

**Final report on the potential effects of Jeffrey Energy Center effluent on the  
fish community of Lost Creek and unnamed receiving tributary**

**Kansas Biological Survey Report No. 163**

**July 2010**

by

Donald G. Huggins, Robert C. Everhart, and Adam J. Blackwood

Central Plains Center for BioAssessment  
Kansas Biological Survey  
University of Kansas

For

Westar Energy

Prepared in fulfillment of contract #90193, KUCR project IND63623

## Introduction

The Central Plains Center for BioAssessment (CPCB) monitored physical, chemical, and biological aspects of Lost Creek and an unnamed tributary that receives downstream cooling water from the Jeffrey Energy Center (JEC) north of St. Marys, KS (Figure 1). The objectives of this project were:

1. Monitor and assess the ecological health of Lost Creek pre- and post-JEC discharge.
2. Monitor and assess the physical habitat conditions of Lost Creek pre- and post-JEC discharge.
3. Monitor and assess the general water quality of Lost Creek pre- and post-JEC discharge.
4. Assess seasonal and hydrological influences (i.e. discharge) on the effects of JEC discharge on the ecology of Lost Creek.
5. Assess the observed and potential overall ecological impact(s) of the JEC discharge to Lost Creek. This was originally to be done using a BACIP (Before-After Control-Impact paired) study design, however, since only one of the four study periods came after the use of the new wet scrubbers, as opposed to two, CPCB used an analysis of variance (ANOVA) approach.

To accomplish these objectives, CPCB monitored three sites (i.e. stream segments) on Lost Creek both above and below its confluence with the unnamed tributary as well as three sites on the tributary itself. Thus a total of nine sites were sampled once during the summer and fall periods of 2008 and 2009. Monitoring activities included habitat assessment, water quality testing, and benthic macroinvertebrate and fish sampling. CPCB also measured a series of *in situ* parameters and analyzed water samples for chloride and sulfate.

## Methods and Materials

Study sites were defined as a stream reach equal to 20 times the wetted width (or 500 meters whichever is shorter) of the stream at the three *a priori* selected sites determined for each of the three study areas (3 study areas x 3 sites = 9 study sites). CPCB sampled each site once during the summer and fall seasons of 2008 and 2009. This sampling scheme allowed for the general temporal assessment of the biology and chemistry associated with study sites during the summer and fall when minimum flows and high biological activity is expected and thus discharge “effects” might be the highest. These sites were sampled at base or “normal” flow to avoid or minimize possible influences of high water events.

To assess the habitat at each site, CPCB used both the Habitat Development Index (HDI) used by the Kansas Department of Health and Environment and the Ohio EPA’s Qualitative Habitat Evaluation Index (QHEI). Huggins and Moffet (1988) developed the HDI specifically for Kansas streams as a quantifiable and standard method of quantifying and characterizing the stream habitat sampled for macroinvertebrates. The QHEI was created by the Ohio EPA and “is a physical habitat index designed to provide an empirical, quantified evaluation of the general lotic macrohabitat characteristics that are important to fish communities” (Ohio EPA 2006, [http://www.epa.state.oh.us/dsw/document\\_index/docindx.html](http://www.epa.state.oh.us/dsw/document_index/docindx.html)).

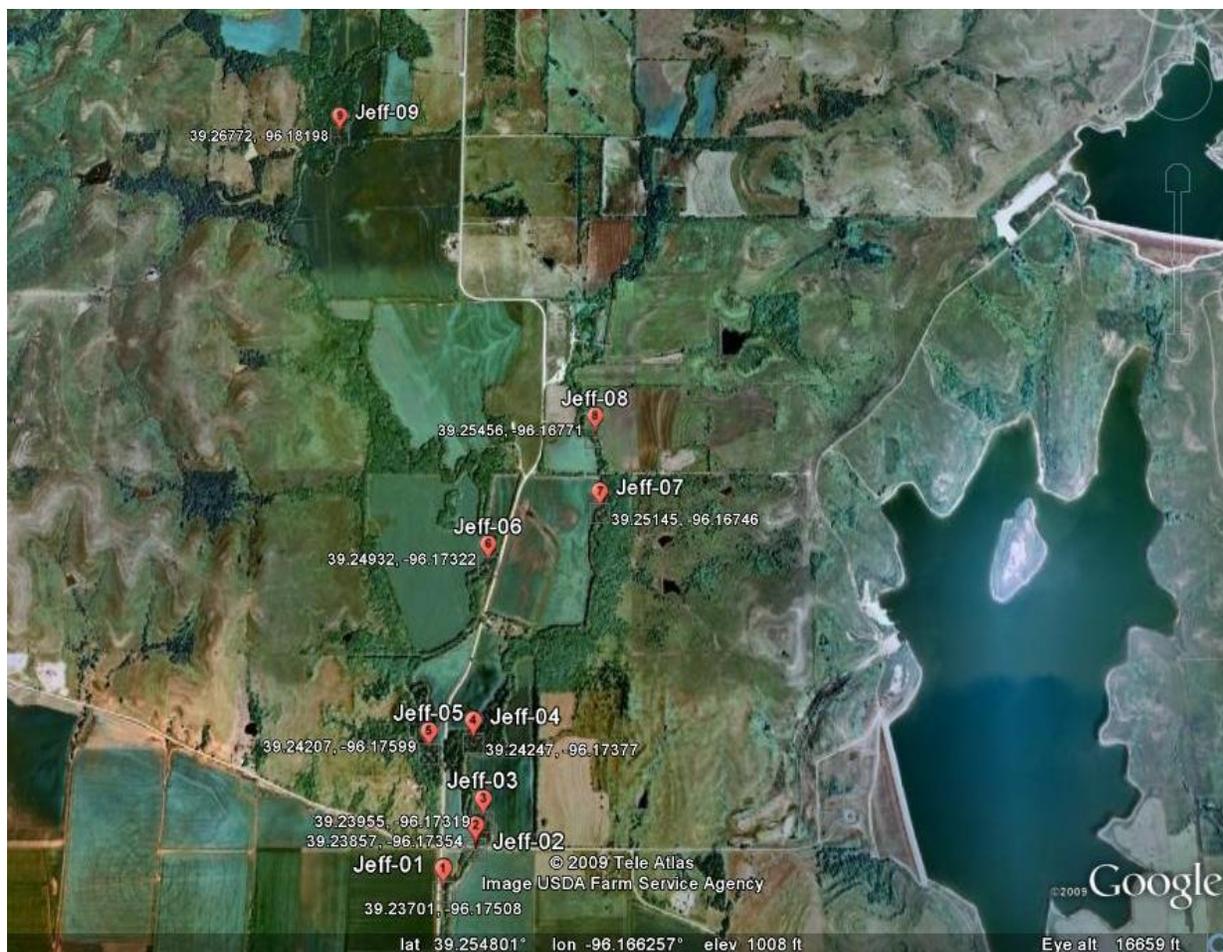


Figure 1. Sampling design at the Jeffrey Energy Center (JEC) in St. Marys, KS. Sites 1, 2, and 3 are on Lost Creek below the confluence with the unnamed tributary, Sites 5, 6, and 9 are on Lost Creek above the confluence, and Sites 4, 7, and 8 are on the tributary downstream of the JEC discharge.

Using a Horiba U-10 Water Checker, CPCB recorded *in situ* measurements of the following parameters at each site's center transect: air temperature, water temperature, dissolved oxygen, pH, specific conductance, salinity and turbidity (Table 1). CPCB maintained and calibrated all testing tools and equipment to ensure their proper function for sampling activities. CPCB also analyzed water samples for chloride and sulfate (Table 2).

Table 1. Summary of analytical methods and instrument detection limits of *in situ* water-quality parameters analyzed by CPCB.

Parameter	Container	Instrument	Method Citation	Detection Limit
Flow Velocity	none	Swoffer <sup>®</sup> Model 2100 Flow Meter	Swoffer Model 2100 Operation Manual	0.01-0.03 m/sec
pH	none	Horiba U-10 Water Quality Checker	21 <sup>st</sup> Ed. Standard Methods (APHA) 4500-H <sup>+</sup>	0.1
Specific Conductance	none	Horiba U-10 Water Quality Checker	21 <sup>st</sup> Ed. Standard Methods (APHA) 2510 A-B	0.001 mS/cm
Salinity	none	Horiba U-10 Water Quality Checker	21 <sup>st</sup> Ed. Standard Methods (APHA) 4500-O G	0.01%
DO	none	Horiba U-10 Water Quality Checker	21 <sup>st</sup> Ed. Standard Methods (APHA) 4500-O G	0.1 mg/L
Turbidity	none	Horiba U-10 Water Quality Checker	21 <sup>st</sup> Ed. Standard Methods (APHA) 2130-B	1 NTU
Water and Air Temperature	none	Horiba U-10 Water Quality Checker	21 <sup>st</sup> Ed. Standard Methods (APHA) 2550-B	0.1°C

Table 2. Summary of analytical methods, instrument detection limits, and sample holding time of additional water-quality parameters analyzed by CPCB.

Parameter	Container	Instrument	Method Citation	Detection Limit	Holding Time	Preservation
Chloride	1L Amber Glass	Lachat QuikChem 8500	21 <sup>st</sup> Ed. Standard Methods (APHA) 4500-Cl G	0.2 mg/L	28 days	4°C
Sulfate	1L Amber Glass	Lachat QuikChem 8500	21 <sup>st</sup> Ed. Standard Methods (APHA) 4500-SO <sub>4</sub> <sup>2-</sup> G	1.8 mg/L	28 days	4°C

At each site CPCB used HDI protocols to collect macroinvertebrate samples (Huggins and Moffet 1988). Within the site, an aquatic kick net (500- $\mu$ m mesh opening) was used to collect macroinvertebrates from a variety of habitats. On bottom substrates, approximately 0.09 m<sup>2</sup> (1ft<sup>2</sup>) of substrate was disturbed to a depth of 1-2 cm. A sweep of similar area was used in vegetated habitats, root wads and areas associated with woody debris. Habitats within each macrohabitat (i.e. pool, riffle, run or glide) in each site were sampled in proportion to its

occurrence in the site. The samples from a site were combined into a sample jar and preserved with 10% buffered formalin and rose bengal solution.

The samples were returned to the CPCB lab for sorting and identification using the CPCB Standard Operating Procedures (available to download from the CPCB webpage at <http://www.cpcb.ku.edu/datalibrary/assets/library/protocols/BenthicLabSOP.pdf>). Samples were sorted to remove  $500 \pm 10\%$  organisms from the sample, using a modified Caton gridded tray. Each sample was sorted until the number of organisms met the subsample requirements or the entire sample was sorted. Sorted organisms were placed into 80% ethanol for storage and later identification to the lowest practical taxonomic level. Merritt *et al.* (2008), Needham *et al.* (2000), Westfall and May (1996), Stewart and Stark (2002), Wiggins (1996), and Epler (2001) were the primary references used for the insect identifications. Wiederholm (1983) and (1986) were used as supporting references for the Chironomidae identifications. Thorp and Covich (2001) was the primary reference used for the crustacean identifications, and Smith (2001) and Pflieger (1996) served as supporting references. Mackie and Huggins (1983), Oesch (1984), Couch (1997), Bleam *et al.* (1999), Burch (1982), Wu *et al.* (1997), and Turgeon *et al.* (1998) were used for snail and bivalve identifications, in addition to past surveys of mussels by KBS (e.g. Liechti and Huggins 1977, Schuster and DuBois 1979, DuBois 1981). Voucher specimens of difficult to identify taxa and well as rare taxa will be retained for a minimum of three years.

The fish community within a site was sampled by electrofishing with a backpack unit, and by seining where possible. A one-pass electrofishing effort was used in each site starting along the right bank at the downstream end of the site and proceed up the right bank to the upper end of the designated site and then down the left bank until the starting point is reached. However, where the stream width permitted electrofishing the total stream width on the upstream pass then this method of sampling was used instead. The easily identified fish were held on site, identified to species, and then released outside the site. Juvenile and small, hard to identify specimens as well as fish taxa that are difficult to identify in the field (mainly Cyprinidae) were preserved in a buffered formalin solution and returned to the CPCB lab for identification. Cross (1967), Cross and Collins (1995), and Pflieger (1997) were the primary references used for fish identifications. Voucher specimens of difficult to identify species and well as rare or unique occurring species will be retained for a minimum of three years.

For analysis of biological community data, CPCB used the following seven general metrics: Species Richness, Family Richness, Richness/Abundance, Total Abundance, Shannon-Weiner Index (Shannon 1948, Wiener 1948), Gleason's Index (Gleason 1922), and Standard Deviation. The Shannon-Weiner Index is one of the most widely used standard indices for biotic diversity. For fish data, Adjusted Abundance (for 100m stream length), Sunfish Richness, Sunfish Abundance, Darter and Madtom Richness, and Darter and Madtom Abundance were included. Sunfish Richness and Sunfish Abundance are both used because they can help to indicate pool condition of a stream, and Darter and Madtom Richness and Darter and Madtom Abundance are both used because they can help indicate riffle condition of a stream (Karr *et al.* 1986). Macroinvertebrate community data were also characterized using Ephemeroptera, Plecoptera, and Trichoptera richness and abundance, as well as richness and abundance of Chironomidae. These macroinvertebrate metrics are commonly used to characterize stream condition, with increasing EPT / decreasing Chironomidae suggesting higher quality waters and decreasing EPT

/ increasing Chironomidae suggesting poorer quality waters (Karr *et al.* 1986). Both the general and taxa specific metrics are also commonly used and accepted in the Midwest region by state and federal monitoring agencies (Goodrich *et al.* 2005).

All data was entered into one database (MS Access), using a dual-entry system of one person entering the data from field and bench sheets, and another person checking all records for accurate entry. Data from the relational database were then used to construct data files that were analyzed using the statistical and power analysis software NCSS (NCSS 2004). All graphic analyses and statistical analyses were performed using 2007 upgrade to NCSS.

## Results and Discussion

An analysis of variance (ANOVA) approach was taken to identify changes or trends in observed data. Because some of the variables of interest did not have normally distributed values, both the Model I GLM (General Linear Model) ANOVA and its nonparametric analog the Kruskal–Wallis one-way analysis of variance by ranks were performed on selected variables. The Kruskal-Wallis test is a non-parametric method for testing equality of population medians among groups. It is identical to a one-way analysis of variance (ANOVA) with the data replaced by their ranks, and is an extension of the Mann–Whitney U test to three or more groups. Since it is a non-parametric method, the Kruskal–Wallis test does not assume a normal population, unlike the analogous one-way analysis of variance. However, the test does assume an identically-shaped and scaled distribution for each group, except for any difference in medians.

We have reported only the outcomes of the GLM ANOVA, since the test results of both the parametric and nonparametric tests were identical, and since GLM ANOVA is a more robust and dependable test that allows for *post hoc* multiple comparison tests to identify potential groups. We used the Tukey-Kramer multiple comparison test to examine possible groups when significant differences ( $\alpha \leq 0.05$ ) were observed between either “Treatment” (i.e., whether the stream site was located above, below, or on the unnamed tributary to Lost Creek) or “Time” (i.e., the sampling period). All ANOVA models were two-way Model I models, since the both time and treatment were considered “fixed” variables. The Sample Periods were: July 2008 (Sample period 1); October 2008 (Sample period 2); July 2009 (Sample period 3); and October 2009 (Sample period 4). Sample period 4 was considered a time period after the new scrubber effluent had become part of the tributary flows.

A summary of these two-way ANOVAs and multiple comparison tests are presented below (Table 3). For indicators of water quality and fish assemblage, time (i.e. sample period) was seldom a significant factor on its own, but when it was the interaction term, it was also significant, whereas time was often a significant factor for macroinvertebrate assemblage metrics, both on its own and as the interaction term. Thus, the interpretation of significant treatment as well as time effects should be done with care.

Table 3. Table of two-way ANOVA and Tukey-Kramer results for selected variables. The threshold for significance was  $\alpha = 0.05$ . P-values higher than this threshold were reported as not significant (NS). The Tukey-Kramer *post hoc* results are represented by stating groups by their treatment memberships (e.g. Tributary/Lower would be a group whereas Upper is the single member of the other group formed when using species richness as the “effects” variable). The Sample Periods were: July 2008 (Sample period 1); October 2008 (Sample period 2); July 2009 (Sample period 3); and October 2009 (Sample period 4). Sample period 4 was considered a time period after the new scrubber effluent had become part of the tributary flows.

<b>Variables</b>	<b>Factors</b>	<b>p values</b>	<b>Tukey-Kramer Range Test (<math>\alpha \leq 0.05</math>) significant groups</b>
<b><i>Water Quality</i></b>			
<b>Chloride (mg/L)</b>	Treatment	0.00001	Upper, Lower, and Tributary all different from each other
	Time	0.00001	Sample period 4 different from all other sample periods
<b>Sulfate (mg/L)</b>	Treatment	0.00001	Upper, Lower, and Tributary all different from each other
	Time	0.00001	Sample period 3 different from all other sample periods
<b><i>Fish Assemblage Metrics</i></b>			
<b>Gleason diversity</b>	Treatment	NS	Not applicable
	Time	NS	Not applicable
<b>Shannon/Wiener diversity</b>	Treatment	NS	Not applicable
	Time	NS	Not applicable
<b>Species Richness</b>	Treatment	0.0011	Upper different from Tributary/Lower groups
	Time	NS	Not applicable
<b>Total abundance</b>	Treatment	0.00001*	Upper different from Tributary/Lower
	Time	NS	Not applicable
<b>Darter/madtom richness</b>	Treatment	0.0116	Upper different from Tributary/Lower
	Time	NS	Not applicable
<b>Sunfish Richness</b>	Treatment	.0032	Upper different from Tributary/Lower
	Time	NS	Not applicable
<b><i>Macroinvertebrate Assemblage Metrics</i></b>			
<b>Gleason diversity</b>	Treatment	<0.0001	Upper different from Tributary/Lower
	Time	0.021	Sample period 3 different from all other sample periods
<b>Shannon/Wiener diversity</b>	Treatment	<0.0001	Upper different from Tributary/Lower
	Time	0.014	Sample period 3 different from sample periods 1 and 2
<b>Species Richness</b>	Treatment	<0.0001	Upper different from Tributary/Lower
	Time	0.023	Sample period 3 different from sample periods 1 and 4
<b>Total abundance</b>	Treatment	NS	Not applicable

Variables	Factors	p values	Tukey-Kramer Range Test (alpha $\leq$ 0.05) significant groups
	Time	NS	Not applicable
<b>Percent Ephemeroptera, Plecoptera, and Trichoptera (EPT) Taxa</b>	Treatment	NS	Not applicable
	Time	<0.0001	Sample period 3 different from all other sample periods
<b>Percent Chironomid Taxa</b>	Treatment	NS	Upper different from Tributary/Lower
	Time	<0.0001	Sample period 3 different from sample periods 1 and 4

For water quality metrics, the results of these ANOVA and *post hoc* comparison tests indicated that there were three distinct treatment groups based on chloride and sulfate concentrations; an Upper Lost Creek group, a Lower Lost Creek group, and the receiving Tributary. While the Tukey-Kramer *post hoc* test indicated that Sample period 3 is different than the other sample period, there appears to be a lot of overlap in the 25th and 75th quartiles (Figure 2). The same situation is seen in Figure 3 for chloride. There appears to be a downward trend in chloride based on the median values show in Figure 3.

Results from the two-way ANOVA tests show that there was no time effect on any of the fish metrics (Table 3). Neither measure of fish community diversity (Shannon\Wiener and Gleason diversity indices) was different among the treatment groups suggesting that the tributary effluent was not causing a change in fish diversity (Figure 4-7). However, there appears to be a treatment effect on species richness, total abundance (standardized for 100m stream reach), darter/madtom richness and sunfish richness (see Table 3 and Figures 8-15).

All macroinvertebrate metrics showed significant differences with time (Table 3). However, the difference observed for all metrics was between sampling period 3 (July 2009) and the other sampling periods suggesting that addition of scrubber effluent to the Tributary was not causing changes to macroinvertebrate metrics through time. Gleason diversity (Figures 16-17), Shannon\Wiener diversity (Figures 18-19), and macroinvertebrate species richness (Figure 20-21) were all significantly different in Upper Lost Creek compared to Lower Lost Creek and the receiving Tributary, which were not significantly different from each other. Total abundance showed no significant differences with treatment or time (Figures 22-23), and neither percent EPT taxa richness (Figures 24-25) nor percent Chironomid taxa richness (Figures 26-27) significantly varied with treatment.

In order to account for the effects of differential water flows and habitats among treatments, an analysis of covariance (GLM ANCOVA) was also performed using total discharge (in cubic feet per second) and the Habitat Development Index (HDI) as covariates (Table 4). The inclusion of covariates did not change interpretation of variability in water quality metrics. However, some of the variability in fish species richness, macroinvertebrate species richness, and percent EPT taxa was significantly explained by differences in total discharge, and addition of covariates more precisely identified differences among treatment types. In general, treatment effects from the tributary appear to be associated with differences in the flow, and more specifically to be associated with (1) higher flow in the Tributary than in Upper Lost Creek during summer/baseflow conditions, and (2) year-round higher flow in Lower Lost Creek, which is likely the result of the confluence of the two upstream branches. Higher flow conditions correlate with decreased EPT taxa richness, increased Chironomid taxa richness, and increased species richness and diversity of both fish and macroinvertebrates.

Table 4. Table of two-way ANCOVA and Tukey-Kramer results for selected variables. The threshold for significance was alpha = 0.05. P-values higher than this threshold were reported as not significant (NS). The Tukey-Kramer *post hoc* results are represented by stating groups by their treatment memberships (e.g. Tributary/Lower would be a group whereas Upper is the single member of the other group formed when using species richness as the “effects” variable). The Sample Periods were: July 2008 (Sample period 1); October 2008 (Sample period 2); July 2009 (Sample period 3); and October 2009 (Sample period 4). Sample period 4 was considered a time period after the new scrubber effluent had become part of the tributary flows.

Variables	p values				Tukey-Kramer Range Test (alpha ≤ 0.05) significant groups
	Total Discharge (cfs)	Habitat Development Index	Treatment	Time	
<i>Water Quality</i>					
Chloride (mg/L)	NS	NS	<0.0001*	<0.0001*	Upper, Lower, and Tributary all different; Sample periods 1, 2/3, and 4 all different
Sulfate (mg/L)	NS	NS	<0.0001*	0.037*	Upper, Lower, and Tributary all different; Sample period 3 different from others
<i>Fish Assemblage Metrics</i>					
Gleason Diversity	NS	NS	NS	NS	Not applicable
Shannon/Wiener Diversity	NS	NS	NS	NS	Not applicable
Species Richness	0.01	NS	NS	NS	Lower different from Upper/Tributary; Sample period 3 different from others
Total abundance	NS	NS	0.04	NS	Lower different from Tributary; Sample period 3 different from others
Darter/madtom richness	NS	NS	NS	NS	Not applicable
Sunfish Richness	NS	NS	NS	NS	Not applicable
<i>Macroinvertebrate Assemblage Metrics</i>					
Gleason Diversity	0.041	NS	0.003	NS	Lower different from Upper/Tributary; Sample period 3 different from others

Variables	<u>p values</u>				Tukey-Kramer Range Test ( $\alpha \leq 0.05$ ) significant groups
	Total Discharge (cfs)	Habitat Development Index	Treatment	Time	
Shannon/Wiener Diversity	NS	NS	0.003	NS	Tributary different from Upper/Lower
Species Richness	NS	NS	0.007	NS	Tributary different from Lower; Sample period 3 different from others
Total abundance	NS	NS	0.042*	NS	Lower different from Upper/Tributary; Sample period 3 different from others
Percent Ephemeroptera, Plecoptera, and Trichoptera (EPT) Taxa	0.056	NS	0.006	NS	Upper different from Lower/Tributary; Sample period 3 different from sample period 1
Percent Chironomid Taxa	NS	NS	0.013	NS	Lower different from Upper/Tributary; Sample period 1 different from others

\*Interaction term between treatment and time is also significant.

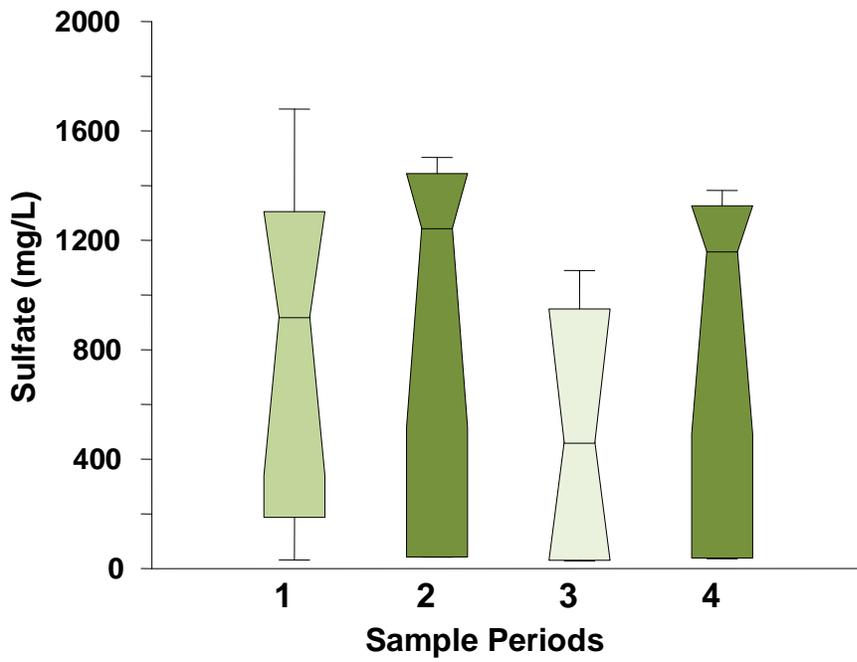


Figure 2. Box plots of sulfate concentrations for each sampling period.

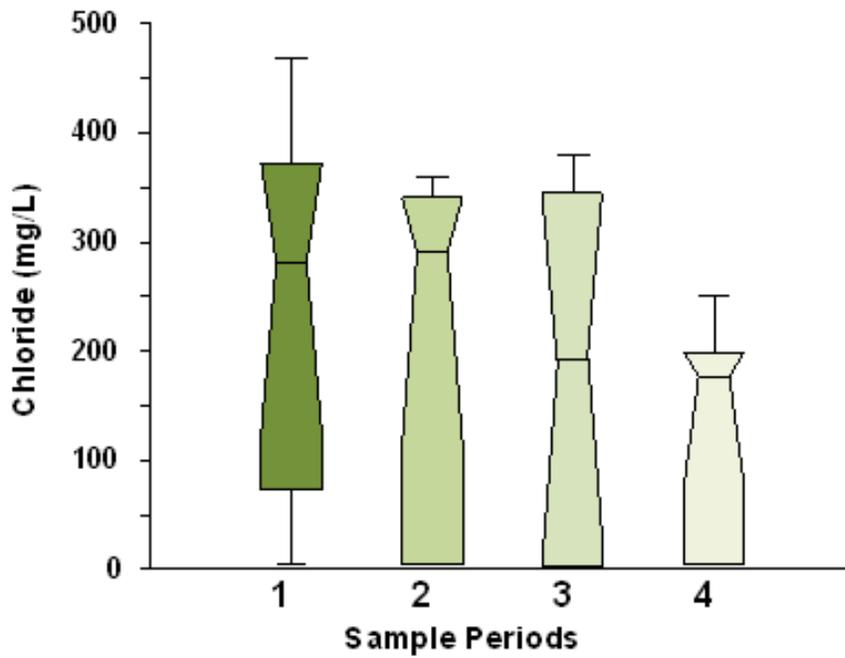


Figure 3. Box plots of chloride concentration for each sampling period.

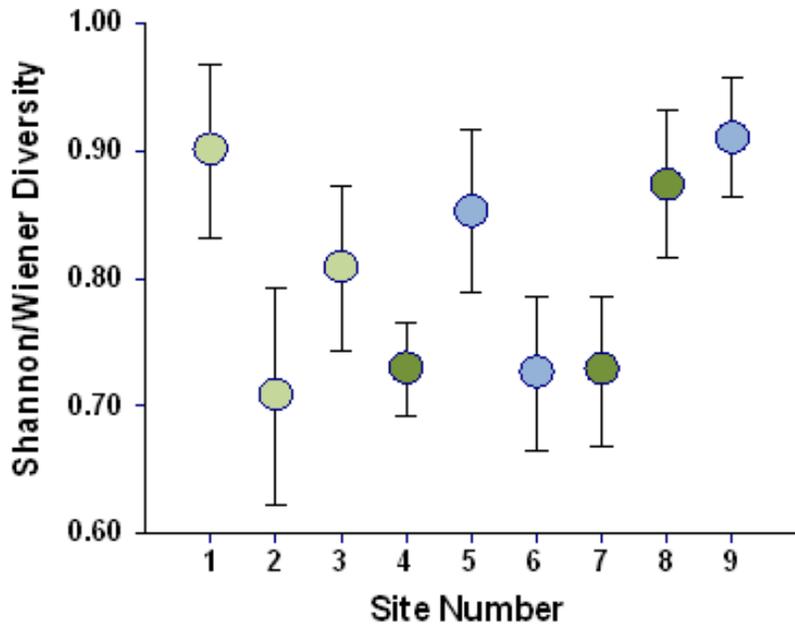


Figure 4. Error bar plots of Shannon/Wiener diversity values for stream sites.

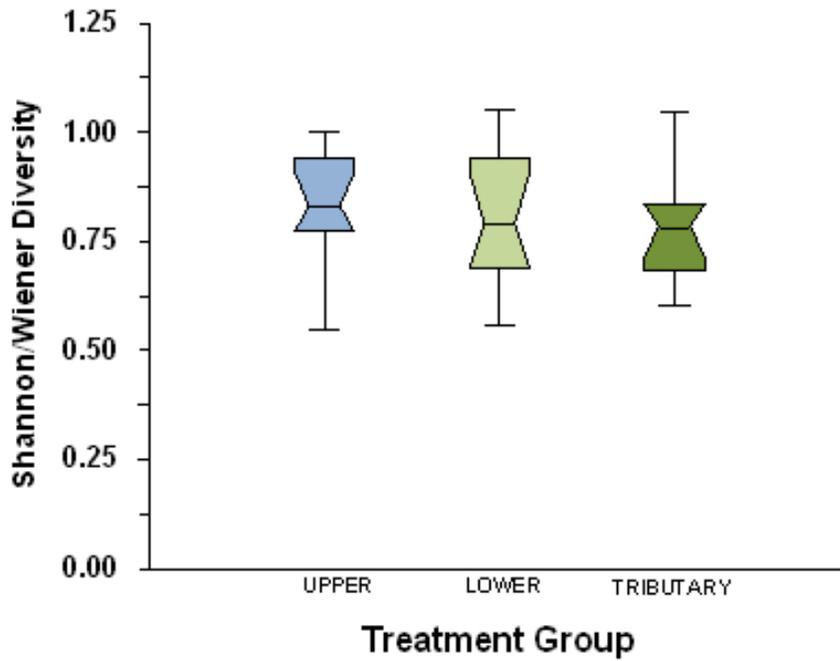


Figure 5. Box plots of Shannon/Wiener diversity values for treatment groups.

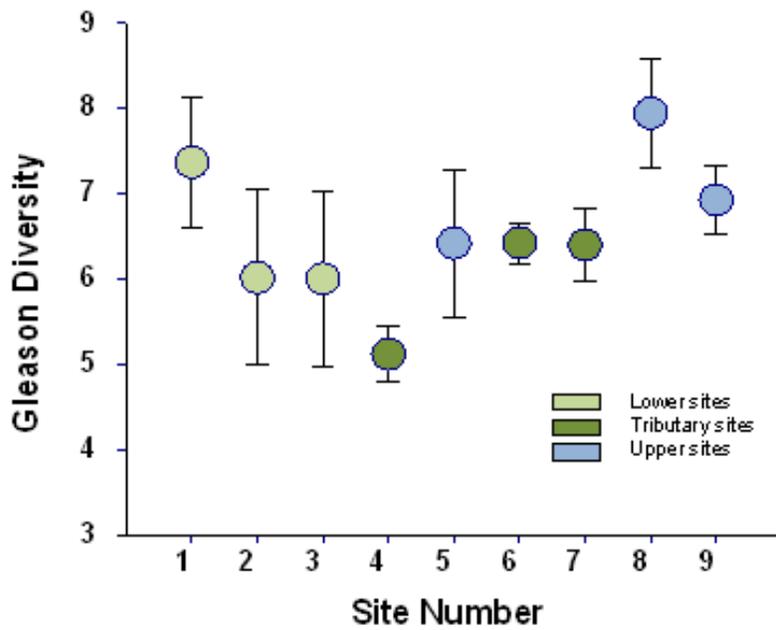


Figure 6. Error bar plots of Gleason diversity values for stream sites.

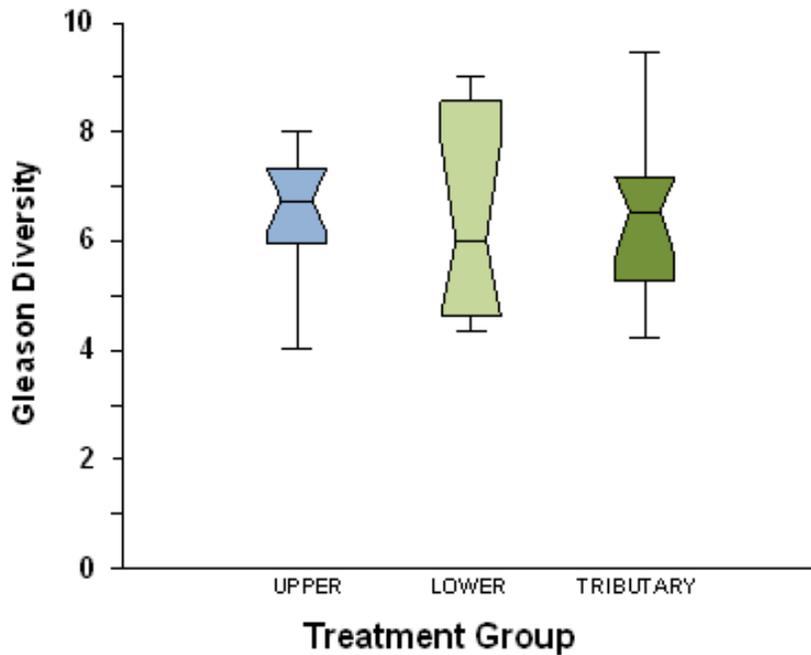


Figure 7. Box plots of Gleason diversity values for treatment groups.

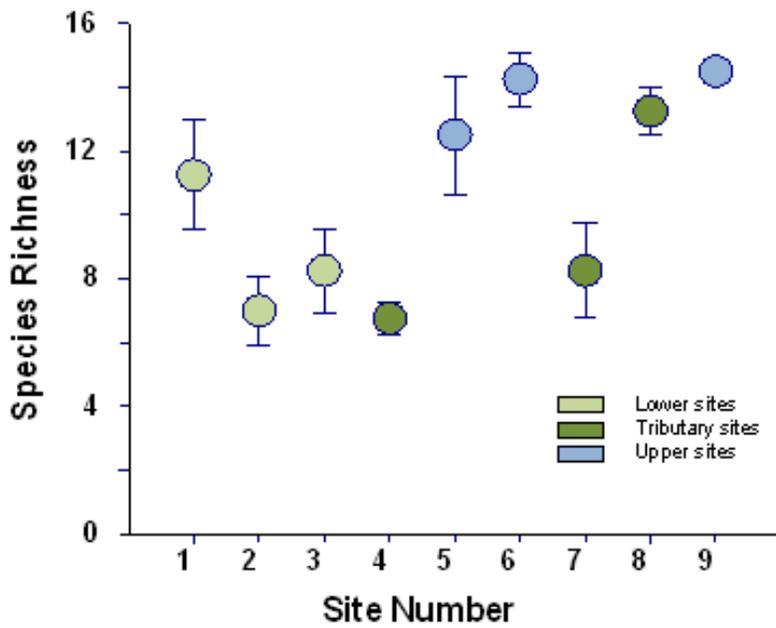


Figure 8. Error bar plots of fish species richness values for stream sites.

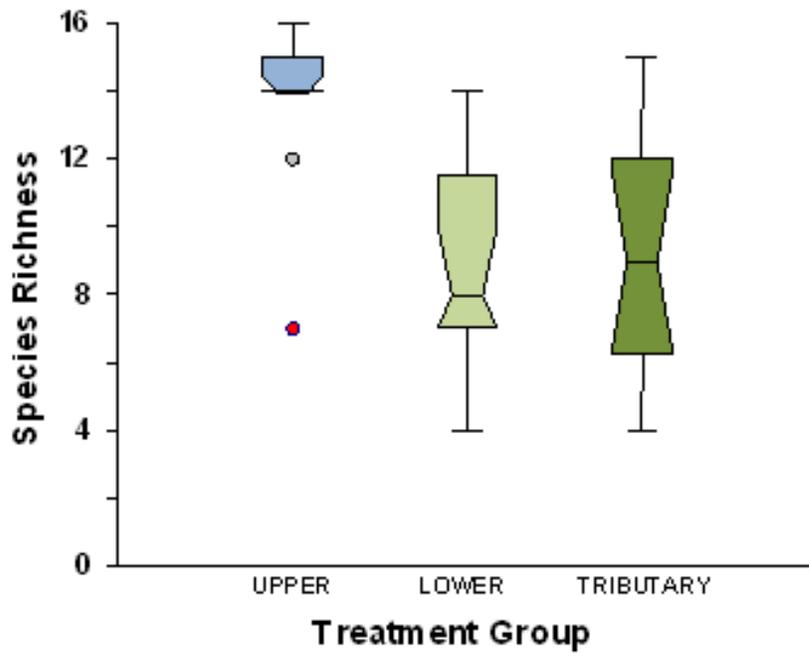


Figure 9. Box plots of fish species richness values for treatment groups.

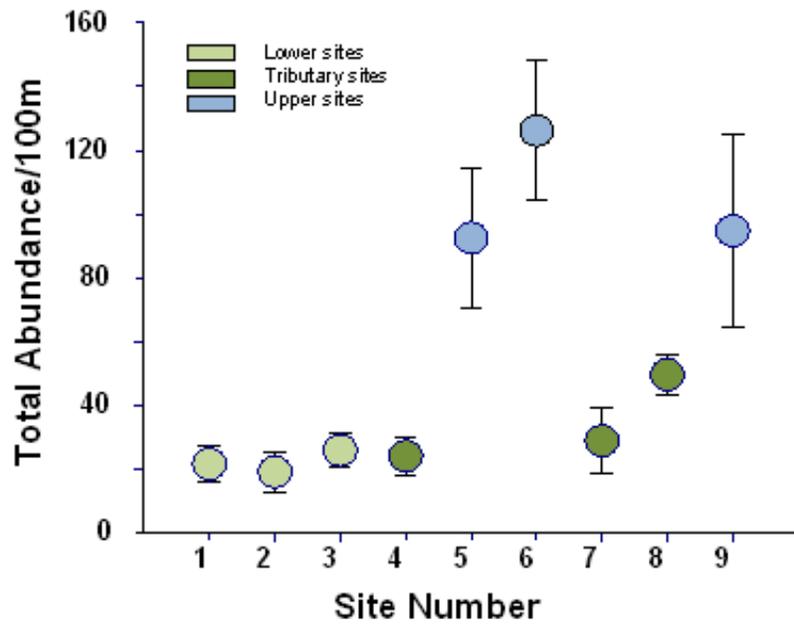


Figure 10. Error bar plots of total fish abundance for stream sites.

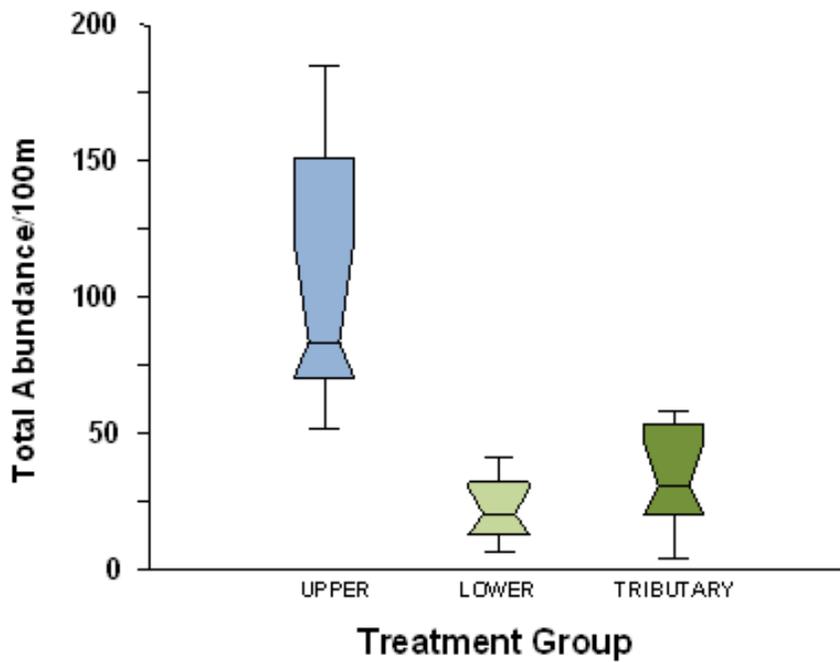


Figure 11. Box plots of total fish abundance for treatment groups.

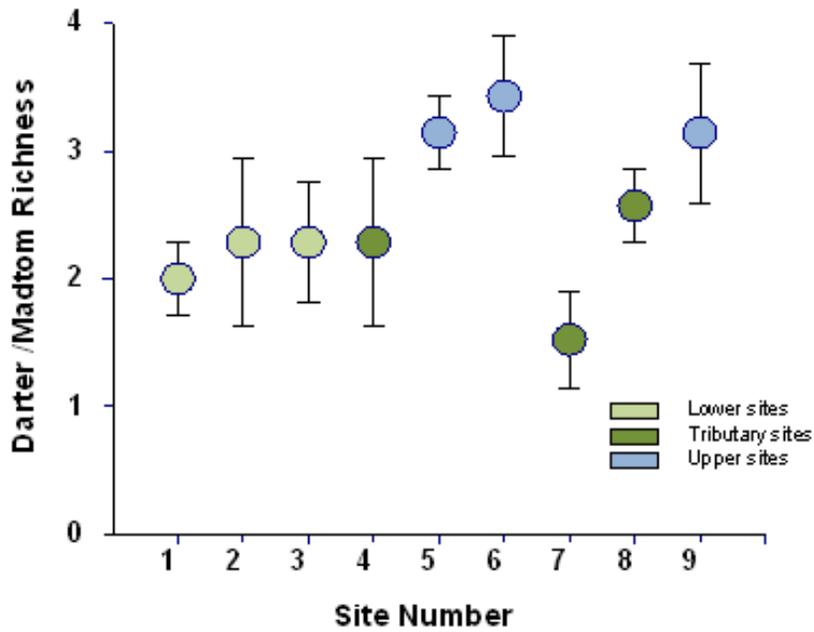


Figure 12. Error bar plots of darter/madtom richness for stream sites.

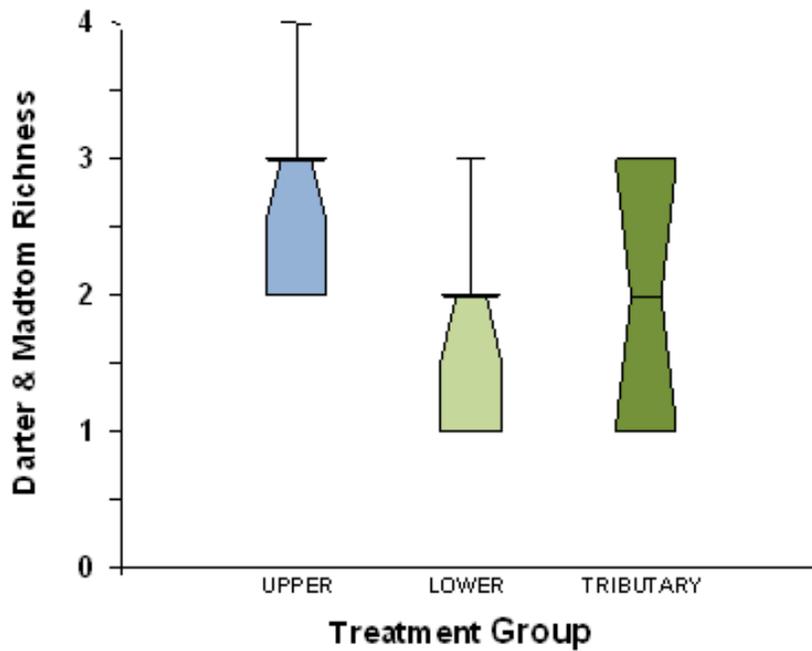


Figure 13. Box plots of darter/madtom richness for treatment groups.

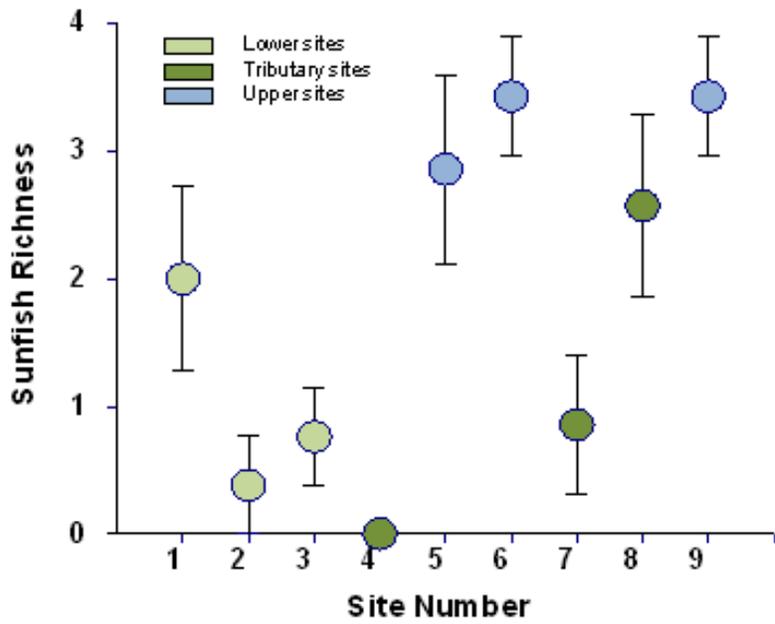


Figure 14. Error bar plots of sunfish richness for stream sites.

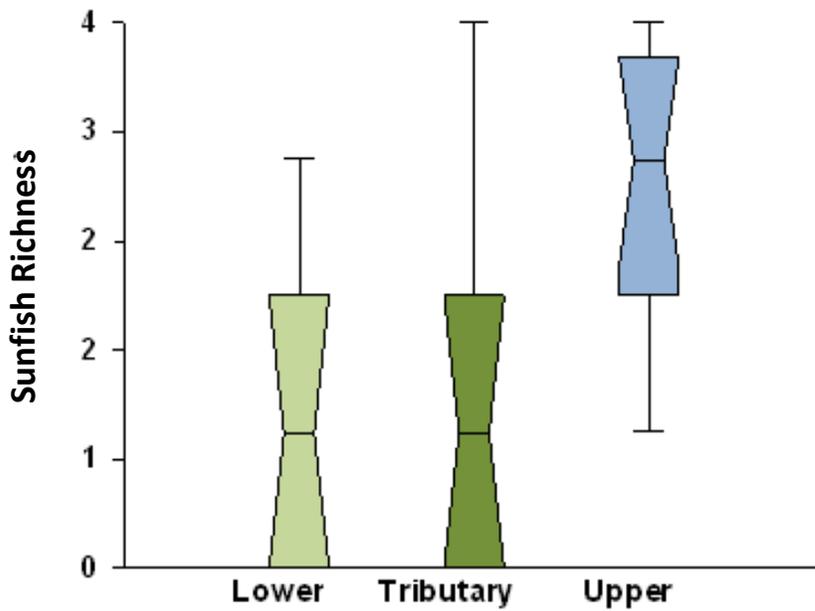


Figure 15. Box plots of sunfish richness for treatment groups.

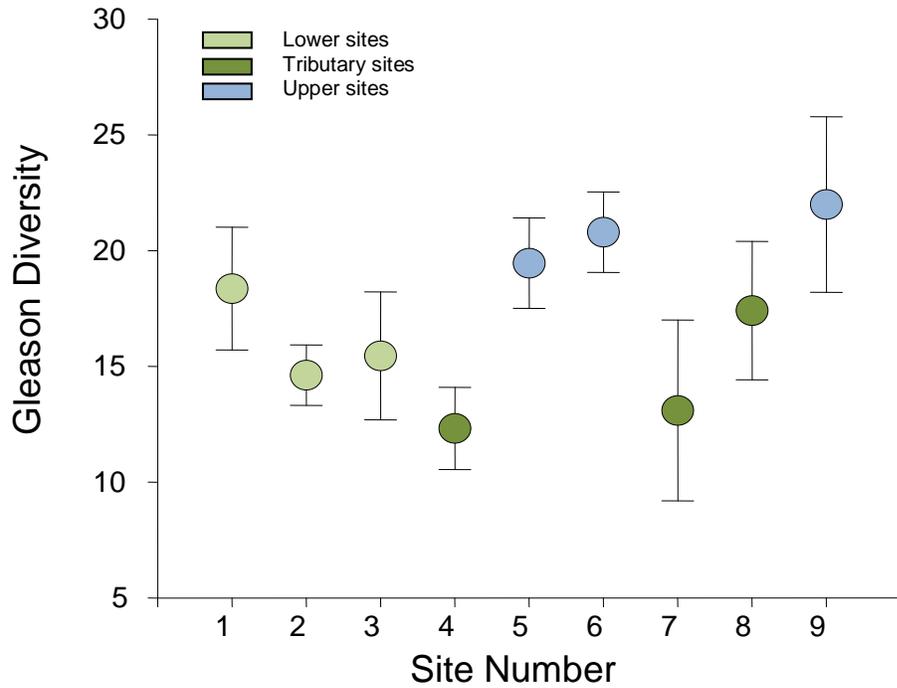


Figure 16. Error bar plots of macroinvertebrate Gleason Diversity for stream sites.

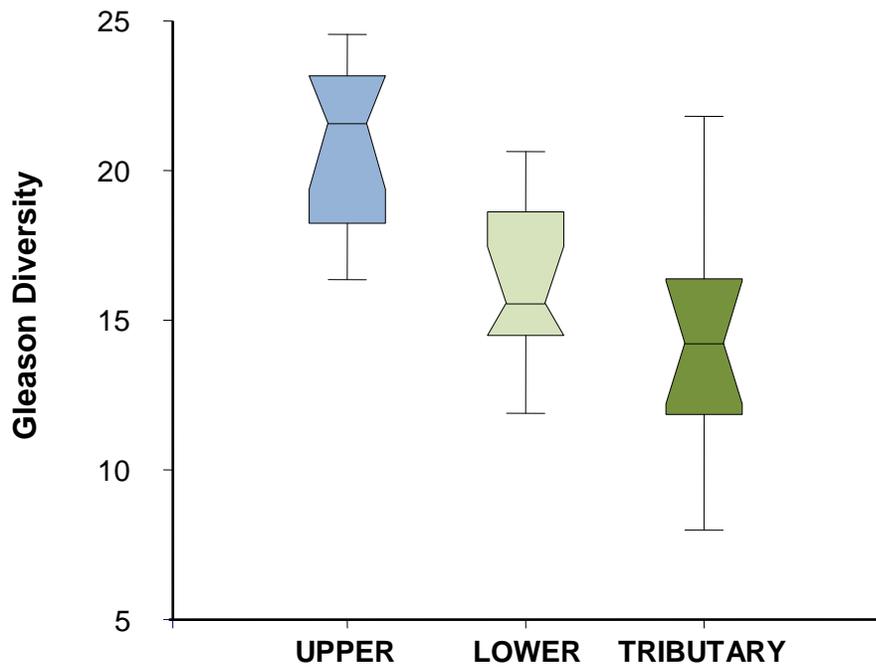


Figure 17. Box plots of Gleason Diversity of macroinvertebrates for treatment groups.

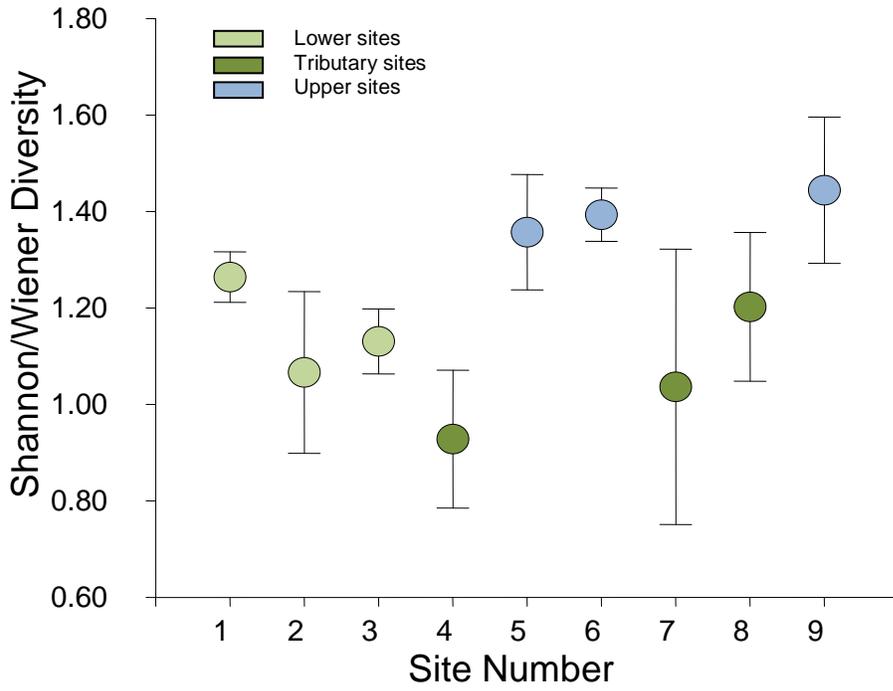


Figure 18. Error bar plots of macroinvertebrate Shannon/Wiener Diversity for stream sites.

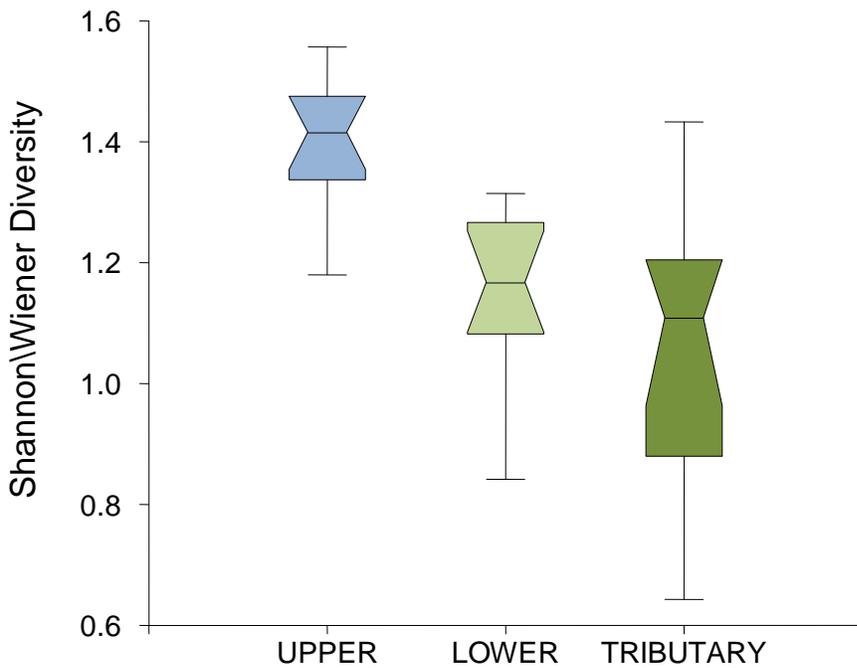


Figure 19. Box plots of macroinvertebrate Shannon/Wiener diversity for treatment groups.

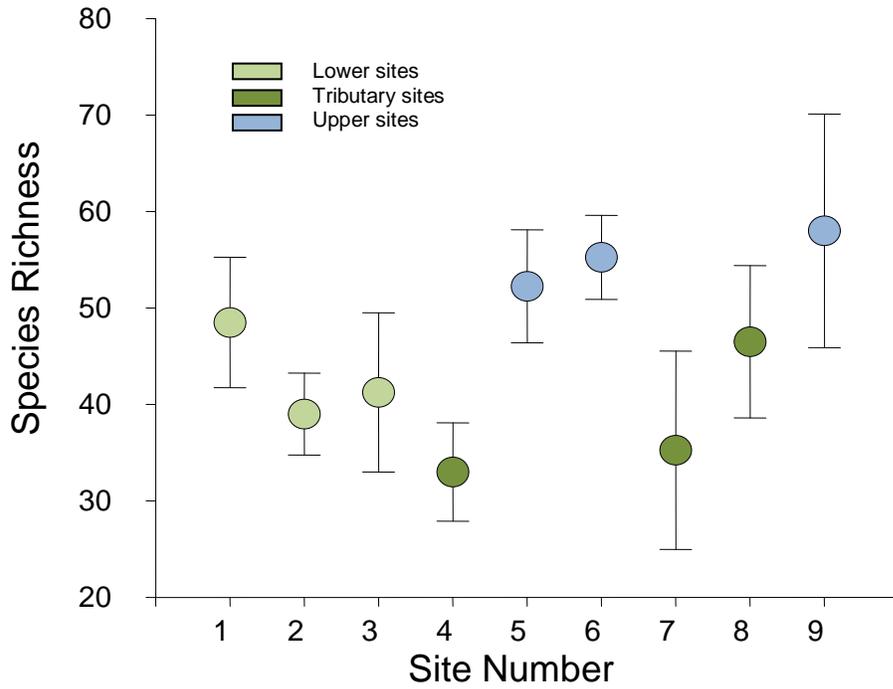


Figure 20. Error bar plots of macroinvertebrate species richness for stream sites.

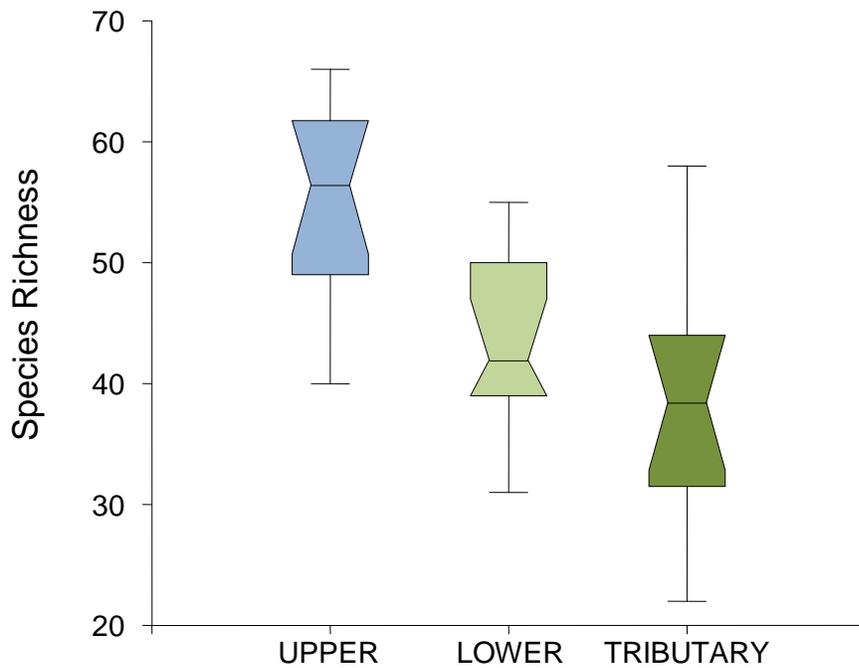


Figure 21. Box plots of macroinvertebrate species richness for treatment groups.

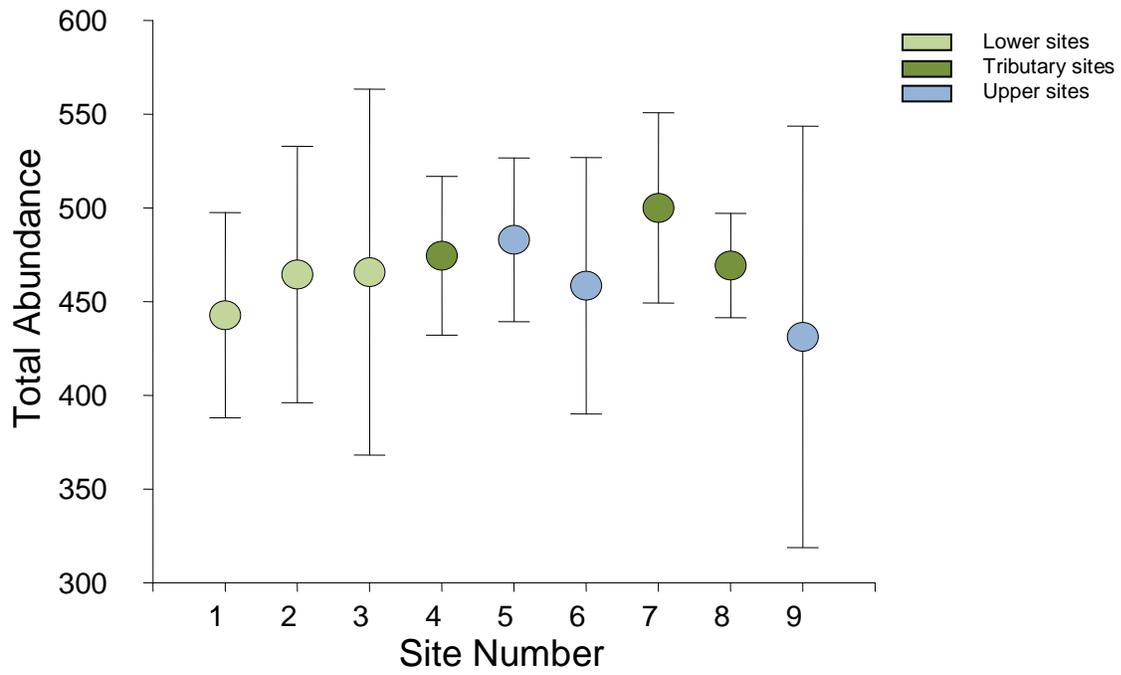


Figure 22. Error bar plots of total macroinvertebrate abundance for stream sites.

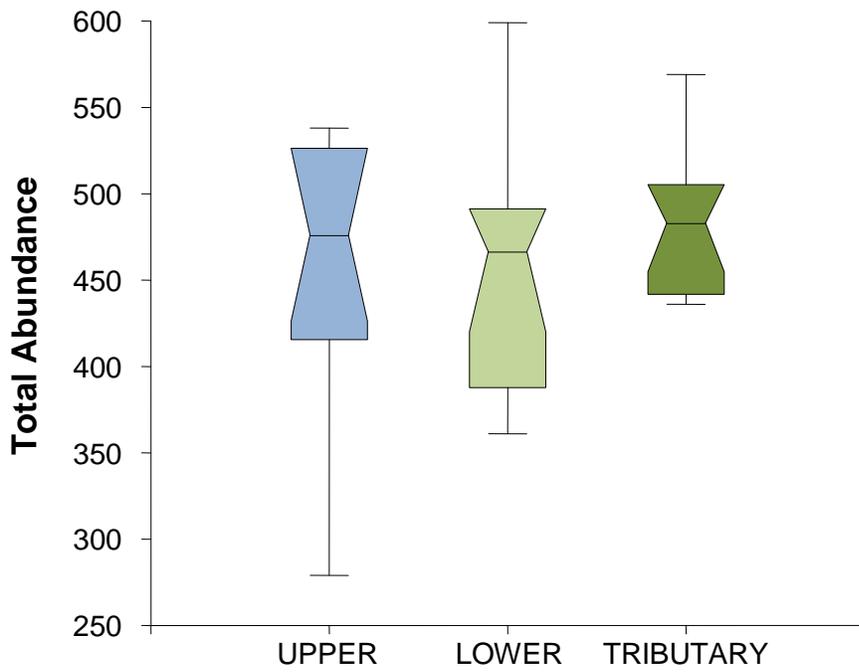


Figure 23. Box plots of total macroinvertebrate abundance for treatment groups.

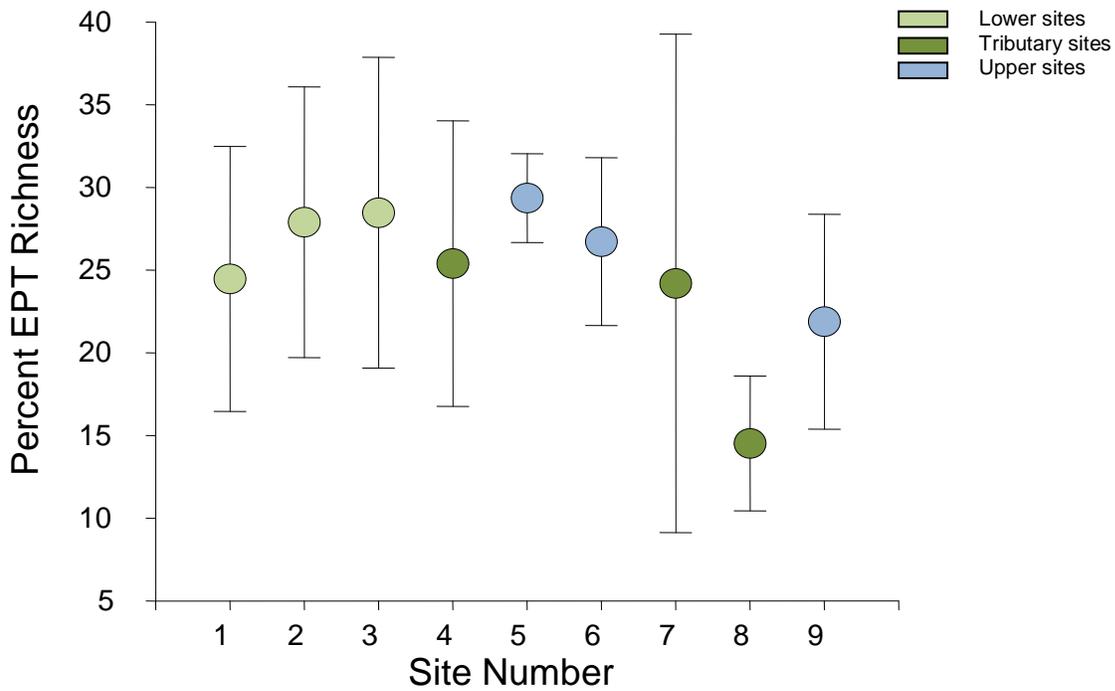


Figure 24. Error bar plots of Percent Ephemeroptera, Plecoptera, and Trichoptera taxa richness for stream sites.

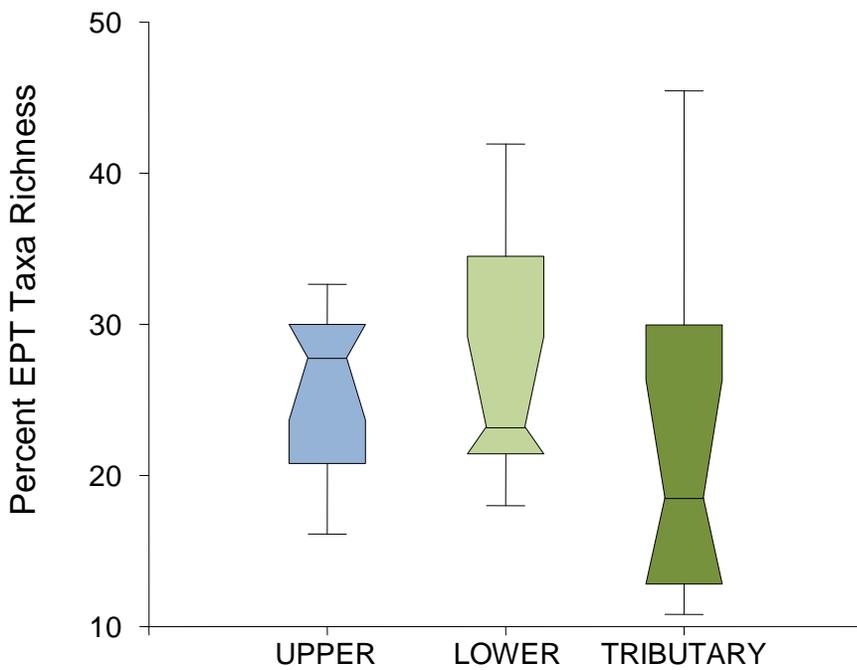


Figure 25. Box plots of Percent Ephemeroptera, Plecoptera, Trichoptera taxa richness for treatment groups.

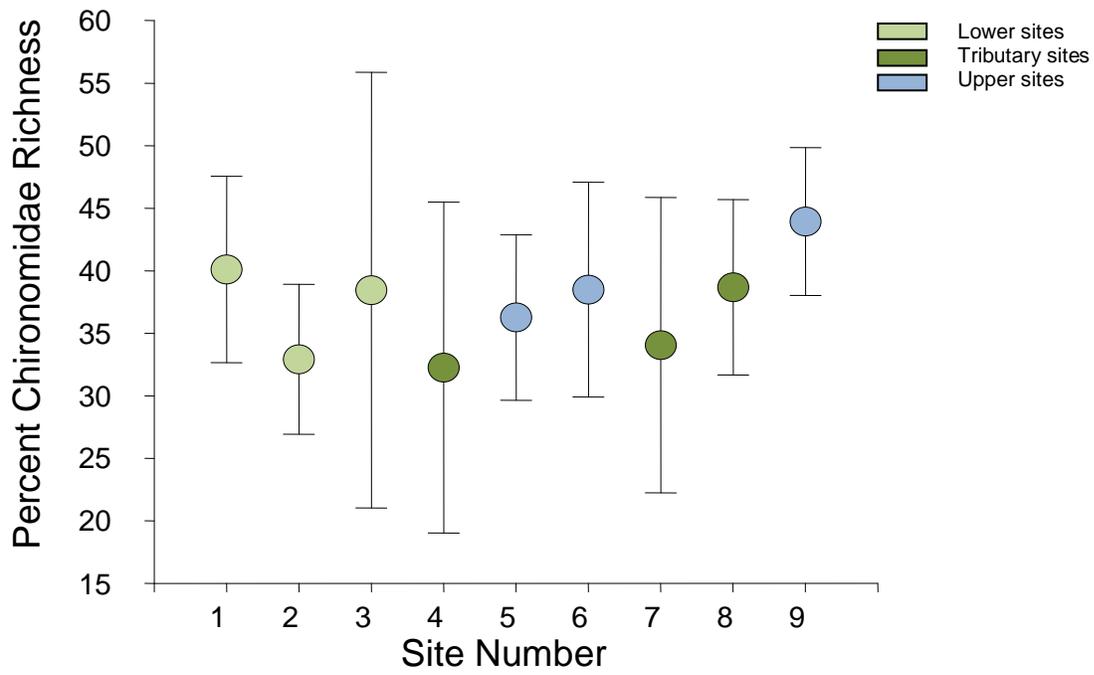


Figure 26. Error bar plots of Percent Chironomid Taxa Richness for stream sites.

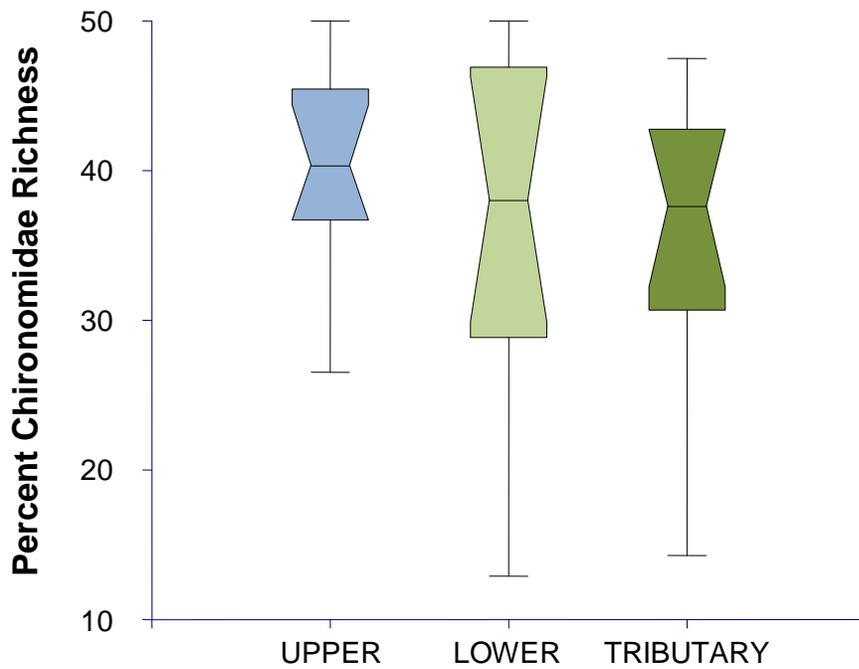


Figure 27. Box plots of Percent Chironomid Taxa Richness for treatment groups.

Cluster analysis is another way to examine variance among groups. By comparing the relative differences (i.e., “Dissimilarity”) among various group factors, patterns and treatment effects may be identified. Using the group average (unweighted-pairs) hierarchical clustering technique (Sneath and Sokal 1973), several dendrograms were produced based on water quality, discharge, habitat, and biological response data from Lost Creek and its Tributary.

The initial dendrogram (Figure 28) was produced using the chemical attributes of sulfate, chloride, conductivity and salinity to describe the chemical nature of these stream sites. The three clusters formed as a result of this analysis are very distinct and their stream site memberships clearly indicate that the *a priori* treatment groups are true chemical groupings. These differences are most likely the result of discharge from the Jeffrey Energy Center entering first the tributary and eventually mixing with Lost Creek flows to create three chemically different study groups (i.e. above, below, and within receiving tributary).

A second cluster dendrogram was constructed of factors that were thought to describe the hydrologic and habitat nature of these stream sites (Figure 29). This dendrogram was used to identify the possible presence of stream site clusters that were similar to the treatment groups. It is clear that only two prominent clusters were produced from the flow and habitat factors used to construct this dendrogram. Cluster 1 is composed of all the Lower Lost Creek study sites, which are below the confluence with the receiving tributary. These sites also lie within the Kansas River floodplain. It is not surprising that the downstream sites clustered together due to increased flows and the low gradient characteristic that these floodplain streams commonly share. There appears to be some naturally occurring categories (clusters) that mirror the treatment groups, and thus might complicate interpretation of the effects of the Jeffrey Energy Center effluent on Lost Creek. However, the use of habitat covariates in the GLM ANOVA testing did not alter the interpretation of test outcomes that were presented in Table 3, suggesting that these naturally occurring differences between treatment groups were not masking chemical impacts on the fish or macroinvertebrate community metrics that were examined in this study.

Biological responses were also characterized using cluster analysis. The fish metric dendrogram (Figure 30) clearly indicates the occurrence of three clusters. Cluster 1 is comprised of both a Tributary and Lower stream site, while a much larger grouping (Cluster 2) consists of all other Tributary and Lower stream sites. The third cluster of all Upper stream sites suggests that those Lost Creek sites that do not receive effluent flows from the Jeffrey Energy Center are quite different than those sites that do receive some effluent. In contrast, the macroinvertebrate metric dendrogram (Figure 31) shows two major clusters, with the first consisting of the Upper stream sites, the uppermost Tributary site, and the lowest Lower stream site and the second comprised of the lower two Tributary sites and the upper most two Lower stream sites. In other words, the first cluster represents the upper and lower most stream sites, while the second cluster represents the sites in between. Rather than reflecting differences in effluent, the macroinvertebrate clusters seem to reflect differences in habitat type and complexity associated with the riffle-run-pool complexes of Lost Creek and its Tributary.

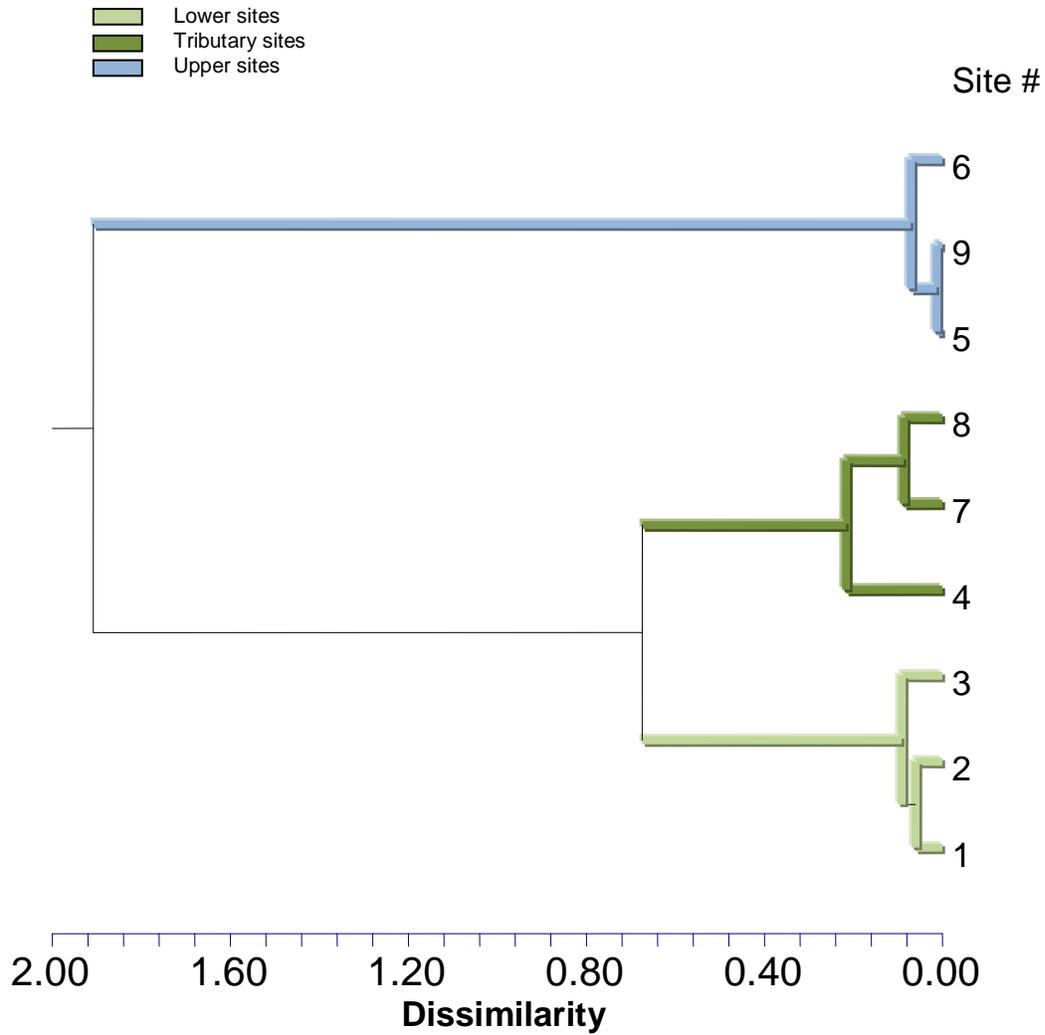


Figure 28. Group average (unweighted pair-group) cluster dendrogram based on sulfate, chloride, conductivity and salinity levels associated with each sampling site. Mean values were for all study measures (4) for each of the four stream parameters.

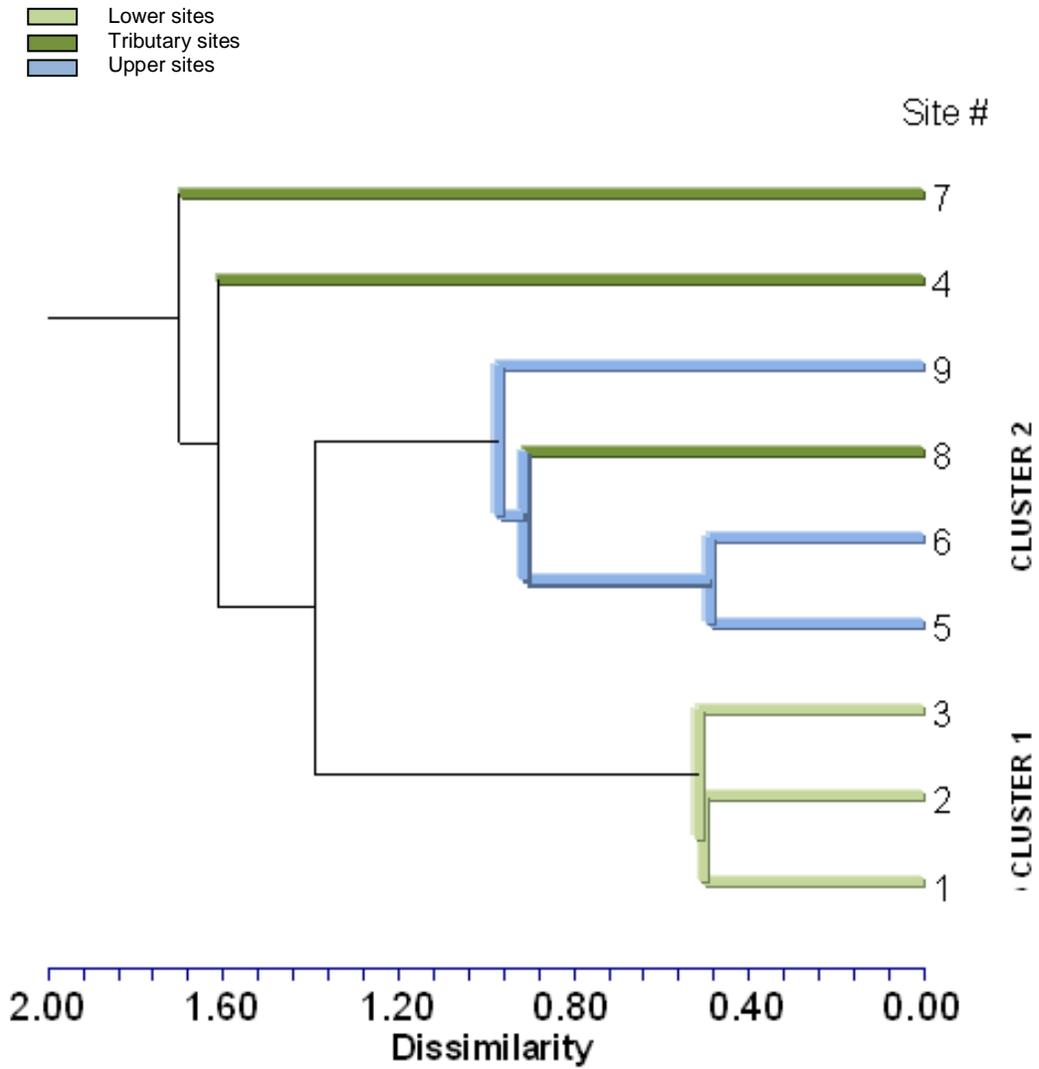


Figure 29. Group average (unweighted pair-group) cluster dendrogram based on mean discharge, velocity and depth and habitat index (QHEI) associated with each sampling site. Mean values were for all study measures (4) for each of the four stream parameters.

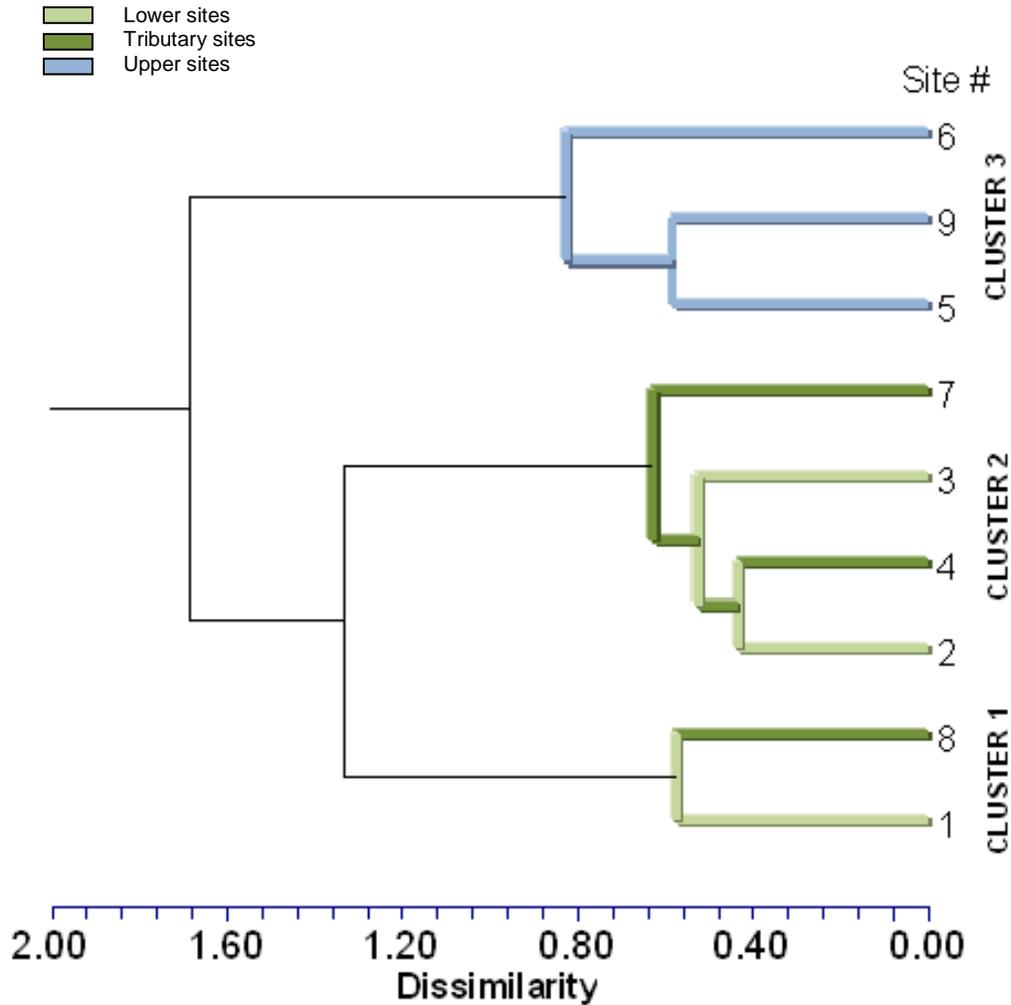


Figure 30. Group average (unweighted pair-group) cluster dendrogram based on eight fish metrics associated with each sampling site. Mean values were for all study measures (4) for each of the eight metrics. These metrics were species richness, Gleason and Shannon/Wiener diversity indices; sunfish richness and abundance; darter plus madtom richness and abundance and total abundance.

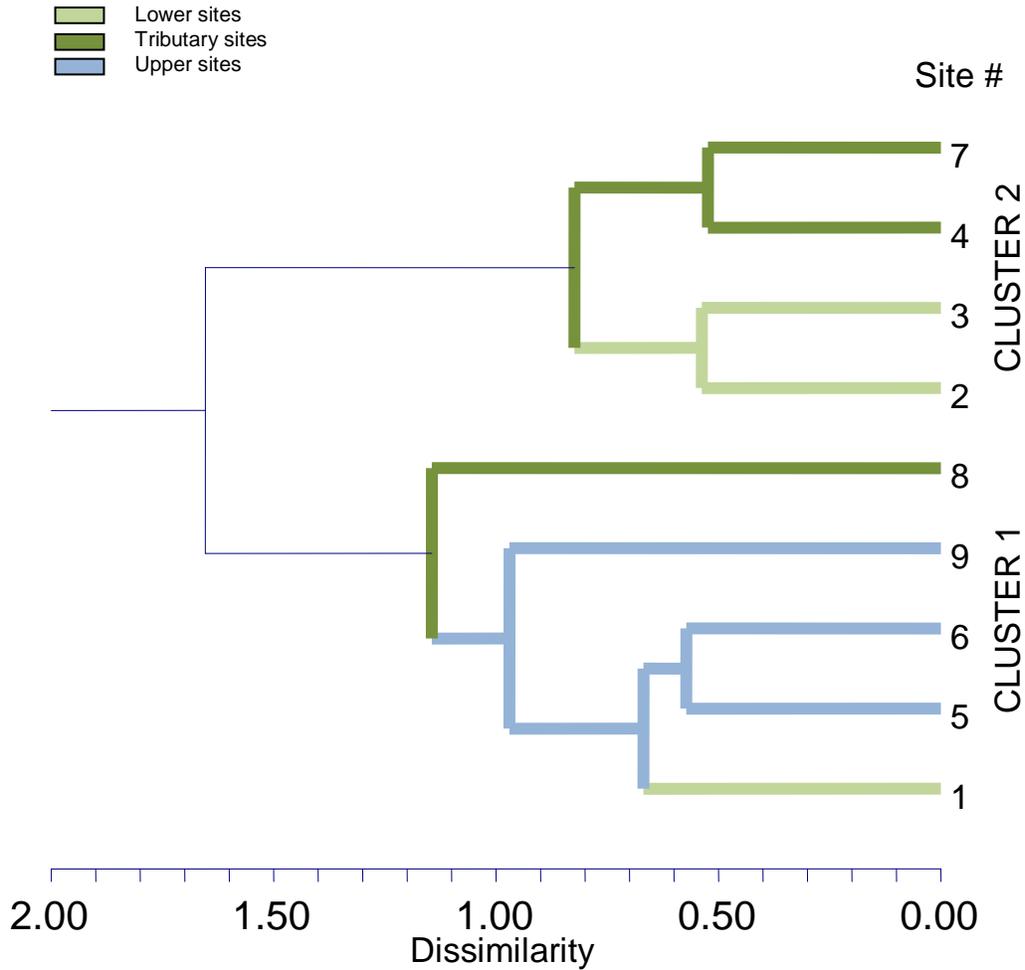


Figure 31. Group average (unweighted pair-group) cluster dendrogram based on six macroinvertebrate metrics associated with each sampling site. For each of the six metrics, group means were calculated across all four sampling periods for each site. These metrics were species richness, Gleason and Shannon/Wiener diversity indices; Total Abundance, Percent EPT Taxa richness, and Chironomid Taxa richness.

## Conclusions

The observed data suggest that there are effluent effects on the Lost Creek fish and macroinvertebrate communities, but these effects are not strong enough to alter the communities' natural diversity. Further, it appears that those effects which are present are tied to alterations in the timing and amount of flow (i.e., more constant flows in the tributary leading to increased base flows and lower peak flows, coupled with an alteration in the timing of pulses), rather than chemical additions to the water. The natural variation in physical habitat and biological condition among sites does not appear to show any treatment effects.

## Literature Cited

Gleason, H.A. 1922. On the relationship between species and area. *Ecology* 3:158-162.

[Goodrich, C., D.G. Huggins, R.C. Everhart, E.F. Smith. 2005. Summary of State and National Biological and Physical Habitat Assessment Methods with a Focus on US EPA Region 7 States. Open-file Report No. 135. Kansas Biological Survey, Lawrence, KS. 87 pp.](#)

[Huggins, D. G. and M. F. Moffett. 1988. Proposed biotic and habitat indices for use in Kansas streams. Open-file Report No. 35. Kansas Biological Survey, Lawrence, KS. 128 pp.](#)

Karr, J.R., K.D. Fausch, P.L. Angermeier, P.R. Yant, and I.J. Schlosser. 1986. Assessing biological integrity in running waters: A method and its rationale. *Ill. Nat. Hist. Surv. Spec. Pub. 5*. Champaign, IL.

Shannon, C.E. 1948. A mathematical theory of communications. *Bell. Systems. Tech. J.* 27:379-423, 623-656.

Wiener, N. 1948. *Cybernetics*. John Wiley and Sons, Inc. New York, NY. 194 pp.

### [Found in CPCB Macroinvertebrate Lab Protocols:](#)

Merritt *et al.* (2008), Needham *et al.* (2000), Westfall and May (1996), Stewart and Stark (2002), Wiggins (1996), Epler (2001), Wiederholm (1983) and (1986), Thorp and Covich (2001), Smith (2001), Pflieger (1996), Mackie and Huggins (1983), Oesch (1984), Couch (1997), Bleam *et al.* (1999), Burch (1982), Wu *et al.* (1997), Turgeon *et al.* (1998), Liechti and Huggins (1977), Schuster and DuBois (1979), DuBois (1981).