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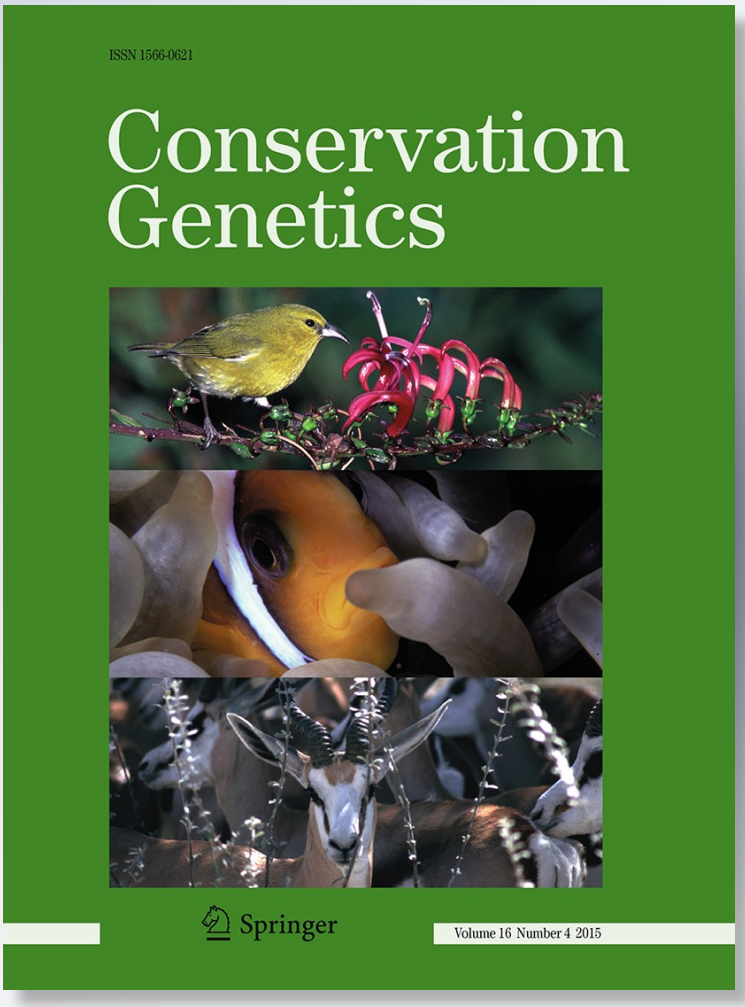

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RESEARCH ARTICLE

Comparative analysis of riverscape genetic structure in rare, threatened and common freshwater mussels

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Abstract Freshwater mussels (Bivalvia: Unionoida) are highly imperiled with many species on the verge of local extirpation or global extinction. This study investigates patterns of genetic structure and diversity in six species of freshwater mussels in the central Great Lakes region of Ontario, Canada. These species vary in their conservation status (endangered to not considered at risk), life history strategy, and dispersal capabilities. Evidence of historical genetic connectivity within rivers was ubiquitous across species and may reflect dispersal abilities of host fish. There was little to no signature of recent disturbance events or bottlenecks, even in endangered species, likely as a function of mussel longevity and historical population sizes (i.e., insufficient time for genetic drift to be detectable). Genetic structure was largely at the watershed scale suggesting that population augmentation via translocation within rivers may be a useful conservation tool if needed, while minimizing genetic risks to recipient sites. Recent interest in population augmentation via translocation and propagation may rely on these results to inform management of unionids in the Great Lakes region.

Keywords Unionid mussels · Scale · Multi-species comparison · Conservation genetics

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Introduction

Contemporary loss of biodiversity is a major global concern, particularly for freshwater species (Dudgeon et al. 2006). As the number of endangered species rises and habitat becomes increasingly sparse and fragmented, it is imperative to recognize the appropriate scale at which to focus conservation efforts (Vaughn 2010). Populations of endangered species are small and often have relatively low genetic diversity compared to common species (Meffe 1986). Because of already reduced genetic diversity in small populations, restoration becomes difficult without a notion of historical distributions and connectivity. Comparative analyses between endangered and common species may be one means of circumventing this problem (Edwards and Wyatt 1994; Maki et al. 2002). Furthermore, comparisons of genetic diversity and structure among threatened and endangered species may provide insight into mechanisms of species decline, loss of diversity, and insight into potential recovery strategies.

Freshwater mussels (Bivalvia: Unionoida) are among the world's most critically imperiled faunas due to recent habitat degradation (impoundments, channelization, sedimentation), pollution, and invasive species (Williams et al. 1993; Lydeard et al. 2004; Strayer et al. 2004), although population declines

over the last 5000 years are also evident for some taxa (Peacock et al. 2005). Although the greatest diversity of unionids occurs in North America (~ 300 species), more than 200 of these species were considered extinct, possibly extinct, critically imperiled, imperiled or vulnerable when last evaluated nearly 10 years ago (Master et al. 2000; Lydeard et al. 2004). Mussels have a unique life history strategy in which their larvae (glochidia) spend a short time parasitizing one or more host fish species to complete transformation to the juvenile stage (McMahon and Bogan 2001; Barnhart et al. 2008). This relationship increases mussel extirpation risk, as they are susceptible to both the direct effects of anthropogenic disturbances and indirectly impacted by effects on host fish populations (Spooner et al. 2011). As glochidia dispersal is dependent on movements of infected host fish, host specificity, dispersal potential, and barriers to fish movement (dams, unsuitable habitat) may have profound effects on connectivity among mussel populations (Newton et al. 2008; Schwalb et al. 2011a, 2011b). Mussels provide important services to ecosystems including coupling benthic and pelagic compartments through filtration, biodeposition, and nutrient excretion and by providing habitat to algae and benthic macroinvertebrates (Spooner and Vaughn 2006; Vaughn 2010). Because of their threatened status and importance to freshwater ecosystems, propagation and recovery initiatives may be critical to restore mussel populations, especially for small, extremely isolated populations (COSEWIC 2003, 2010; Morris and Burridge 2006).

Because much of the mussel life cycle is poorly understood or difficult to study (e.g., host fish use, dispersal distances), population genetics has become a useful tool for understanding the basic ecology of freshwater mussels and defining distinct populations (e.g., Berg et al. 1998; Kelly and Rhymer 2005; Elderkin et al. 2007). There is considerable interest in population augmentation through artificial propagation and adult translocation as a means of mitigating declines in mussel populations (The National Native Mussel Conservation Committee 1998; Morris and Burridge 2006; Kurth et al. 2007). Prior to augmentation of natural populations, however, it is necessary to define conservation units and evaluate current genetic diversity in order to ensure the maintenance of that diversity and adaptive potential (Jones et al. 2006; Hoftyzer et al. 2008).

Patterns of genetic structure in mussels could occur at multiple spatial scales, as mussel populations can be patchy on a scale of centimeters to hundreds of kilometers (Strayer et al. 2004). Connectivity within and among these patches has been shown to be a function of sperm dispersal, host fish dispersal, and/or patterns of biogeographic history (Strayer et al. 2004; Zanatta and Murphy 2007, 2008; Berg et al. 2008; Galbraith 2009; Schwalb et al. 2011a; Zanatta and Wilson 2011; Ferguson et al. 2013). While total variation in mussel genetic structure is likely a sum of all of these effects, understanding the primary scale of population structure is useful information for appropriate conservation and management. Genetic structure among freshwater mussels likely reflects all of these hierarchical scales along with more recent disturbances and habitat fragmentation. The extent to which these factors are evident in the genetic signature of mussels has been addressed in several individual species (e.g., Berg et al. 1998; Kelly and Rhymer 2005; Elderkin et al. 2007; Zanatta et al. 2007; Zanatta and Murphy 2007); however, comparative studies of population genetics among multiple species are rare (Berg et al. 2007; Elderkin et al. 2008).

The goal of this project was to describe and compare the spatial genetic structure and diversity of three at-risk unionid species to a rare, but widely distributed mussel species and two abundant and widespread species to determine if at-risk species have similar genetic structure to their apparently secure counterparts and to determine the scale of management for Ontario freshwater mussel species. This study addressed the following questions: (1) Do at-risk mussels exhibit similar patterns of genetic variation (spatial structure and diversity) and do these patterns allude to potential mechanisms of decline? (2) Do at-risk species have similar spatial genetic structure (population distributions) to their apparently secure counterparts? By delineating population structure and distribution, these data can help inform appropriate spatial scales for unionid recovery activities.

Materials and methods

Study area and species

The spatial genetic structure of six broadly co-occurring unionid species was assessed in four Great Lakes tributaries in southwestern Ontario: the Ausable, Grand, Sydenham and Thames rivers (Fig. 1). This region supports the greatest aquatic diversity, including the richest freshwater mussel fauna, in Canada (Metcalfe-Smith et al. 1998; Staton et al. 2003). The six species of freshwater mussels on which this study focused (Supplemental Table 1) are distributed across the eastern half of North America and range in jurisdictional-level (state or province) conservation status from "presumed extirpated" to "secure" (NatureServe 2011). In Canada, snuffbox (*Epioblasma triquetra*) and kidneyshell (*Ptychobranchus fasciolaris*) have been assessed as endangered, and occur only in the Ausable and Sydenham Rivers; the Great Lakes population of mapleleaf (*Quadrula quadrula*) has been assessed as threatened, and are found in few numbers in several Lake St. Clair tributaries including the Grande, Sydenham, and Thames Rivers; and wavy-rayed lampmussel (*Lampsilis fasciola*) has been assessed as special concern by the

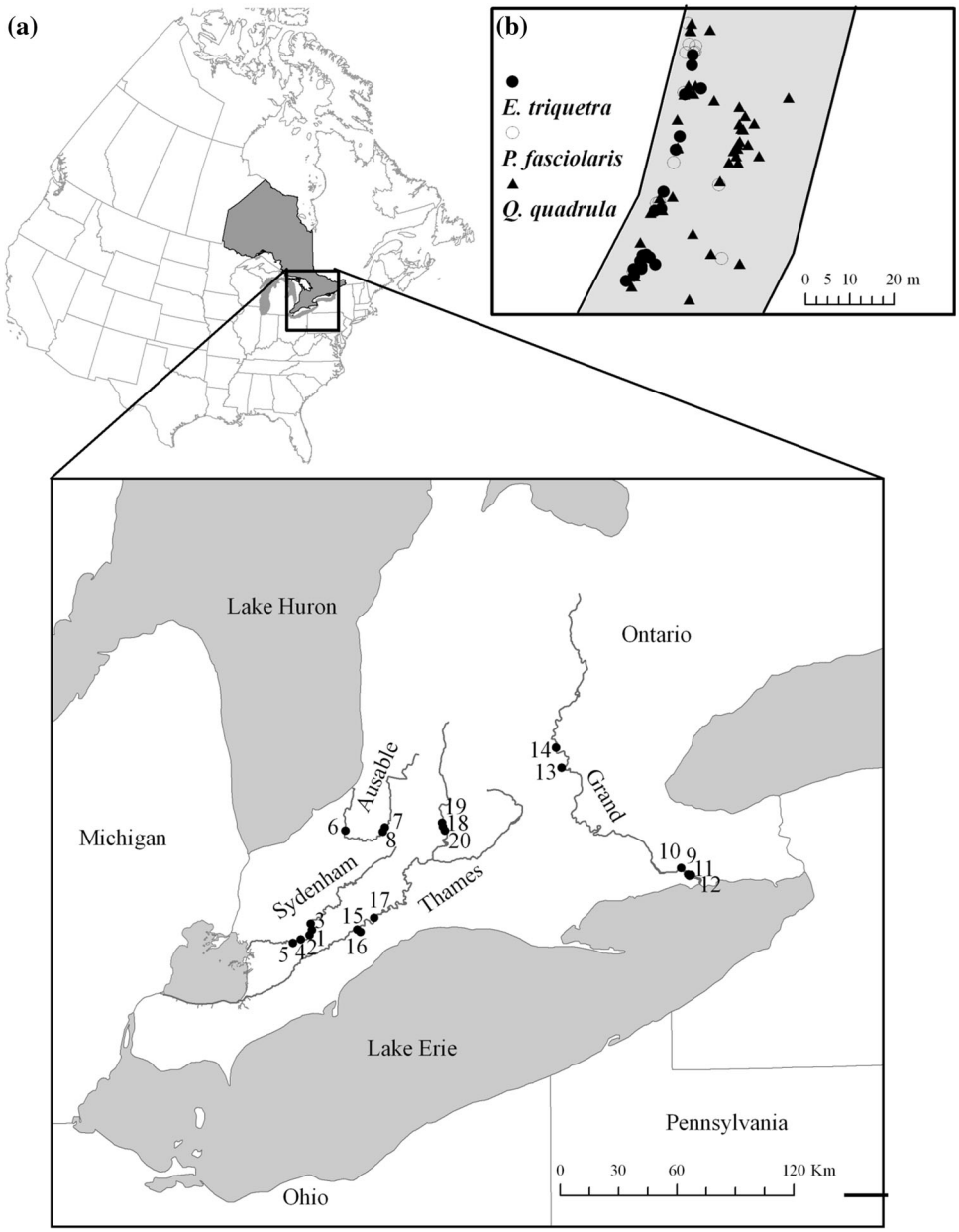

Fig. 1 Rivers and sites in southwestern Ontario, Canada that were sampled for this study showing **a** 20 collection sites from four Great Lakes tributaries. **b** Example of high resolution spatial data collected for 3 species within a single stretch of river

Committee on the Status of Endangered Wildlife in Canada and is found in the Grande, Maitland, Ausable, and Thames Rivers (COSEWIC 2003, 2006, 2010; Morris and Burridge 2006). Flutedshell (*Lasmigona costata*) and threeridge mussels (*Amblema plicata*) in the Great Lakes region of Canada have not been assessed, but are not on the priority list for status assessment and are thus thought to be secure (COSEWIC 2012).

Tissue sampling and genetic analysis

Tissue samples were collected from all mussel species at multiple sites within the four rivers between the summer of 2008 and fall of 2009 (Supplemental Table 1; Fig. 1). Upon their detection, high-resolution spatial data were also recorded for each individual mussel from the endangered, threatened, or special concern species (*E. triquetra*, *L. fasciola*, *P. fasciolaris*, and *Q. quadrula*) using a Trimble GeoXT GPS (Fig. 1b). *E. triquetra* collections were made using the visceral swab method of Henley et al. (2006) and samples were preserved in cell lysis buffer. Tissue from all other species was sampled via mantle clipping (Berg et al. 1995) and stored in 95 % ethanol. DNA was extracted from swab samples using a modification of the Puregene (Gentra) extraction kit for buccal brushes. DNA was extracted from tissue samples using a modified

isopropanol extraction method (Sambrook et al. 1989) detailed in Wilson et al. (2007).

Microsatellites were used for all species to assess population genetic diversity and structure. Although analysis of mitochondrial DNA is useful for addressing genetic questions on macrogeographic or evolutionary scales, microsatellites have been shown to be more useful for inferring population structure in unionids inhabiting recently glaciated regions (Zanatta and Murphy 2007, 2008). Microsatellite loci for *L. costata* (11 loci, N = 241 individuals from 4 rivers) and *P. fasciolaris* (9 loci, N = 155 individuals from 2 rivers) were amplified in PCR reactions using the conditions reported in Galbraith et al. (2011a, b), run on an ABI 3730 sequencer (Applied Biosystems), and scored using GeneMapper v. 4 (Applied Biosystems). Microsatellite loci for *E. triquetra* (11 loci, N = 94 individuals from 2 rivers; Zanatta and Murphy 2006, 2008) and *L. fasciola* (8 loci, N = 175 individuals from 2 rivers; Eackles and King 2002; Zanatta et al. 2007) were optimized in multiplexed PCR reactions (see Supporting Information) and genotyped and scored as described above. Microsatellite loci designed for *Q. fragosa* (Hemmingsen et al. 2009) were optimized for amplification in *Q. quadrula* (6 loci, N = 405 individuals from 3 rivers; Supporting Information), and loci designed for *A. neislerii* (Díaz-Ferguson et al. 2011) were optimized for amplification in *A. plicata* (8 loci, N = 92 individuals from 2 rivers; Supporting Information).

Statistical analysis

All sites with fewer than ten individuals were removed from population-based analyses to avoid bias from small sample sizes, although individuals from these sites were retained as unknowns for individual-based population assignment tests. MICROCHECKER (van Oosterhout et al. 2004) was used to test data for genotyping errors, allelic dropout, and estimating frequency of null alleles for each locus-population combination (Brookfield 1996). FSTAT v. 2.9.3 (Goudet 1995) was used to calculate mean and standardized allelic richness per locus (A and A_r, respectively) to estimate within-population diversity before and after correcting for sample size using rarefaction (Petit et al. 1998). Expected and observed heterozygosity (H_e and H_o) for each locus and population, as well as tests for deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium within sites, were calculated in GENEPOP′007 (Raymond and Rousset 1995; Rousset 2008), using sequential Bonferroni adjustment for multiple comparisons. The fixation index, F, was quantified within sites as $[(H_e\_H_o)/H_e]$. Because of the limitations of F_{ST} for comparing among species (Jost 2008), genetic differentiation among conspecific populations was quantified with D_{est}, which was explicitly designed to facilitate comparisons between species (Jost 2008). D_{est} estimates were calculated using SMOGD (Crawford 2010). To facilitate comparison of genetic structuring across species, an analysis of molecular variance (AMOVA) was conducted for each species in GENALEX v. 6.41 (Peakall and Smouse 2006), with river as the grouping variable, to partition the observed intraspecific variance within and among rivers and populations.

Analysis of spatial structure was completed via individual-based population assignment using STRUCTURE v. 2.3.3 (Pritchard et al. 2000; Falush et al. 2003) allowing for admixture among genetic groups (K) and assuming independent allele frequencies to minimize potential bias towards finding apparent substructure. Ten iterations were run for each value of K (number of clusters) which was defined based on the number of collection locations for each species: the maximum K for each species was calculated by adding 3 to the number of collection locations to allow detection of substructure within sampling locations. Each trial used an initial burn-in period of 100,000 replicates followed by an additional 100,000 replicates after burn-in. STRUCTURE HARVESTER v. 0.6.8 (Earl and vonHoldt 2012) was used to calculate ΔK from STRUCTURE output in combination with 'the log probability of the solution for each value of K', to determine optimal solutions for potential numbers of genetic groups (K) within each species (Evanno et al. 2005). CLUMPP v. 1.1.2 (Jakobsson and Rosenberg 2007) was used to generate the optimal alignment of STRUCTURE replicates based on the number of clusters identified from HARVESTER, and data were graphically represented using DISTRUCT v. 1.1 (Rosenberg 2004). The level of assignment success was estimated as the proportion of all individuals with probability of assignment $Q > 0.8$.

Genetic isolation by distance was analyzed for each species by comparing pairwise genetic divergences among populations (D_{est}, Jost 2008) to their intervening geographic distances. Distances among sites were calculated in river kilometers using ARCMAP v. 9.3 (Environmental Systems Research Institute, Redlands, California). Distance between river drainages was estimated as the shortest water distance between the mouths of each river. Genetic isolation-by-distance was also analyzed within sites at two locations in the Sydenham River (sites 1 and 5) where multiple at risk species were collected in sufficient numbers to enable statistical testing. Statistical significance was tested using a Mantel test in GENALEX v. 6.4.1 (Peakall and Smouse 2006).

Following the determination of spatial genetic units, evidence of recent population declines was assessed using BOTTLENECK (Cornuet and Luikart 1996). Sites belonging to the same genetic cluster were grouped and

analyzed for evidence of bottlenecks using the Wilcoxon test implemented in BOTTLENECK under the two-phase mutation model (Di Rienzo et al. 1994).

Results

It was not possible to statistically compare allelic richness (A_r) among species due to their phylogenetic separation and lack of shared loci among species. Despite this, all species generally exhibited substantial genetic diversity within sites, with the highest values of A_r observed in *L. costata* and lowest A_r in *Q. quadrula* (Table 1). Average ($\pm$ SE) A_r across sites was similar for *A. plicata* (7.28 $\pm$ 0.07), *E. triquetra* (8.48 $\pm$ 0.31), *L. costata* (8.20 $\pm$ 0.31), and *L. fasciola* (8.32 $\pm$ 0.24), but substantially lower in *P. fasciolaris* (5.51 $\pm$ 0.14) and *Q. quadrula* (4.57 $\pm$ 0.13). As MICROCHECKER results revealed no evidence of genotyping error due to stuttering or large allelic dropout, null alleles were presumed to be the only source of genotyping error. Null allele frequencies varied by locus-population combinations but were generally low for all species: mean frequency of null alleles across loci ranged from 0.01 to 0.08 for all species. Maximum frequency of null alleles ranged as high as 0.36 in *A. plicata* and 0.32 in *L. costata* for some locus-population combinations, but was lower for all other species (range of maximum locus-population combinations: 0.10–0.24). Single-locus testing for HWE showed that the majority of loci were within HWE expectations within individual sampling locations for most species, although significant heterozygote deficits for multiple loci were observed in *A. plicata* and *P. fasciolaris* at all sampling sites (Table 1). Four of the six species (*E. triquetra*, *L. costata*, *L. fasciola*, *Q. quadrula*) were within HWE across sites, with the exception of *L. costata* at two locations and *L. fasciola* at one location (Table 1). The only evidence for potential linkage disequilibrium was observed in two populations of *L. fasciola* where apparent linkage was detected between two loci (*Ecap*1 and *Ecap*5) in two of five populations.

For all species, the AMOVA results showed the greatest variation within populations, with little to no explained variation among populations within rivers (<3 %), although still significant for *E. triquetra* and *A. plicata* (Table 2). Variation among rivers was significant for all species and ranged from 3 % in *A. plicata* to 13 % in *E. triquetra* (Table 2). Partitioning among rivers was slightly greater for endangered species and declined with lessening conservation concern (Table 2), although this pattern was weak. Within population variation (Φ_{PT}) was greater for rare versus common species, resulting in lower within population variation, although these differences were not significant (Table 2). Greater genetic divergence generally

Table 1 Genetic diversity metrics for the six mussel species for sites with >10 sampled individuals, showing mean and standardized allelic richness per locus (A and A_r), observed and expected heterozygosity (H_o and H_e), and fixation index (F) for each species and sampling location in southwestern Ontario, Canada

River	Site	N	A	A_r	H_O	He	F
E. triquetra							
Ausable	6	30	8.55	7.87	0.7109	0.7156	0.007
Sydenham	1	23	8.82	8.63	0.7738	0.7596	−0.019
Sydenham	5	27	9.45	8.93	0.7388	0.7728	0.044
P. fasciolaris							
Ausable	7	25	6.56	5.38	**0.5411**	0.5949	0.091
Ausable	8	25	6.78	5.60	**0.5670**	0.6261	0.094
Sydenham	1	17	6.11	5.63	**0.4743**	0.6073	0.219
Sydenham	2	14	5.89	5.74	**0.4304**	0.6772	0.364
Sydenham	3	16	5.11	4.87	**0.4954**	0.5986	0.172
Sydenham	5	49	8.56	5.82	**0.5612**	0.6623	0.153
Q. quadrula							
Grand	9	20	4.83	4.78	0.6250	0.5748	−0.0874
Grand	10	19	4.67	4.67	0.6404	0.5913	−0.0830
Grand	12	21	4.50	4.43	0.6429	0.5625	−0.1428
Sydenham	1	68	4.83	4.11	0.6137	0.5493	−0.1174
Sydenham	2	38	4.83	4.31	0.5769	0.5476	−0.0536
Sydenham	3	54	5.33	4.42	0.5910	0.5551	−0.0647
Sydenham	4	23	4.33	4.17	0.5501	0.5120	−0.0743
Sydenham	5	47	4.67	3.96	0.5770	0.5370	−0.0744
Thames	15	21	5.33	5.17	0.6563	0.5687	−0.1540
Thames	16	25	5.50	5.23	0.6212	0.5635	−0.1025
Thames	17	67	6.67	5.03	0.6542	0.5484	−0.1930
L. fasciola							
Grand	13	18	7.88	7.88	0.7986	0.7966	−0.002
Grand	14	38	10.63	8.88	**0.7308**	0.8073	0.095
Thames	18	32	10.13	8.59	0.7545	0.7757	0.027
Thames	19	50	12.25	8.63	0.8050	0.7567	−0.064
Thames	20	37	9.38	7.64	0.7804	0.7496	−0.041
L. costata							
Ausable	6	24	13.27	9.00	0.8235	0.8317	0.010
Ausable	8	25	12.91	8.75	0.8235	0.8255	0.002
Grand	13	20	9.27	7.20	0.7682	0.7558	−0.016
Grand	14	19	9.27	7.20	0.7376	0.7539	0.022
Sydenham	1	19	11.36	8.87	**0.7547**	0.8470	0.109
Sydenham	4	10	9.91	9.91	0.8273	0.8321	0.006
Sydenham	5	23	12.91	8.87	0.7950	0.8279	0.040
Thames	18	18	9.09	7.25	0.7141	0.7652	0.067
Thames	19	28	11.27	7.68	0.7318	0.7701	0.050
Thames	20	27	10.45	7.26	**0.6908**	0.7665	0.099
A. plicata							
Ausable	6	25	11.63	7.37	**0.7406**	0.8728	0.151
Ausable	7	10	8.25	7.25	**0.7219**	0.8680	0.168
Ausable	8	20	10.63	7.14	**0.7125**	0.8426	0.154
Sydenham	1	11	9.13	7.49	**0.6440**	0.8692	0.259
Sydenham	3	10	8.38	7.16	**0.7429**	0.8577	0.134

Bold values indicate significant heterozygote deficits at $\alpha = 0.05$ after Bonferroni correction. See Fig. 1 for site locations

Table 2 Results of analysis of molecular variance (AMOVA) for six mussel species in southwestern Ontario, Canada

Source of variation	df	SS	Percent variation (%)	Statistics	*p value*
E. triquetra					
Among rivers	1	64.310	13	$\Phi_{RT} = 0.126$	**0.001**
Among populations within rivers	1	16.575	3	$\Phi_{PR} = 0.038$	**0.004**
Within populations	77	641.240	84	$\Phi_{PT} = 0.159$	**0.001**
P. fasciolaris					
Among rivers	1	54.614	10	$\Phi_{RT} = 0.097$	**0.001**
Among populations within rivers	4	29.102	0	$\Phi_{PR} = 0.004$	0.243
Within populations	140	933.797	90	$\Phi_{PT} = 0.100$	**0.001**
Q. quadrula					
Among rivers	2	82.703	10	$\Phi_{RT} = 0.099$	**0.001**
Among populations within rivers	8	29.374	1	$\Phi_{PR} = 0.007$	0.034
Within populations	392	1152.881	89	$\Phi_{PT} = 0.105$	**0.001**
L. fasciola					
Among rivers	1	57.136	9	$\Phi_{RT} = 0.094$	**0.001**
Among populations within rivers	3	23.178	1	$\Phi_{PR} = 0.007$	0.035
Within populations	170	1052.111	90	$\Phi_{PT} = 0.101$	**0.001**
L. costata					
Among rivers	3	132.554	7	$\Phi_{RT} = 0.070$	**0.001**
Among populations within rivers	6	51.716	0	$\Phi_{PR} = -0.003$	0.779
Within populations	203	1853.324	93	$\Phi_{PT} = 0.067$	**0.001**
A. plicata					
Among rivers	1	18.324	3	$\Phi_{RT} = 0.031$	**0.002**
Among populations within rivers	3	31.220	2	$\Phi_{PR} = 0.018$	**0.004**
Within populations	71	578.317	95	$\Phi_{PT} = 0.049$	**0.001**

Bold values indicate that Φ is significantly different from 0 at $\alpha = 0.01$

occurred between the Ausable River and the three other rivers for all species, and also between the Grand and Thames in *L. fasciola* (Table 3).

Individual-based population assignment revealed that all mussel species grouped into two distinct genetic populations. For *A. plicata*, *E. triquetra*, *L. fasciola*, and *P. fasciolaris*, genetic structure was primarily evident among rivers, with genetic divergence among rivers ranging between 3 and 15 times greater than within-river divergences for these species (Fig. 2; Table 3). *Q. quadrula* showed evidence of partitioning between the Sydenham River and the Grand and Thames River sites, with the latter two rivers falling out as a single population (Fig. 2). *L. costata* from the Ausable River sites were distinct from those in the Grand, Thames, and Sydenham rivers (Fig. 2). Further analysis for $K = 3$ showed evidence of substructuring among these latter rivers: the Grand and Thames river sites clustered separately, while those from the Sydenham River exhibited fractional assignment to both groups (Fig. 2). Percentage of individuals assigned with a probability of >0.8 varied according to species, but was generally high for *E. triquetra* (91 %), *L. costata* (85 %), and *L. fasciola* (87 %), lower for *A. plicata* (55 %) and *Q. quadrula* (54 %), and extremely low for *P. fasciolaris* (27 %). For species in which population assignment grouped individuals according to watershed, percent assignment to watershed of origin was estimated as the proportion of individuals with $p > 0.8$ that were "correctly" assigned to the watershed from which they were collected. Again, this varied according to species but was high for *E. triquetra* (89 %) and *L. fasciola* (83 %) and much lower for *A. plicata* (48 %) and *P. fasciolaris* (26 %).

Isolation-by-distance analysis among populations indicated significant positive relationships between geographic distance and genetic distance for *L. costata* and *P. fasciolaris*; however, trends in all species were primarily a function of separation among rivers (Fig. 3a–f). There was no significant isolation by distance within rivers (Fig. 3a–f) or within sites (Fig. 3g–l). Despite documented population declines in several of the species, little genetic evidence of bottlenecks was seen except in *A. plicata* ($p > 0.05$ for all species and sites except *A. plicata*: Ausable $p = 0.014$, Sydenham $p = 0.027$).

Discussion

These results indicate that the relevant spatial scale for genetic management of freshwater mussel populations in

Table 3 Mean (± SE) pairwise genetic divergence estimates (D_{est}) for conspecific populations within and among rivers for mussels in southern Ontario, Canada

	Ausable	Grand	Sydenham	Thames
Ausable				
E. triquetra	–			
P. fasciolaris	0.0000			
Q. quadrula	–			
L. fasciola	–			
L. costata	−0.0021			
A. plicata	0.0157 (0.0108)			
Grand				
E. triquetra	–	–		
P. fasciolaris	–	–		
Q. quadrula	–	0.0036 (0.0045)		
L. fasciola	–	0.0123		
L. costata	0.1255 (0.0130)	−0.0005		
A. plicata	–	–		
Sydenham				
E. triquetra	0.1981 (0.0223)	–	0.0193	
P. fasciolaris	0.0373 (0.0057)	–	0.0054 (0.0039)	
Q. quadrula	–	0.0173 (0.0015)	−0.0009 (0.0005)	
L. fasciola	–	–	–	
L. costata	0.0797 (0.0207)	0.0534 (0.0202)	−0.0034 (0.0032)	
A. plicata	0.0939 (0.0230)	–	0.0429	
Thames				
E. triquetra	–	–	–	–
P. fasciolaris	–	–	–	–
Q. quadrula	–	0.0095 (0.0032)	0.0481 (0.0033)	−0.0006 (0.0020)
L. fasciola	–	0.1216 (0.0115)		0.0037 (0.0037)
L. costata	0.149 (0.0025)	0.0838 (0.0088)	0.0472 (0.0071)	−0.0002 (0.0002)
A. plicata	–	–	–	–

southwestern Ontario is at the watershed (i.e., river) scale for both rare and common species. No genetic structuring was evident at smaller spatial scales (i.e., no evidence of multiple genetic groups within rivers or within sites), suggesting historical connectivity and/or recent gene flow among habitat patches for each river and species. However, patterns of smaller-scale genetic structuring may have been masked by larger-scale patterns of differentiation or were not detectable by the methods used in this study. There was also no evidence of recent fragmentation or population bottlenecks due to anthropogenic disturbances.

Freshwater mussel populations in the Great Lakes region have been drastically reduced and fragmented (Metcalfe-Smith et al. 1998; Schloesser et al. 2006). Despite this recent geographic and demographic fragmentation, there was little evidence of genetic bottlenecks in any of the species examined here. Correspondingly, impoundments have been shown to have little to no effect on current patterns of genetic distance in other mussel species (Kelly and Rhymer 2005; Reagan 2008; Szumowski et al. 2012). This is likely a function of some recent gene flow combined with historically large population sizes and mussel longevity (i.e., little time since disturbance for genetic drift to have occurred) (DeHaan et al. 2006). Unless appropriately managed, future genetic diversity and structure in mussels will likely reflect current demographic fragmentation and could result in drastic losses of genetic variability and adaptive potential, inbreeding depression, accumulation of deleterious mutations, and local or global extinction (Keyghobadi 2007).

Patterns of genetic structuring were generally consistent across all six unionid species and were not related to taxonomic or local rarity, despite their differences in life history, body size, and host use. However, patterns of genetic structure were not always entirely congruent among species, and could only have been revealed using a multi-species approach (Maki et al. 2002; Whitely et al. 2006; Berg et al. 2007). For example, divergence among rivers was most pronounced in *E. triquetra*, which may reflect regional-scale host dispersal of generally non-vagile hosts

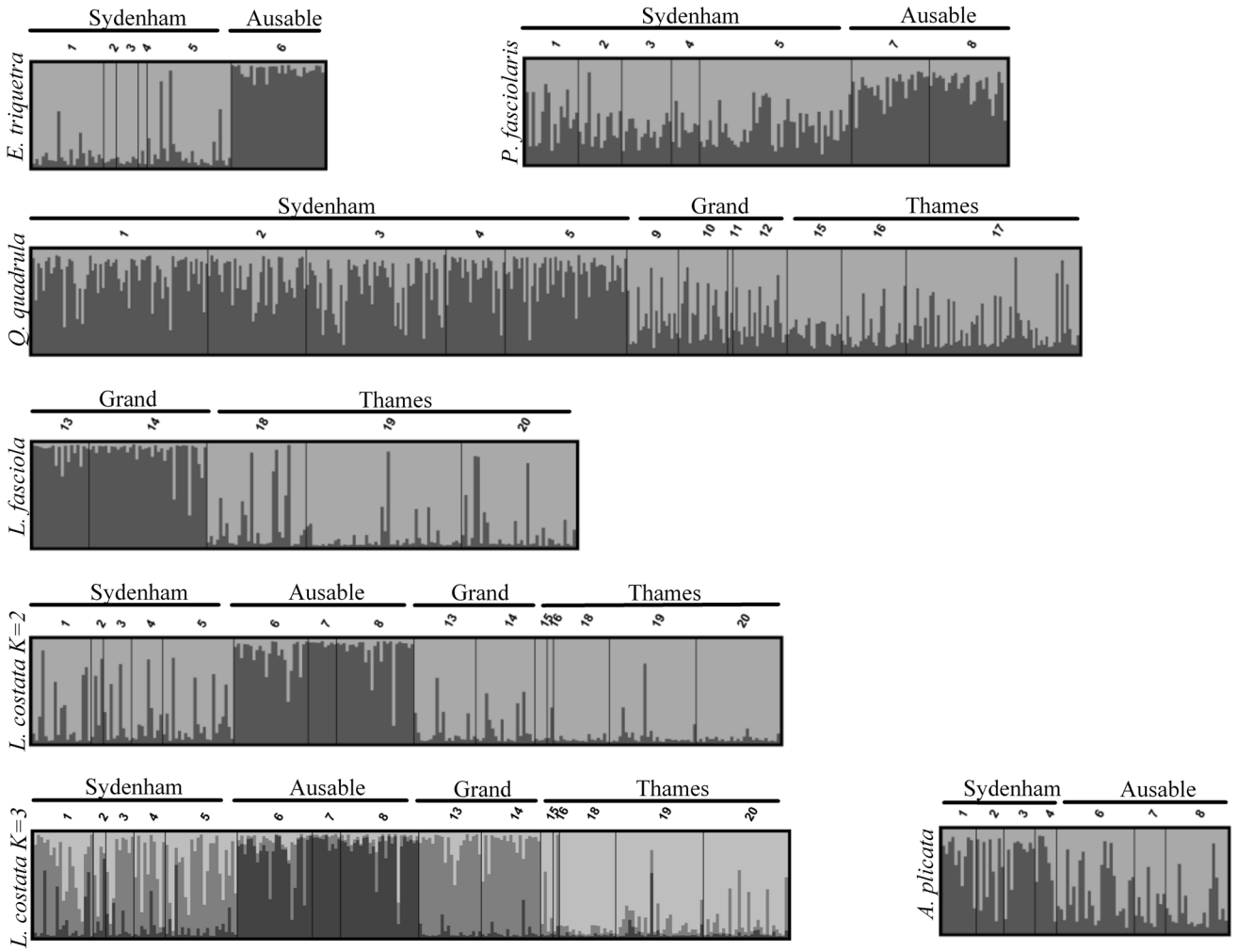

Fig. 2 Results of individual-based population assignment for each of six freshwater mussel species from southwestern Ontario, Canada. *Plots* show STRUCTURE outputs for $K = 2$ within each species unless otherwise noted

(darters and stickleback, Table 4; McNichols 2007; Schwalb et al. 2011a) on contemporary genetic structure; however, this remains to be explicitly evaluated (but see Zanatta and Wilson 2011). Despite parasitizing small host fish with limited dispersal capabilities, *P. fasciolaris*, exhibited comparatively lower divergence among rivers. The moderately- (e.g., sunfish, bass) to highly-vagile (e.g., catfish, suckers) host fish of *A. plicata*, *L. costata*, *L. fasciola*, and *Q. quadrula* (in general, given the lack of Ontario-specific host data) may be responsible for the greater gene flow in these mussels and warrants further investigation. Similarly high levels of gene flow have been elsewhere noted for three of these species and have been partly attributed to host dispersal (Berg et al. 1998; Elderkin et al. 2007; Zanatta et al. 2007); this is the first published record of population genetic structure in *L. costata*. Further analysis of the effects of host dispersal on mussel genetic structure is warranted. Similarly, assessing genetic patterns among host fish may add additional insight for restoration of endangered mussels, potentially identifying historic mussel locations that have been extirpated where host fish remain (Zanatta and Murphy 2007; Zanatta and Wilson 2011).

While the results of this study provide useful insight into the population genetics of a suite of mussel species, caution should be used in applying them to other areas. One limitation of this research lies in its narrow, relatively young (post-glacial) geographic range (southwestern Ontario) and relatively small distance between sampling sites warranting caution when extrapolating these results to older ecosystems. This may partially explain the non-significant isolation by distance relationships (both across and within rivers) that have been shown to exist in other mussel species at larger spatial scales (Berg et al. 1998; Elderkin et al. 2007). Additionally, this study was conducted for several species inhabiting the extreme northern edge of their geographic range (Parmalee and Bogan 1998). These species may be limited by habitat constraints that elsewhere are not limiting, potentially resulting in genetic

Table 4 Mussel host fish families and species according to the Mussel/Host Database (Cummings and Watters 2010). Listed fish species include those historically or currently found in Ontario, Canada (Mandrak and Crossman 1992; Scott and Crossman 1978)

Mussel	Host family	Host species
E. triquetra	Cottidae	*Cottus bairdii* (Mottled sculpin)
	Gasterosteidae	***Culaea inconstans* (Brook stickleback)**
	Percidae	***Percina caprodes* (Logperch)**[a]; *Percina maculata* (Blackside darter)
P. fasciolaris	Gasterosteidae	***Culaea inconstans* (Brook stickleback)**
	Percidae	*Etheostoma caeruleum* (Rainbow darter); ***Etheostoma flabellare* (Fantail darter)**; ***Etheostoma exile* (Iowa darter)**; ***Etheostoma nigrum* (Johnny darter)**; ***Percina maculata* (Blackside darter)**
Q. quadrula	Ictaluridae	*Ictalurus punctatus* (Channel catfish)
L. fasciola	Centrarchidae	*Lepomis megalotis* (Longear sunfish); ***Micropterus dolomieu* (Smallmouth bass)**[a]; ***Micropterus salmoides* (Largemouth bass)**[a]
	Cottidae	***Cottus bairdii* (Mottled sculpin)**
L. costata	Amiidae	*Amia calva* (Bowfin)
	Catastomidae	*Hypentelium nigricans* (Northern hogsucker); *Moxostoma carinatum* (River redhorse)
	Centrarchidae	*Ambloplites rupestris* (Rock bass); *Lepomis cyanellus* (Green sunfish); *Lepomis gibbosus* (Pumpkinseed); *Lepomis macrochirus* (Bluegill); *Lepomis megalotis* (Longear sunfish); *Micropterus salmoides* (Largemouth bass)
	Clupeidae	*Dorosoma cepedianum* (Gizzard shad)
	Cyprinidae	*Campostoma anomalum* (Central stoneroller); *Carrasius auratus* (Goldfish)*; *Cyprinus carpio* (Common carp)*; *Rhinichthys cataractae* (Longnose dace); *Semotilus atromaculatus* (Creek chub)
	Esocidae	*Esox lucius* (Northern pike)
	Ictaluridae	*Ameiurus nebulosus* (Brown bullhead)
	Percidae	*Etheostoma caeruleum* (Rainbow darter); *Etheostoma flabellare* (Fantail darter); *P. flavescens* (Yellow perch); *Sander vitreus* (Walleye)
A. plicata	Catastomidae	*Hypentelium nigricans* (Northern hogsucker); *Moxostoma duquesnei* (Black redhorse); *Moxostoma erythrurum* (Golden redhorse)
	Centrarchidae	*Ambloplites rupestris* (Rock bass); *Lepomis cynanellus* (Green sunfish); *Lepomis gibbosus* (Pumpkinseed); *Lepomis gulosus* (Warmouth); *Lepomis macrochirus* (Bluegill); *Micropterus salmoides* (Largemouth bass)
	Cyprinidae	*Cyprinella spiloptera* (Spotfin shiner); *Hybopsis x-punctata*(Streamline chub); *Notropis atherinoides* (Emerald shiner)
	Esocidae	*Esox lucius* (Northern pike)
	Hiodontidae	*Hiodon tergisus* (Mooneye)
	Ictaluridae	*Ictalurus punctatus* (Channel catfish)
	Moronidae	*Morone chrysops* (White bass)
	Percidae	*Perca flavescens* (Yellow perch); *Percina caprodes* (Logperch); *Pomoxis annularis* (White crappie); *Pomoxis nigromaculatus* (Black crappie); *Sander canadensis* (Sauger)
	Sciaenidae	*Aplodinotus grunniens* (Freshwater drum)

Species listed in bold are confirmed host fish in at least one of the four rivers assessed in this study

* Introduced species are indicated with asterisks

[a] Signifies the best (statistically higher transformation success) host fish species of those in bold (McNichols 2007)

structure that is not reflective of patterns observed elsewhere across the species' range (Sexton et al. 2009; Rasic and Keyghobadi 2012). Finally, there was evidence of potential null alleles as a source of genotyping error in some species. *Amblema plicata* and *P. fasciolaris* showed significant deviations from HWE (Table 1); however, this was not evident in assignment analysis (Fig. 2) suggesting that deviations from HWE may be attributed to null alleles (Pemberton et al. 1995). Although null alleles can induce artificial structure in assignment analysis, this was surprisingly not the case in this study, suggesting that the effects of possible genotyping error due to null alleles may be negligible.

The results of this study illustrate the importance of landscape genetics in resolving spatial genetic structure and identifying historically connected populations, as well as its limitations for detecting population declines, at least in the species examined here. As patterns of genetic diversity did not vary predictably as a function of mussel rarity, this study suggests that common species could serve

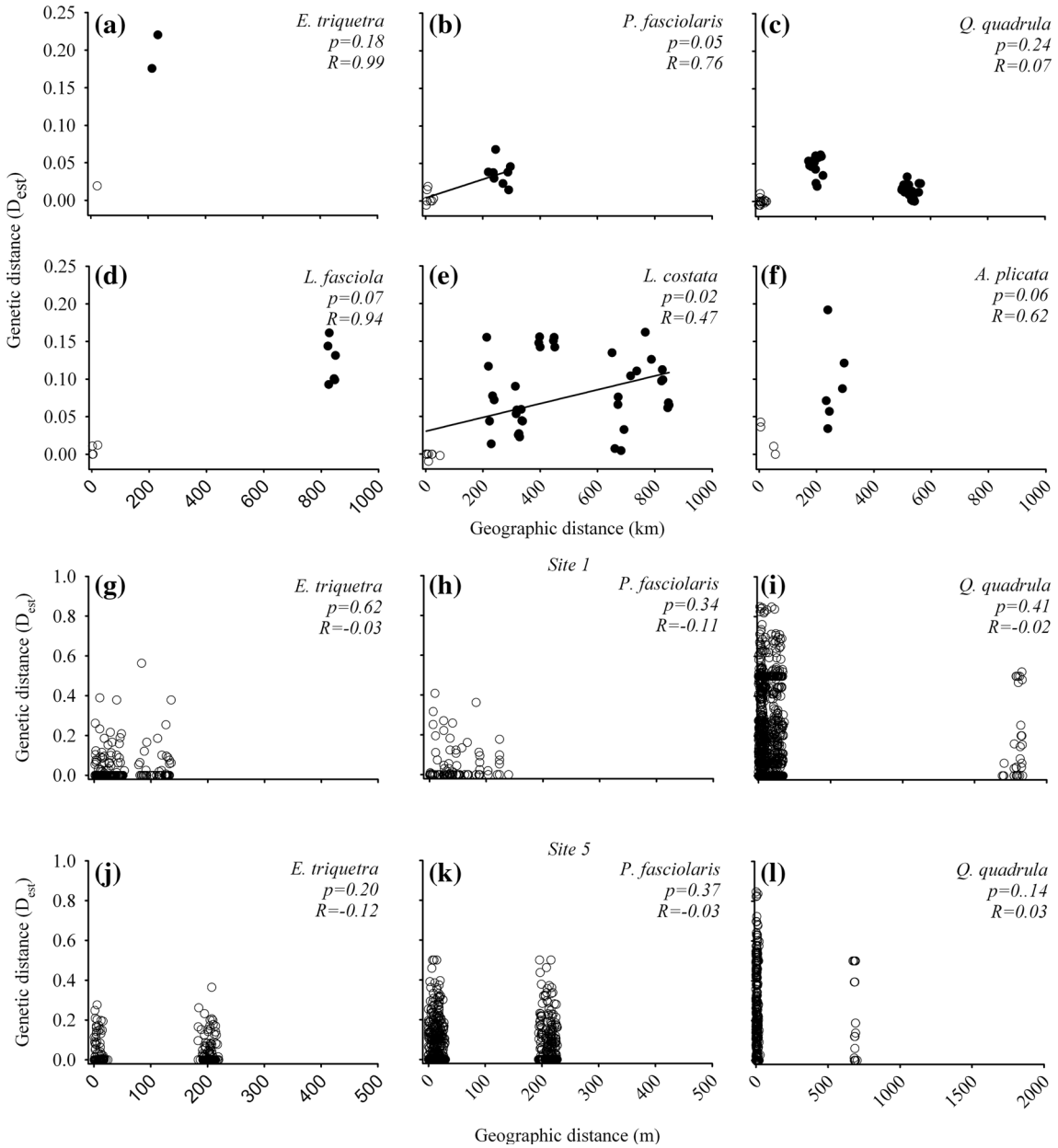

Fig. 3 Relationship between geographic distance (km) and genetic divergence within and among conspecific populations at sites in the Sydenham, Ausable, Grand, and Thames Rivers in southwestern Ontario, Canada. Open circles represent sites within the same river; closed circles represent sites from different rivers. *Panels* **a–f** show population-level divergence (D_{est}; Jost 2008) among sites (localized habitat patches); *panels* **g–l** represent pairwise relatedness coefficients between individuals within sites as a function of microgeographic distance (m) within two sites in the Sydenham River, southwestern Ontario, Canada

as useful surrogates for understanding genetic structure of threatened and endangered species. Additionally, local population (i.e., single site) losses within individual rivers may not result in an irreversible loss to the species, and other mussel beds within the same watershed could be used as sources for restoration via propagation or translocation (providing that patterns in adaptive diversity are similar to those of molecular diversity). However, more meaningful organismal metrics (e.g., changes in mussel density, recruitment, physiological condition) need to be quantified, ideally in conjunction with spatial genetic structure, to fully understand and monitor declines in freshwater mussel populations as genetic structure was not reflective of demographic declines. Combined analyses of mussel demographics and population genetics should offer more robust and effective insights for assessing mussel decline and extinction than either approach in isolation (Lande 1988; Berg et al. 2008).

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