meters as the shortest distance via water between any two site pairs. Model 2 evaluated the influence of IBD and total number of barriers on genetic distance among all sampled sites. Model 3 evaluated the influence of IBD and a random effect of drainage comparison (a factor indicating the two drainages involved in the pairwise estimate of distance) on genetic distance among all sampled sites. The purpose of model 3 was to determine if other unmeasured differences among drainages explained more variance than distance alone. Category 2 models limited the dataset by removing pairwise comparisons between drainages and analyzing only within-drainage pairwise comparisons (referred to as within-drainage models hereafter). Six within-drainage models were evaluated. Model 4 was our null within-drainage model and evaluated the influence of just IBD on genetic distance within each drainage, ignoring the presence or absence of barriers. Model 5 evaluated the influence of IBD and barrier type (no barrier, dam, causeway, fall line, or combination of a dam and a fall line) on genetic distance. Model 6 assumed all barrier types were equal and evaluated the influence of IBD and total number of barriers (0–2) on genetic distance. Model 7 evaluated the influence of IBD and barrier age on genetic distance measured as the age of the oldest barrier in years separating two populations (0–12,000) and Model 8 assumed genetic distance was drainage-specific, and evaluated the influence of IBD and drainage size (km^2) on genetic distance. To account for variation in units among predictors, all parameter estimates were standardized by dividing them by two standard deviations (Gelman 2008). All but our two null models included only distance and a single additional predictor to avoid issues associated with collinearity between barrier metrics which can confuse model interpretation (Zuur et al. 2010).

Model selection was conducted separately in each of the two model categories and was based on three principal metrics. First, models were ranked using Akaike's Information Criterion (AIC) whereby a larger absolute value AIC indicates more support for a given model (Akaike 1992). Second, to test if added predictors improved a model beyond that of a null model of isolation by distance, we used likelihood ratio tests calculated using the anova() function in the stats package of R. Third, the adjusted R^2 was calculated for each model to provide a directly interpretable metric of the variance explained by each model. To help with independent model interpretation, null and residual deviance and residual degrees of freedom were also reported, but not used directly in model selection.

Results

The reported heights of dams and estimated heights of fall lines were roughly equivalent. Therefore, all barriers were considered to be effective barriers to tessellated darters. A total of 482 tessellated darters were sampled during July and August 2016 and an additional 24 darters were collected during August 2017. Tessellated darters were successfully sampled from all targeted locations other than above and below the natural fall line in the Missisquoi River where tessellated darters have been reported to be less common possibly due to the presence of fantail darters (*Etheostoma flabellare;* Rich Langdon personal communication). Thus, only samples from above and below the dam in Missisquoi River were collected and evaluated. Evidence of null alleles was found in 9 out of 162 locus-site comparisons. However, no locus was identified to have null alleles in more than 3 of 18 populations and there were no consistent deviations from HWE among loci or within populations following Bonferroni corrections. Because evidence of null alleles and deviations from HWE was infrequent and inconsistent, the complete dataset was analyzed for population analysis moving forward. Tests for statistical power indicated the probability of detecting genetic differences of F_{ST} of 0.005 and greater was 100% (all simulations detected differentiation; Table S2). Therefore, the current loci and sample sizes should be sufficient to detect all but small differences which are likely not biologically meaningful in the context of this study and therefore interpretation of their effect should be avoided (Hedrick 1999; Richardson et al. 2016).

Allelic richness, H_E, and H_O differed significantly among sampled drainages (ANOVA $p < 0.001$ for all comparisons)

and were consistently higher in Lake Champlain and Missisquoi River than sites in Indian Brook or Lewis Creek (Table 2; Fig. 2). In contrast, effective population size was estimated to be infinity for at least one site in every drainage and the jackknifed confidence interval included infinity in all but three sites, with no clear pattern by drainage. F_{IS} was variable but generally low (range = −0.09 to 0.14, mean = 0.02) across all sites. Allelic richness, H_E, and H_O also decreased slightly with distance upstream from Lake Champlain. When Lake Champlain was included in the analysis, allelic richness, H_E, and H_O all a had significant negative relationship with upstream distance (Fig. 2); however, when only river populations were analyzed, only allelic richness maintained a significant negative relationship with upstream distance, though a negative, non-significant, relationship was still apparent between H_E and H_O and upstream distance.

Allele frequencies differed among sampling drainages. STRUCTURE and DAPC analysis revealed three distinct clusters grouped by sampling drainage (Fig. 3). Lake Champlain and Missisquoi River samples clustered into a single, admixed group while Lewis Creek and Indian Brook formed separate, more divergent populations with very little overlap with other clusters. Lewis Creek and Indian Brook clusters had higher definition than the Missisquoi and Lake Champlain cluster as indicated by the high density of points along discriminant function 1 of the DAPC analysis (Fig. 3).

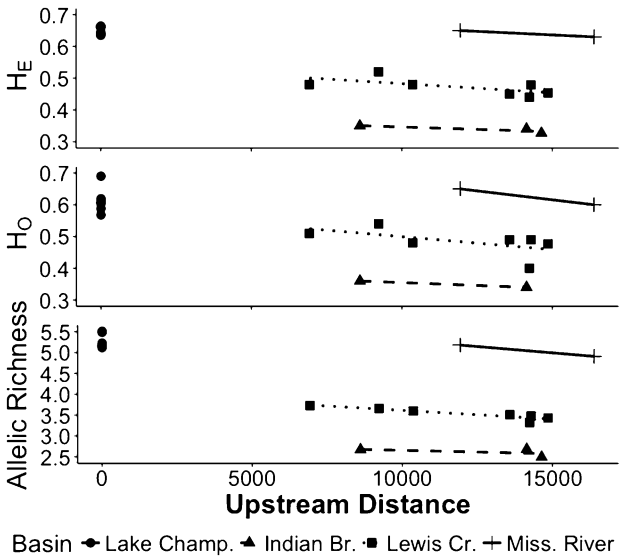

Fig. 2 Average observed (H_O) and expected (H_E) heterozygosity, and allelic richness for tessellated darters collected from Lake Champlain, Indian Brook, Lewis Creek and the Missisquoi River as a function of upstream distance from Lake Champlain. Each dot represents a single sample location

Estimates of pairwise G'_{ST} corroborated observed clusters whereby G'_{ST} values between pairs of drainages were much higher than within drainages.

Table 2 Number of tessellated darters genotyped (N), mean effective sample size (efN), observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F_{IS}), allelic richness (AR), and estimated effective population size (Ne). Site codes indicate location (e.g. Ch = Champlain) followed by location in relative to barriers for river sites (e.g. AD = Above Dam, BF = Below Fall line) or basin for Lake Champlain sites (e.g. Mal = Malletts Bay). Full site names and codes can be found in Table S4

	N	efN	H_o	H_e	F_{IS}	AR	Ne
Lake Champlain							
ChIS	39	33.0	0.619	0.662	0.07	5.17	360.5 (44.5–∞)
ChML1	24	23.3	0.609	0.645	0.03	5.16	∞ (96.4–∞)
ChMalE1	12	10.9	0.568	0.635	0.09	5.49	∞ (17.5–∞)
ChMalE2	24	22.3	0.588	0.638	0.09	5.23	∞ (127.8–∞)
ChMalW	35	33.9	0.604	0.664	0.07	5.51	2678.3 (101.8–∞)
ChML2	13	12.6	0.690	0.660	−0.05	5.12	19.5 (10.4–56.9)
Indian Brook							
IBADAF	48	41.3	0.297	0.327	0.08	2.49	65.3 (19.7–∞)
IBADBF	24	19.9	0.340	0.340	−0.02	2.68	∞ (13.4–∞)
IBBDBF	47	41.3	0.360	0.350	0.03	2.67	50.4 (21.6–485.7)
Lewis Creek							
LADAF1	23	22.7	0.490	0.479	−0.03	3.48	223.0 (26.3–∞)
LADAF2	24	24.0	0.477	0.454	−0.04	3.43	∞ (74.1–∞)
LBDAF1	24	22.0	0.480	0.480	0.02	3.60	35.5 (12.5–∞)
LBDAF2	12	11.0	0.490	0.450	−0.09	3.51	∞ (15.6–∞)
LBDAF3	12	10.6	0.400	0.440	0.14	3.32	7.9 (2.5–46.2)
LBFBD1	23	21.2	0.510	0.480	−0.08	3.73	44.9 (16.0–∞)
LBFBD2	24	23.7	0.540	0.520	−0.02	3.66	∞ (33.7–∞)
Missisquoi River							
MissAD	48	43.1	0.600	0.63	0.04	4.91	549.2 (82.3–∞)
MissBD	50	44.4	0.650	0.65	−0.01	5.18	∞ (121–∞)

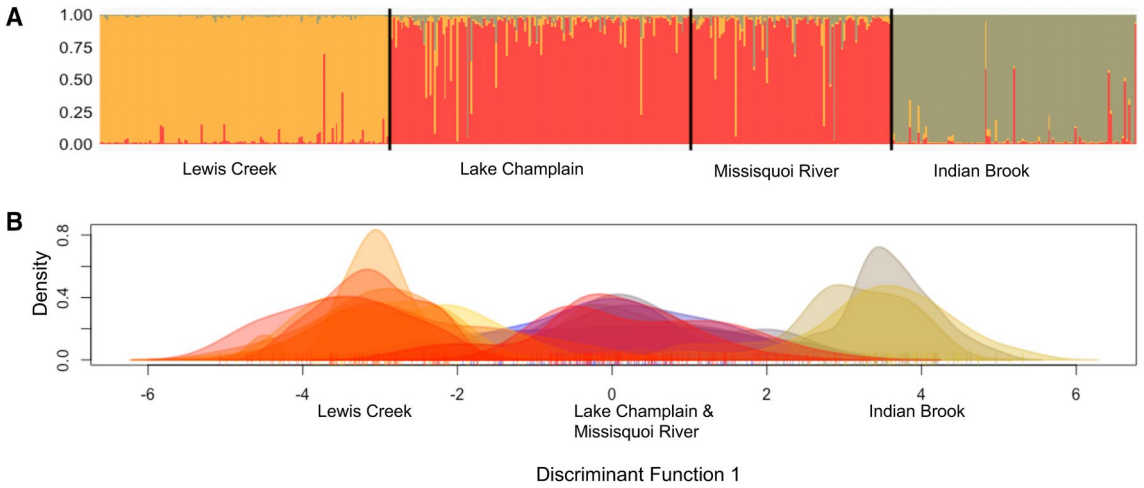

Fig. 3 Two types of cluster analysis of tessellated darters sampled from 18 sites. **a** Barplot of STRUCTURE results for the most likely number of clusters (k = 3). Each bar represents a single individual with color representing the relative likelihood an individual is from a given colored cluster, vertical black lines indicate separation between drainages. **b** Clustering of darters along the most descriptive discriminant function of a DAPC. Colored peaks refer to specific sampling locations in the drainages Lewis Creek (oranges), Lake Champlain and Missisquoi River (reds and blues), and Indian Brook (beige). (Color figure online)

The influence of barriers on genetic diversity and population structure was less defined than the influence of drainages. Cluster analysis conducted within each drainage did not show any clustering that would indicate the presence of more than a single, panmictic population within each drainage (Fig. S1). Estimates of pairwise G'_{ST} corroborated the observed lack of clusters whereby G'_{ST} values between pairs of sites within the same drainage were universally low and confidence intervals almost always included zero (Fig. S2). Within drainages, allelic richness, H_O, and H_E did not change as the number of downstream barriers increased ($p > 0.1$ for all; Table 2). The change in allelic richness differed among barrier types ($F_{4,35} = 4.65$, $p = 0.004$) and was significantly greater across fall lines and the combination of dams and fall lines than across causeways (Tukey HSD $p = 0.008$ and 0.015) but similar among all other barrier types. The same main effect was found for H_O ($F_{4,35} = 2.73$, $p = 0.045$) and H_E ($F_{4,35} = 6.80$, $p < 0.001$). However, Tukey HSD test revealed no significant pairwise differences in H_O among barrier types (Tukey HSD $p > 0.05$ for all) but did reveal that H_E was significantly greater across fall lines and the combination of dams and fall lines than across causeways or dams (Tukey HSD $p = 0.002$, 0.040, 0.004, and 0.046 respectively; Fig. 4). The change in diversity from downstream to upstream of a barrier was greater across fall lines than dams, but similar to populations separated by causeways or no barrier at all (Fig. 4). Overall, estimated migration was higher in the downstream direction (mean = 0.45; SD = 0.23) than upstream (mean = 0.35; SD = 0.15) for river samples ($p = 0.014$) but was similar in both directions across causeways for lake samples ($p = 0.78$). The relative amount of estimated migration did not vary by barrier type ($p = 0.77$).

Generalized linear models

Of the three global models of genetic distance, Model 3 which contained the predictors of waterway distance and a random effect, basin combination, performed significantly better than the other two models (Table 3) and appeared to predict almost all the variation among sites (adjusted $R^2 = 0.97$). Model 2, which included the total number of barriers separating two sites as a predictor, performed slightly but not significantly worse than our null IBD model (Model 1). Models 1 and 2 explained identical amounts of variation (adjusted $R^2 = 0.21$), further indicating that the number of barriers between two sites did not substantially influence genetic distance. Of the five within-drainage models of genetic distance, no predictor was found to significantly improve the performance from the null model of IBD (Table 3). However, this does not indicate that the IBD model explained a high amount of variation in genetic distance (adjusted $R^2 = 0.02$, Fig. 5). Additionally, there was low overall null deviance in G'_{ST} within drainages, and therefore little deviance for any predictive variable to explain (Table 3).

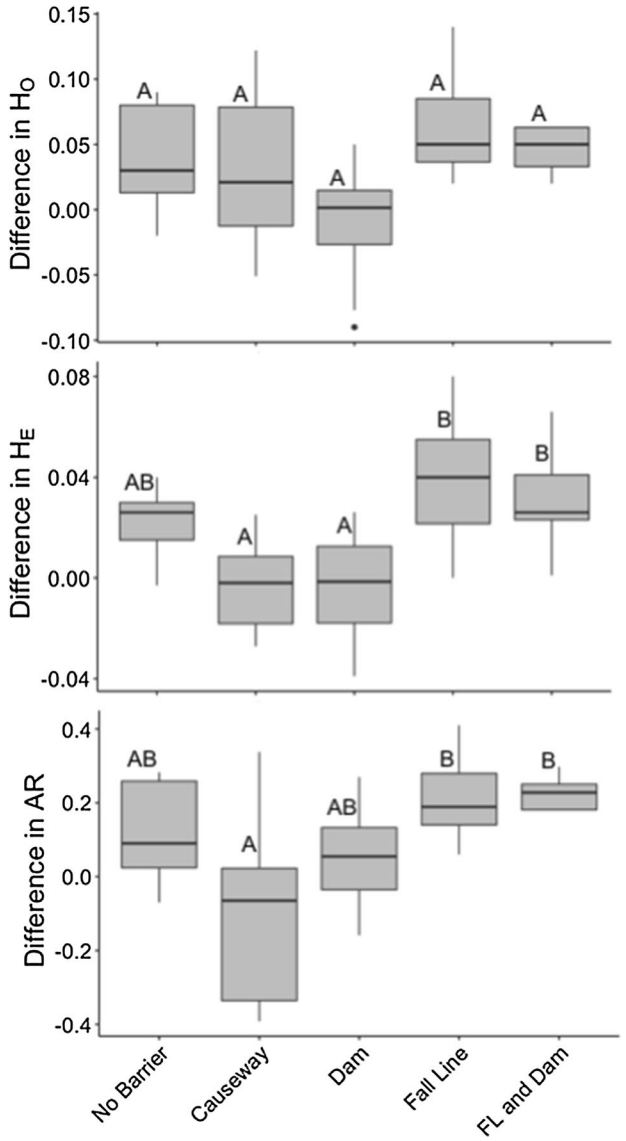

Fig. 4 Average change (downstream to upstream) in observed (H_O) and expected (H_E) heterozygosity, allelic richness (AR) between sites within drainages for tessellated darters collected on either side of five barrier treatments (x-axis). *FL* = fall line

Discussion

Tessellated darters in the Lake Champlain watershed were characterized by a high amount of variation among drainages but low variation in genetic diversity and allele frequency within drainages. Populations within individual drainages maintained genetic connectivity even across strong dispersal barriers and had limited loss of diversity with upstream distance and increased fragmentation. These findings are indicative of distinct sub-populations residing in river drainages with exchange of individuals across barriers within drainages.

Tessellated darter populations had drainage-specific patterns of genetic diversity. Estimates of allelic richness, H_E, and H_O, were more than twice as high in Lake Champlain and Missisquoi River than in Indian Brook and more than 50% higher than in Lewis Creek. These observations are consistent with a patch size hypothesis of genetic diversity whereby genetic diversity increases with area occupied by the population (Vellend 2003). The Missisquoi River drains over 80 times the area of land and discharges 70 times more volume of water than Indian Brook, and is 11 times larger in drainage area and discharge than Lewis Creek. If the size of the drainage is proportional to the population size and patch size, our results are consistent with other studies (Frankham 2008). Knaepkens et al. (2004) found that observed and expected heterozygosity of European bullhead (*Cottus gobio*) nearly doubled as patch size doubled from 3000 to 6000 m. Additionally, Whiteley et al. (2013) found that variation at eight microsatellites was related to habitat fragmentation and patch size in brook trout. Finally, Vellend (2005) used simulations to evaluate how genetic diversity varied with patch size that and found that not only did genetic diversity increase with patch size, but that the relationship was stronger for common species than rare species. Therefore, because tessellated darters are common in all four of the drainages we analyzed, the relationship between patch size and genetic diversity may have a larger effect size and therefore be more detectable in our study compared to studies in which populations sizes are small.

Not surprisingly, drainage also had a large influence on the population structure of tessellated darters and drainage combination was the strongest predictor of genetic distance in our global model. Drainage often explains much of the variation in other darter species; for example, greenside darter populations (*Etheostoma blennioides*) in Ontario were structured by drainage ($F_{ST} = 0.079$) and similar results were found for the fountain darter (*Etheostoma fonticola*), Okaloosa darters (*Etheostoma okaloosae*) and others (Beneteau et al. 2009; Austin et al. 2011; Olsen et al. 2016). In addition to drainage effects, we found that waterway distance had a moderate effect on genetic distance at a global scale and almost no effect of distance among sites within basins. Similarly, distance explained 40–85% of genetic divergence among drainages in a recent invasion of greenside darter populations (Beneteau et al. 2009). The strong divergence of the greenside darters in that study may be partially explained by a strong founder effect related to the recent invasion. While distance explained about 20% of the variation of G'_{ST} in global models in the present study, the IBD pattern showed a notable break in suggesting that other, unmeasured differences among drainages also influence genetic distance. The observed break in the IBD pattern is exemplified by the apparent lack of genetic divergence between of Missisquoi River and Lake Champlain darters but large

Table 3 Models used to describe connectivity of tessellated darters across the Lake Champlain basin and within individual drainages

Model ID	Model	Rank	AIC	RDF	Residual deviance	Null deviance	Adj_R^2	LRT p
Global models								
Model 1	G'_{st} ~ dist	2	−417.34	151	0.563	0.723	0.216	
Model 2	G'_{st} ~ total barriers + distance	3	−415.38	150	0.563	0.723	0.211	0.84
Model 3	G'_{st} ~ basin combination + distance	1	−914.12	142	0.019	0.723	0.971	**< 0.01**
Within-drainage models								
Model 4	G'_{st} ~ distance	2	−297.31	38	0.001	0.001	0.017	
Model 5	G'_{st} ~ barrier type + distance	3	−296.90	34	0.001	0.001	0.091	0.13
Model 6	G'_{st} ~ drainage area + distance	5	−295.31	37	0.001	0.001	−0.010	0.98
Model 7	G'_{st} ~ isolation time + distance	4	−295.76	37	0.001	0.001	0.002	0.52
Model 8	G'_{st} ~ total barriers + distance	1	−298.81	37	0.001	0.001	0.075	0.07

Bolded values indicate the level of significance found by likelihood ratio tests for model improvement form a null model of IBD

Model selection metrics included: Akaike Information Criteria (AIC), residual degrees of freedom (RDF), residual deviance, null deviance, adjusted R2, and likelihood ratio test Chi square p-value (LRT p)

genetic divergence between Indian Brook and Lake Champlain darters. The Missisquoi River is 44 km from the closest Lake Champlain population we sampled, while Indian Brook empties into the lake only 10 km from the nearest Lake Champlain sample site. If distance alone predicts genetic distance, darters from Indian brook should be genetically more similar to Lake Champlain darters than we observed, while Missisquoi River darters should be genetically more distant. These patterns could indicate that Missisquoi River functionally acts as a continuation of Lake Champlain, while smaller drainages like Indian Brook and Lewis Creek contain isolated sub-populations with little migration to or from Lake Champlain. Overall, our results suggest that, in a large, stable population of tessellated darters, genetic structure and diversity may be almost entirely determined by river drainage, with low migration between sub-populations regardless of distance or physical barrier, partially refuting hypothesis 1 that distance and barriers influence population structure.

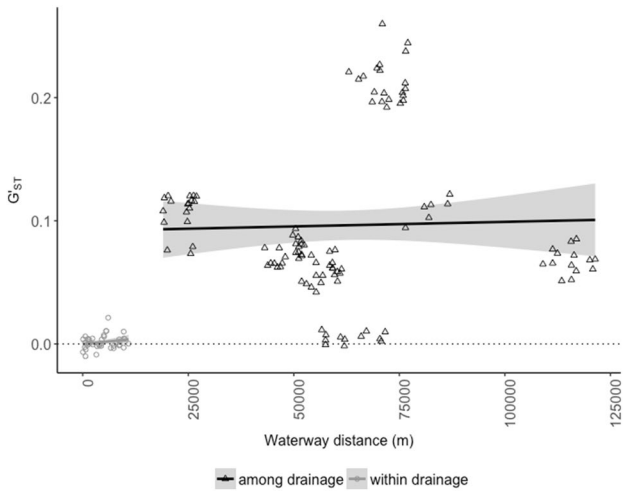

Fig. 5 Pairwise G'_{ST} by waterway distance. Points indicate individual pairwise comparisons, lines indicate linear relationship among within drainage (circles) and between drainage (triangles) comparisons. Grey area around lines indicate standard error

Within drainages, neither natural nor man-made fragmentation had a large influence on the genetic structure and diversity of darter populations, giving no support to hypotheses 3 (that natural and manmade barriers increase population structure) and 5 (the magnitude of a barrier's effect is related to barrier age and type). Incomplete samples from the Missisquoi River led to decreased statistical power to detect differences barrier types; however, genetic diversity and distance within all rivers showed little change across any barrier, suggesting the inclusion of fall line samples in Missisquoi River would not likely influence the conclusions of this study. Because all but one within-drainage pairwise G'_{ST} estimate had a 95% confidence interval that included zero, the level of genetic distance among sites within drainages was functionally zero. Therefore, the inability to detect clusters of individuals within drainages or explain variance in pairwise distance across different barrier types was not surprising. Some genetic structure may exist within drainages that could not be detected with the loci used in in this study. However, our power analysis indicated that levels of F_{ST} greater than 0.005 should have been detectable. Therefore, any genetic structure present is small and unlikely to be of conservation concern or have major evolutionary consequences (Richardson et al. 2016). The lack of genetic distance among fish separated by barriers in our study is in direct contrast to research on many other species including yellow perch (*Perca flavescens*), bull trout (*Salvelinus confluentus*), and log-perch (*Percina caprodes*), where dams were one of the strongest predictors of population structure

(Leclerc et al. 2008; Meeuwig et al. 2010; Roberts et al. 2013). We assessed population structure across three dams ranging from 37 to 117 years old, of which all had a height of at least 1 m and formed strong upstream barriers for small fish such as tessellated darters (Porto et al. 1999). If the barriers we evaluated truly isolated darter populations, our power analysis indicated that even at a relatively large effective population size (2000 individuals), significant genetic distance should be detectable after 20 generations of isolation and drift. Given that tessellated darters likely mature at 1–2 years old and only live to age 4 or 5 years old, even 37 years of isolation could be enough to result in population structure (Fahy 1954). Barriers of similar age and size have been shown to result in observable genetic structure in populations of other small fish, some with abundant populations. For example, the European chub (*Squalius cephalus*) had higher genetic differentiation and a larger decline in allelic richness across regions separated with many small weirs or large dams than in un-fragmented sections (Gouskov and Vorburger 2016). Other species, such as the Yazoo darter (*E. raneyi*), with compromised or endangered populations also show signs of increased genetic distance among sites separated by dams (Sterling et al. 2012). However, the effect of multiple small dams on European chubs was relatively small, indicating that either migration across barriers was possible, the population size was high enough to limit genetic drift, or both. Also, much of the difference among Yazoo darter populations could be explained by strong bottlenecks associated with small population size. Therefore, the impact of a barrier may be more strongly linked to life history and population demography of a species than the age or size of the barrier.

Population demography and life history are strongly linked to estimates of genetic distance (Cano et al. 2008). The level of genetic distance among populations is often attributed to the level of dispersal associated gene flow (Ward et al. 1994). However, effective population size strongly influences genetic distance (Hedrick 2005b). Simulations indicate that when Ne is large, (6000–20,000 individuals), hundreds to thousands of generations are needed to reach low levels of genetic distance (Cano et al. 2008). Although, tessellated darters are common throughout our study area, the effective population size at sampled sites was often much smaller than a thousand individuals. Thus, we suggest that while population size likely plays an important role in reducing the effects of genetic drift compared to endangered populations, some gene flow would still be necessary to explain the level of homogeneity observed within drainages.

Though dams often influence population structure of fish, there are many examples where they do not. Mottled sculpin (*Cottus bardi*), which are common in the Nantahala River (North Carolina, USA), show patterns of strong isolation by distance across just 5 km, but very little evidence of any isolation by barrier (Lamphere and Blum 2012). The population structure of six species of fish in the Truckee River of California and Nevada was found to be significantly structured by barriers during a low-flow year, but the structure disappeared the following year when high river discharge re-distributed fish and broke down the observed structure (Peacock et al. 2016). These examples suggest that small, instream barriers do not necessarily result in genetic differentiation of fish populations, even if they limit fish movement. For tessellated darters, downstream migration across barriers may be sufficient to homogenize populations. Especially, if upstream populations are large enough to reduce the effects of genetic drift. We found very low levels of genetic distance among sites within drainages and evidence of strong downstream migration, supporting hypothesis 4 (that migration would be primarily downstream). Tessellated darters were found within a few hundred meters above and below most barriers. Individuals directly above barriers would be particularly vulnerable to being washed downstream, especially during storm events. Additionally, we found only a small decrease in genetic diversity with upstream distance (hypothesis 2), indicating that upstream populations are not suffering from stronger genetic drift or inbreeding than downstream populations. Therefore, downstream migration combined with low inbreeding and genetic drift may be enough to maintain darter population homogeneity across barriers.

Implications for barrier management and fish conservation

Instream barriers have been a conservation concern and focus of research for decades, with the general consensus that dams and other barriers have long-term, negative effects on genetic diversity (Helfman 2007). As interest in barrier removal continues to grow (Mclaughlin et al. 2013), identifying the highest-impact barriers to target for removal and understanding the potential impacts of new barriers is increasingly important. However, efforts to identify and predict the influence of barriers on fish populations has had mixed success; some investigators have found a strong relationship between barrier type and connectivity (Gouskov and Vorburger 2016) and others found only limited relationships between barriers and connectivity (Chick et al. 2006). Our research and number of other studies indicate some populations may be resistant to the effects of habitat fragmentation. However, predicting which taxa or populations are most sensitive to habitat fragmentation can be problematic (McLaughlin et al. 2006). Therefore, we suggest future studies of aquatic fragmentation focus on assessing the influence of a common barrier on multiple taxa, rather than multiple barrier types on a single taxon as we presented here.

Our results indicate that high population structure between drainages and variable genetic diversity may be normal for darters and therefore sufficient for a sustainable population. Many darter species are endangered and are the focus of population restoration or reintroduction (e.g., Shute et al. 2005; Olsen et al. 2016). Our results provide a baseline level of genetic structure and diversity for a non-endangered species of darter and can therefore be used to help establish target conservation goals for endangered darters with similar ecology. Although we did not find that barriers had an influence on the population structure of tessellated darters, many studies on other threatened or endangered species have found that barriers can have a large effect on the dispersal, diversity, and genetic structure of populations (e.g., Austin et al. 2011; Beneteau et al. 2012; Roberts et al. 2013). Therefore, the influence of habitat fragmentation may be species-specific and amplified by small population sizes inherent in endangered species.

Acknowledgements We thank Dr. Matthew Wargo and Dr. Steven Keller for the use of their laboratories for processing samples. We also would like to thank our field technician, Bethany Alger, and the captain Steve Cluett and crew, Brad Roy for help collecting darter samples. Special thanks to Alan Howard, University of Vermont for help with statistical support. Finally, we would like to thank anonymous reviewers whose suggestions helped to improve the final manuscript. This study was supported by funds made available by Senator Patrick Leahy through the Great Lakes Fishery Commission and the USGS Vermont Water Resources and Lakes Study Center.

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