Discrete longitudinal variation in freshwater mussel assemblages within two rivers of central Michigan, USA

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Discrete longitudinal variation in freshwater mussel assemblages within two rivers of central Michigan, USA

Amanda J. Chambers · Daelyn A. Woolnough

Abstract A primary focus in the study of lotic ecosystems involves understanding the relationship between changes in the physical environment and changes in biota along a longitudinal gradient. Previous studies examining riverine organisms, primarily fish and aquatic insects, have noted two commonly occurring upstream-to-downstream patterns: either the restriction of species to distinct zones (i.e., discrete variation), or the gradual accumulation of species with increasing distance from the headwaters (i.e., continuous variation). Like other riverine organisms, freshwater mussels exhibit longitudinal patterns in distribution and abundance; however, few studies have quantified these patterns and the environmental variables that influence them. This study examined longitudinal patterns in the assemblage structure of mussels in the Chippewa and Pine rivers, Michigan, United States. A stratified random sampling design was used to sample mussels at 54 sites and timed searches allowed for the characterization of mussel assemblages. Multivariate regression tree models revealed differences in mussel assemblage structure that are consistent with upstream-to-downstream changes in surficial geology and wetland habitat. Certain species were restricted to specific geologies suggesting discrete variation with k-means partitioning and non-metric multidimensional scaling supporting these results. Because the results were consistent between rivers, we expect these patterns to prevail in similarly glaciated regions.

Keywords Unionidae · Surficial geology · Distribution · Continuous variation

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Introduction

An integral first step in the conservation and management of stream organisms involves understanding the composition and structure of communities within lotic systems. Stream ecologists have long debated whether the longitudinal nature of assemblage variation in rivers is through the formation of distinct communities (Huet, 1959; Lasne et al., 2007; McGarvey, 2011) or whether communities vary continuously along an upstream-to-downstream gradient (Vannote et al.,

1980; Heino et al., 2014). These two phenomena are commonly referred to as discrete variation (i.e., turnover, exclusion, zonation; Rahel & Hubert, 1991; Legendre, 2014) and continuous variation (i.e., nestedness, continual addition, species gain or loss; Rahel & Hubert, 1991; Baselga, 2010). In systems that are characterized by discrete variation, species are gradually replaced along the longitudinal extent of a stream or river (Whittaker, 1952; Legendre, 2014). Some of the processes that are often attributed to discrete patterns are environmental filtering, competition, and various spatial and biological constraints (Qian et al., 2005; Leprieur et al., 2011). A classic example of discrete variation was described by Huet (1959) for fish communities in western European rivers. Huet discovered four zones which were identified by the dominant fish species present and were termed the brown trout (*Salmo trutta fario*; Linnaeus, 1758), the grayling (*Thymallus thymallus;* Linnaeus, 1758), the barbell (*Barbus barbus*; Linnaeus, 1758), and the bream (*Abramis brama*; Linnaeus, 1758) zones. These zones corresponded mainly to abrupt upstream-to-downstream changes in river width and slope (Huet, 1959). Ultimately, determining whether patterns of discrete variation exist is useful from a management perspective because species that occupy the same zone often exhibit similar life-history traits, and respond in an analogous fashion to biological and anthropogenic threats thus requiring zone-specific management actions (McGarvey, 2011; Marion et al., 2015).

Contrary to discrete variation, patterns of continuous variation are observed when the species present at a given site represents a subset of the species occurring at richer sites (Baselga, 2010). In fluvial systems, an additive pattern is often observed whereby species richness increases with increasing distance from the headwaters, and upstream communities represent subsets of downstream communities (Rahel & Hubert, 1991; Daniel & Brown, 2014). This pattern is typically linked to the species–area relationship which ascribes a gradual downstream increase in species richness to greater habitat area and presumably, greater habitat heterogeneity facilitating higher diversity (Connor & McCoy, 1979; Rahel & Hubert, 1991). Consequently, patterns of continuous variation are often observed in systems with overall low levels of habitat heterogeneity thus allowing widespread species to occupy habitats throughout the entire longitudinal extent of a system resulting in assemblage homogenization (Rahel & Hubert, 1991). An alternative explanation to the species–area relationship is that the upstream reaches of a river represent a periodically variable environment characterized by increased incidences of spates and higher frequencies of dry down events during periods of low flow (Rahel & Hubert, 1991; Atkinson et al., 2012). In turn, only highly mobile and physiologically tolerant species can survive in upstream reaches relative to a greater number of species that can occupy more benign downstream environments (Rahel & Hubert, 1991). It is essential to gather baseline information pertaining to the number and structure of communities that exist in a river so that managers can assess the status and trends of those communities over time (Marion et al., 2015).

Following the assessment of patterns in the composition and structure of communities, it is necessary to explore the underlying environmental variables responsible for the structure of riverine communities. Historically, in riverine systems, studies have focused on quantifying local reach-scale environmental variables with limited regard to processes or practices occurring upstream or on the surrounding landscape (Allan, 2004). However, rivers represent hierarchical systems where local habitat variables are influenced by geomorphological processes that are ultimately governed by larger-scale patterns in climate, geology, and topography (Frissell et al., 1986; Fausch et al., 2002; Allan, 2004). Failing to account for the hierarchical nature of river systems may result in missing processes or failing to attribute the proper mechanisms responsible for patterns in organismal distribution that may only be evident at coarser spatial scales (Fausch et al., 2002).

Freshwater mussels (Bivalvia; family: Unionidae) are a group of organisms well suited to study longitudinal variation in assemblage structure in rivers. Like other aquatic organisms, freshwater mussels have long been known to exhibit longitudinal patterns in abundance and distribution (Ortmann, 1913, 1920). However, few studies have explicitly quantified the nature of variation in mussel assemblages, specifically whether mussels exhibit discrete or continuous variation in assemblage structure. Studies that have examined longitudinal variation, generally report patterns of continuous variation typically noting an additive pattern with downstream distance from the headwaters (Haag & Warren, 1998; McRae et al., 2004; Daniel & Brown, 2014). Although

the species–area relationship has been used to successfully predict longitudinal changes in mussel assemblage structure in some streams (Watters, 1992), mussel assemblages are influenced by many factors (Strayer, 2008; Haag, 2012); therefore, the species–area relationship may not be appropriate for all riverine mussel communities (Haag, 2012). As an example, Strayer (1983) found that the downstream succession of mussels in a Lake Erie tributary was additive; however, species richness and stream size were weakly correlated. In fact, certain species were associated with distinct geological formations suggesting evidence of discrete variation.

Moreover, there is a recent shift in the understanding of the environmental factors that structure mussel assemblages has taken place. Historically, studies that focused on local microhabitat variables such as sediment and current velocity were only able to explain a small amount of the variation in assemblage structure (Holland-Bartels, 1990; Strayer & Ralley, 1993). Following the general trend in community ecology, contemporary studies suggest that distribution patterns are more likely to be influenced by multi-scale spatial and temporal variables (Atkinson et al., 2012). For example, catchment-scale slope and geology (Arbuckle & Downing, 2002; McRae et al., 2004) and buffer-scale slope and land cover variables (Atkinson et al., 2012) can be important predictors of mussel assemblage structure. At the present time, few studies exist focusing on broad-scale environmental influences on mussel assemblages.

Understanding patterns in assemblage structure, as well as the environmental factors responsible for these patterns, is imperative for the implementation of effective conservation measures for highly imperiled taxa such as freshwater mussels (Williams et al., 1993; Haag & Williams, 2014). Therefore, the objective of this study was to determine if the freshwater mussel assemblages in two Michigan rivers, within the same subwatershed, exhibit distinct and complementary patterns along a longitudinal gradient. This study will attempt to answer the following questions: (i) What is the nature of longitudinal variation (i.e., continuous or discrete) of freshwater mussel assemblages in the Chippewa and Pine rivers? and (ii) What are the multi-scale environmental variables that contribute to the observed patterns and are these variables consistent with past studies that have been conducted on nearby streams with similar geology and land use practices?

Methods

Study sites

The Chippewa River and the Pine River are located in the central portion of Michigan's Lower Peninsula and are part of the larger Tittabawassee River (sixth order stream) watershed which drains into Lake Huron (Fig. 1). The Chippewa River subwatershed (excluding the Pine River) drains an area of about 1,567 km^2 while the Pine River subwatershed drains about 1,088 km^2. The rivers flow roughly parallel to one another from west to east until the Pine River reaches a confluence with the Chippewa River just upstream of the city of Midland. Both rivers have similar geology and land use. The surficial geology in the upper basin of both rivers consists of glacial deposits of outwash, moraines, and till (Schrouder et al., 2009). In the lower basin, the rivers flow through predominately lake plain sediments consisting of lacustrine sand, gravel, and clay (Schrouder et al., 2009). Agriculture is the dominant form of land use in both watersheds constituting over 50% of the catchment area. The remaining land use is upland forest (32 and 21% in the Chippewa and Pine river watersheds, respectively) with only a small fraction characterized by urban land use (<2% in both watersheds; Schrouder et al., 2009). Urban land use is concentrated in the cities of Mt. Pleasant (population [pop] ∼26 K) and Midland (pop ∼42 K) on the Chippewa River, and Alma (pop ∼9 K) and St. Louis (pop ∼7 K) on the Pine River (Schrouder et al., 2009, United States Census Bureau, 2014).

Major anthropogenic impacts affecting the rivers are primarily associated with agriculture. Artificial drainage (i.e., tiling) of farm fields is common throughout both watersheds and affects nutrient loading and patterns of stream discharge (Schrouder et al., 2009). Additionally, both rivers are known to experience seasonally high levels of *Escherichia coli* in the summer months due to runoff from crop production and cattle farms (Schrouder et al., 2009). Moreover, water quality is further impaired in the Pine River downstream of the City of St. Louis as a result of contamination from the former Velsicol Chemical Company. Historically, the Velsicol Chemical Company was a major manufacturer of polybrominated biphenyls (PBB), which was used in the production of flame retardants, and dichlorodiphenyltrichloroethane
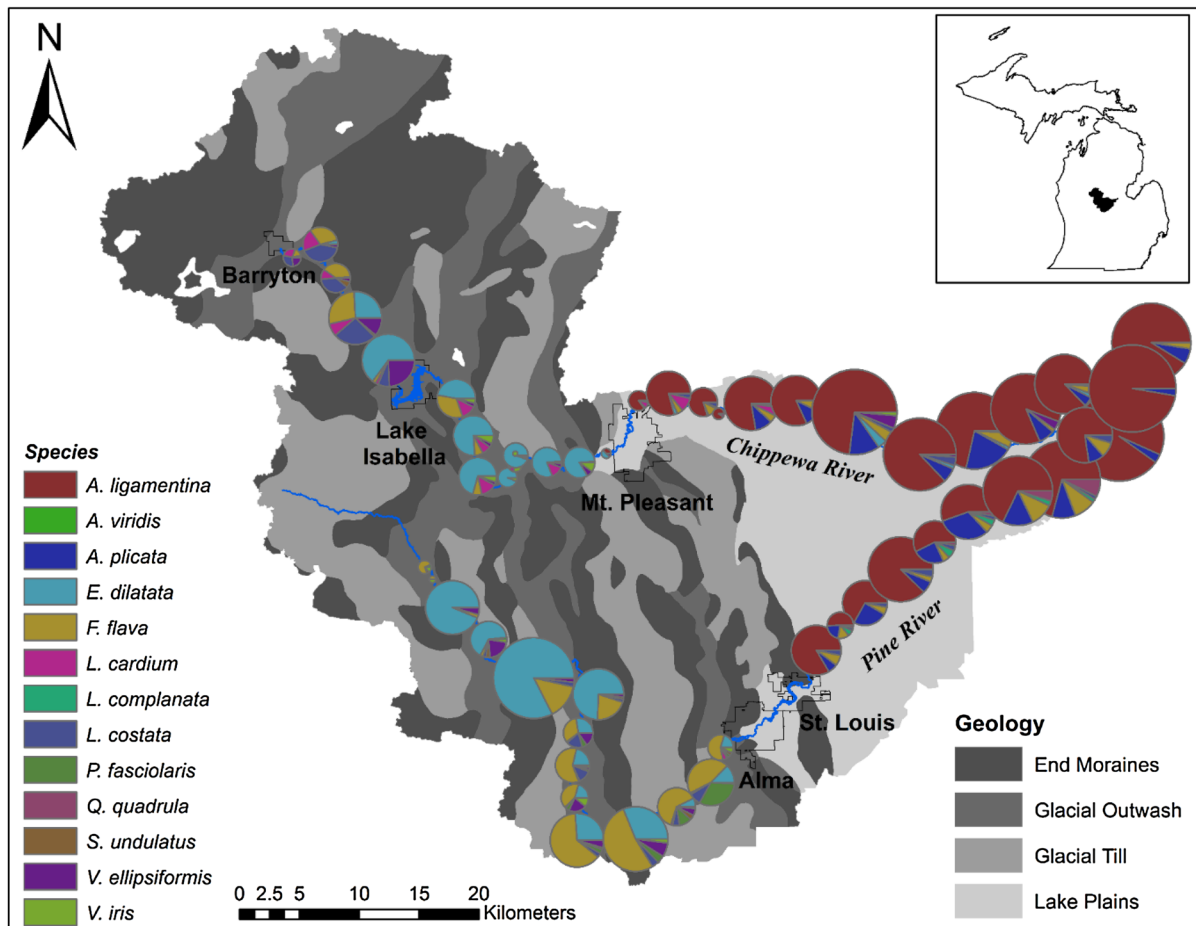
Fig. 1 Map of study sites on the Chippewa and Pine rivers, MI, USA in relation to major surficial geology formations. *Pie charts* represent total relative abundance (number per person-hour) of mussels found in the Chippewa and Pine rivers, MI, USA. *Black* in key map represents the watersheds of the study within the State of Michigan, USA

(DDT), which were used in the production of pesticides. In 1983, due to poor waste management practices and direct discharge of chemicals into the river, the site was designated by the U. S. Environmental Protection Agency (EPA) as a Superfund Site (EPA, 1999). Although water quality downstream of St. Louis has improved, consumption advisories are still in place for fish due to high tissue concentrations of PBB and DDT (Schrouder et al., 2009).

Mussel sampling

Sampling for mussels was conducted from June through October of 2015. A weighted, stratified random sampling design was used to apportion sites in the Chippewa and Pine rivers (Strayer & Smith,

2003). Sites were stratified among four surficial geology types: end moraines, glacial outwash, glacial till, and lake plains, and were allocated proportional to the length of river flowing through each geology type (Fig. 1). The sites were randomly assigned using ArcMap (Version 10.3.1, ESRI, Redlands, CA, USA). A total of 54 sites were sampled, 28 in the Chippewa River and 26 in the Pine River. This stratified design was chosen because geology has been found to be a major environmental factor influencing the distribution of freshwater mussels elsewhere in Michigan (Strayer, 1983; McRae et al., 2004). At each site, a 2,500 m^2 area was delineated within which a semiquantitative, three person-hour timed search was performed. The area surveyed was determined by measuring the average width of the river at the furthest

downstream reach that was considered sampleable (i.e., wadeable at summer baseflow) and multiplying it by 50 so that the longitudinal distance surveyed for each site was at least 50 m. This allowed the area sampled at sites to be standardized to account for greater river widths at downstream sites as a result of increasing distance from the headwaters. Mussels were sampled using a timed search approach because it allows for a more complete assessment of assemblage structure relative to quadrat sampling (Vaughn et al., 1997; Metcalfe-Smith et al., 2000). Sampling was carried out using snorkeling equipment and proceeded from downstream to upstream until the entire area was searched and the allotted time had expired. All mussels found were identified to species, enumerated, and returned to the approximate location where they were detected.

Additionally, a Wolman pebble count (Wolman, 1954) was conducted to characterize the bed sediment at each site. Substrate composition often provides a useful surrogate for position in the catchment, and high levels of substrate heterogeneity are often associated with greater organismal diversity (Williams, 1980; Atkinson et al., 2012). Ten evenly spaced transects were placed perpendicular to stream flow along the length of the survey reach. Along each transect, ten sediment particles obtained across the wetted width of the river channel were measured using the Wentworth scale (Wentworth, 1922). A total of 100 sediment particles were obtained from each site and were used to estimate percent substrate composition.

Landscape analyses

Variation in mussel abundance data was compared to landscape-scale variables which were calculated for multiple spatial scales using ArcMap (Version 10.3.1, ESRI, Redlands, CA, USA). Spatial scales were defined following Atkinson et al. (2012) and included: catchment (entire drainage area), buffer (100 m riparian buffer of the entire catchment), and reach (100 m riparian buffer extending 1 km upstream from the sampling site) scale (Fig. 2). Landscape variables were mean topographic slope, six Anderson level I land cover classes (urban, barren, forest, grass/pasture, agriculture, and wetland; Fry et al., 2009), and bedrock and surficial geology. These landscape layers were chosen because they have been found to explain a

substantial amount of variation in mussel assemblage structure in previous studies (Arbuckle & Downing, 2002; McRae et al., 2004; Atkinson et al., 2012). The land cover layer was obtained from the National Land Cover Database (Homer et al., 2015) while the remaining layers were obtained from the State of Michigan's Center for Geographic Information (Michigan Department of Information Technology, 2002). Prior to analysis, an arcsine square root transformation was applied to the proportional data for each land cover class along with each surficial and bedrock geology class. Site location (i.e., geographic coordinates) was also used as an explanatory variable that was entered into the models.

Statistical analyses

The relative abundance of mussel species, calculated as catch-per-unit effort (CPUE; number of mussels per person-hour), was used to summarize assemblage structure at each site. Abundance was Hellinger transformed to reduce the influence of highly abundant species (Heino, 2014). Furthermore, rare species (i.e., occurring at only a single site or comprising <5% of the total catch) were removed from the following analyses. Rare species were removed because based on the methods we used, we cannot guarantee adequate detection of rare species (Metcalfe-Smith et al., 2000).

Multivariate regression trees (MRT) were used to determine the relationship between the landscape-scale environmental variables and mussel abundance among sampling sites (De'ath, 2002). Multivariate regression trees relax many of the assumptions of generalized linear models (e.g., homoscedasticity, linearity), are robust to data transformations, and account for higher-order interactions making them well suited for modeling assemblage–environment relationships (De'ath, 2002; Heino et al., 2014). Multivariate regression trees seek to create groups of sites by iteratively splitting the data based on a single value of one of the explanatory variables. At each split, two groups (i.e., leaves) are formed which are defined based on the environmental variable that best minimizes the within-group sum of squares and maximizes the between-group sum of squares. The splitting procedure continues until each site is its own group or until a user-defined splitting threshold has been reached. Ultimately, the tree must be "pruned" to

Fig. 2 Map of reach (*bottom inset*), buffer (*middle*), and catchment (*middle* and *top inset*) scales used for multivariate regression tree models of freshwater mussel assemblage structure in the Chippewa and Pine rivers, MI. This example depicts site 30 on the Pine River, MI, USA
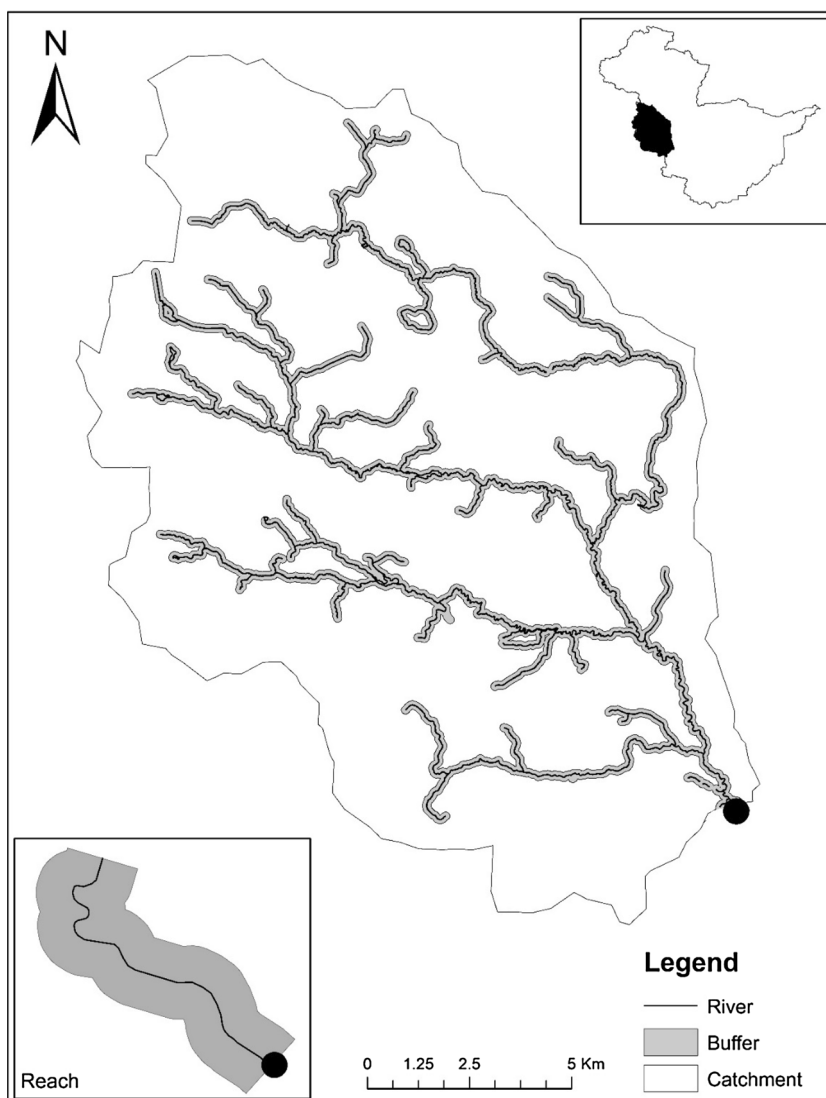
produce the best predictive tree—the one that explains the most amount of variation in the data with the least number of binary partitions. The best tree is the one that produces the lowest cross-validated relative error (CVRE). MRTs were developed for each spatial scale using R statistical software (packages: mvpart; Therneau & Atkinson, 2012; MVPARTwrap; Ouellette & Legendre, 2012). The statistical significance of the environmental variables that produced the final groups in the best MRT models were tested using a non-parametric Kruskal–Wallis test (package: Rcmdr; Fox, 2005). If the global test was significant, pairwise comparisons were made among the groups using a Dunn's test followed by a Bonferroni correction to reduce the Type-I error rate (package: dunn.test; Dinno, 2012). Differences among groups were visualized using boxplots.

To detect the existence of continuous or discrete variation, abundance data were ordinated using nonmetric multidimensional scaling (NMDS). An NMDS was carried out for 400 iterations using a Bray–Curtis distance measure, an instability criterion of 0.0001, and a step length of 0.20. A Monte Carlo test ($P < 0.05$; 999 permutations) was used to determine the optimal number of dimensions based on the final stress value. Multi-response permutation procedure (MRPP) was used to determine if the MRT-formed groups were statistically different. MRPP was carried

out for 999 permutations using a Bray–Curtis distance measure. A weighting factor $((n)/\text{sum}(n))$ was applied to the groups to account for differences in group size, and pairwise comparisons were made among groups if the MRPP was significant. Non-metric multidimensional scaling and MRPP were carried out using PC-ORD v. 6 (McCune & Mefford, 1999). Linear regression was then used to determine if mussel species richness increased with downstream distance from the headwaters both within rivers, and within MRT-formed groups. Regression analyses were performed in R (package: stats; R Core Team, 2005).

To assess the robustness of the groups created by the MRT analysis, the MRT-formed groups were compared to groups formed by an unconstrained clustering technique (i.e., *k*-means partitioning; package: Rcmdr; Fox, 2005). While MRT seeks to partition the response variable into groups based on values of the explanatory variables, *k*-means seeks to form clusters based solely on the response variable. If the results of the unconstrained analysis differ from the MRT, this could indicate that other explanatory variables may be more important in explaining variation in the data (De'ath, 2002). Like MRT, *k*-means attempts to form groups of sites by minimizing the within-group sum of squares. Unlike other unconstrained cluster analyses, *k*-means clustering requires that the number of groups to partition the data into be specified a priori. Therefore, the number of groups chosen was equivalent to the number of final groups formed by the MRT analysis. Similarities between the groups formed by MRT and *k*-means were visualized by plotting the sites within each group in NMDS ordination space.

Indicator species analysis was used to define the species that were most characteristic of each MRT-formed group (Dufrene & Legendre, 1997; package: labdsv; Roberts, 2015). Indicator species analysis takes into account both how abundant a species is (i.e., specificity), and how frequently it is found in a particular group and not in another (i.e., fidelity). Indicator values range from 0 to 1 (0 = poor indicator, 1 = perfect indicator). Species that are good indictors are both highly abundant and are found at most sites within a group. Indicator values were assessed for statistical significance using a Monte Carlo test ($P < 0.05$; 999 permutations).

Last, because sediment was not measured at multiple spatial scales, a separate MRT model was developed for percent substrate composition. Prior to analysis, an arcsine square root transformation was applied to the percent substrate composition data for each size class.

Results

In total, 10,812 live mussels represented by 18 species were collected from the Chippewa and Pine rivers. Both rivers were found to be relatively speciose with 14 species represented by live individuals detected in the Chippewa River, and 18 species detected in the Pine River. An additional four species in the Chippewa River and three species in the Pine River were only represented by shells. Catch-per-unit effort of mussels at each site ranged from 0 to 223 ($\bar{x} = 68 \pm 8.4$) mussels/person-hour. Two species, *Elliptio dilatata* (Rafinesque, 1820) and *Fusconaia flava* (Rafinesque, 1820) were numerically abundant at the upstream sites of both rivers while *Actinonaias ligamentina* (Lamark, 1819), and to a lesser extent, *Amblema plicata* (Say, 1817), were numerically abundant at downstream sites (Fig. 1).

Between 49 and 66% of the variation in the mussel assemblage structure in the Chippewa and Pine rivers was explained by five environmental variables operating at three spatial scales (Table 1). At the buffer and catchment scale, the proportion of lacustrine geology was consistently among the most important predictor variables (Table 1). Models for the buffer and catchment scale had better predictive ability than the reach-scale model. Due to the large number of statistical analyses that were performed at each scale, only results from the scale that explained the most amount of variation in mussel assemblage structure, the buffer-scale model, will be described further (see Supplemental Information for reach- and catchment-scale analyses).

The buffer-scale model resulted in three leaves. The first node was separated based on the proportion of lacustrine clay and silt (LCS), and resulted in the formation of leaf 2 (Fig. 3). Leaf 2 which had ≥2% LCS in the buffer and included 23 sites all of which were located in the lower portion of the Chippewa and Pine river watersheds (Supplemental Information). Sites that had <2% LCS in the buffer, which were located in the upper portions of the watersheds, were further bifurcated by the proportion of wetland land

Table 1 Results of multivariate regression tree models for evaluation of longitudinal variation in freshwater mussel assemblages at three spatial scales in the Chippewa and Pine rivers, MI

Scale	R^2	CVRE	SE	Important variables
Reach	0.49	0.56	0.08	Site location
Buffer	0.66	0.45	0.08	Lacustrine clay and silt geology, wetland land cover
Catchment	0.65	0.46	0.08	Lacustrine sand and gravel geology, urban land cover

Values of R^2, cross-validated relative error (CVRE), and standard error (SE) are also provided
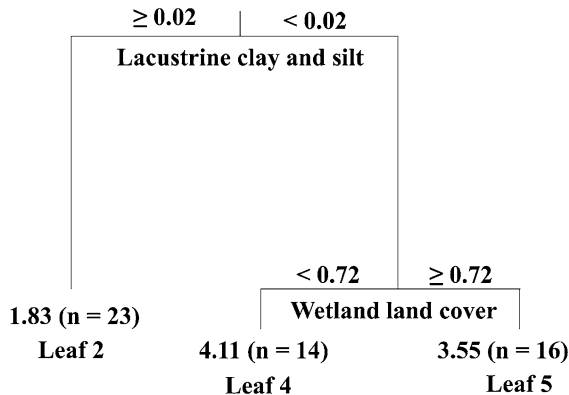
Fig. 3 Model of environmental variables influencing abundance of freshwater mussels at the buffer scale in the Chippewa and Pine rivers, MI. The proportion of the two envrionmental variables discriminating each node is given along with the threshold values for those environmental variables. Three leaves were produced by the model (2, 4, 5). For each leaf the mean value of the response variable is given along with number of sites (*n*) contained within that leaf. Model cross-validated relative error was 0.45 with a standard error of 0.08

cover, and formed leaf 4 and leaf 5 (Fig. 3; Supplemental Information). Leaf 4 which included sites having <72% wetland land cover in the buffer consisted of 14 sites while leaf 5 which had >72% wetland land cover was made up of 16 sites. There were significant differences in both the proportion of lacustrine clay and silt geology ($\chi^2 = 46.82$, d.f. = 2, $P < 0.001$) and wetland land cover ($\chi^2 = 45.22$, d.f. = 2, $P < 0.001$) at each node of this model (Fig. 4).

Patterns among sites as represented by buffer-scale MRT model group affiliation were further visualized using NMDS. The NMDS resulted in a 3-dimensional solution with a final stress value of 6.20. The first two axes explained 81% of the variation in the data. Discrete differences among sites were visually apparent when sites were represented by buffer-scale MRT-formed groups (Fig. 5). Leaf 2 sites (i.e., sites
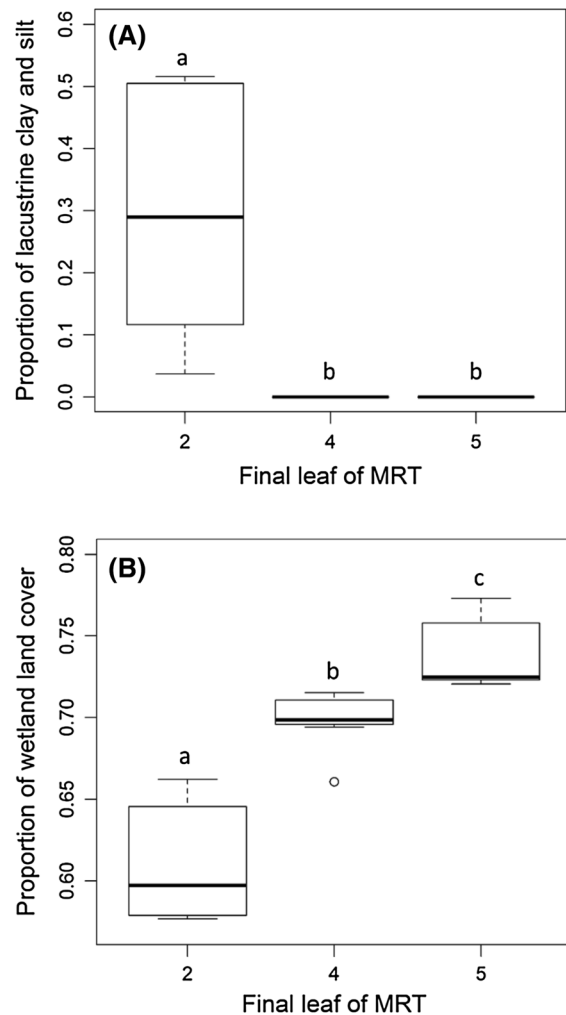
Fig. 4 *Boxplots* of the environmental variables discriminating each node of the final MRT model for the buffer scale. Environmental variables most important to the model were the proportion of lacustrine clay and silt geology (**A**) and wetland land cover (**B**). *Horizontal line* median, *box* 25th and 75th quartiles, *whiskers* the highest and lowest values, excluding outliers, *circles* outliers. *Letters adjacent to boxes* indicate statistical significance (Dunn's test, $\alpha = 0.05$, Bonferonni corrected for multiple comparisons)
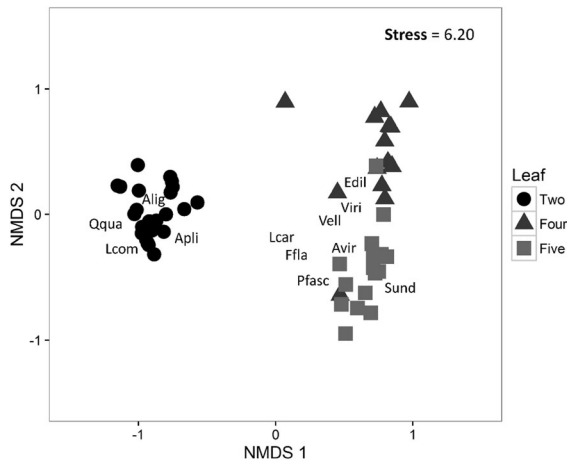
Fig. 5 NMDS ordination plot of all sites in the Chippewa and Pine rivers, MI. Represented by the three groups (leaves 2, 4, and 5) formed during mulitvariation regression tree models. Species scores are plotted along with site scores. Codes for species are as follows: Alig, *Actinonaias ligamentina*; Apli, *Amblema plicata*; Avir, *Alasmidonta viridis*; Edil, *Elliptio dilatata*; Ffla, *Fusconaia flava*; Lcar, *Lampsilis cardium*; Lcom, *Lasmigona complanata*; Pfasc, *Ptychobranchus fasciolaris*; Qqua, *Quadrula quadrula*; Sund, *Strophitus undulatus*; Vell, *Venustaconcha ellipsiformis*; Viri, *Villosa iris*

characterized by higher levels of LCS) grouped together apart from leaves 4 and 5. Leaves 4 and 5 appeared to separate out distinctly from one another, but there was a higher level of integration between sites characterized by these two leaves relative to sites belonging to leaf 2 (Fig. 5). Average differences in assemblage structure varied significantly among MRT-created groups (MRPP: $P < 0.00$, $A = 0.43$, $T = -27.5$). Pairwise comparisons showed that all leaves were significantly different from one another, but values of within-group homogeneity (A) and between-group separation (T) were much lower between leaves 4 and 5 ($A = 0.17$, $T = -11.45$), relative to leaf 2 (2 vs. 4: $A = 0.40$, $T = -22.54$; 2 vs. 5: $A = 0.43$, $T = -24.83$). A comparison between clusters formed by the buffer-scale MRT analysis and k-means partitioning resulted in nearly identical group structure of sites when plotted side-by-side in ordination space (Fig. 6). The relationship between species richness and distance from the headwaters was not significant either for the comparison within rivers or within MRT-formed groups ($P > 0.05$; Fig. 7).

There were significant indicator species for each buffer-scale MRT-formed group. Leaf 2 which was characterized by downstream sites that were high in
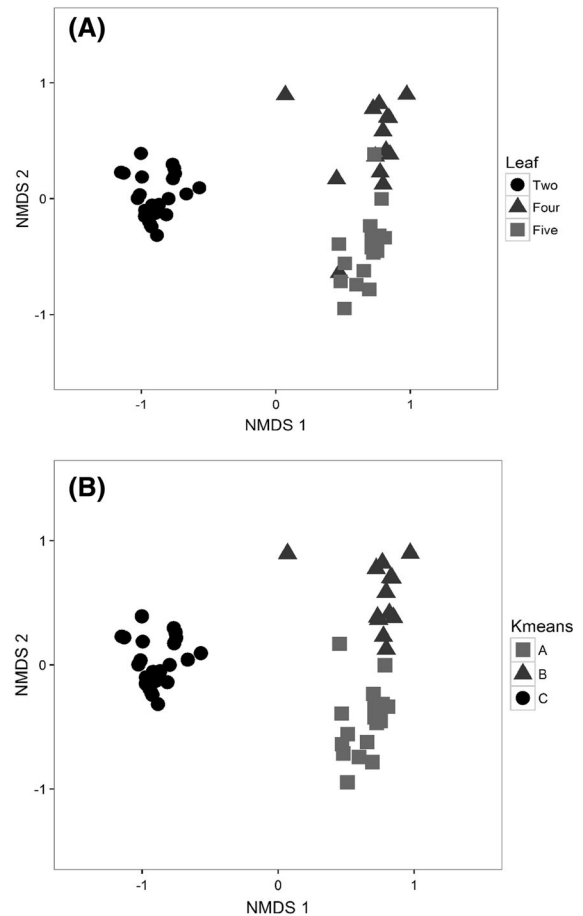

Fig. 6 Comparison of groups formed by multivariate regression trees (**A**) versus k-means partitioning (**B**) for the buffer scale plotted in NMDS ordination space. Plotting groups formed by k-means partitioning, an unconstrained clustering technique, provides a means of assessing the robustness of groups formed by multivariate regression trees

lacustrine clay and silt had four significant indicator species. Two species, *A. ligamentina* and *A. plicata*, were almost perfect indicators with indicator values of 0.93 and 0.87, respectively. Although *Quadrula quadrula* (Rafinesque, 1820) and *Lasmigona complanata* (Barnes, 1823) were only found at sites in leaf 2, these species were found in low abundance and, therefore, had lower indicator values (Table 2). Leaf 4 which included sites that had lower levels of wetland land cover (<72%) had two significant indicator species, *E. dilatata* and *Villosa iris* (Lea, 1829), which had intermediate indicator values of 0.54 and 0.55, respectively. These species were both numerically abundant but were also found in leaf 5 which
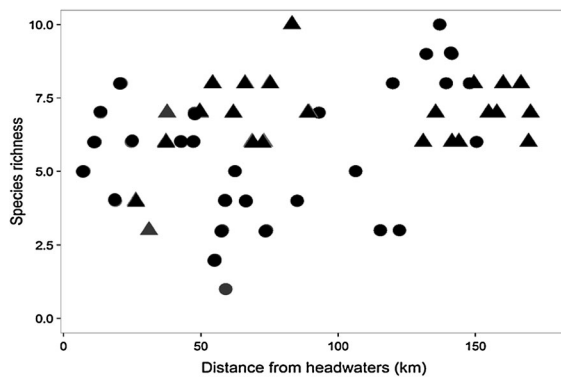
Fig. 7 Mussel species richness versus distance from headwaters for freshwater mussels in the Chippewa (*circles*) and Pine (*triangles*) rivers, MI, USA. No significant relationships were observed within rivers or within MRT-formed groups (*P* > 0.05)

accounted for their lower indicator values. Leaf 5 sites which had higher levels of wetland land cover (>72%) had five significant indicator species: *F. flava*, *Strophitus undulatus* (Say, 1817), *Ptychobranchus fasciolaris* (Rafinesque, 1820), *Venustaconcha ellipsiformis* (Conrad, 1863), and *Lasmigona costata* (Rafinesque, 1820). Similar to leaf 4, these species had intermediate indicator values due to lower levels of fidelity.

The substrate composition model resulted in two final leaves that were partitioned based on the percentage of silt substrate. Sites having <24% silt (*n* = 19) formed the first leaf and sites having ≥24%

silt (*n* = 34) formed the second leaf. Overall model performance was poor ($R^2 = 0.15$, CVRE = 1.07).

Discussion

In contrast to previous work focusing on longitudinal variation in freshwater mussel assemblages, the results of this study suggest the existence of discrete variation in mussel assemblage structure in the Chippewa and Pine rivers as opposed to a pattern of continuous variation along a longitudinal gradient. Strong structural patterns revealed by a combination of statistical analyses (i.e., MRT, ordination, and indicator species analysis) indicated three major assemblage clusters in each river that were separated based on the proportion of lacustrine clay and silt geology and wetland land cover at the buffer scale. Further, there was no evidence of increasing species richness with increasing distance from the headwaters either within rivers or within MRT groups which has been previously reported in the watersheds of similar size (Strayer, 1983; Haag, 2012). Patterns of assemblage structure were nearly identical in both rivers adding to the strength of evidence in support of discrete variation. These findings are in partial agreement with work conducted by Strayer (1983) and McRae et al. (2004) who observed that certain mussel species in southeastern Michigan exhibited strong fidelity to portions

Table 2 Results of indicator species analysis for buffer-scale groups formed by multivariate regression tree (MRT) analysis of mussels in the Chippewa and Pine Rivers, MI

Species	MRT leaf	Indicator value	*P* value
Actinonaias ligamentina	2	0.93	0.001
Amblema plicata	2	0.87	0.001
Quadrula quadrula	2	0.48	0.001
Lasmigona complanata	2	0.35	0.004
Villosa iris	4	0.55	0.001
Elliptio dilatata	4	0.54	0.002
Fusconaia flava	5	0.63	0.001
Lasmigona costata	5	0.55	0.002
Venustaconcha ellipsiformis	5	0.46	0.003
Ptychobranchus fasciolaris	5	0.44	0.004
Strophitus undulatus	5	0.43	0.007

Indicator values are ordered from highest indicator value to lowest within each MRT leaf. Indicator values were assessed for statistical significance using a Monte Carlo test (*P* < 0.05; 999 permutations)

of rivers flowing through particular geological formations in spite of the fact that their host fish was found in abundance outside of those formations. Strayer (1983) specifically noted that species such as *A. plicata* were predominately associated with stream reaches flowing through lake plains while *E. dilatata* occupied reaches in outwash and moraines which corroborates the findings of this study. Indeed, in the present study, geology appeared to be the strongest discriminating factor in separating assemblages in the Chippewa and Pine rivers at both the buffer and catchment scale.

Even though 30 years have passed since Strayer's work, and changes in land use have occurred in many parts of the upper Midwest, including Michigan (Michigan Land Use Leadership Council, 2003; Hamilton et al., 2014), geology still appears to be the overarching feature of the landscape contributing to the structure of mussels in the Chippewa and Pine rivers. Although the mechanisms by which surficial geology, an enduring aspect of habitat, influences mussel assemblage structure are still poorly understood, geology is known to exert hierarchical control of a number of important habitat variables, namely, discharge (Strayer, 1983). Discharge, in turn, affects other in-stream variables including current velocity, bed sediment material, temperature variability, and aspects of water quality and chemistry (Strayer, 1983; Johnson et al., 1997; McRae et al., 2004) which can affect the transport of gametes and settlement of juveniles (Morales et al., 2006; Daraio et al., 2012), movement of adults (Di Maio & Corkum, 1995), and delivery of food particles (Rypel et al., 2008). Recent studies have found recruitment to be strongly related to patterns of spring and summer discharge (Peterson et al., 2011; Ries et al., 2015). For some species, fertilization success has been reported to be higher in years of below average spring flows (Payne & Miller, 2000). It has been speculated that lower than average spring discharge may enhance fertilization by preventing the over-dispersion of sperm (Ries et al., 2015). Variable patterns in discharge among geology types may account for differential recruitment success of certain mussel species and may help to explain observed patterns of species distribution. A better understanding of species- or guild-specific in-stream flow requirements at various times of the year is necessary to inform management actions and enhance conservation success of mussels (Gates et al., 2015).

In addition to surficial geology, wetland land cover was also important in explaining patterns of mussel

assemblage structure in the Chippewa and Pine rivers. Similarly, Atkinson et al. (2012) found wetland land cover to be an important variable for explaining patterns of distribution and abundance of mussels in Oklahoma rivers. Because wetlands are not easily scoured by high flow events, it is likely that wetland habitat along the margins of river systems may attenuate the effects of high upstream flows on downstream river reaches (Mitsch & Gosselink, 2000; Atkinson et al., 2012). This may lead to greater habitat stability allowing mussel species to persist during flood events. Contrary to Atkinson et al. (2012) who found that species such as *S. undulatus* and *F. flava* were not associated with wetland habitat, these species were important indicator species for sites with higher levels of wetland land cover in the present study. Due to the extensive geographic separation between Michigan and Oklahoma, differences in habitat association might be explained by other hierarchical biogeographic factors that act as filters to limit these species from occurring in Oklahoma river reaches where adjacent riparian habitat has higher levels of wetland land cover (Poff et al., 1997; Haag, 2012). Even though wetlands provide many ecosystem services, the amount of wetland habitat world-wide has undergone substantial reductions since the early 1900s (Mitsch & Gosselink, 2000). The results of this study indicate the importance of wetland habitat in the riparian buffer to mussels in the Chippewa and Pine rivers and provides yet another reason why wetland habitats should be maintained and protected.

Contrary to numerous previous studies (Strayer, 1983; Haag & Warren, 1998; Daniel & Brown, 2014), there was no evidence of increasing species richness with increasing distance from the headwaters. There are several reasons that the strong downstream additive pattern in mussel species richness that seems to be so pervasive in other systems was not observed in this study. First, although overall levels of urbanization are low (<2%) in the Chippewa and Pine river watersheds, centers of urban development along both rivers (i.e., Mt. Pleasant, Alma, and St. Louis) coincide with the major geological change from well-drained morainal-outwash soils to poorly drained lake plain soils. Correspondingly, these locations are where strong discrete changes in mussel assemblage structure were observed. Alterations to riverine habitat imposed by urban development may create conditions less

suitable for mussels and their host fish by increasing sediment, contaminant, and nutrient loads, increasing storm-water runoff, increasing peak discharge and frequency of bank-full discharge, and reconfiguring natural stream channels (Klein, 1979; Biabanaki et al., 2011). Along with limiting or precluding mussels from river reaches within or downstream of urbanized areas, the combined effects of urbanization may limit the dispersal of host fish which could prevent colonization of mussel species in upstream reaches (Lonzarich et al., 2000; Wang et al., 2001). Alterations to the flow regime created as a result of urban development may have a negative impact on small-bodied minnow and darter species with already limited dispersal capabilities (Crawford et al., 2016). In this study, urban land use was an important predictor of mussel assemblage structure at the catchment scale. There is still debate as to how urban land use specifically affects mussel populations. Haag (2012) suggests that severe point source pollution, for example, can often act to non-discriminately reduce mussel populations regardless of the life-history traits exhibited by mussel species. Previously, it was believed that only more tolerant, generalist species could persist following disturbance from urban development (Haag, 2012); however, mussels as a faunal group are highly sensitive and the single greatest factor associated with persistence following disturbance is more likely to be the initial relative abundance of a species prior to the perturbation rather than the traits exhibited by a particular species (Warren & Haag, 2005). Better quantification of the fish assemblage structure and analysis of fish movement patterns may help to elucidate the role in which urbanization might play in limiting certain species of freshwater mussels upstream and downstream of urban areas in the Chippewa and Pine rivers.

Furthermore, additive patterns may not have been observed in this study because neither the Chippewa nor Pine river is completely free flowing over the longitudinal extent examined. On the Pine River, the city of Alma maintains a 57 ha impoundment while further downstream the city of St. Louis has a 637 ha impoundment to provide electricity. Because these dams lack fish passage structures, the dams may fragment downstream from upstream populations of mussels by limiting the movement of host fish (Watters, 1996; Porto et al., 1999; Bednarek, 2001). Several studies have shown species richness of mussels to be reduced downstream of dams (Williams et al., 1992; Layzer et al., 1993; Vaughn & Taylor, 1999), and extensive downstream distances are often required in order for mussels to fully recover from the temperature and hydrological impacts of dams (Vaughn & Taylor, 1999). However, even though a 295 ha impoundment occurs on the Chippewa River greater than 25 km upstream of the city of Mt. Pleasant, discrete changes in mussel assemblage structure were not observed from upstream to downstream of this dam. It is difficult to adequately separate out the role that geology plays in driving discrete patterns of assemblage structure when a number of natural and anthropogenic changes occur concomitantly. Unfortunately, both rivers have limited historical data on mussel assemblages to tease apart these factors.

Historically, studies attempting to explain patterns in mussel assemblages using simple, local-scale variables have had variable success in predicting mussel–habitat relationships (Holland-Bartels, 1990; Strayer & Ralley, 1993). Future studies in the Chippewa and Pine rivers should explore the role that complex hydraulic variables (e.g., Froude and Reynolds number, shear stress) play in structuring mussel assemblages. Several studies have found complex hydraulic variables to be important in structuring the abundance and richness of mussels elsewhere (Layzer & Madison, 1995; Steuer et al., 2008; Zigler et al., 2008; Allen & Vaughn, 2010). Specifically, low shear stress and high substrate stability at high flows are needed to prevent dislodgement and smothering (Allen & Vaughn, 2010), while minimum flows are needed to transport gametes, waste, and nutrients (Layzer & Madison, 1995; Steuer et al., 2008). Moreover, these variables may better represent how changes in geology affect aspects of hydrology that are important to mussels at a local scale and can be measured in situ. Although potentially more tedious to measure than traditional, simple local-scale variables (e.g., current velocity, depth), complex hydraulic variables may be more useful when trying to understand the specific mechanisms responsible for patterns in assemblage structure, and for establishing flow criteria for mussels in hydrologically altered systems (Gates et al., 2015).

It would be beneficial to explore life-history relationships of the species that characterized the clusters that were detected in this study. Only recently has a life-history framework been developed to

classify mussels into life-history strategies (i.e., opportunistic, periodic, equilibrium) based on suites of life-history traits (Haag, 2012). Life-history frameworks convey important information concerning the relative stability of the environment in which organisms persist, and the role of density-dependent and density-independent processes in structuring assemblages (Winemiller & Rose, 1992; Winemiller, 2005; Haag, 2012). Based on the broad, genus-level classifications suggested by Haag (2012) many of the species we found occupying the lower portions of the Chippewa and Pine rivers characterized by lacustrine clay and silt geology were equilibrium strategists (e.g., *Actinonaias, Amblema, Quadrula*). Conversely, species in the upper portions of the watershed were primarily periodic strategists (e.g., *Lampsilis*, *Lasmigona*, *Strophitus*). Typically, populations of equilibrium strategists are driven by density-dependent processes (e.g., competition, predation) while the role of density-independent processes (e.g., spates, drought) is stronger in structuring populations of periodic strategists (Winemiller, 2005; Haag, 2012; Daniel & Brown, 2014). However, mussels can exhibit extensive regional morphometric variation, and populations of the same species may be characterized by different life-history strategies (Haag, 2012). Furthermore, life-history information is currently lacking for many mussel species in North America (Haag, 2012). Therefore, it would be necessary to quantify the life-history traits of local populations to accurately determine their affiliation to a particular life-history strategy, and in turn, the role of processes that structure these populations. Information concerning the life-history traits and the abundance of mussel populations has been found to be important in predicting the extinction risk of mussels (Vaughn, 2012), and can ultimately be used to prioritize conservation and management efforts for these highly imperiled organisms (Haag, 2012; Vaughn, 2012).

The results of this study convey the importance of considering processes and practices that occur on the surrounding landscape when trying to understand patterns in the distribution and abundance of mussels. Similar to previous studies, environmental variables measured at the buffer and catchment scale explained the greatest amount of variation in mussel assemblage structure (Arbuckle & Downing, 2002; McRae et al., 2004; Atkinson et al., 2012). Freshwater mussels are long lived, essentially sessile organisms. As such,

mussels are perceived to incorporate stressors over greater spatial and temporal extents (Atkinson et al., 2012) than short-lived, highly vagile organisms which are often more strongly affected by their local environment (Lammert & Allan, 1999). Ultimately, processes occurring at broader scales should hierarchically influence process occurring at smaller scales which affect aspects of assemblage structure (Poff et al., 1997). How factors operating at larger spatial extents manifest to influence mussels in their local environment is poorly understood. Our data revealed differences in mussel assemblage structure consistent with upstream-to-downstream changes in surficial geology and wetland habitat and certain species were restricted to specific geologies suggesting discrete variation. These findings may have important implications in terms of the conservation and management of freshwater mussels. For example, efforts to reintroduce mussels to a new or formerly occupied section of river should consider the geological setting of the river. In doing so, biologists may avoid placing mussels in a location they are ill-adapted for. Furthermore, efforts to implement riparian improvement projects may have limited success in bolstering mussel populations if the underlying structuring force occurs over a much greater spatial extent, as is the case with geology. The results of this study will provide information necessary for the effective conservation and management of mussel assemblages in the Chippewa and Pine rivers and a framework to better understand mussel assemblage structure in similarly glaciated regions.

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