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A Comparative analysis of water quality and invertebrate diversity in a primarily agricultural stream and a trout stream in southeastern Minnesota

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Abstract: As agricultural activity expands and intensifies across the Midwest, its associated environmental effects increase in severity. Agricultural runoff is linked with surface water pollution, causing significant effects on both local watersheds and downstream areas. As a result, the number of healthy streams in the Midwest has decreased. Trout streams, characterized by high water quality and an array of pollution-sensitive species, have become threatened in large part due to agricultural activity. In this study, I compare water quality between Rice Creek, a trout stream, and Mud Creek, a primarily agricultural stream. For this comparison, I gathered data on water quality indices such as conductivity, pH, temperature, dissolved oxygen, and nutrient levels. Biotic characteristics of both streams were assessed through macroinvertebrate samples to determine species richness, diversity, and sensitivity. Although I expected Rice Creek to demonstrate greater health compared to Mud Creek, my results suggested that Mud Creek performed the same or slightly better in the indices under consideration. Only one chemical and one biotic parameter showed a significant difference between the streams. Given these unexpected results, it is necessary to examine the factors contributing to Rice Creek's degradation while also searching for positive attributes that keep Mud Creek at a higher quality than might be expected from a primarily agricultural stream. These further explorations may be used to inform future management practices in the area.

Across the United States, rising awareness of agricultural pollution has contributed to the development of an activist movement of conservation biologists, hydrologists, ecologists, farmers, and citizens concerned about agricultural effects on water quality. Agricultural activity across Minnesota and other Corn Belt states has significantly reduced the number of trout streams and other healthy small watersheds across the Midwest. Southeastern Minnesota specifically is part of the Driftless Region, an area largely untouched by glacial drifts of sand, rock, and soil from prehistoric periods of glaciation. The Driftless Region contains many small, groundwater-fed trout streams. Trout streams are characterized by cool water temperatures in summer, water temperatures above air temperatures during the winter, and year-round streamflow stability which promotes the wellbeing of adult trout, their eggs, and their insect food sources (MNDNR 2018).

In recent decades, increasing urbanization and agricultural activity have posed significant threats to the health of trout streams throughout southeastern Minnesota. Row crop agriculture is especially harmful to trout streams, since its associated lack of perennial vegetation allows for increased runoff. Greater runoff volumes disrupt both streamflow stability and stream temperatures, making streams less hospitable to trout and their prey. Agricultural runoff contains sediment that can smother trout eggs and nutrient loads that create inadequate conditions for adult fish and insects. Livestock overgrazing and farming up to the edge of streams compromises streambank stability as well, giving rise to conditions ripe for erosion (MNDNR 2018).

The effects of stream and watershed degradation are well documented across the Midwest. Streams in agricultural and urban areas alike often experience the negative effects of eutrophication and changing water chemistry from nutrients in runoff entering the watershed. Common approaches to the evaluation of stream and watershed health include the study of biological indices such as aquatic invertebrates, fish populations, and bacteria (Wang et al 2006). In a number of streams throughout the Midwest, the effects of agricultural and urban runoff have been studied in relation to frequency and diversity of invertebrate and fish populations (Herringshaw et al. 2011). Studies have found that high nitrogen concentrations in rural watersheds are linked with declines in taxonomic diversity among invertebrate populations (Wang et al. 2006, Houghton 2012, MPCA 2014).

While past studies have extensively covered the effects of agricultural and urban pollution on specifically trout streams and streams in general, there are few comparisons of the two environments. Especially in southeastern Minnesota, many trout streams flow in part through agricultural lands and receive substantial amounts of nutrient and sediment laden runoff throughout their path. Widespread degradation of trout streams is increasingly prevalent, and it is worth comparing their ecosystem characteristics to those of primarily agricultural streams in order to assess differences between the two. Such a comparison can provide examples of changes that might be expected with continued trout stream degradation, if agricultural streams model the future of formerly healthy streams. Making note of differences between trout streams and agricultural streams may also help identify areas for watershed conservation, changes in agricultural practices, and methods not only for the protection of current trout streams, but also for the restoration of agricultural streams.

In order to assess stream quality, this study analyzed various parameters related to stream chemistry (e.g. dissolved oxygen, pH, conductivity) and biological composition (e.g. species diversity, species richness, presence of indicator species). These factors are used to conduct a comparative analysis of a southern Minnesota trout stream in Rice County and a primarily agricultural stream in Dakota County. I gathered water chemistry data on each site using a variety of field instruments, and I assessed biological composition by sampling macroinvertebrates from all sites. The lower the species richness and species diversity, the less biologically robust and healthy the stream. I also considered the presence and absence of indicator organisms, which are classified according to their tolerance of pollution in aquatic ecosystems, with the presence of more sensitive biota indicating greater water quality. In terms of stream chemistry, lower dissolved oxygen (DO) levels, higher temperature, high conductivity (indicating high levels of particulate matter), more extreme pH (determined by distance from neutral), and lower clarity were associated with more unhealthy streams. These measurements provided both biological and chemical indices for stream quality. Given the patterns associated with greater stream health, I expected that Rice Creek would exhibit lower temperatures, higher DO, lower conductivity, greater clarity, and less extreme pH than Mud Creek, the primarily agricultural stream. From a biological point of view, I expected to observe greater species diversity and species richness in Rice Creek, as well as a greater frequency of pollution-sensitive biota.

Methods:

For this study, I used four different sites to assess the chemical and biotic indices that I needed to determine water quality. Two sites were used for both Rice Creek (trout stream, Figure 1) and Mud Creek (primarily agricultural stream, Figure 2). Water quality was determined using a variety of chemical characteristics measured directly on site. At each location and for each sampling date, water samples were drawn, filtered, and bottled. They were then frozen until later analysis. Nitrate and ammonium levels were determined for each of these water samples and compared between all sites and sampling dates.

Other water chemistry characteristics were measured for each site using a variety of digital meters. Conductivity was measured using a YSI Model 30 Handheld Salinity, Conductivity, and

Temperature System. I measured pH with a EcoSense pH10A Pen Tester from YSI, and dissolved oxygen was measured using a YSI ProODO Handheld Dissolved Oxygen Meter. A manual Secchi tube was used to measure water clarity for all sites. All of these measurements were taken in the middle of the stream in water that I had not yet disturbed by walking through.

For measurement of biotic indices in the two streams, I referred to invertebrate sampling procedures utilized by the Minnesota Pollution Control Agency. In each stream I sampled invertebrates from leaf packs, which form deposits near stream banks, log jams, and other areas protected from the current. In order to do this, I used a D net to take approximately one meter sweeps of the leaf packs on the sides of the stream. In the case of the first Mud Creek site (MC 1), I performed the same procedure using the submerged reeds on the stream bank in the absence of leaf pack. For each site, I took four sweeps with successful invertebrate yields. On the first Rice Creek site (RC 1) and the second Mud Creek site (MC 2), I also sampled invertebrates from large rocks on the stream bottom. Four rocks were randomly selected and extracted at each of these sites for sampling. This procedure was repeated for two of the three sampling dates. After collection, invertebrates were then euthanized onsite using 70% ethanol and transported back to the lab for identification.

To assess the level of biological diversity and pollution tolerance in each stream, I identified invertebrates in the samples according to their taxonomic order and/or class. Special attention was paid to species known to have a low tolerance to water pollution. Indicator species serve as benchmarks of water quality, with the presence or absence of highly sensitive taxa demonstrating levels of pollutants. For example, the commonly used EPT index assesses the species richness of highly tolerant aquatic invertebrates and is used to determine water quality in rivers worldwide (NRCS, n.d.).

For this study, I used the Shannon (H') and Simpson (D_1) indices to calculate species diversity for Rice Creek and Mud Creek. Each stream site was analyzed separately using these diversity measures. The values were then input into a contingency table in R Commander module (R version 3.5.1) to determine significant differences between the streams. Both the Shannon and Simpson indices as well as the Simpson variance were analyzed with a Chi-square test of significant difference. Finally, the Simpson variance values (Ds) were analyzed through pairwise comparisons and fit with t-values, which were tested for significant difference using a predetermined algorithm. The raw numbers for each taxonomic group were also input into a contingency table in order to assess the relationships between the counts.

Chemical characteristics of the streams were assessed primarily through analysis of variance (ANOVA) tests. ANOVAs were conducted separately for all chemical characteristics and divided both by sampling time and by sampling location. Nutrient levels for both nitrate and ammonium were also tested using separate ANOVAs for sampling time and location. These tests determine whether there are significant differences in means of the measured characteristics for each site.

Results:

According to my observations, there were very few significant differences between the variables under consideration. My analysis began with ANOVAs for the water chemistry characteristics at all of the sites. My first ANOVA considered water chemistry parameters divided by site, with all of the sampling times averaged into a mean for each site (Table 1). Of the measurements for conductivity, pH, dissolved oxygen, and temperature, only conductivity showed a significant difference between the sites, with a p-value of 0.00112. The second ANOVA that I conducted divided water chemistry characteristics by sampling date rather than location, tracking significant changes over the sampling period by considering the mean values for all sites combined (Table 2). This ANOVA yielded only one value of significance, with significant differences between water temperature measurements for the three sampling dates.

The next ANOVA test addressed nutrient levels from each sampling site (Table 3). Nitrate and ammonium concentrations were calculated using a SMARTCHEM analysis for each water sample from the study. For the ANOVA table I took means for each site from all of the samples. Ammonium measurements did not show any significant differences between the sites, with a p-value of 0.868. Nitrate levels, however, were significantly different with a p-value of 0.0142. As Table 3 shows, Mud Creek had significantly lower nitrate levels compared to Rice Creek.

The final three tables address the biological characteristics of the two streams. Table 4 contains raw counts of the different taxa sampled at each site. No particular index or statistical test is used to evaluate these data, so raw counts are compared between sites to make conclusions. Particular attention is paid to the Ephemeroptera and Trichoptera taxa, since they comprise part of the highly sensitive EPT taxa that are commonly used as indicator species for water quality in freshwater ecosystems. For the two Rice Creek sites, a total of 21 Trichoptera were collected compared to a combined 17 for the Mud Creek sites. The magnitude of this difference is not especially significant, so there does not appear to be an observable difference between the number of Trichoptera present at each site. Ephemeroptera, on the other hand, were represented by a total of four individuals for Rice Creek compared to 24 for Mud Creek, suggesting a notable difference between the two streams in terms of their host potential for this particular taxonomic group.

The Shannon and Simpson diversity indices were then used to assess the taxonomic compositions of all of the sites (Table 5). The values in the top row of Table 5 indicate that the two sites differ very little from one another with respect to species richness. Two significant differences appear in the following table, however, which contains pairwise comparisons of the Simpson Index values (Table 6). These pairwise results involve comparison between two sites in different combinations. The first significant difference appears between RC 1 and MC 2, with a t-value of -2.136150 and a corresponding p-value < 0.05 indicating statistical significance. The second of the significant results involves both Mud Creek sites, with a t-value of -2.580508 and a statistically significant p-value less than 0.05.

Discussion:

Water chemistry characteristics:

My first set of ANOVA tests between sites for water chemistry characteristics (Table 1) indicated only one statistically significant relationship, with a significant difference between conductivity in Rice Creek and Mud Creek. The values in this table show that conductivity levels were significantly higher in Mud Creek than Rice Creek. This is surprising because Mud Creek is a primarily agricultural stream, and I expected that its proximity to agricultural land would imply higher conductivity as a result of runoff. Since conductivity measures water capacity in carrying an electrical current, the presence of inorganic dissolved solids in water leads to increased conductivity. This includes nitrate and phosphate ions, which are often present at high levels in agricultural runoff (EPA 2012). In this case, it appears as though Mud Creek actually feels fewer effects of agricultural runoff and pollution compared to Rice Creek.

The other water chemistry characteristics, which I expected to vary between the two streams, also surprised by failing to show statistical significance according to the ANOVA tests. Dissolved oxygen, for example, is typically measured at high levels for trout streams, serving as an indicator for high water quality. In this case, both Rice Creek sites meet expectations by containing greater dissolved oxygen than the Mud Creek sites. However, these differences are not statistically significant, so I cannot conclude that the trout stream has greater water quality than the agricultural stream, at least on the basis of dissolved oxygen. Trout streams are also characterized by low water temperatures in summer and warmer than air temperatures in winter (MNDNR 2018). Since my sampling took place in mid to late fall, I decided that lower water temperature would be used to indicate high water quality as expected during the summer. Unfortunately, I could not draw conclusions from this measurement since water temperature also failed to show any significant differences between sites. According to the raw numbers in Table 1, Rice Creek showed minimally lower water temperatures than the Mud Creek sites, with a very high p-value indicating no statistical significance. This result appears to counteract some of the defining characteristics of trout streams, suggesting concerning patterns in Rice Creek's water quality and overall stream health.

When water chemistry characteristics were compared based on sampling time rather than by stream site, only one significant relationship appeared again. While it may seem as though comparing times rather than specific sites might be irrelevant, I wanted to determine whether there were any noticeable changes in any of the parameters over the sampling period, since those changes might affect any differences that I noticed between sites. When chemistry was considered by sampling time, however, the only significant difference appeared for temperature, which makes sense because my first two sampling dates were much warmer than the third date, when I had to break up the ice on the edge of the stream in order to sweep for invertebrate samples.

Nutrient levels:

Nitrate and ammonium concentrations were also calculated using water samples taken from all four sites on three different occasions. As mentioned in the introduction, I expected that the agricultural stream would have significantly higher nutrient levels than the trout stream due to its close proximity to farmland and the supposedly greater health in the trout stream. These expectations correspond with the findings of Herringshaw et al. in a similar study on highly altered Midwestern watersheds. In this study, higher nitrate levels were positively related to agricultural land use (Herringshaw et al. 2011).

Test results for my water samples, however, showed a different pattern. Although there were no significant differences in ammonium levels between the four sites, nitrate levels were significantly different among the four sample sites (Table 3). Just by examining the numbers for nitrate concentrations, it is apparent that Rice Creek contains significantly higher nitrate levels than Mud Creek in spite of its status as a trout stream. These results are consistent with other studies that have shown relatively low nitrate levels in Mud Creek (according to my personal research over the past two years) and high nitrate levels in Rice Creek (as calculated for the BIO 371 lab at St. Olaf College). These results carry several implications for both watersheds. First of all, this indicates that Rice Creek may be receiving greater amounts of agricultural runoff than I originally expected. Secondly, high nutrient levels in Rice Creek may bear notable consequences for aquatic species throughout the watershed.

Biotic indices:

In addition to water chemistry and nutrient levels, I also considered biological characteristics of the two streams as a means of assessing water quality and overall watershed health. Since my sampling results showed significantly higher nitrate levels in Rice Creek compared to Mud Creek, I might expect a reversal of my original prediction that biodiversity of aquatic invertebrates would be higher in Rice Creek. Instead, given my new data, I expect that the higher nitrate levels in Rice Creek will contribute to lower biodiversity among invertebrates. This expectation is in line with findings from previous research, which indicate that invertebrate populations in rural watersheds tend to decrease in taxonomic diversity as nitrate concentrations increase (Wang et al 2006, Houghton 2012, MPCA 2014).

When looking at the raw numbers of each taxonomic group sampled by site, particular attention is necessary for the Trichoptera and Ephemeroptera taxa. These taxa are two of the three groups used for the EPT Index, which analyzes species richness in aquatic invertebrate communities and uses Trichoptera, Ephemeroptera, and Plecoptera as indicators of high water quality due to their low tolerance of water pollution (NRCS n.d.). In this case, the lack of significant difference between Trichoptera values and the observably higher counts for Ephemeroptera in Mud Creek indicate that the trout stream failed to demonstrate water quality high enough to produce greater species richness among highly sensitive taxa.

Furthermore, the Shannon/Simpson diversity indices do not show many significant differences between the sites. This contradicts my original prediction that Rice Creek would have much greater species richness and diversity than Mud Creek. Instead, it appears as though the agricultural stream under consideration is no less healthy than its trout stream counterpart.

Limitations:

In the context of this study, a number of factors introduced the possibility of error in sampling procedures and results. First of all, my original plan was to sample biweekly a total of three times for all sites. Because I was hesitant to be in the field for the beginning of hunting season, I ended up missing one of my intended sampling dates and going much later than anticipated. This led to some of the significant temperature differences that I observed between sampling times, as well as changing conditions in the surrounding agricultural fields due to changes in harvest status. I also took my last two samples for Rice Creek and Mud Creek on different days, which may have introduced some error in terms of differences in sampling and field conditions between the two days.

In future studies, I would like to consider more sites on both of these streams in order to determine whether my observed patterns persist for other locations. I would also like to calculate an EPT index in order to have a quantitative measurement for the species richness of highly sensitive invertebrate species. I feel that this would give me a more extensive assessment of the biotic indices for the watersheds in question.

Future management strategies:

Although Rice Creek did not show significantly higher water quality or biological diversity and sensitivity in comparison to Mud Creek, my results still give way to a number of possible management strategies for the future. First of all, the lack of data supporting Rice Creek's greater health suggests that Rice Creek needs to become more of a priority for stream protection. This may be accomplished by mobilizing the general public in the surrounding community, since trout streams are a treasured resource in southern Minnesota and are the focus of extensive conservation efforts. Further studies may also provide some of the information necessary to inform future management practices. For example, studying farming and drainage practices in the fields surrounding Rice Creek may contribute to a greater understanding of the local factors impacting water quality. Examining aquatic habitats within the stream may also help researchers gain an understanding of the types of different aquatic species that they might expect to see regardless of water quality, helping to separate the two variables from one another. Finally, considering stream origins and upstream practices may also inform researchers regarding potentially significant effects that may not be observable in the scope of my study, since I focused primarily on the factors that I could directly observe from my location.

Secondly, the fact that Mud Creek, a primarily agricultural stream, appeared to have greater water quality and potentially more sensitive biological taxa suggests that its specific characteristics should be analyzed to determine what is happening correctly in the Mud Creek watershed. This may be used to inform management practices to preserve habitat in other primarily agricultural streams, or it might be applied toward streams like Rice Creek which, in theory, should be significantly healthier. Land use practices and upstream/origin effects should also be considered for Mud Creek.

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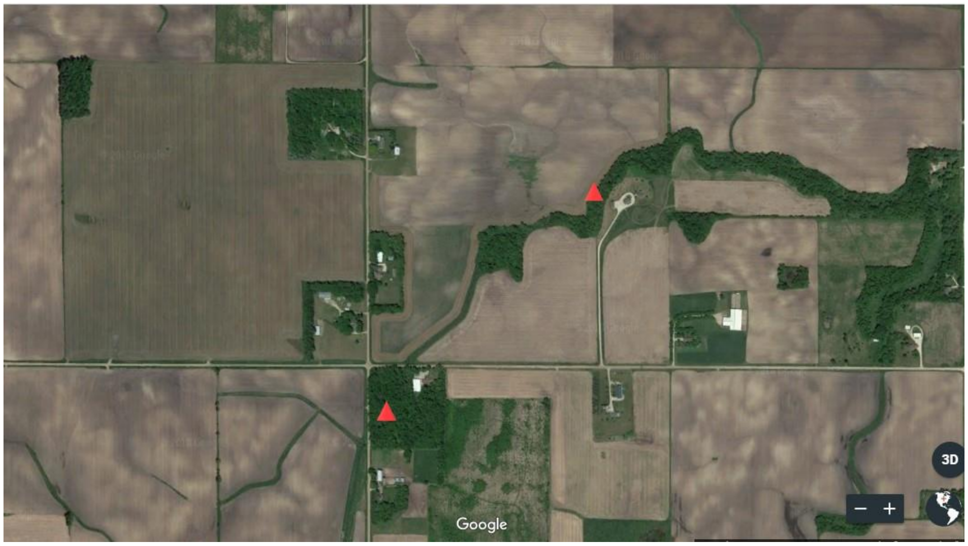

Figure 1: Rice Creek sites. This satellite image shows the two sampling locations that I used on Rice Creek for water testing/sampling and invertebrate collection. Sampling sites are indicated by the red triangles. Site 1 is the one closer to the top of the image, and was located on the stream at the edge of a farmer’s cornfield. Site 2 was part of a county ditch that ran under the road and into a forested area where I sampled.

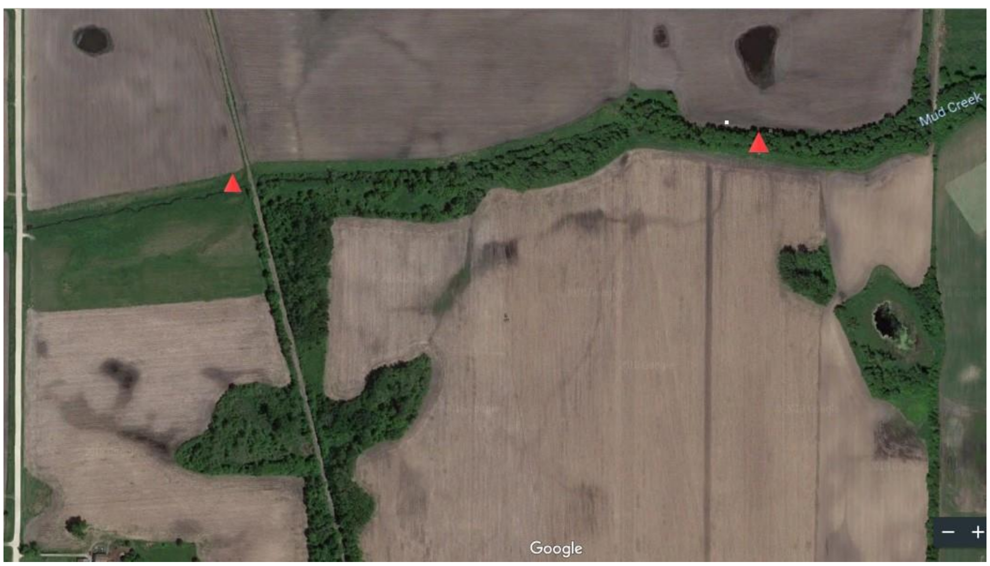

Figure 2: Mud Creek sites. This satellite image shows the two sampling locations that I used on Mud Creek. Again, sites are identified by the red triangles. The furthest left site was located next to a railroad bridge on corn/soybean farmed land. The rightmost site was about a quarter of a mile downstream and was located near a corn/soybean rotation no-tilled field with a saturated buffer between the field and the stream.

Table 1: ANOVA for water chemistry indices by site. The table below shows the results of four separate ANOVA tests of the statistics for conductivity, dissolved oxygen, pH, and temperature. Each column labeled by site contains the average of three measurements taken on three different dates. Of these tests of significant difference, only the measure of conductivity showed a significant difference between the sites, with a p-value of 0.00112 indicating a significantly higher conductivity for the Rice Creek sites compared to the Mud Creek sites.

	MC 1	**MC 2**	**RC 1**	**RC 2**	**Pr(>F)**
Conductivity (ppm)	663.6667	653.6667	766.6667	761.3333	0.00112**
DO (mg/L)	9.946667	11.056667	11.936667	12.776667	0.137
pH	7.793333	7.753333	7.936667	8.200000	0.153
Temperature (℃)	7.703333	6.900000	6.533333	6.500000	0.998

Table 2: ANOVA for water chemistry indices by sampling time. This table presents four separate ANOVAs for conductivity, dissolved oxygen, pH, and temperature. Each value represents the average for each parameter taken from all four sites at a given sampling time. Of these tests, only temperature shows a significant difference between the times, with a p-value of 2.35e-08.

	Time 1	**Time 2**	**Time 3**	**Pr(>F)**
Conductivity	686.75	709	738.25	0.586
DO (mg/L)	11.29500	8.3425	12.40000	0.502
pH	7.8275	7.8275	8.1075	0.443
Temperature	8.625	9.375	2.225	2.35e-08***

Table 3: ANOVA for nutrient levels by site. This table displays the nutrient levels calculated for the water samples collected at each site. Each value represents the average nutrient level for all sampling dates. For the Rice Creek sites, ammonium levels are averaged from two dates instead of three. Nitrate levels were significantly different between the sites, with Rice Creek significantly higher in nitrate than Mud Creek and a p-value of 0.0142.

	NO_3	**NH_4**
MC 1	**0.7924**	**0.02467**
MC 2	**1.1668**	**0.03033**
RC 1	**5.8360**	**0.03800**
RC 2	**6.9934**	**0.03400**
p-values	**0.0142***	**0.868**

Table 4: Counts of sampled taxa by site. The table below shows the raw counts for each taxonomic group sampled in each site. Highlighted rows indicate the especially pollution-sensitive taxa within this group, with no major observable difference between sites for Trichoptera and significantly more Ephemeroptera observed for Mud Creek than for Rice Creek.

Taxon name	**RC 1**	**RC 2**	**MC 1**	**MC 2**
Amphipoda	40	42	95	35
Trichoptera	18	3	6	11
Odonata	1	3	2	2
Gastropoda	4	9	33	39
Bivalvia	0	0	2	70
Megaloptera	0	1	0	1
Diptera	2	4	0	2
Ephemeroptera	3	1	20	3
Coleoptera	1	0	4	0
Hemiptera	0	4	2	1

Table 5: Shannon/Simpson indices by site. The following table shows species richness, Shannon Index (H'), Simpson Index (Ds), and variation within the Simpson index.

	MC 1	**MC 2**	**RC 1**	**RC 2**
Richness	**8**	**9**	**7**	**8**
Shannon (H')	**0.55**	**0.63**	**0.52**	**0.57**
Simpson (Ds)	**0.60**	**0.59**	**0.61**	**0.71**
Variance of Ds	**0.002566**	**0.004226**	**0.001213**	**0.000425**

Table 6: Pairwise comparisons of Ds values. The table below includes the t values calculated for each pair of values. Numbers highlighted in yellow indicate significant differences in Ds values at P<0.05.

	RC 1	RC 2	MC 1	MC 2
RC 1				
RC 2	0.144138			
MC 1	-0.201557	-0.329079		
MC 2	-2.136150	-1.887240	-2.580508	

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