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Inventory and Assemblage Classification of the Freshwater Mussels (Mollusca: Unionidae) of the Strawberry River, Arkansas, USA, with Implications for Conservation Planning

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Abstract: Spatial hierarchical approaches to classify freshwater systems can add to our understanding of biogeographical patterns and can be used for biodiversity conservation planning. The Strawberry River is located primarily in the Ozark Highlands Central Plateau of north central Arkansas, USA, with a small downstream portion in the Mississippi Alluvial Plain and has been designated an Extraordinary Resource Water, an Ecologically Sensitive Water Body, and a Natural Scenic Waterway. The goals of this study were to document Strawberry River, Arkansas freshwater mussels to aid in conservation planning. Our first objective was to inventory freshwater mussel species in the Strawberry River. Our second objective was to use this stream-wide dataset to classify the freshwater mussel assemblages. We used unpublished survey data of 59 sites distributed from the headwaters to the mouth to inventory species occurrence and abundance, classified mussel assemblages using non-metric multi-dimensional scaling (NMS), and conducted indicator species analysis on resulting assemblages. We observed 39 taxa across the 59 survey sites including two S1, five S2, 16 S3, 11 S4, four S5, and one state non-ranked conservation rank species. Furthermore, our assemblage NMS revealed two distinct freshwater mussel assemblages roughly organized by an upstream (Sites 1–31) to downstream (Sites 32–59) gradient. There were five upstream indicator species and 13 downstream indicator species. This study provides a case study on using existing datasets with NMS and indicator species analyses to classify mussel assemblages and adds to our understanding of freshwater mussel fauna classification at smaller spatial scales. Both NMS and indicator species outcomes can aid in conservation planning for freshwater mussels.

Keywords: Strawberry River; Arkansas; biogeography; faunal groups; hierarchical spatial classification; conservation planning

Citation: Christian, A.D.; McCanty, S.T.; Poudel, S.; Chordas, S.W.A.; Harris, J.L. Inventory and Assemblage Classification of the Freshwater Mussels (Mollusca: Unionidae) of the Strawberry River, Arkansas, USA, with Implications for Conservation Planning. *Diversity* **2021**, *13*, 86. https://doi.org/10.3390/d13020086

Academic Editor: Bert W. Hoeksema

Received: 21 January 2021
Accepted: 16 February 2021
Published: 18 February 2021

1. Introduction

Spatial hierarchical approaches to classify freshwater systems can be used for biodiversity conservation planning, and one such approach is a four-level zoogeographic classification which includes an aquatic zoogeographic unit (AZU; 10,000–100,000 km^2), ecological drainage units (EDU; 1000–10,000 km^2) within AZU, aquatic ecological systems (AES; 100–1000 km^2) within EDU, and macrohabitats (i.e., valley segments; 1–10 km length) within AES [1]. North American freshwater mussels have been classified into four major faunal regions and 17 smaller faunal provinces [2]. When comparing the two classification approaches, the freshwater mussel faunal regions represent AZU, while freshwater mussel provinces represent EDU with regional biodiversity distinctions within AZU [1,2]. While larger hierarchical freshwater mussel classification patterns are useful for conservation

planning, additional case studies at the AES and macrohabitat scales are needed that (1) illustrate techniques to classify freshwater mussels at these scales and (2) aid conservation efforts by identifying spatial units characterizing aquatic ecosystem diversity. Accordingly, opportunities exist to classify mussel assemblages at smaller spatial scales of AES and macrohabitats based on evidence that mussels are distributed by small-, mid-, and large-sized stream guilds and to a lesser extent macrohabitat [3].

The Strawberry River is located primarily in the Ozark Highlands Central Plateau of northeast Arkansas, USA, with a small downstream portion in the Mississippi Alluvial Plain [4], and it has been designated an Extraordinary Resource Water, an Ecologically Sensitive Water Body, and a Natural Scenic Waterway [5]. However, recent water quality assessments have indicated decreasing water quality due to reach and sub-watershed scale habitat degradation [6,7]. The Strawberry River is known to support diverse aquatic macroinvertebrate [8] and fish [9,10] communities, and it has been considered a hotspot for at-risk fish and mussel species and a critical watershed for conservation of these species [11]. Furthermore, the Strawberry River was ranked among the top five hydrologic units in the Ozark and Ouachita highlands based on species richness, species density, and habitat availability for rare species used to calculate a relative importance index value [12]. While there have been freshwater mussels reported from a few sampling sites in the Strawberry River [8], no stream-wide surveys have been conducted exclusively for freshwater mussels that could aid in conservation planning.

The goal of this study was to document Strawberry River mussels to aid in conservation planning by identifying spatial units that characterize mussel diversity. Our first objective was to use available surveys to inventory freshwater mussel species inhabiting the Strawberry River. Our second objective was to use this stream-wide dataset to classify the freshwater mussel assemblages at finer scale than the Mississippian faunal region and Interior Highlands faunal province hierarchical classification. Based on previous studies on stream size guilds [3], we expect the Strawberry River mussel assemblages to classify into distinct groups along the stream-size gradient, especially small- and mid-size stream guilds, and to have identifiable dominant (>10% relative abundance at >3% site occurrences) species indicators. Further, our results may be useful in considering conservation planning for freshwater mussels [13].

2. Materials and Methods

2.1. Study Area

The Strawberry River in north central Arkansas, USA, is a tributary of the Black River within the White River basin (Figure 1) and has a 1970 km^2 watershed. The free-flowing Strawberry River winds approximately 185 km through the Ozark Central Plateau Ecoregion, before joining the Black River at the western edge of the Mississippi Alluvial Plain northeast of Swifton, Arkansas [4]. The Strawberry River is predominantly a spring-fed, clear stream consisting of wide, shallow pools separated by riffles [8]. The Strawberry River watershed is dominated by dolomite (50.6%) and sandstone (43.3%), has 1.3% Quaternary alluvial deposits, and has a land cover of 41% deciduous forest, 29.2% pasture area, 5.5% urban development, and 4.4% open land [14].

2.2. Sampling Methods

In order to ensure geographical coverage, we used a total of 59 headwater-to-mouth sites that were sampled between 1983 and 2016 (Figure 1) with seven sites from the 1980s (3, 8, 14, 26, 44, 48, and 54), 32 sites from the 1990s (4–7, 9–13, 15–20, 27–42, 52), 18 sites from the 2000s (1–2, 21–25, 45–48, 49–51, 53, 55–57, and 59), and two sites from the 2010s (43 and 58). A total of 57 sites were collected using qualitative methods and two sites (51 and 52) were collected using semi-quantitative methods. Qualitative methods consisted of searching the stream, based on walk-in or canoe access, from bank to bank in a zig-zag pattern by two or more surveyors in which mussels were visually located and collected by hand through a recorded amount of time. The two semi-quantitative survey sites results

were obtained through 1 m^2 quadrat sampling in highest density areas within a sampling reach [15,16]. All live and fresh dead individuals were identified in the field, enumerated, and most returned to the sampling area from where they were collected. Live and dead collected voucher specimens for most species are being curated into the Arkansas State University Museum of Zoology.

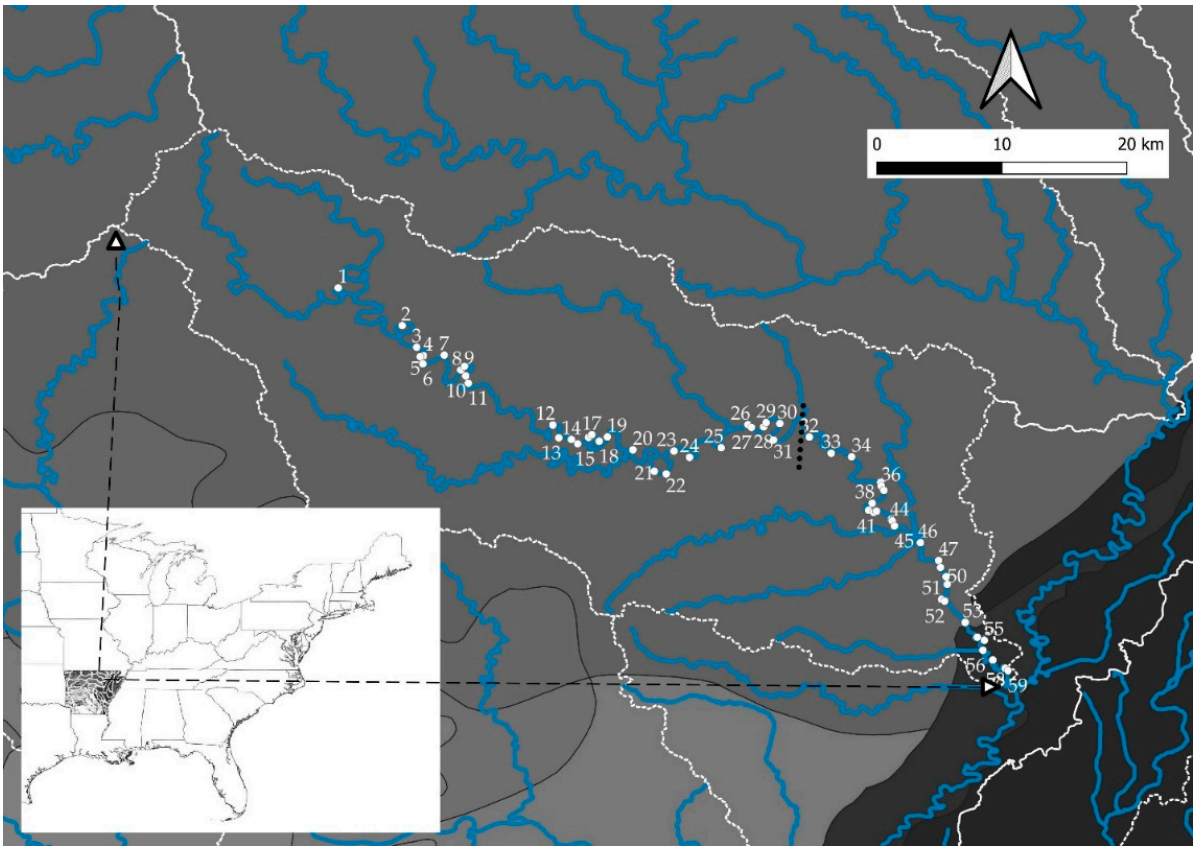

Figure 1. Map of the Strawberry River watershed, Arkansas, USA, sampling sites, and ecoregions. The inset map shows the location of the study area in the southcentral U.S.A. The blue lines are streams, the white dashed outline shows the watershed boundary, the shaded backgrounds with solid black lines are ecoregions (Ozark Highlands Central Plateau, gray shade; Mississippi Alluvial Plain, black), and white circles are the Strawberry River mussel sampling sites in numerical order upstream to downstream. Due to cartographic labeling issues, a few sites do not have labels. Black dotted line between sites 31 and 32 represents demarcation between the non-metric multi-dimensional scaling analysis upstream and downstream assemblage groupings.

2.3. Data Analysis

For data analysis, species names followed Williams et al. [17]. Species conservation ranks were assigned as global G1 to G5 (G1 = critically imperiled globally, G2 = imperiled globally because of rarity, G3 = very rare or found locally, G4 = apparently secure globally, G5 = demonstrably secure globally) and state S1 to S5 and SNR (S1 = extremely rare, S2 = very rare, S3 = rare to uncommon, S4 = common, apparently secure, and S5 = common and secure, SNR = not ranked) [18]. Where there was a split designation (e.g., S3/S4), we used the lowest "S" score (e.g., S3) in our reporting.

We assessed patterns in mussel assemblage data with non-metric multi-dimensional scaling (NMS) based on Bray–Curtis dissimilarity using the vegan package [19] in Program R [20]. To correct for differences in sampling methods and effort, we transformed assemblage data to relative abundance by dividing the number of individuals of each species collected at a site by total number of individuals (all species) collected at each site. Based on visual inspection of initial NMS plots, two clusters were assigned as an a posteriori

hypothesis of geographic grouping, representing upstream and downstream assemblage groups. Significance was evaluated by determining if between group variation, measured as the distance between geographic assemblage group centroids, was significantly greater than within group variation, based on a simulated distribution drawn from resampled data (analysis of similarity, ANOSIM). NMS clusters are displayed with 95% confidence interval ellipses for group centroid location, using the vegan package in Program R [18,19].

We used indicator species analysis to identify species that were uniquely characteristic of the identified geographic groups [21]. We conducted this analysis using the Indicspecies package in Program R [20,22]. This analysis assigns an indicator value to each species in each group from 0 to 1, where 0 indicates that a species was not observed at any site in the group, and 1 indicates a species was observed at every site in the group and never outside of the group. Indicator species were identified as those having indicator values that were significant at $p \leq 0.05$ based on a permutation test.

We calculated the percentage of sites each species was observed for the a posteriori upstream and downstream grouping sites discussed above. We also calculated mean relative abundance for each species for all study sites and the two a posteriori upstream and downstream assemblage grouping sites by averaging the site relative abundances overall and within each grouping for each species.

3. Results

3.1. Species Inventory

A total of 39 species were observed across the 59 survey sites resulting in two S1, five S2, 16 S3, 11 S4, four S5, and one SNR conservation rank species (Tables 1 and 2). Study-wide species occurrence ranged from a low of ≤0.2% (*Leptodea leptodon*, *Potamilus ohiensis*, *Plectomerus dombeyanus*, and *Pyganodon grandis*) to a high of 77.9% (*Lampsilis cardium)* at the 59 sites (Table 2). The overall relative abundance across all 59 survey sites ranged from a low of 0.03% (*Obovaria arkansasensis* and *Leptodea leptodon*) to a high of 13.97% (*Amblema plicata*) (Table 2).

Table 1. Species list of Strawberry River freshwater mussels collected at 59 stations and stream size which a species is strongly associated as reported by Haag [3]; species not categorized by Haag [3] are indicated with a dash (-); stream size guild designation listed from highest to lowest dominance. Nomenclature follows Williams et al. [17]. G1 = critically imperiled globally, G2 = imperiled globally because of rarity, G3 = very rare or found locally, G4 = apparently secure globally, G5 = demonstrably secure globally; Q in the global rank indicates the element's taxonomic classification is uncertain, S1 = extremely rare, S2 = very rare, S3 = rare to uncommon, S4 = common, apparently secure, S5 = common and secure, SNR = Unranked, the state rank not yet assessed, T = subranks are given to global ranks when a subspecies, variety, or race is considered at the state level. The subrank is made up of a "T" plus a number or letter (1, 2, 3, 4, 5, H, U, X) with the same ranking rules as a full species. Global (G) and State (S) rankings obtained from Arkansas Natural Heritage Commission database [18].

Taxon	G Rank	S Rank	Stream Size Haag (2012)
Actinonaias ligamentina (Lamarck 1819)	G5	S5	Mid and Large
Alasmidonta marginata (Say 1818)	G4	S3	Small
Amblema plicata (Say, 1817)	G5	S5	Mid, Large, and Small
Cyclonaias pustulosa (Lea, 1831)	G5T5	SNR	Large and Mid
Cyclonaias tuberculata (Rafinesque, 1820)	G5	S3	Mid
Cyprogenia aberti (Conrad, 1850)	G2G3Q	S2	Mid
Ellipsaria lineolata (Rafinesque, 1820)	G4G5	S3	Large
Eurynia dilatata (Rafinesque, 1820)	G5	S4	Mid, Small, and Large
Fusconaia flava (Rafinesque, 1820)	G5	S4	Mid, Large, and Small
Fusconaia ozarkensis (Call, 1887)	G3G4	S3	Mid and Small
Lampsilis cardium (Rafinesque, 1820)	G5	S4	Small and Mid
Lampsilis reeveiana (Call, 1887)	G3	S3	Small and Mid
Lampsilis siliquoidea (Barnes, 1823)	G5	S3	Small and Mid

Table 1. *Cont.*

Taxon	G Rank	S Rank	Stream Size Haag (2012)
Lampsilis teres (Rafinesque, 1820)	G5	S4	Large
Lasmigona complanata (Barnes, 1823)	G5	S3S4	Small and Mid
Lasmigona costata (Rafinesque, 1820)	G5	S3	Small and Mid
Leptodea fragilis (Rafinesque, 1820)	G5	S4	Mid
Leptodea leptodon (Rafinesque, 1820)	G1G2	S1	-
Ligumia recta (Lamarck, 1819)	G4G5	S2	-
Obliquaria reflexa (Lea, 1845)	G5	S4	Large
Obovaria arkansasensis (Lea, 1862)	G2	S2	Small and Mid
Plectomerus dombeyanus (Valenciennes, 1827)	G4	S4	-
Pleurobema sintoxia (Rafinesque, 1820)	G4G5	S3	Mid
Potamilus ohiensis (Rafinesque, 1820)	G5	S3	
Potamilus purpuratus (Lea, 1831)	G5	S4	Large
Ptychobranchus occidentalis (Conrad, 1836)	G3G4	S3	Mid and Small
Pyganodon grandis (Say, 1829)	G5	S5	Mid
Quadrula quadrula (Rafinesque, 1820)	G5	S5	Mid
Reginaia ebenus (Lea, 1831)	G4G5	S3S4	Large
Strophitus undulatus (Say, 1817)	G5	S3	Small
Theliderma cylindrica (Say, 1817)	G3G4T3	S2	-
Theliderma metanevra (Rafinesque, 1820)	G4	S3S4	-
Toxolasma lividum (Rafinesque, 1831)	G3Q	S2	Small
Tritogonia verrucosa (Rafinesque, 1820)	G4G5	S4	Mid
Truncilla truncata (Rafinesque, 1820)	G5	S4	Large
Utterbackia imbecillis (Say, 1829)	G5	S4	-
Venustaconcha pleasii (Marsh, 1891)	G3G4	S3	-
Villosa iris (Lea, 1829)	G5Q	S2/S3	Small and Mid
Villosa lienosa (Conrad, 1834)	G5	S3	Small and Mid

Table 2. Strawberry River freshwater mussel species occurrence at upstream and downstream assemblage group sites (*n* = number of sites), mean % relative abundance of a posteriori upstream and downstream assemblage sites and total sites, and upstream and downstream assemblage group species indictor analysis correlations with associated *p* values. Nomenclature follows Williams et al. [17].

Species	Upstream Sites (*n*)	Downstream Sites (*n*)	Mean Upstream Abundance (%)	Mean Downstream Abundance (%)	Mean Total Abundance (%)	Upstream Indicator Value	Downstream Indicator Value	*p* Value
Actinonaias ligamentina	11	20	1.76	13.03	7.11		0.817	0.001
Alasmidonta marginata	12	7	1.63	0.52	1.10			
Amblema plicata	24	21	19.76	7.55	13.97			
Cyclonaias pustulosa	3	22	0.14	13.97	6.70		0.881	0.001
Cyclonaias tuberculata	7	3	0.97	0.12	0.56			
Cyprogenia aberti	8	10	0.74	1.58	1.14			
Ellipsaria lineolata	0	2	0.00	0.09	0.04			
Eurynia dilatata	20	13	7.33	4.37	5.92			
Fusconaia flava	12	22	0.96	5.23	2.99		0.806	0.001
Fusconaia ozarkensis	17	0	4.57	0.00	2.40	0.741		0.001
Lampsilis cardium	25	21	11.52	7.42	9.57			
Lampsilis reeveiana	24	0	9.02	0.00	4.74	0.880		0.001
Lampsilis siliquoidea	16	4	2.99	0.40	1.76	0.678		0.002
Lampsilis teres	0	16	0	3.74	1.78		0.756	0.001
Lasmigona complanata	0	8	0	0.28	0.13		0.535	0.002
Lasmigona costata	20	13	5.7	1.69	3.80			
Leptodea fragilis	1	10	0.02	2.37	1.14		0.584	0.003
Leptodea leptodon	0	1	0	0.06	0.03			
Ligumia recta	6	7	2.26	0.51	1.43			
Obliquaria reflexa	1	15	0.08	1.76	0.87		0.709	0.001
Obovaria arkansasensis	0	2	0.00	0.07	0.03			
Plectomerus dombeyanus	1	0	0.17	0.00	0.09			
Pleurobema sintoxia	10	14	1.3	3.32	2.26			
Potamilus ohiensis	0	1	0	0.09	0.04			

Table 2. *Cont.*

Species	Upstream Sites (*n*)	Downstream Sites (*n*)	Mean Upstream Abundance (%)	Mean Downstream Abundance (%)	Mean Total Abundance (%)	Upstream Indicator Value	Downstream Indicator Value	*p* Value
Potamilus purpuratus	14	24	6.58	14.59	10.38		0.778	0.005
Ptychobranchus occidentalis	22	2	9.22	0.07	4.87	0.837		0.001
Pyganodon grandis	0	1	0	0.08	0.04			
Quadrula quadrula	0	6	0	0.49	0.23		0.463	0.009
Reginaia ebenus	0	8	0	1.27	0.60		0.535	0.002
Strophitus undulatus	3	8	0.82	0.5	0.66			
Theliderma cylindrica	4	11	0.18	2.39	1.23		0.598	0.004
Theliderma metanevra	6	18	0.81	4.04	2.35		0.756	0.001
Toxolasma lividum	4	1	0.1	0.20	0.15			
Tritogonia verrucosa	23	19	7.3	5.63	6.51			
Truncilla truncata	2	14	0.09	1.82	0.91		0.689	0.001
Utterbackia imbecillis	2	2	0.14	0.18	0.16			
Venustaconcha pleasii	10	0	2.99	0.00	1.57	0.568		0.002
Villosa iris	5	0	0.35	0.00	0.18			
Villosa lienosa	7	6	0.51	0.57	0.54			

3.2. Assemblage Classification

The NMS analysis revealed a geographic pattern of two clusters representing an upstream assemblage group from sites 1–31 and a downstream assemblage group from sites 32–59 (Figure 2). The two groupings were significantly different based on the standard deviation of sites to their geographic group centroids (ANOSIM, R = 0.3931, $p < 0.001$). We created 95% confidence interval ellipses for each group centroid based on standard deviation (Figure 2). There are three sites (1, 29, and 47) which fall outside of their group's 95% CI.

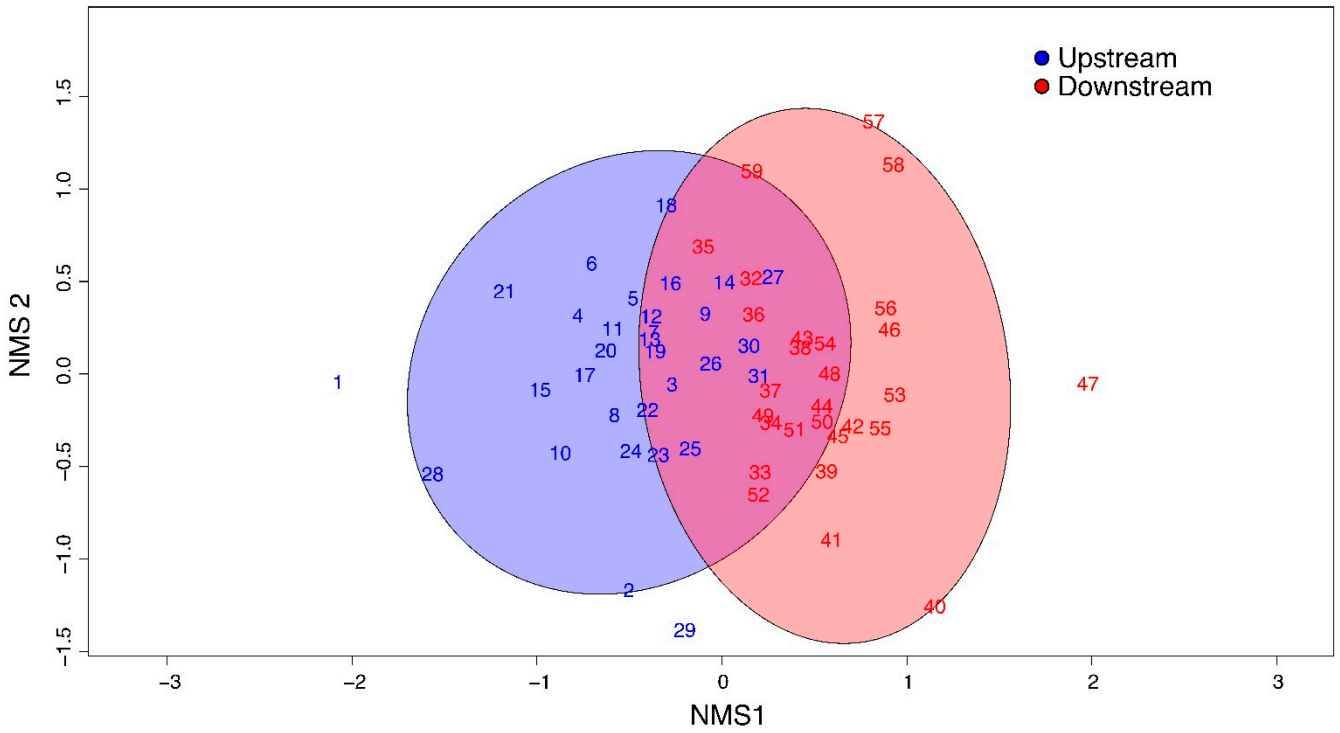

Figure 2. Non-metric multi-dimensional scaling (NMS) plot of mussel assemblages at 59 Strawberry River sampling sites. Ellipses represent 95% confidence intervals around group centroids for two geographic groupings representing upstream (blue) and downstream (red) assemblage groups. There are three sites (1, 29, and 47) which occur outside of the 95% confidence interval ellipses.

Indicator species analysis identified 18 characteristic species across both geographic groups (Figure 3). The upstream assemblage group had 5 significant indicator species that were, in order of highest correlation, *Lampsilis reeveiana* (0.880; $p = 0.001$), *Ptychobranchus occidentalis* (0.837; $p = 0.001$), *Fusconaia ozarkensis* (0.741; $p = 0.001$), *Lampsilis siliquoidea* (0.678; $p = 0.001$), and *Venustacocha pleasii* (0.568; $p = 0.002$). These taxa are indicative of small- or small- and mid-sized streams guild species and composed 2.99 to 9.22% of mean relative abundance of upstream assemblage group sites (Tables 1 and 2). The downstream assemblage group had 13 species that were significant indicators, with the significant correlations being *Cyclonaias pustulosa* (0.881; $p = 001$), *Actinonaias ligamentina* (0.817; $p = 0.001$), *Fusconaia flava* (0.806; $p = 0.001$), *Potamilus purpuratus* (0.778; $p = 0.005$), *Lampsilis teres* (0.756; $p = 0.001$), *Theliderma metanevra* (0.756; $p = 0.001$), *Obliquaria reflexa* (0.709; $p = 0.001$), *Truncilla truncata* (0.689; $p = 0.001$), *Theliderma cylindrica* (0.598; $p = 0.004$), *Leptodea fragilis* (0.584; $p = 0.003$), *Lasmigona complanata* (0.535; $p = 0.002$), *Reginaia ebenus* (0.535; $p = 0.002$), and *Quadrula quadrula* (0.463; $p = 0.009$). The downstream indicator taxa are indicative of mid-and large-sized streams, with the notable exception of *Lampsilis teres* and *Lasmigona complanata* (both small- and mid-sized stream guild species) and composed 0.49 to 14.59% of mean relative abundance for downstream assemblage group sites (Tables 1 and 2).

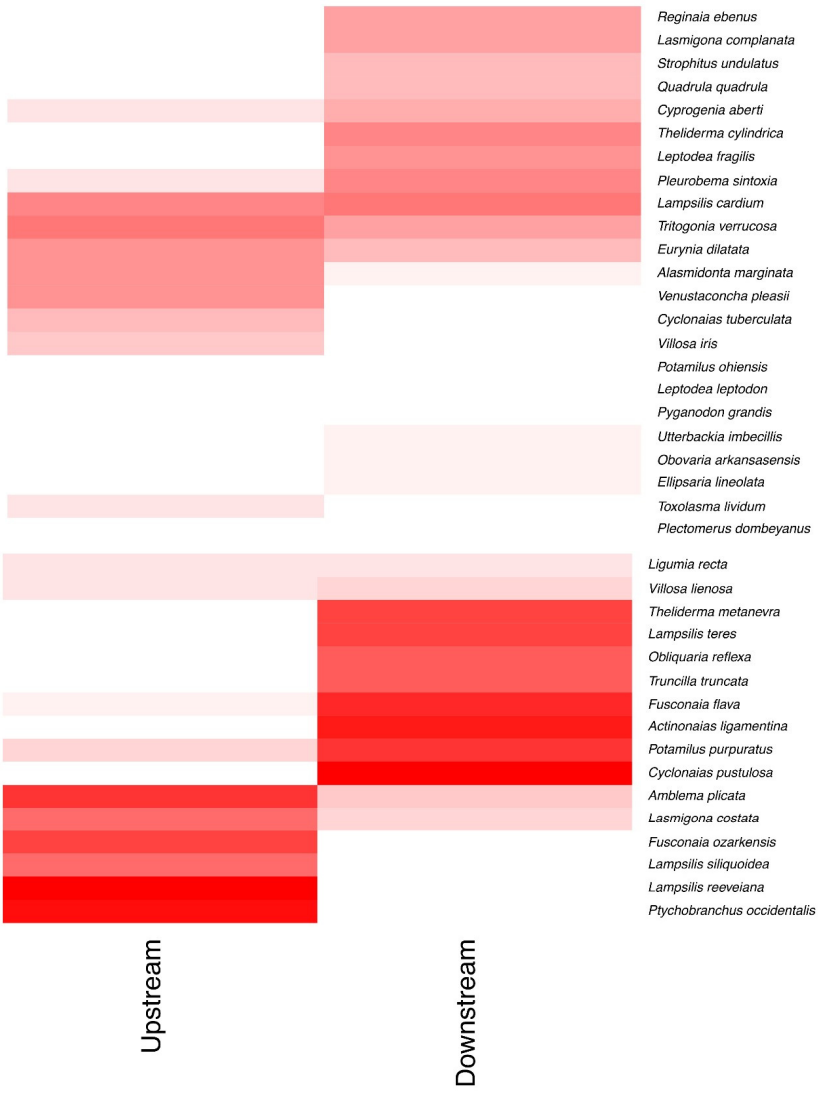

Figure 3. Heat map showing results of indicator species analysis for upstream and downstream mussel assemblage groups in the Strawberry River. Deeper red coloring indicates stronger indicator relationships within each group.

4. Discussion

Our results revealed two major findings. First, the Strawberry River has relatively high species richness (39) and a large percentage (n = 23) of these species are of high state-level conservation concern (S1–S3). Second, our assemblage classification revealed two distinct freshwater mussel assemblage groupings generally organized by an upstream to downstream gradient.

4.1. Species Inventory

The results of our study indicated that the Strawberry River has just over 50% of the 75 taxa currently considered native to Arkansas [23] and nearly 60% of these 39 taxa are vulnerable (S3) to critically imperiled (S1) in Arkansas [18]. Additionally, two species found during this study are federally protected and included *Leptodea leptodon* (endangered) and *Theliderma cylindrica* (threatened). It is noteworthy from our upstream and downstream grouping species site occurrence and abundance results that S1-S3 taxa were observed at multiple sites in our study and typically were represented by more than one individual at a site (Table 2). Other taxonomic group studies also have reported high aquatic species richness in the Strawberry River watershed such as 100+ fish species from 18 families [9,10] and 313 aquatic macroinvertebrate species including 23 freshwater mussel taxa from seven sites [8]. All 23 of these freshwater mussel taxa were found in our study.

4.2. Assemblage Classification

Our NMS classification revealed two distinct freshwater mussel groupings organized as upstream and downstream assemblages. Combined with the species indicator analysis, these results were mostly consistent with the expectations for small, mid, and large stream size guilds [3]. Upstream assemblage relative abundance was dominated by *Amblema plicata* (19.76%) and *Lampsilis cardium* (11.52%); however, *Ptychobranchus occidentalis* (9.22%), and *Lampsilis reeveiana* (9.02%) also were highly abundant. Upstream indicator species were predominantly small- to mid-sized stream guild species and were, in relative abundance order, *Ptychobranchus occidentalis*, *Lampsilis reeveiana*, *Fusconaia ozarkensis*, *Lampsilis siliquoidea, and Venustachoncha pleasii* [3]. It is noteworthy that *Amblema plicata* (stream size generalist) and *Lampsilis cardium* (small- and mid-sized stream species) were dominant in relative abundance upstream but were not upstream assemblage group species indicators. It also is noteworthy that upstream indicator species *Lampsilis reeveiana*, *Fusconaia ozarkensis*, and *Venustaconcha pleasii* were exclusively found at upstream assemblage grouping sites.

The downstream assemblage group relative abundance was dominated by *Potamilus purpuratus* (14.59%), *Cyclonaias pustulosa* (13.97%), and *Actinonaias ligamentina* (13.03%). The downstream indicator species were dominated (relative abundance >10%) by mid- to large-sized stream species and were, in relative abundance order, *Potamilus purpuratus*, *Cyclonaias pustulosa*, and *Actinonaias ligamentina* [3]. Four downstream indicator species were observed only within the downstream assemblage sites and included *Lampsilis teres*, *Lasmigona complanata, Quadrula quadrula,* and *Reginaia ebenus*. Furthermore, five non-indicator species, in relative abundance order, *Amblema plicata*, *Lampsilis cardium*, *Tritogonia verrucosa, Eurynia dilatata, and Pleurobema sintoxia* were abundant at both upstream and downstream sites, indicating they are widespread generalists.

While we observed two distinct mussel assemblage groups based on NMS, we did not observe the dominant species in each assemblage corresponding exclusively to the expected stream-size guild, namely that upstream sites were expected to be dominated by small-sized stream species while the downstream sites expected to be dominated by mid-sized stream species [3]. Contrary to our stream-size guild expectations, our findings showed that both assemblage groups had a mixture of mid-size stream guild dominant taxa. Many of our sampling sites in the middle portion of the river correspond to sites in the overlap of our 95% confidence ellipses (upstream assemblage sites 22, 23, 25, 26, 27, and 30; downstream assemblage sites 32–38, 43–44, 48–52, and 54) that may represent a lengthy transition zone between the mussel assemblages extending downstream from

the confluences of Piney Fork (downstream of Site 20), North Big Creek (downstream of Site 25), and Mill Creek (between sites 31–32) (Figures 1 and 2). We conducted a post hoc stream order classification which determined that the Strawberry River at Site 1 is a 5th order stream, becomes a 6th order stream after the entry of the Little Strawberry River (between Sites 1 and 2), and remains a 6th order stream until its confluence with the Black River [24]. Further investigation revealed that drainage area changes substantially from 588 km^2 (29.8% of total watershed) upstream of the Piney Fork confluence (Site 20), to 992 km^2 (50.3 % of total watershed) at the North Big Creek confluence (between Sites 25–26), to 1321 km^2 (67% of total watershed) at the Mill Creek confluence (between Sites 31–32), a drainage area increase of 225% over approximately 30 river km in length [14]. This lack of change in stream order but increase in drainage area results in corresponding changes in mean discharge, water depth, and wetted channel which likely influences the mussel composition along the Strawberry River.

We also observed three sites (1, 29, and 47) which occurred outside of their respective centroid confidence interval ellipses; however, all three of these sites were characterized by low richness and abundance, even though the sampling effort at these sites was similar to our other sites. Site 1 consisted of two species observed, *L. reeveiana* and *V. pleasii*, and both taxa are upstream indicator species in our study, found exclusively in the upstream section of the Strawberry River. Site 29, which falls within the upstream assemblage group site distribution, also only had 2 species observed, *L. cardium* and *Ligumia recta*. While *L. cardium* is an upstream assemblage group indicator in our study, it also is abundant in the downstream portion of Strawberry River, and the same was observed for *L. recta* distribution and abundance. At Site 47, we only observed two individuals of *Cyclonaias pustulosa*. *Cyclonaias pustulosa* is a downstream group indicator species in our study, and Site 47 falls within the downstream assemblage group site distribution.

5. Conclusions

Recent water quality and best management practices implementation assessments have indicated decreasing habitat and water quality in the Strawberry River watershed [6,7]. Considering these deteriorating conditions and with the Strawberry River state designated as an Extraordinary Resource Water, an Ecologically Sensitive Water Body, and a Natural Scenic Waterway [5], knowing the inhabiting mussel fauna and associated biodiversity classification can aid in conservation planning. This case study illustrates the utility of NMS as a classification tool and serves as a reference for use of existing survey data to classify freshwater mussels at hierarchical levels smaller than faunal regions and provinces. Using the combination of North American freshwater mussel faunal region and province classification [2] and the four-level zoogeographic classification [1], the freshwater mussels of the Strawberry River can be classified into the Mississippian faunal region-AZU, the Interior Highlands province-EDU, and upstream and downstream Strawberry River AES. This classification, along with the associated inventory, provides an additional case study adding to our understanding of freshwater mussel fauna ecological classification [2,25] that can be used for conservation planning aimed at conserving biodiversity across spatial and biogeographical scales [13]. Resource managers may use the upstream and downstream assemblages to prioritize and enforce habitat and water quality efforts; reduce or establish policies on land use, land cover, and fragmentation; prioritize preservation or restoration activities; or identify stream sections for mitigation activities such as translocating and augmenting populations based on historical distributions and assemblage associations [13]. Where possible, using source populations from the same AES (e.g., Strawberry River upstream or downstream) as proposed conservation efforts could enhance the success of future augmentation, restoration, relocation and/or translocation activities [26,27]. Using source populations from the same assemblage should help minimize negative effects to species unable to acclimate to novel environments [28,29].

Author Contributions: Conceptualization, A.D.C. and J.L.H.; methodology, A.D.C., S.W.A.C., J.L.H.; software, S.T.M.; validation, A.D.C., J.L.H., S.T.M.; formal analysis, S.T.M.; investigation, A.D.C., J.L.H., S.W.A.C.; resources, A.D.C., J.L.H., S.W.A.C.; data curation, J.L.H.; writing—original draft preparation, A.D.C. and S.P.; writing—review and editing, A.D.C., S.T.M., S.P., S.W.A.C., J.L.H.; visualization, A.D.C. and S.T.M.; supervision, J.L.H.; project administration, J.L.H., A.D.C.; funding acquisition, J.L.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Arkansas Game and Fish Commission, Arkansas Natural Heritage Commission, the U.S. Army Corps of Engineers, and the U.S. Fish and Wildlife Service for funding portions of this study.

Institutional Review Board Statement: Freshwater mussels are invertebrate animals, thus are not governed by any of our Institutional Animal Care and Use Committees (IACUC). IACUC ensures the human and appropriate care and use of all live vertebrate animals used or intended for use in research, research training, experimentation, biological testing, or teaching.

Informed Consent Statement: Not applicable.

Data Availability Statement: This data is held with A.D.C. and J.L.H.

Acknowledgments: We thank P. J. Rust, J. H. Seagraves and M. E. Gordon for field work assistance.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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