



# White River Macroinvertebrate Analysis, 2017 – 2019

January 2021



# White River Macroinvertebrate Analysis, 2017-2019



Submitted to:  
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Meeker, Colorado

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Project Manager

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# 1. Introduction

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In recent years, there has been increased interest in using the condition of the macroinvertebrate assemblages to infer the overall health of watersheds such as the White River. As a result, macroinvertebrate samples were collected from multiple sites on the White River and two White River tributaries near Meeker, Colorado by Colorado Parks and Wildlife (CPW) biologists and Trout Unlimited (TU) in collaboration with the White River Conservation District (the District). The U.S. Geological Survey (USGS) has conducted a related study on the White River focused on algae in conjunction with the macroinvertebrate study (Day et al. 2020). The White River has suffered from nuisance algal blooms in recent years, most notably from dense growths of the green filamentous algae, *Cladophora glomerata* (hereafter referred to as *Cladophora*). The USGS study is investigating the driving factors behind these blooms, and the macroinvertebrate study was designed in part to assess the interactions between these blooms and the macroinvertebrate assemblages in the White River and its tributaries.

Further goals of the macroinvertebrate study are to characterize the macroinvertebrate communities within this watershed and to document differences in the macroinvertebrate assemblages among sites and the years sampled, including differences that may be attributed to *Cladophora* growths. In addition, aerial insecticides were sprayed in the vicinity of the North Fork White River (NFWR) in summer 2018; additional sample collection was conducted at control sites upstream of the spraying on the NFWR and on the unaffected South Fork White River (SFWR) and at impact sites downstream of the spraying on the NFWR to investigate if the insecticides had an effect on the macroinvertebrate communities.

The macroinvertebrate samples were collected in summer and/or early fall of 2017, 2018, and 2019 from sites on NFWR, SFWR, and the White River mainstem (WRM). Samples were collected using two different methods, with qualitative samples collected using a kick net at some sites, quantitative samples collected using a Hess sampler at other sites, and both methods used at some sites. Sampling methods are described further below. Samples were then sent to one of two laboratories for analysis, with the 2017 samples being processed by Timberline Aquatics (Timberline) and the 2018 and 2019 samples processed by Utah State University (USU). GEI Consultants, Inc. (GEI) was then contracted to analyze the data produced from the sample collection to answer the questions posed by these studies. GEI submitted our approach for data analysis and interpretation to CPW, TU, and the District in late August.

## 2. General Approach to Analysis

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GEI's proposed approach to data analysis and interpretation is discussed in more detail under each separate part of the study, but, in general, we intended to characterize the macroinvertebrate communities in the White River basin upstream of Meeker through evaluation of the taxonomic composition and computation of various metrics describing different traits and attributes of the assemblages. Changes in the taxonomic composition and metric values over time or among sites were detected by visual observations of graphical data, calculating mean and median values, and performing statistical analyses when sufficient data were available. Where possible, statistical analyses focused on determining if any observed changes or differences were related to *Cladophora* growths and/or aerial insecticide spraying, dependent on the data set. We also formulated an approach to determine if differences observed in the data that could be attributed to the two different sampling methods or the two different laboratories utilized for taxonomic analysis. All statistical analyses were conducted in NCSS 12 (NCSS, 2018), and all graphical representations of data were made with Sigma Plot 14.0 (2020).

Macroinvertebrate metrics vary with respect to their specificity. Some can be used to determine general traits such as diversity, while others can be used to determine specific community traits such as tolerance to pollution or disturbance. All of these metrics can be used to identify differences in communities between sites or over time. The specific metrics included in each individual analysis were selected because they were best suited to answer the questions relevant to that part of the study (Table 1), as explained below.

Standard metrics such as number of taxa, number of Ephemeroptera (mayfly), Plecoptera (stonefly), and Trichoptera (caddisfly) taxa (collectively referred to as EPT taxa), Shannon-Weaver diversity ( $H'$ ) values, and Hilsenhoff Biotic Index (HBI) values are informative in both characterizing the macroinvertebrate communities and highlighting differences between sites potentially affected by *Cladophora* growth or pesticide use. Abundance and number of taxa collected at a site would often be expected to decrease in response to disturbance or pollution as some taxa disappear or persist in lower numbers. EPT taxa in particular are generally considered to be sensitive groups of macroinvertebrates that are less tolerant of stressors. The  $H'$  value generally ranges up to about 4.50 and indicates how balanced a community is; this value decreases when one or a few (often tolerant) taxa dominate the macroinvertebrate assemblage in comparison to a community in which the taxa are more evenly distributed. The HBI value is a measure of the community's tolerance to organic pollution; this value increases when organic pollution-tolerant taxa are relatively abundant at a site.

Other metrics that describe macroinvertebrate assemblages in terms of their feeding and habit preferences are also useful, because different environmental conditions favor different food

collection and habitat use strategies. Macroinvertebrate taxa are classified by their Functional Feeding Group (FFG) and habit in EDAS. The FFG classification describes the mechanisms by which a taxon obtains its food and the particle size of their food, in some cases. The habit classification describes a taxon’s mode of existence, and, in some cases, locomotion. FFG and habitat metrics included in our analysis describe either the number of taxa within each classification or the relative abundance of each classification (Table 1). All of the selected metrics in Table 1 are included in Colorado’s Water Quality Control Division (WQCD) Policy 10-1: Aquatic Life Use Attainment (2017).

**Table 1: List of metrics utilized in each analysis. All metrics except the similarity metrics were calculated through Ecological Data Application System (EDAS), and all metrics calculations were based on the EDAS-generated subsample data. Percent metrics calculated as the percent individuals of the group specified of all individuals. Metrics are described in WQCD (2017).**

Number of taxa based- metrics	Spatial Analysis	Temporal Analysis	Duplicate Analysis	Method Comparison Analysis	Cladophora Analysis	Insecticide Analysis
<b>Basic Metrics</b>						
Number of Taxa	X	X	X	X	X	X
Number of EPT Taxa	X	X	X	X	X	X
Multi-Metric Macroinvertebrate Index	X	X	X	X	X	X
Shannon Weaver Diversity Index (Base 2)	X	X	X	X	X	X
Hilsenhoff Biotic Index	X	X	X	X	X	X
<b>Taxa Composition Metrics</b>						
% Dominant Taxon	X	X	X	X	X	X
# of Chironomid Taxa	X	X	X	X	X	X
% Chironomidae	X	X	X	X	X	X
# of Diptera Taxa	X	X	X	X	X	X
% Diptera	X	X	X	X	X	X
# of Ephemeroptera Taxa	X	X	X	X	X	X
% Ephemeroptera	X	X	X	X	X	X
% EPT Excluding Baetidae	X	X	X	X	X	X
# of Plecoptera Taxa	X	X	X	X	X	X
% Plecoptera	X	X	X	X	X	X
# of Trichoptera Taxa	X	X	X	X	X	X
% Trichoptera	X	X	X	X	X	X
# of Coleoptera Taxa	X	X	X	X	X	X
% Coleoptera	X	X	X	X	X	X
# of Insect Taxa	X	X	X	X	X	X



# of Non-Insect Taxa	X	X	X	X	X	X
% Non-Insects	X	X	X	X	X	X
% Non-insect Taxa	X	X	X	X	X	X
<b>Tolerance Metrics</b>						
# of Intolerant Taxa	X	X	X	X	X	X
% Intolerant	X	X	X	X	X	X
% Intolerant Taxa of All Taxa	X	X	X	X	X	X
# of Tolerant Taxa	X	X	X	X	X	X
% Tolerant	X	X	X	X	X	X
<b>Functional Feeding Group Metrics</b>						
# of Collector Taxa	--	--	X	--	X	X
% Collectors	--	--	X	--	X	X
# of Filterer Taxa	--	--	X	--	X	X
% Filterer Taxa	--	--	X	--	X	X
# of Predator Taxa	--	--	X	--	X	X
% Predators	--	--	X	--	X	X
# of Scraper Taxa	--	--	X	--	X	X
# of Shredder Taxa	--	--	X	--	X	X
% Shredders	--	--	X	--	X	X
<b>Habit Metrics</b>						
# of Burrower Taxa	--	--	X	--	X	X
% Burrowers	--	--	X	--	X	X
# of Climber Taxa	--	--	X	--	X	X
% Climber Taxa	--	--	X	--	X	X
# of Clinger Taxa	--	--	X	--	X	X
% Clingers	--	--	X	--	X	X
# of Sprawler Taxa	--	--	X	--	X	X
% Sprawler Taxa	--	--	X	--	X	X
# of Swimmer Taxa	--	--	X	--	X	X
% Swimmer Taxa	--	--	X	--	X	X
<b>Similarity Indices</b>						
Percent Common Taxa	X	--	X	--	--	X
Bray-Curtis Dissimilarity Index	X	--	X	--	--	X

In Colorado, attainment of the aquatic life use for streams and some rivers is determined using the Macroinvertebrate Multi-Metric Index (MMI) bioassessment tool, which is calibrated separately for each of the three analytically defined Biotypes in Colorado (WQCD 2017). Biotypes are defined by the ecoregion, stream slope, and elevation at a site. The MMI is composed of eight separate metrics selected to represent categories of community characteristics including richness, composition, FFG, habit, and pollution tolerance. Metrics were chosen based on their ecological meaningfulness, ability to describe various attributes

of the assemblages, and their ability to discriminate between reference and stressed sites. Each metric is scored separately, with all eight metric scores then averaged to calculate the final MMI score. Thresholds were developed for each biotype that then determine if the MMI score is in attainment of the Aquatic Life Use or impaired. MMI samples are to be collected utilizing specific protocols described in WQCD (2017); briefly, a kick sample is collected in riffle habitat over an estimated area of 1 meter squared ( $m^2$ ) and a time period of 60 seconds.

The WQCD utilizes a program, the Ecological Data Application System (EDAS), to calculate all of the metrics that are used in the MMI, as well as many additional metrics descriptive of the macroinvertebrate assemblages. Most of the metrics that EDAS outputs are included in Table 1, as well as in WQCD (2017). Protocols for processing MMI samples include targeting the collection of 300 ( $\pm 20\%$ ) organisms to standardize for sample abundances. When analysis of sample data is conducted through EDAS, the program uses random resampling to reduce all organism counts that are greater than 300 ( $\pm 20\%$ ) to this target number of organisms.

Based on the raw data provided to GEI, the samples processed by both labs appeared to target 500 or 600-counts. In addition, some samples were collected using a Hess sampler and were therefore not collected using the standard MMI protocol of the kick sample. However, 303(d) guidance in Colorado allows for the use of Hess replicates and references a study that demonstrated that the Hess method can produce similar MMI scores if certain rules and modifications are followed. The Hess samples were collected in riffle habitat as the kick samples were, and eight Hess samples were collected from each site, resulting in a total area sampled at each site of 0.688  $m^2$ . However, these samples were composited into a single sample per site prior to being processed; the 303(d) guidance requires that the samples be processed separately.

As higher abundances in some samples and sample areas that differ can both influence metric values in terms of the number of taxa and abundances of organisms collected, all samples utilized for this study, even those that were not collected following MMI protocols, were run through EDAS first to be reduced to 300-count ( $\pm 20$  percent) prior to EDAS calculating the array of metric values. This allowed for the consistent calculation of metrics quickly through the EDAS program, and also allowed for more valid comparisons of sample data collected using different methods and analyzed by different labs by minimizing the impact of the variability in total abundances and areas sampled. **All metrics discussed and comparisons made between samples below were based on the subsample data. In addition, all data in tables and figures were generated from the EDAS data.**

Two additional metrics were calculated for these data to determine what sites had more similar macroinvertebrate composition to one another than others: the Percent Common Taxa (PCT) and the Bray-Curtis Dissimilarity Index (BCDI) (Table 1). The PCT value varies from 0 to 100 and is based on the number of taxa the two sites have in common compared to the total number of taxa present at both sites. This metric only utilizes data on what taxa are

present or absent and does not account for how abundant each taxa was within the samples. Values for this metric increase when sites are more taxonomically similar. The BCDI ranges from 0 to 1; higher values for this metric indicate the sites are less similar in terms of the macroinvertebrate communities. This index not only incorporates how many taxa are common to both sites but also utilizes abundances. The equations for the calculation of these indices are included in Appendix A, while the larger tables including PCT and BCDI values for each pair of sites for each year and sample type of data were extensive and are thus included in Appendix B.

While data sets were limited in terms of number of sample sites and sampling events, statistical analyses were run when possible to compare samples collected with differing methods, sites among streams, those with and without *Cladophora* growths. All statistical analyses were conducted using NCSS. When two groups were compared to each other, two-sample or paired t-tests were utilized if the data met the assumptions of normality. When those assumptions were not met, the Mann Whitney U-test or Wilcoxon Signed Rank test, respectively, were utilized as non-parametric alternatives. Likewise, when more than two groups were compared, One-Way Analysis of Variance (ANOVA) or its non-parametric alternative, the Kruskal-Wallis One Way ANOVA on Ranks, were utilized. Linear Regression analysis was used to detect trends over time in metrics for sites that were sampled in each of the three years.

### 3. Available Data

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GEI received the following data in Xcel spreadsheets from CPW in September 2020. Data are described separately for the two different studies.

#### Cladophora Study

- Kick samples were collected from nine sites in September 2017, with an additional duplicate sample collected from one of the sites (Table 2). Data provided included taxa lists, abundances, MMI scores, and all EDAS-calculated metrics for 2017, as analyzed by Timberline.
- Kick and Hess samples were collected from ten sites in September 2018, with one duplicate kick and one duplicate Hess sample collected from one of these sites (Table 2). Data included complete taxa lists and some or all metrics, dependent on data set, as analyzed by USU.
  - MMI scores were provided for kick and Hess data; however, the MMI scores for the Hess data were provided in two different locations within the spreadsheet and did not match each other. The MMI scores in the EDAS sheet were determined to be correct.
  - Metrics utilized in the MMI were provided for the Hess data as calculated through EDAS, but the complete list of EDAS metrics was not provided. The complete list was provided for the kick samples.
  - At the direction of the CPW (M. May, CPW, personal communication, September 2020), Chris Theel at the Colorado Department of Public Health and Environment (CDPHE) was contacted and sent GEI the full list of metric values that he had previously calculated through EDAS for the Hess samples.
- Kick samples were collected from three sites and Hess samples from 11 sites in October 2019. (Table 2) Three duplicate Hess samples were collected: one each at a NFWR, SFWR, and WRM site. Data provided included complete taxa lists and some metrics, as analyzed by USU.
  - MMI scores were provided, but not the individual metric scores that are used to calculate the MMI and not the other EDAS metrics.
  - Trip Armstrong at USU was contacted and provided the raw taxa lists for both the Hess and Kick samples. These taxa lists were run through EDAS by GEI taxonomists to provide MMI scores, and other EDAS metrics were provided.

**Table 2: Sites sampled on the North Fork White River, South Fork White River, and the mainstem White River from 2017 to 2019. Sites within each stream are listed from upstream to downstream. Samples were collected in September of each year unless otherwise noted. H = Hess samples collected, K = Kick sample collected, H-P = Hess samples collected for pesticide study (months sampled in parentheses), D = Duplicate sample collected.**

Stream/Site	Year Sampled		
	2017	2018	2019
<b>North Fork White River</b>			
North Fork White River Below Trappers Lake (3079)	--	H, K	H
North Fork White River below Missouri Creek (445)	--	--	H
North Fork White River Below Lost Creek (6111)	K	H, K, H-P (June), H-P (July), H-P (August)	H, HD, K
North Fork River at County Road 14 (6110)	K	H, HD, K, KD, H-P (August)	H
North Fork River above Bufurd (6108)	K	--	--
North Fork River at Bel Aire (6107)	K	H, K, H-P (June), H-P (July), H-P (August)	H
<b>South Fork White River</b>			
South Fork White River at USFS Campground (3077)	--	H, K	H
South Fork White River above Buckeye Creek (3078)	--	H, K	H, HD, K
South Fork White River above Bel Aire (6106C)	--	H-P (June), H-P (July)	--
South Fork White River at Bel Aire (6106)	K	H, K, H-P (June), H-P (July)	H
<b>White River (Mainstem)</b>			
White River at Sleepy Cat (6105)	K	H, K	H
White River above Coal Creek (6104)	K, KD	H, K	H
White River at Meeker Pasture (6103)	K	H, K	H, HD, K
White River at 5 <sup>th</sup> Street (531)	K	--	--

### Pesticide Study

- Hess samples were collected from four sites in June, four sites in July, and three sites in August 2018. Data provided included complete taxa lists and some metrics, as analyzed by USU.
  - Metric results provided did not appear to be calculated through EDAS and were a more limited list than the metrics standardly calculated through EDAS.
  - While MMI scores were not necessary for this study, GEI utilized the taxa lists provided to run these samples through EDAS, with all metrics calculated by the program, based on the standard 300-organism ( $\pm 20\%$ ) count.

## 4. Macroinvertebrate Community Characterization

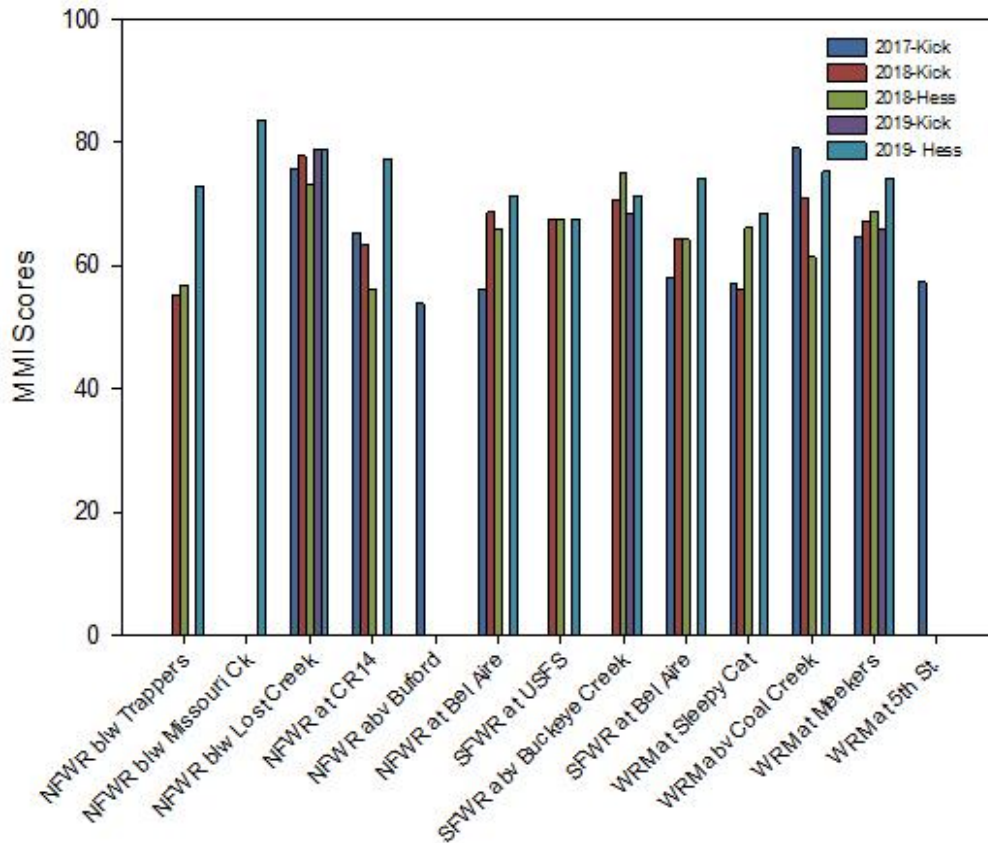
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Macroinvertebrate assemblages sampled in the NFWR and SFWR were dominated by caddisflies (Trichoptera) and true flies (Diptera) in almost every sample collected from 2017 through 2019. Multiple caddisfly genera were abundant in one or more sample in the NFWR and SFWR sites, including *Lepidostoma*, *Microsema*, *Glossosoma*, *Oligophlebodes*, *Brachycentrus*, and *Hydropsyche*. Abundant true fly taxa included the chironomid midge *Cricotopus nostocicola* and the crane fly *Antocha monticola*. In addition, the riffle beetle (Coleoptera) *Optioservus* and the ephemereid mayfly (Ephemeroptera) *Ephemerella* were collected in high abundances in one or more samples as well.

The overall composition of the WRM sites differed somewhat from the tributary sites. True flies continued to be a dominant group in many of the samples collected from 2017 through 2019, but mayflies were the most numerically abundant group in other samples, with *Ephemerella* sp. and *Baetis tricaudatus/bicaudatus* being the dominant taxa in one or more samples. The most abundant true fly taxa were *Orthocladius* sp. and *Eukiefferiella* sp, both of which are chironomid midges.

MMI scores for all samples collected via kick or Hess sampling from 2017 through 2019 ranged from 53.8 at the NFWR above Buford (kick sample) to 87.7 at the NFWR below Missouri Creek site (Hess sample) in 2019 (Figure 1: Multi-metric Index (MMI) values for sites on the North Fork White River (NFWR), South Fork White River (SFWR), and White River Mainstem (WRM) sampled in 2017 through 2019. All sites except the NFWR below Trappers site are located within Biotype 1, with an attainment threshold of 45. The NFWR below Trappers site is located within Biotype 2, with an attainment threshold of 48.

), with all samples having scores greater than the threshold indicating attainment of the aquatic life use. Of note, the Hess samples may not be appropriate to determine aquatic life use attainment from a regulatory perspective. While not all samples were collected following the specific MMI protocols, they do as a whole suggest that the macroinvertebrate assemblages in the NFWR, SFWR, and WRM are not impaired.



**Figure 1: Multi-metric Index (MMI) values for sites on the North Fork White River (NFWR), South Fork White River (SFWR), and White River Mainstem (WRM) sampled in 2017 through 2019. All sites except the NFWR below Trappers site are located within Biotype 1, with an attainment threshold of 45. The NFWR below Trappers site is located within Biotype 2, with an attainment threshold of 48.**

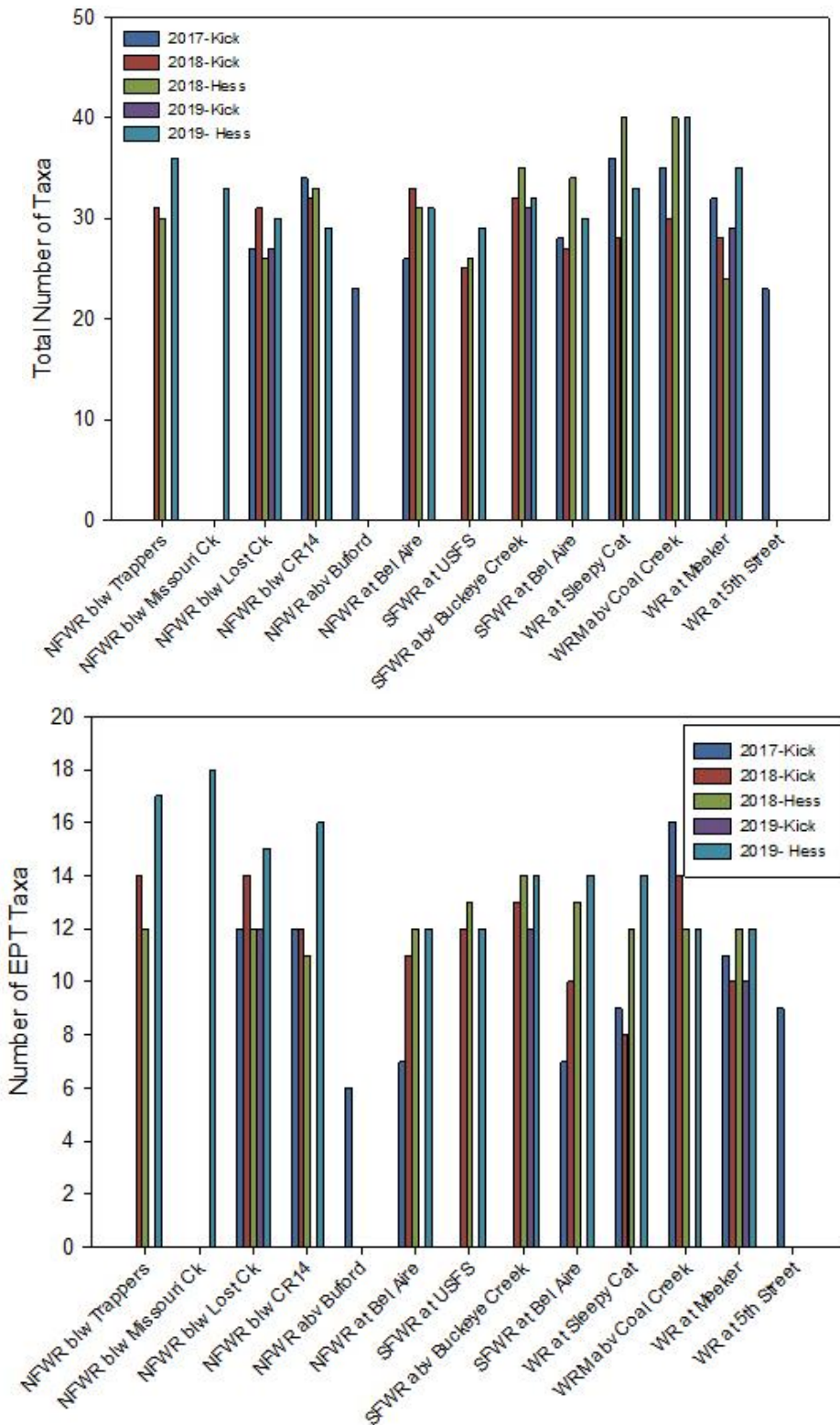
Other metrics describing the macroinvertebrate communities confirm that the assemblages are healthy. From 23 to 40 macroinvertebrate taxa were present at each site; 6 to 18 of these taxa were EPT taxa, which are generally considered sensitive taxa (Figure 2). EPT organisms also comprised 24 to 86 percent of the total abundance (Figure 3), with almost all samples having EPT organisms making up a third or more of the total abundance, suggesting further that the macroinvertebrate communities in the NFWR, SFWR, and WRM are not impaired. Of these three groups, Ephemeroptera (mayflies) can be the most sensitive group, dependent on the type of disturbance or pollution. Mayfly relative abundance varied greatly among sites, with this group comprising 10% or less of the total abundance at multiple sites, mostly on the SFWR. At other sites, they comprised up to 64% of the total abundance (Figure 3). While there is substantial diversity within chironomid midges (true flies), this group is often considered one of the more tolerant groups of organisms; chironomids comprised 1 to 47% of the total abundance, with this group making up less than a third of the total abundance in most samples (Figure 4).

Shannon-Weaver diversity values (log base 2) indicated that macroinvertebrate communities in the NFWR, SFWR, and WRM were diverse and balanced, ranging from 2.96 to 4.42 (Figure 4). In general, values greater than 2.50 are considered indicative of balanced and diverse communities. In addition, HBI values ranged from 1.46 to 4.86, indicating that the macroinvertebrate communities were largely dominated by taxa with low pollution tolerance values (Figure 5). Hilsenhoff (1987) categorizes such values as indicative of excellent, very good, or good water quality. Six to 19 intolerant taxa, defined as those with tolerance values from 0 to 3, were present at each site (Figure 5). In contrast, from 2 to 11 tolerant taxa (with tolerance values from 7 to 10) were observed at each site, with most sites having 5 or less of these taxa.

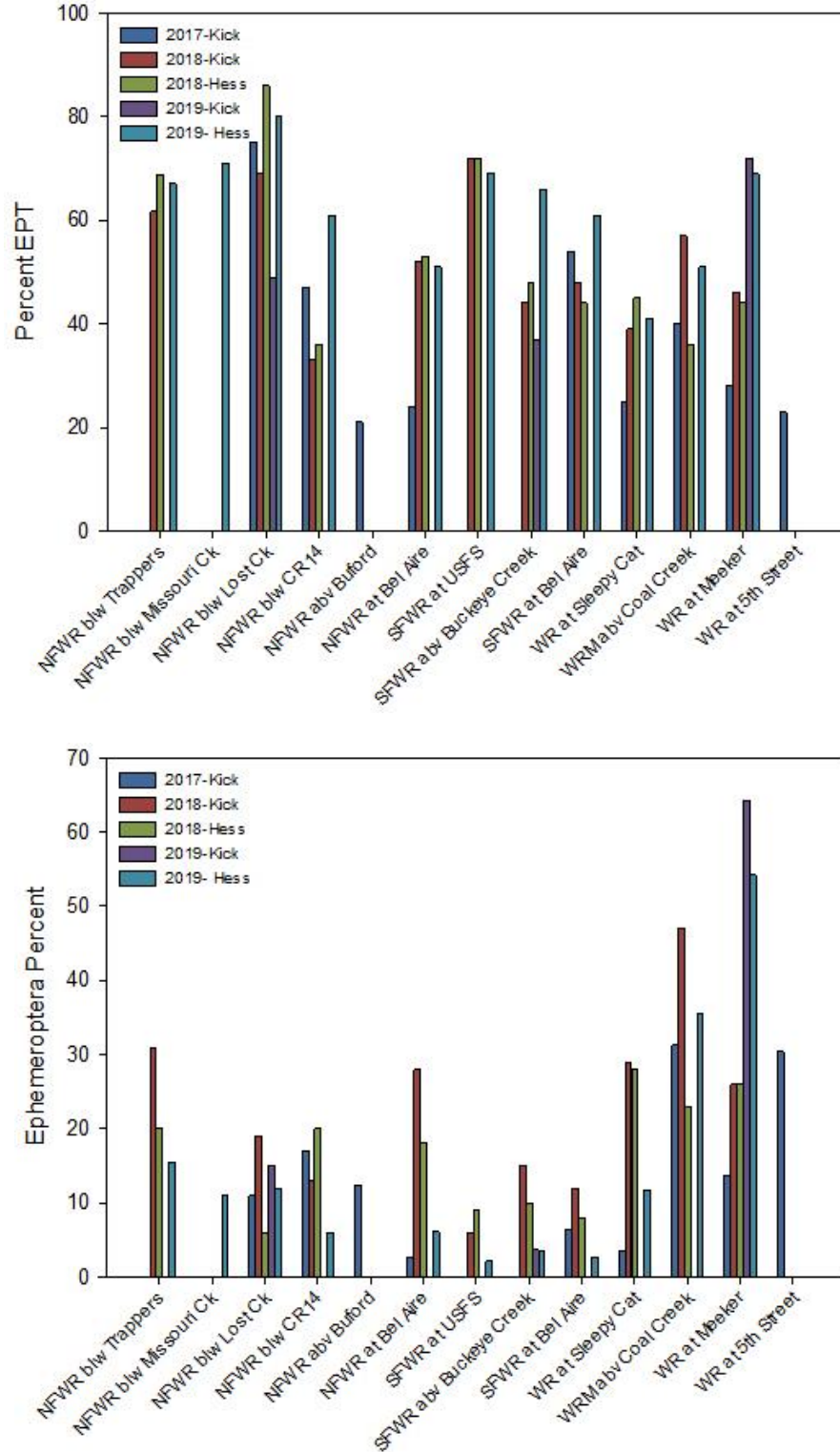
**In summary:**

- The MMI and other metrics suggest that the macroinvertebrate assemblages in the NFWR, SFWR, and WRM are overall healthy and not impaired. Often, a caddisfly or mayfly taxon was the most abundant taxon at sites.
- While a few sites and sampling events had low percentages of mayflies and somewhat high percentages of chironomids, most samples contained numerous sensitive species, as evidenced in the number and percent of EPT taxa, low HBI scores, and numbers of intolerant taxa.
- In addition, the diversity values were above 2.50 at all sites, indicating that balanced communities were present.





**Figure 2: Number of total taxa (above) and Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa (below) collected at sites on the North Fork White River (NFWR), South Fork White River (SFWR), and White River Mainstem (WRM) sampled in 2017 through 2019.**



**Figure 3: Percent Ephemeroptera Plecoptera, and Trichoptera (EPT) individuals of all individuals (above) and % Ephemeroptera of all individuals (below) collected at sites on the North Fork White River (NFWR), South Fork White River (SFWR), and White River Mainstem (WRM) sampled in 2017 through 2019.**

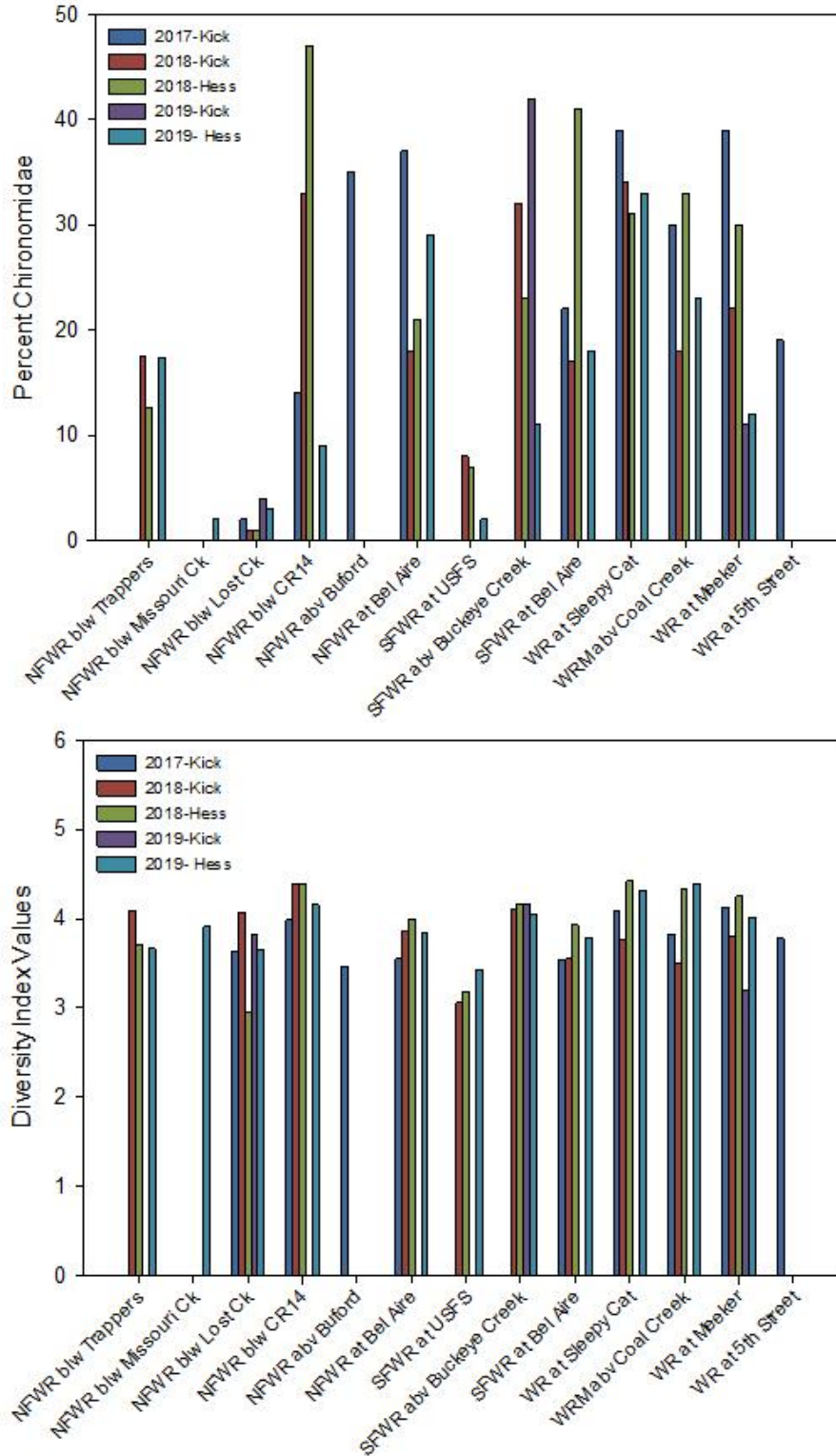
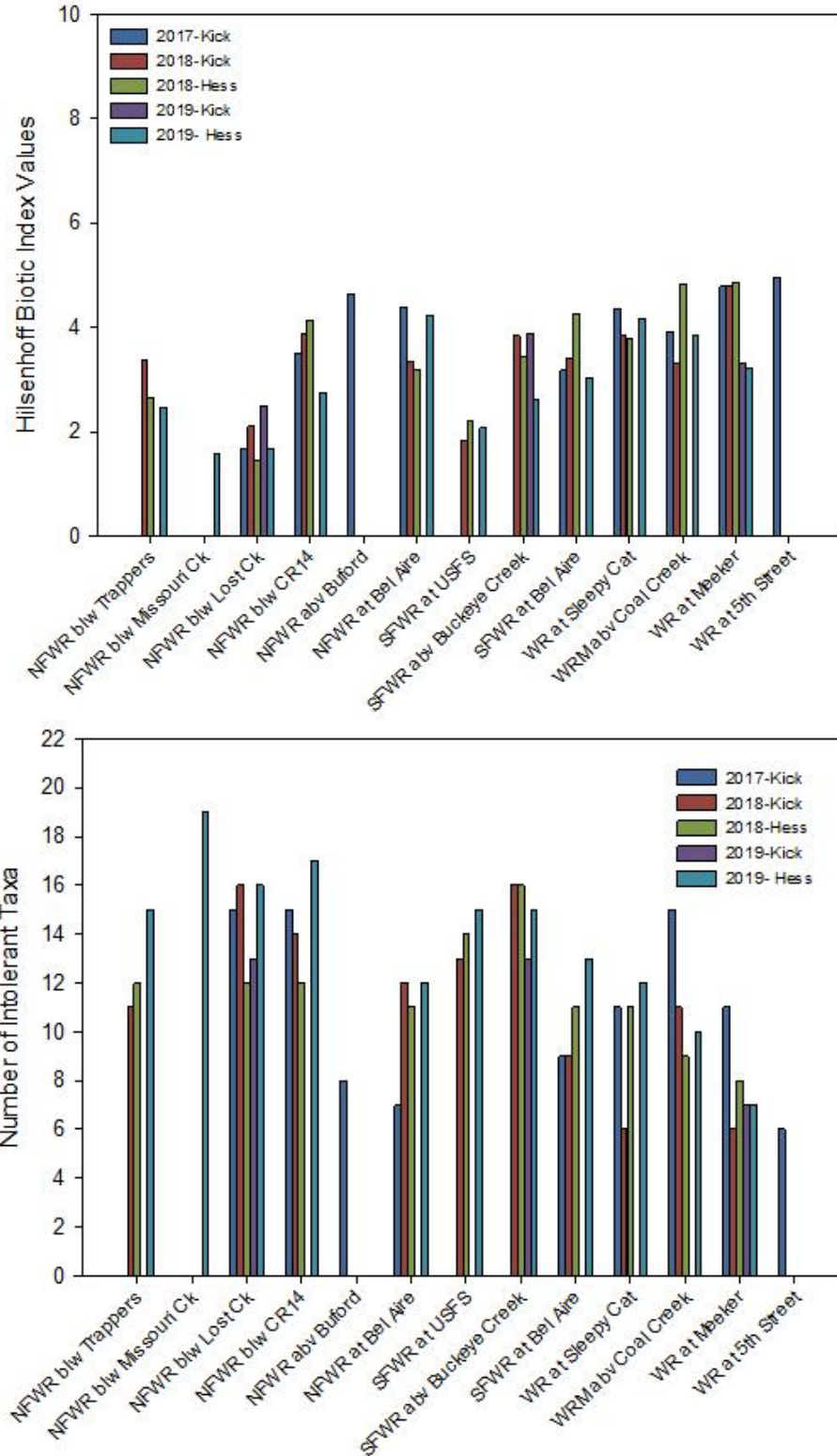


Figure 4: Percent Chironomidae individuals of all individuals (above) and diversity values (below) for samples collected at sites on the North Fork White River (NFWR), South Fork White River (SFWR), and White River Mainstem (WRM) sampled in 2017 through 2019.



**Figure 5: Hilsenhoff Biotic Index (HBI) values (above) and number of intolerant taxa (below) for samples collected at sites on the North Fork White River (NFWR), South Fork White River (SFWR), and White River Mainstem (WRM) sampled in 2017 through 2019.**

## 5. Duplicate Sample Analysis

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From 2017 to 2019, six duplicate samples were collected (Table 2), including one kick sample duplicate each collected in 2017 and 2018, one Hess sample duplicate collected in 2018, and three Hess sample duplicates collected in 2019. The kick and Hess sample duplicates collected in 2018 were collected from the same site. Prior to proceeding with the analyses, the similarity of the duplicate samples were evaluated to provide information on how much variability exists in the macroinvertebrate assemblages even when the samples are collected on the same day at the same site using the same methods.

On a broad scale, some of the duplicate samples differed from one another in terms of the dominant taxon and dominant group present within each sample. For example, the mayfly taxon, *Ephemerella* sp., was the most abundant taxon in both duplicate samples collected in the kick samples collected from a WRM site in 2017 and the Hess samples from another WRM site in 2019, but, when abundances were summed for each order or group of macroinvertebrates, one sample had more mayflies and the other sample had more true flies. In the duplicate Hess samples collected from the NFWR in 2019, caddisflies were the dominant group, but the caddisfly *Lepidostoma* sp. was most abundant in one sample, while a caddisfly from a different family, *Glossosoma* sp., was most abundant in the other sample. The 2018 kick duplicates collected from the NFWR site differed the most, with the true fly *Antocha* sp. dominating in one sample, and true flies overall being the most abundant group, while within the other sample, *Lepidostoma* was the dominant taxon and caddisflies were the most abundant group.

The two similarity indices, the PCT and the BCDI, were also used to evaluate the duplicate samples. The PCT metric had values ranging from 40 to 67% (Table 3), indicating that of all the taxa collected at both sites, from 40 to 67% of these taxa were found in both samples. At the site at which both types of samples and their duplicates were collected, the kick samples were less taxonomically similar. The enclosed area sampled by the Hess samples may be a factor in the higher similarity in the Hess sample. However, the duplicate set of samples collected via kick samples in 2017 were the most similar pair of duplicates. Of note, these percentages may be biased low in some cases based on differences in the taxonomic level that some taxa could be identified to. As an example, in the Hess duplicates taken at the NFWR below Lost Creek site and the kick sample duplicates taken at the NFWR at County Road 14 site, mayflies were identified at the genus level as *Paraleptophlebia*; however, in the duplicate sample, individuals that were likely also *Paraleptophlebia* were only identified at the family level as Leptophlebiidae, likely because these individuals were immature or damaged.

Bray-Curtis Dissimilarity Index values between the duplicate samples ranged from 0.17 (most similar) to 0.57 (least similar, Table 3). The pattern with this index differed from the

one observed with the PCT, with the most similar and least similar duplicate sample sets as assessed with the BCDI differing from those assessed with the PCT. This difference could be expected since the BCDI includes abundance data while the PCT is based on the presence and absence of taxa.

**Table 3: Common taxa percent and Bray-Curtis dissimilarity index values for duplicate samples collected in the White River drainage in 2017 through 2019.**

Sites	Sample Type	Sample Date	Percent Common Taxa	Bray-Curtis Dissimilarity Index
White River above Coal Creek (6104)	WQCD Kick	9/6/2017	66.7	0.20
North Fork White River at County Road 14 <sup>1</sup> (6110)	WQCD Kick	9/25/2018	40.0	0.34
North Fork White River at County Road 14 (6110)	Hess Composite	9/25/2018	61.4	0.52
South Fork White River above Buckeye Creek (3078)	Hess Composite	10/1/2019	66.7	0.36
White River at Meeker Pasture (6103)	Hess Composite	10/1/2019	46.8	0.57
North Fork White River below Lost Creek (6111)	Hess Composite	10/1/2019	58.3	0.17

<sup>1</sup> The duplicate sample within the CDPHE EDAS database was labeled as “North Fork White River at Maggot Hollow (RW-3).”

Differences in metric values between duplicates were also assessed using a % difference metric; the equation for this metric is included in Appendix A. Of all the EDAS metrics for which the % Difference was scored, some of the metrics that scored lowest and highest when the % differences were averaged across all duplicate sets were of limited ecological relevance, as these metrics were related to macroinvertebrate groups that were uncommon at these sites, resulting in either a % difference of 0 or a very large value that was based on little actual compositional difference between sites. An example of this occurred with metrics related to taxa categorized as “swimmers” (organisms adapted for “fishlike” swimming; Cummins et al. 2008) or “collectors”, as generally zero to two taxa were present at these sites that were categorized as swimmers or collectors, so if one was collected in one duplicate sample but not another, the % difference metric calculation was high. However, this difference did not equate to a large difference in taxonomic composition between the two duplicate samples.

Common metrics that are often utilized to evaluate differences in macroinvertebrate communities such as the MMI, number of taxa, number of EPT taxa, number of total taxa, Shannon-Weaver diversity index values, HBI values, number of intolerant taxa, % intolerant taxa, number of chironomid midge taxa, % Diptera, number of true fly taxa, and number of Trichoptera taxa differed at most by 33% and often by much less, indicating that duplicate samples were relatively similar. In addition, some of the FFG and habitat metrics also had maximum differences in this range. The majority of the other metrics in Table 1 also had

average % differences of 33% or less, suggesting that while one or more set of duplicate samples may have had a higher % difference, most duplicate sets had more moderate differences. A few of the metrics, most of which were based on relative abundances differed more. Of note, more variability should be expected within macroinvertebrate duplicate sample analysis compared to the analysis of other media such as sediment or surface water samples, as biotic and abiotic factors (i.e., substrate size, substrate diversity, flow velocity, water depth, food resources, and other factors) can vary within a riffle and determine macroinvertebrate distributions on a very fine scale.

**In summary:**

- Even with samples that are taken at a site on the same day from what were presumably locations within the stream that appeared similar to one another from the human perspective, some variability in taxonomic composition and metric values occurs, particularly in those metrics that are based on relative abundances.
- This variability should be considered when evaluating the differences observed in the remaining analyses. The ranges observed in similarity in the PCT and BCDI provide a baseline to compare to for the following analyses as well.
- Many of the basic descriptive metrics had maximum percent difference values of 33% or less, and most of the other metrics in Table 1 had average values that were also within this range. This suggests that while some variability exists in the taxa richness and abundances between two duplicate samples, they are relatively similar. A few metrics, often those based on relative abundances, differed more. Sometimes a single taxon can be abundant in one sample but represented at lower abundances in the second samples, resulting in more variability in some of the metrics based on relative abundances.

## 6. Comparison of Sampling and Laboratory Protocols

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As discussed previously, two differing methods of sample collection were used over the course of this study from 2017 to 2019, with only WQCD kicks collected in 2017 and both WQCD kicks and Hess samples collected in 2018 and 2019. In addition, the samples collected in 2017 were sent to Timberline to be processed, while the samples collected in 2018 and 2019 were sent to USU.

### 6.1 Comparison of Sampling Protocols

Both of these sampling methodologies utilized for this study focus on targeting the same type of stream habitat (riffles, when present). However, while the habitat targeted is similar, the sampling methodologies differ in other ways.

The Hess sampler encloses a defined area of substrate and is placed on the bottom of the stream channel in a manner that seals off the designated area for sampling. There is no specific time spent taking each sample; instead, sampling is complete when the substrate is disturbed sufficiently to have dislodged all organisms. The WQCD kick samples are collected following protocols in Policy 10-1 (WQCD 2017). As mentioned briefly previously, this protocol states that a riffle area of 1 m<sup>2</sup> that is considered representative of the reach is selected. Substrate is disturbed by kicking for one minute. While the area to be sampled with a WQCD kick sample is estimated to be 1 m<sup>2</sup> and the effort is timed, these kick samples are considered semiquantitative as the actual area sampled is not enclosed as it is with a Hess sample; thus, there is some potential for dislodged organisms to be washed around the kick net or for organisms drifting in from upstream of the sampling area to be included in the sample. The estimated area sampled with a kick sample is also substantially larger than the defined area sampled with a Hess sampler. However, for this study, eight Hess samples, each of which enclosed 0.086 m<sup>2</sup> of stream substrate, were collected at each site, equaling a total area sampled of 0.688 m<sup>2</sup>.

There were 13 sites at which a Hess composite and a kick sample were both collected during the same sampling events, including ten sites in 2018 and three sites in 2019 (Table 2). Upon an initial review of the macroinvertebrate composition within the paired samples, we observed that in 7 of the 13 samples sets, the dominant taxon and group within the Hess samples was identical to that in the kick samples. For example, at the WRM at Meeker Pasture site in 2019, the most abundant single taxon present in both the Hess and kick samples was the mayfly, *Ephemerella* sp., and most abundant group as a whole within these samples were mayflies. This occurred most frequently in the White River mainstem sites, but also in one and two of the SFWR and NFWR samples, respectively.



In the remaining six pairs of samples, the most abundant taxon and/or the most abundant group differed. Of note, this also occurred in some of the duplicate samples collected with the same method. As another example, the NFWR below Lost Creek site in 2019, the riffle beetle, *Optioservus* sp., was the most abundant taxon in the kick samples, with beetles (Coleoptera) in general dominating the sample. In the Hess sample, the caddisfly *Lepidostoma* sp. was the most abundant taxon, with caddisflies (Trichoptera) being the most abundant group. As the same habitat type is targeted for both types of samples, likely the discrepancies in some samples are related to the natural variability in microhabitats and the macroinvertebrates that prefer very specific habitat niches within the riffle. Also of note, there was no pattern to these differences, i.e., no taxa or group was consistently identified as more abundant in one type of sample compared to the other.

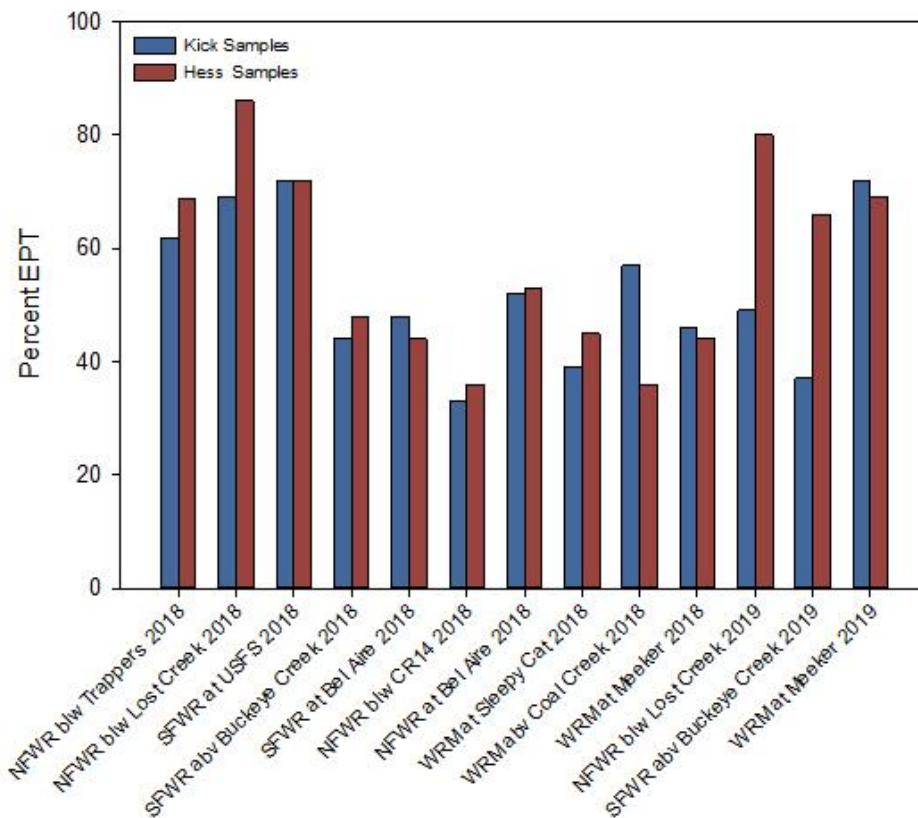
Paired t-tests were conducted on a subset of macroinvertebrate metrics to determine if metric values significantly differed dependent on sample type. When metric values were determined to not demonstrate normal distributions, the Wilcoxon-Signed Rank test was utilized instead as a non-parametric alternative to the paired t-test. Specifically, the metrics assessed included those that are basic descriptors of the assemblages such as total number of taxa, number of EPT taxa, HBI index, and Shannon-Weaver diversity index (base 2), as well as the metrics that describe the composition and tolerance of the macroinvertebrate community (Table 2).

As mentioned previously, taxonomic lists and abundances were standardized by running the data through EDAS first; based on this, sample with abundances greater than 360 (all samples) were reduced to 300 organisms +/- 20% by random subsampling through the EDAS program prior to metrics being calculated. With samples that were subsampled such as these rather than having the entire sample processed and that had differing target and actual organism counts and different areas sampled, standardizing the data to a 300 count, minimizes the potential for over-estimation of taxa richness with higher subsamples, higher target counts, and higher areas sampled (WQCD 2017). The percent of the samples randomly processed to obtain the 600-count target for the 2018 kick and Hess composite samples ranged from less than 2% to 50%, and the number of organisms in the subsamples ranged from 556 to 969 organisms. Larger subsamples and higher counts would tend to contain more taxa than smaller subsamples, as would the greater area sampled with the kick sample compared to the Hess composites. This tendency can result in misinterpretation of the data if fewer taxa at one site in comparison to another is assessed as an actual difference in the communities that could then be attributed to the effects of a stressor when in fact it resulted from differences in the subsample percentage or the number of organisms sorted within the subsample.

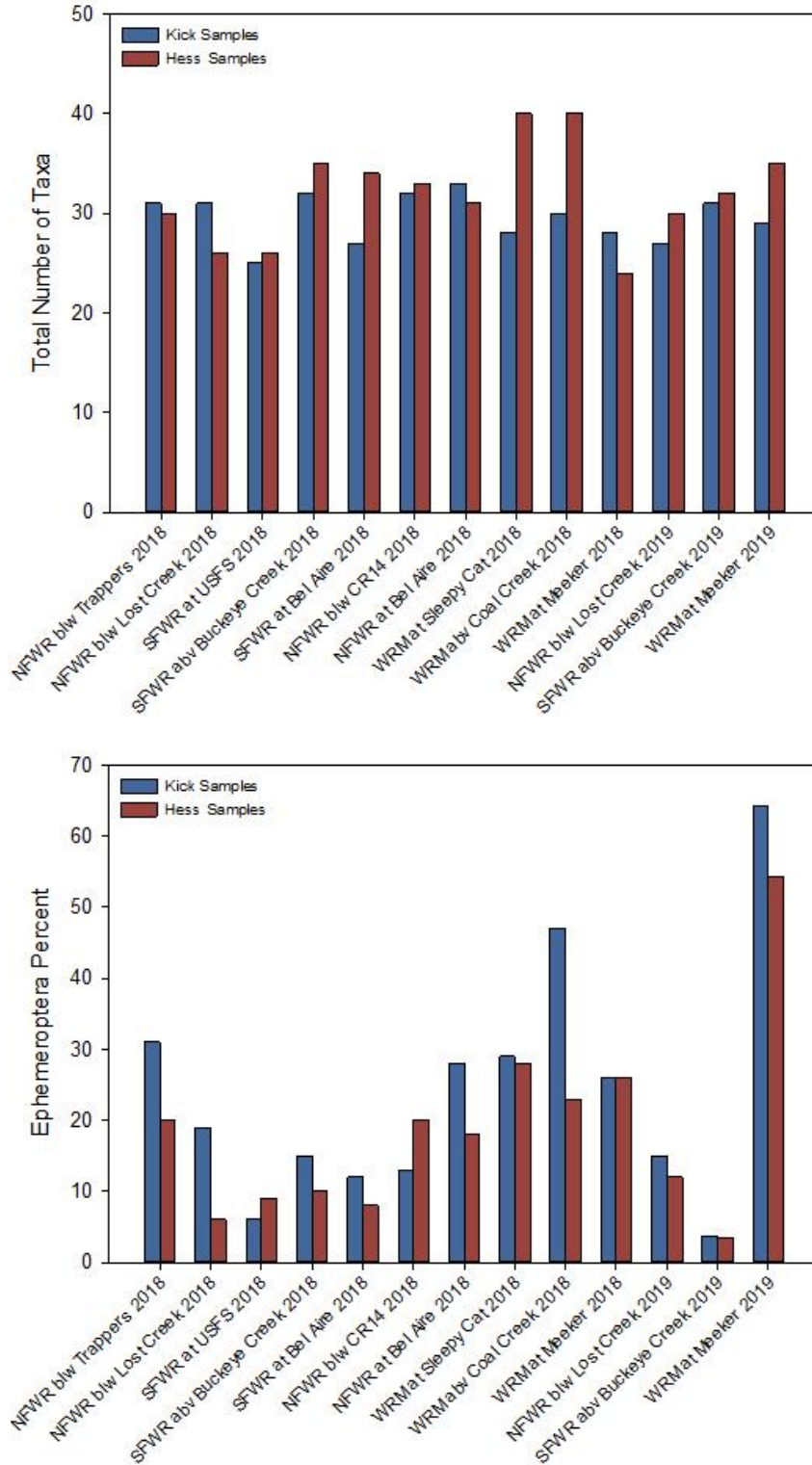
Relatively few metric values (5 of 28 metrics) were identified as being significantly different between the Hess composite and kick samples. Of those that were significant, some were relatively minor, but consistent, differences. Other metrics such as the % Ephemeroptera and Trichoptera individuals metrics were often similar in several sample sets, but differed more

substantially in others, often as a result of high abundances of a single taxon in one type of sample but not another (Figure 6). Four metrics were on average significantly higher in the Hess samples compared to the kick samples, including the total number of taxa (Figure 7), number of insect taxa, number of tolerant taxa, and % Trichoptera individuals ( $p \leq 0.033$ ). Only a single metric, the % Ephemeroptera individuals, was higher in the WQCD kick sample ( $p = 0.035$ ; Figure 7). All other metrics did not differ significantly between the Hess and kick samples ( $p \geq 0.083$ ).

Of note, the duplicate analyses indicated that variability in metric values was present between samples taken at the same site and time; for some metric values, the variability was high, while others had more consistent values between the two duplicate samples. Of the five metric values that were significantly different between the two sample types, all except the % Ephemeroptera individuals had an average percent difference in the duplicate sample analysis of 10% or less, suggesting relatively low variability. The % Ephemeroptera individuals metric had an average percent difference of 16%, indicating this metric was more variable in comparison; metrics based on taxa abundance compared to taxa richness tended to often exhibit higher variability in the duplicate analysis.



**Figure 6: Comparison of the percent Ephemeroptera, Plecoptera, and Trichoptera (EPT) individuals in the paired kick and Hess samples collected in 2018 and 2019 from sites on the North Fork White River (NFWR), South Fork White River (SFWR), and White River Mainstem (WRM).**



**Figure 7: Comparison of the total number of taxa (top) and percent Ephemeroptera individuals (bottom) in the paired kick ad Hess samples collected in 2018 and 2019 from sites on the North Fork White River (NFWR), South Fork White River (SFWR), and White River Mainstem (WRM).**

To further this analysis, the PCT and BCDI were calculated for each of these pairs of samples in 2018 and 2019 (Appendix B). The BCDI values varied from 0.24 at the SFWR at USFS Campground site in 2018 (most similar) to 0.53 at WRM at Sleepy Cat site in 2018. The PCT similarity index that is based only on the presence or absence of taxa ranged from a low similarity of 17.7% at the SFWR above Buckeye Creek site in 2018 to 61.0% at the SFWR at Bel Aire site in 2018. Most values for this metric indicated that roughly 40 to 60 percent of the taxa collected in the paired samples were common to both; these results were similar to the duplicate sample analysis. As mentioned previously this percentage is likely biased low, as damaged or immature specimens are often not identified at the same taxonomic level as intact or more mature specimens, which would lead to an underestimate in similarity. Likewise, differences in taxonomic resolution would affect the BCDI values as well.

### **In summary:**

- Some differences in taxonomic composition were observed between the two sampling methods, but these differences were not consistent and were likely related to the natural variability that occurs even when the effort is made to sample the same general type of habitat, as was observed in the duplicate sample analysis.
- After sample abundances were standardized through EDAS via random resampling, most metric values were relatively similar between the two sample types. While the sample size was not extensive, only 18% of the metrics analyzed differed statistically between the WQCD kicks and the Hess samples after utilizing EDAS to standardize for the target number of organisms. While standardizing for outside factors to the extent possible is always preferable, results in general suggested that the variability in the sampling method used should have minimal impact on the following analyses when utilizing the EDAS-generated data for both types of samples.

## **6.2 Comparison of Lab Outputs**

Samples collected in 2017 were processed by Timberline; processing included presentation of taxa lists and abundances, EDAS subsample lists, and calculation of MMI scores and other EDAS metrics. Data were presented in spreadsheets that included individual site data with abundances presented by the lowest taxonomic level identified. Sample counts targeted 500 organisms, with 507 to 885 organisms included in the original data. In addition, the EDAS subsample dates were provided, along with MMI scores and all other EDAS-produced metrics (all standardized to the  $300 \pm 20\%$  count). Taxa lists were reduced to the operational taxonomic unit (OTUs) utilized by EDAS for this effort; reduction of taxa lists to their OTUs eliminates the potential confusion over identifying identical taxa as unique taxa and ensures that consistent taxonomy is applied across all samples. Large and rare taxa were clearly identified in the individual site data. Large & Rare are defined as taxa that are searched for in the remainder of the sample after the target number of organisms is reached in at least the minimum subsample to be processed, because they are either notably large organisms or are

clearly organisms that were not encountered while picking and sorting macroinvertebrates from the subsample before the target number of organisms is reached.

Samples collected in 2018 and 2019 were processed by USU. Site data were combined in a single spreadsheet with all sites presented in different columns, which provided a complete taxa list of all macroinvertebrates identified during each sampling event. These datasets included both Hess and WQCD kick samples. Regardless of sample type, split counts, defined as the number of organisms randomly subsampled, ranged from 404 to 1,021 organisms for the standard data set collected for the *Cladophora* and insecticide studies. The target count for these samples was defined as 600 organisms.

For the samples processed by USU, both “raw” and “standardized” data were provided for these samples, with the “standardized” data distilling the data into the appropriate OTUs. However, the “raw” data was not the data initially compiled by the taxonomist that provided the actual counts of the organisms identified at the lowest practical level; instead, the raw data appeared to have already factored in for the proportion of the sample identified compared to the entire sample. The raw data also did not clearly identify large and rare taxa. Ecological Data Application System metric lists and subsample data were provided for some but not all of the samples processed by USU (See Section 3). When not provided, the CDPHE or GEI ran the sample data through EDAS to calculate the MMI and other metrics. For the datasets for which the EDAS subsample data were not provided and the EDAS metrics were not calculated, we requested that USU provide the actual “raw” data and/or GEI back-calculated to the raw data using the lab split and split count data.

One potential difference between laboratories who do taxonomic identifications of macroinvertebrate samples is a difference in the level (order, family, genus, species) to which organisms are typically identified. In general, Timberline and USU appeared to consistently be targeting identification of most organisms to the same level, preferably genus or species for most taxonomic orders or groups. A few minor differences that would not affect analysis were noted. For example, the true fly *Antocha* was identified as *Antocha monticola* by USU but left at *Antocha* sp. by Timberline. In addition, only USU identified any *Brachycentrus* spp. as *B. echo*, and only Timberline identified any *Hydropsyche* spp. as *H. occidentalis*. Similarly, Timberline identified the species of *Potthastia* and *Rhyacophila* as *P. longimanus* and *R. coloradensis*, while the likely same organisms were identified as *P. longimanus* group and *R. coloradensis* group by USU. EDAS requires that species level identifications be reduced to the genus level before initiating metric calculation, so these discrepancies were resolved at that step. All of these differences would have been standardized before the MMIs and EDAS metrics were calculated. We would not expect these minor differences to have much impact on data output.

With some groups of organisms, identifications to these levels are difficult or uncertain, and they rely on features that may only be present in mature or intact organisms or that may only be visualized through slide mounts or higher levels of magnification than a standard

dissecting and compound microscope provide. This appeared to result in some discrepancies in the level of identification some organisms were identified by both laboratories; however, this is unavoidable.

**In summary:**

- Both laboratories appeared to identify taxa to the lowest possible level with few differences that could be related to effort or available literature.
- While there were some differences in lab outputs, we do not anticipate that the difference in laboratories unduly influenced the analyses necessary to answer the questions in these studies once all sample data were run through EDAS to standardize for a 300-count for organisms and to calculate all metrics with the standardized data.

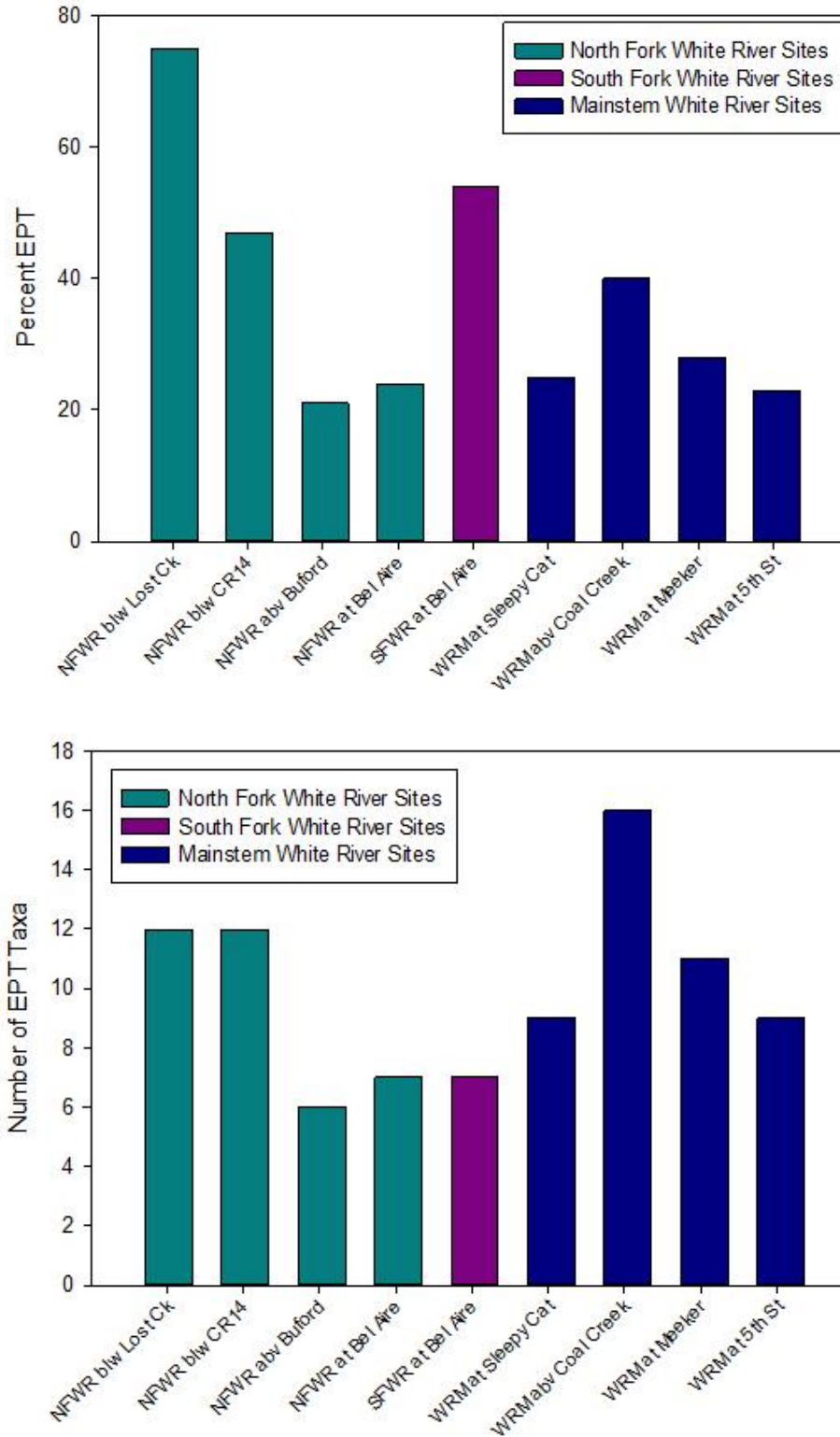
## 7. Spatial and Temporal Differences in Macroinvertebrate Communities

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### 7.1 Spatial Differences

In order to determine if macroinvertebrate communities varied among sites, differences in metric values were examined among data sets collected during the same year and utilizing the same methods, i.e. the three sets of comparisons were: the nine sites sampled with kick nets in 2017, the 10 Hess and 10 kick samples in 2018, and the three kick samples and the 11 Hess samples in 2019. Initially, the metric values and community composition were compared without regard to whether *Cladophora* was present at these sites. Evaluation of the spatial differences among sites focused on the EDAS metrics describing composition and tolerance (Table 1, Figures 1-5).

When looking at various metric values at the sites sampled in 2017, some patterns were apparent. In general, several metrics at the NFWR sites were most favorable at one or both of the upstream sites sampled (the NFWR below Lost Creek site and NFWR below CR14) and often the least favorable at the most downstream site (the NFWR at Bel Aire). Metrics in which this pattern was most notable included the MMI scores, percent EPT individuals (Figure 8), number of EPT taxa (Figure 8), % Chironomidae, HBI, and number of intolerant taxa. Only a single SFWR site (SFWR at Bel Aire) was sampled in 2017; most metric values at this site were within the range observed at the NFWR sites. Metric values such as the MMI, number of EPT taxa, number of intolerant taxa, and diversity index values were similar between the SFWR at Bel Aire and the NFWR at Bel Aire site. At the WRM sites, the WRM above Coal Creek site had the most favorable values for many metrics, such as the MMI, percent EPT individuals, number of EPT taxa, HBI, and number of intolerant taxa. Graphs of the 2017 percent EPT individuals and number of EPT taxa are presented to demonstrate the patterns discussed above (**Error! Reference source not found.**); the graphs in Section 4 that include all the data from 2017 through 2019 also demonstrate these patterns (Figures 1-5).

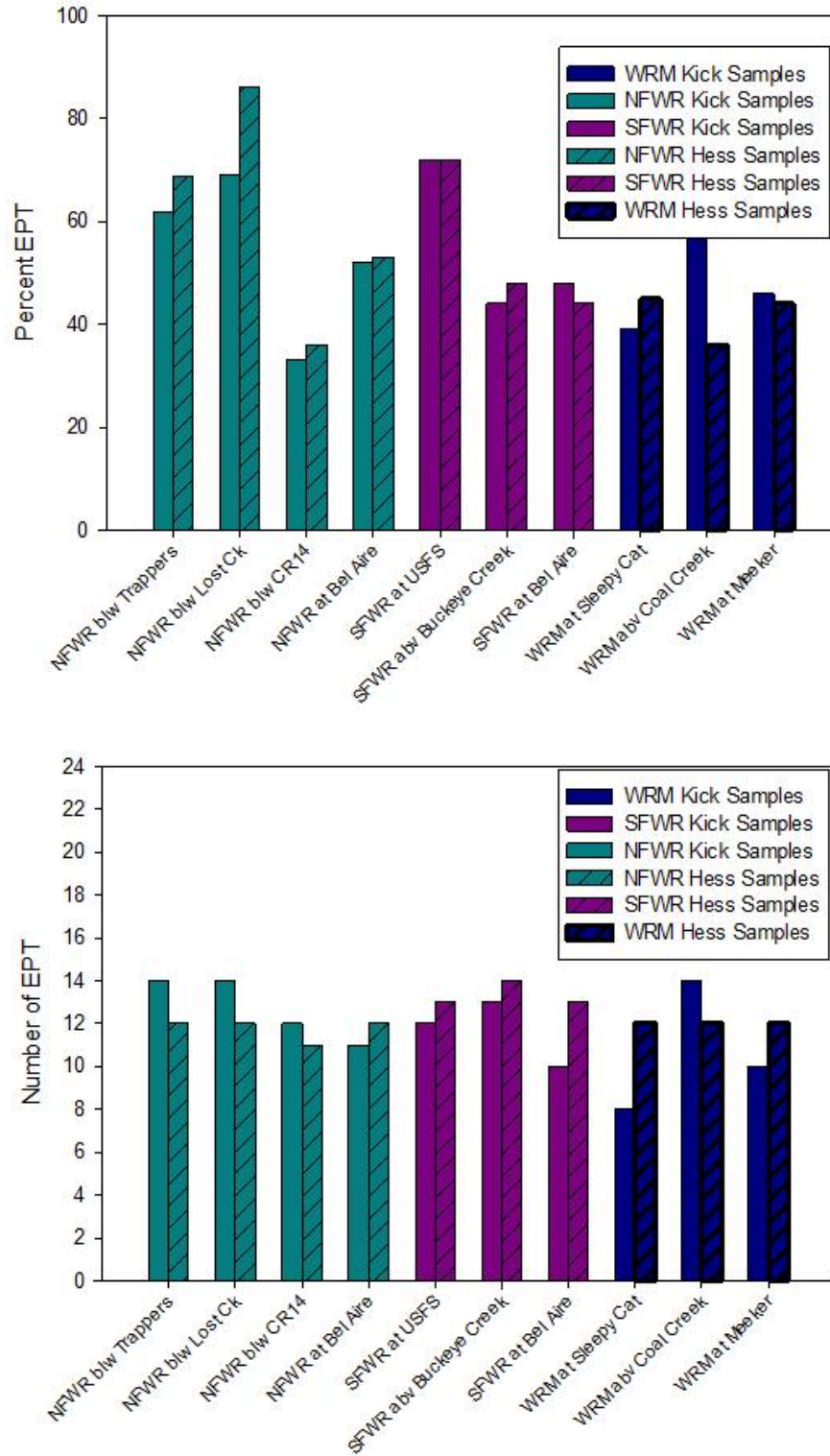


**Figure 8: Comparison of percent Ephemeroptera, Plecoptera, and Trichoptera (EPT) individuals (top) and EPT taxa (bottom) at sites on the North Fork White River (NFWR), South Fork White River (SFWR), and White River Mainstem (WRM) in 2017.**

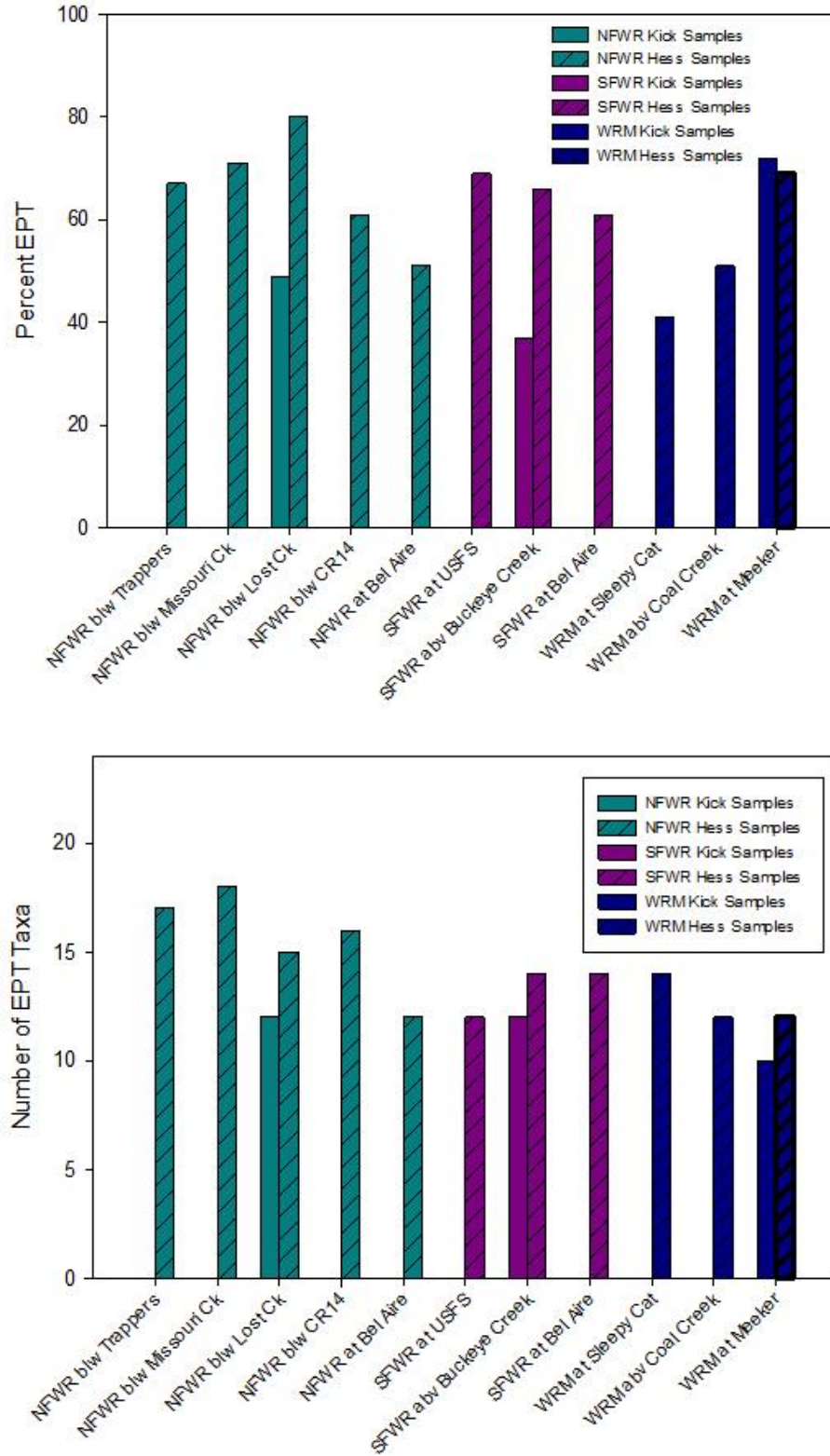


Similar to 2017, multiple metrics in the 2018 Hess and kick samples had more favorable values at the NFWR below Lost Creek compared to the site immediately upstream (which was not sampled in 2017) and the two downstream sites. This pattern was noted in the MMI scores, percent EPT individuals (Figure 9), HBI, and %Chironomidae individuals (Figures 1-5). Other metrics varied more from upstream to downstream, and the most downstream site at Bel Aire did not consistently have the least favorable metric scores as often occurred in 2017 (Figure 9). At the SFWR sites, the patterns in the Hess samples often differed from the patterns in the kick samples. While some metric values were highest at the SFWR above Buckeye Creek site, this pattern was not apparent among all or most metrics. Within the White River mainstem sites, no consistent patterns were observed from upstream to downstream, and no site consistently had more favorable metric values than the other sites. Graphs of the percent EPT individuals and number of EPT taxa are presented to demonstrate the patterns or lack of patterns discussed in the 2018 data (Figure 9); the graphs in Section 4 that include all of the data from 2017 to 2019 also demonstrate trends in the 2018 data (Figure 1-5).

Some spatial trends were observed from upstream to downstream in 2019 (Figures 1-5). Within the NFWR sites, several metric values were most favorable at the NFWR below Missouri Creek site, one of the upstream sites that was only sampled in 2019, and least favorable at the NFWR at Bel Aire site, including the MMI, the number of EPT taxa (Figure 10), HBI, number of intolerant taxa, and % Chironomidae. To a lesser extent, the same pattern from upstream to downstream was detected in the SFWR sites, with percent EPT individuals (Figure 10), HBI, % Chironomidae, and to a lesser extent, the number of intolerant taxa (Figures 1-5). The %Ephemeroptera individuals was low at all three SFWR sites sampled in 2019. The WRM sites displayed less consistent trends. Some metric values were most favorable at the most downstream site, including % EPT individuals (Figure 10), HBI, percent Chironomidae, and % Ephemeroptera individuals of all individuals (Figures 2-5). Other metrics such as the number of EPT taxa (Figure 10) and number of intolerant taxa decreased downstream. As with 2017 and 2018, graphs of the percent EPT individuals and number of EPT taxa are presented as patterns in these two metrics were discussed above for the 2019 data (Figure 10); the graphs in Section 4 that include all of the data from 2017 to 2019 also show patterns or lack thereof in some of the other metrics (Figure 1-5).



**Figure 9: Comparison of percent Ephemeroptera, Plecoptera, and Trichoptera (EPT) individuals (top) and number of EPT taxa (bottom) at sites on the North Fork White River (NFWR), South Fork White River (SFWR), and White River Mainstem (WRM) in 2018.**



**Figure 10: Comparison of percent Ephemeroptera, Plecoptera, and Trichoptera (EPT) individuals at sites on the North Fork White River (NFWR), South Fork White River (SFWR), and White River Mainstem (WRM) in 2019.**

The PCT and BCDI were calculated for these data to determine whether sites that were closer together were more similar than those that were further apart. Tables with the PCT and BCDI values for each pair of sites for each year and sample type of data are included in Appendix B. Over the three years of data with sites of the same sample type (i.e., kick or Hess) compared to every other site within the same year, the PCT metric ranged in value from 10.8 to 57.1%, while the BCDI ranged from 0.25 to 0.92. For comparison, the PCT varied from 40 to 67% in the duplicate samples analysis, while the BCDI ranged from 0.17 to 0.57, suggesting that higher taxonomic similarity was observed in some but not all of the duplicate samples. The two comparisons with the highest BCDI were the WRM above Coal Creek site compared to the SFWR at the USFS campground site (2019 Hess data) and the WRM at Meeker Pasture and the SFWR at the USFS campground site (2018 Hess data). These results suggest that the tributary sites differ from the mainstem sites.

The PCT categorized the most taxonomically similar sites as the NFWR at Bel Aire and SFWR at Bel Aire sites in the 2017 kick data. The BCDI identified the most similar sites (i.e., the comparison with the lowest index value) as the SFWR at the USFS campground site compared to the NFWR below Missouri Creek site in the 2019 Hess data. While these pairs of sites were different, in both cases, these sites were on different tributaries but were similarly placed within the drainages, i.e., both the sites near Bel Aire were just above the confluence with the mainstem on the separate tributaries, while the other two sites were higher up in the drainages. There was also some evidence with the similarity index data that the sites located closer to each other were more similar; however, this pattern was not consistent (Appendix B).

Statistical analyses were conducted to determine if differences between streams were apparent when data from 2017 through 2019 were combined. As most metrics differed little when the two sample types were compared, Hess and WQCD kick data were both utilized in these comparisons to increase the sample size ( $n = 18, 11, \text{ and } 14$  for the NFWR, SFWR, and WRM sites, respectively); however, this may have introduced some variability. Analysis was conducted on the basic metrics, as well as the other metrics describing the composition and tolerance of the macroinvertebrate communities (Table 1), utilizing either ANOVA or its non-parametric alternative, the Kruskal-Wallis ANOVA on ranks.

The following metrics had statistically more favorable average or median values at the NFWR and SFWR sites compared to the WRM ( $p \leq 0.003$ ):

- HBI
- % EPT individuals excluding Baetidae of all individual
- % intolerant taxa, number of intolerant taxa
- % intolerant taxa of all taxa
- % Trichoptera individuals

In contrast, median values for these metrics were significantly greater at the WRM compared to one or both tributaries ( $p \leq 0.009$ ):

- % Diptera
- % Ephemeroptera individuals

In addition, the NFWR and WRM sites had significantly higher median values of Coleoptera (beetle) taxa ( $p = 0.006$ ). Comparisons of the median or average values for all other metrics were not significant ( $p \geq 0.052$ ).

Based on these analyses, the macroinvertebrate assemblages at the tributary sites support more EPT individuals and higher percentages and higher abundances of intolerant taxa, which could be a reflection of differing water quality or other habitat conditions. However, mayflies and true flies, which are generally thought of as intolerant and tolerant groups, respectively, were both higher at the WRM sites. At many of the WRM mainstem sites, the most abundant taxon was the mayfly *Ephemerella* sp.; however, in comparison to many other mayfly genera, the taxon is moderately tolerant. In addition, when abundances for all taxa within each order were combined, true flies were the dominant order.

While the ANOVAs utilized to determine if statistical differences existed among the three streams inherently accounts for the variability in metric values within each stream to an extent, with small data sets, there is a chance of finding “significant” differences that do not actually exist. In addition, these tests do not directly factor in the duplicate analysis results showing high variability in some metrics, so the results of the statistical analysis should be viewed in the context of the other analyses and observations as only one part of the approach to data evaluation.

To assess the significant differences between these streams in the context of the variability observed within the duplicate samples analyses, the mean difference observed in the duplicate samples was compared to the differences in the mean metric values for each stream to provide some context. The magnitude of the differences observed among the streams for the HBI, % intolerant taxa, and % Ephemeroptera individuals metrics exceeded the average and, for percent intolerant taxa, the maximum difference observed in the duplicate samples, supporting further that the streams differed in these respects. However, the differences among streams in the % EPT excluding Baetidae, % Trichoptera individuals, and % Diptera individuals did not have larger differences in mean values among streams than the average differences in these metrics among duplicate samples. Despite the statistical differences noted, the high variability in these metrics even when samples are collected at the same site and time indicate that further data may be needed to determine if the differences among streams detected in the statistical analyses continued to be supported.

**To summarize:**

- Metric values were often more favorable at one of the upstream sites on the NFWR compared to the downstream site; however, this pattern was not consistent across all years or all metrics. Patterns among sites within the SFWR and WRM differed even more among metrics and years. In addition, the high variability in some metric values observed in the duplicate sample analyses suggests that further data collection may be necessary to verify if the patterns that were observed continued to be supported.
- Similarity indices indicated that in general the taxonomic composition at the tributary sites were more similar to one another and less similar to the WRM sites.
- Geographic location also appeared to influence the similarity, as the two sites on the NFWR and SFWR that were near the confluence with the White River had relatively similar macroinvertebrate communities.
  - These sites are similarly situated in the watershed and also geographically close via aerial distance, despite being on different tributaries.
  - Aerial dispersal or comparable habitat conditions could account for the similarity in macroinvertebrate assemblages.
  - Sites located in the upstream regions of both tributaries also were more similar to each other than many of the other sites, and, in some cases, adjacent sites tended to have more similar macroinvertebrate communities to one another than those further off.

## 7.2 Temporal Differences

Initial examination of the range of values for the MMI, number of taxa, number of EPT taxa, HBI, diversity index values, number of intolerant taxa, %Ephemeroptera, and %Diptera (Table 1, Figures 1 – 5), suggested there were no striking differences in the range of values observed for these metrics between years, with values overlapping each year. At most sites, MMI scores for Hess samples were greater in 2019 compared to 2018 (Figure 1), and the %Ephemeroptera individuals in the kick samples was often higher in 2018 than 2017 at most sites, with no consistent increase or decrease in this metric between 2018 and 2019 (Figure 3). No other patterns among years were clearly apparent in the graphical data.

Observations of differences over time were limited as not all sites were sampled in all three years, and sampling protocols changed over time. Three NFWR sites were sampled in all three years, including the sites below Lost Creek, at County Road 14, and at Bel Aire (Table 2). Only one of these sites was sampled in all three years with consistent protocols: the NFWR below Lost Creek, which had kick samples collected in all three years, as well as Hess samples collected in 2018 and 2019. Only the most downstream SFWR site was sampled in all three years, with kick samples collected in 2017 and 2018 and Hess samples collected in 2018 and 2019. Three of the four sites sampled on the WRM were sampled in all three years, albeit with differing methods for all sites except the one at Meeker Pasture,

which had kick samples collected in all three years and Hess samples collected in 2018 and 2019.

To statistically investigate differences in the macroinvertebrate assemblages over time, linear regression analysis was utilized for each of those sites that had three years of data. As with the spatial analysis, data from both kick and Hess samples were utilized to increase the sample size; even so, this analysis is limited in statistical power with sample sizes that range from three to five. Multiple sites, including all of the WRM sites, had no trends over time observed ( $p \geq 0.078$ ). A few sites had significant trends observed ( $p < 0.050$ ) in a few metrics; however, the limited number of years and data points ( $n = 5$  or  $n = 3$ ) over which these trends were tracked, in addition to the high variability observed in some metric values in the duplicate samples, suggests that they should be viewed with caution as only initial indications that any changes are occurring. Further data collection in upcoming years would be necessary to determine if these are true trends or if instead annual variability becomes more apparent over time.

The initial trends detected over time at these sites are as follows; as stated above, further data collection over a longer time period would be necessary to determine if they persist:

- Decreasing trends in the non-insect metrics at the NFWR at Bel Aire site, likely driven in part by higher abundances of water mites (Hydrocarina) in 2017 compared to later years.
- Trends over time at the NFWR at Lost Creek site, particularly in the kick data, that suggested that the numbers of tolerant taxa and abundances of these taxa increased from year to year, while intolerant taxa numbers and abundances decreased. Decreasing relative abundances of caddisflies appear to have contributed to these trends in part.
- MMI scores at the SFWR at Bel Aire site increased by six or more points from 2017 through 2019 from year to year.

Differences in stream flows from year to year can often be a factor influencing macroinvertebrate communities. Peak flow data for the USGS gage on the White River near Meeker indicated that peak flows were substantially higher in 2019 than in 2017 and 2018. Peak flows in 2017 and 2018 were more similar, but flows were somewhat lower in 2018. Higher peak flows might initially impact macroinvertebrate communities adversely, as some organisms would be displaced downstream. However, at the time of sampling in the late summer/early fall after flows have decreased, such effects would likely not be apparent, and, if the higher snowmelt levels resulted in more sustained baseflows, macroinvertebrate communities would likely benefit. The graphical representations of the data (Figures 1-5) indicate that there are no consistent patterns of more (or less) favorable metric values in 2019 compared to the two earlier years of the study, other than MMI scores in Hess samples tending to be higher in 2019 compared to 2018 at many sites (Figure 1).

**In summary:**

- Trends over time are difficult to detect as only three years of data exist for some of the sites, and other sites were only sampled in one or two of these years.
- Based on our professional experience, natural variability in macroinvertebrate communities is often high from year to year and between different locations on the same stream.
- In addition to the high natural variability, macroinvertebrate sampling techniques target a small portion of the substrate. Despite attempts to standardize sample collection for substrate, depth, and flow (all of which not only vary within a site but certainly vary among years), microhabitat requirements for macroinvertebrates are very specific and not necessarily detectable to sampling personnel, resulting in further variation.
  - The eight-Hess composite samples collected in 2018 and 2019 would minimize this variability, but the single kick samples (albeit over a larger area) may be more susceptible to over or under-representing some taxa.
- The trends that were detected were isolated to a single site and did not extend to tributary or mainstem-wide trends. These trends should be considered with caution based on the high variability in the duplicate data for some metrics and the limited amount of data available; additional data collection would be necessary to determine if they persist or if annual variability becomes more obvious with a larger data set. Site-specific factors appear to be influenced these initial trends, but, as such, these factors are difficult to identify based on the data available as they do not appear to relate to broader scale climate or flow-influenced factors that would be expected to effect multiple sites and streams.



## 8. Effects of *Cladophora*

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### 8.1 Literature Review

Dense growths of *Cladophora* have been observed at sites on the NFWR and WRM over the course of this study, and one of the main objectives of the macroinvertebrate sample collection was to determine if these filamentous algal growths are affecting the macroinvertebrate assemblages within this watershed. Prior to conducting these analyses, a literature review was completed to assist in focusing our efforts on the macroinvertebrate taxa and groups that might be expected to be affected by the *Cladophora* growths. Based on the time limitations, this review was not comprehensive, but it did guide metric selection prior to analysis.

In general, excessive growths of *Cladophora* are considered a nuisance and associated with eutrophication of waters (Dodds and Gudder 1992). *Cladophora* growths can adversely impact macroinvertebrate communities through decreased attachment sites, food resources, and habitat, as well as through large diurnal shifts in dissolved oxygen that some organisms cannot tolerate (Patrick et al. 1983; Dodds and Gudder 1992; Ellsworth 2000; Tonkin et al. 2014). However, interactions between *Cladophora* growths and macroinvertebrate communities are complex, with these growths benefiting macroinvertebrate assemblages in other ways. Some macroinvertebrates graze directly on *Cladophora* (Feminella and Resh 1991; Dodds and Gudder 1992; Tinsley et al. 2016; Jansen 2018). In addition, *Cladophora* growths may increase substrate complexity in some cases, providing interstitial habitat and protection from flow disturbance and predation. *Cladophora* also provides substrate for epiphytic growth of other types of algae in some cases; these epiphytic growths serve as a food resource for some invertebrates (Patrick et al. 1983; Dodds and Gudder 1992).

Many species that are present in the White River drainage are known to graze on *Cladophora* or its epiphytes, including the amphipod *Hyaella*; caddisfly genera such as *Agapetus*, *Helicopsyche borealis*, *Hesperophylax*, and *Hydropsyche*; the stonefly family Capniidae; the true bug (Hemiptera) *Sigara*; the snail (Gastropoda) *Lymnaea*, the crane fly *Tipula* sp., the blackfly *Simulium*, and the chironomid *Eukiefferiella* (Feminella and Resh 1991; Dodds and Gudder 1992; Tinsley et al. 2016; Jansen 2018). *Cladophora* may comprise large proportions of the diet of some taxa seasonally; Tinsley et al. (2016) noted that up to 98% of the gut content of the caddisfly genus *Hydropsyche* was comprised of *Cladophora* at certain times of the year. However, some studies suggest that *Cladophora* may be less preferable as a food resource for macroinvertebrate grazers compared with the epiphytes it hosts (Patrick et al. 1983; Dodds and Gudder 1992). A study by Dobbs (1991) indicated that grazers, predominately *Baetis tricaudatus*, *Tricorythodes minutus*, and *Brachycentrus occidentalis* removed up to 75% of the epiphytes within the *Cladophora* growths, suggesting that the primary benefit of filamentous algae to these taxa is that it provides substrate for epiphytic

diatoms. Furthermore, some evidence exists that some macroinvertebrate grazers may remove, without ingesting, unfavorable algal forms to allow favorable forms to remain for grazing (Tonkin et al. 2014).

Mature *Cladophora* is postulated to deter grazing by invertebrates through chemical defenses that are lacking in the initial growth phase (Ellsworth 2000), and there was evidence that when the snail (Gastropoda) *Physa* did consume *Cladophora*, egg production was curtailed (Patrick et al. 1983). Filamentous algae often has high cellulose content and thick walls, which may make digestion difficult (as reviewed in Tonkin et al. 2014).

While taxa that feed upon *Cladophora* or the epiphytic algae associated with it may benefit from these algal growths, other taxa are adversely affected. These complicated interactions have resulted in mixed responses when *Cladophora* proliferates, with some studies reporting negative associations with macroinvertebrate diversity, density, and number of taxa, and others reporting positive associations (Dodds and Gudder 1992; Ellsworth 2000, Ward and Ricciardi 2010; Tonkin et al. 2014; Jansen 2018). For example, Ellsworth (2000) found positive relationships between *Cladophora* density and densities of *Baetis*, *Simulium*, and Chironomidae, but found a negative correlation between *Cladophora* density and overall diversity. The negative relationship with diversity was linked to the *Cladophora* growths favoring the few taxa mentioned, resulting in a decrease in evenness in the macroinvertebrate assemblage as a whole. Tonkin et al. (2014) found that macroinvertebrate diversity in general responded negatively to percent cover with filamentous algae, likely due to competition for space. Percentage of EPTs also declined in this study, but only at the site with the highest level of percent cover. Ward and Ricciardi (2010) also summarized results of a study conducted by Hart (1992) that reported that densities of chironomids as well as several sensitive species, including a heptageniid mayfly, two stonefly taxa, two caddisfly species, and a tipulid species (*Antocha*) were positively associated with *Cladophora* cover. In contrast, other studies noted that the dense growths of filamentous algae lead to displacement of sensitive taxa by more tolerant taxa such as many chironomid midges that can tolerate the large diurnal fluctuations in dissolved oxygen that tend to occur when density of filamentous algae is high (Dodds and Gudder 1992; Tonkin et al. 2014).

The response of one specific taxon, the black fly *Simulium*, points to the complexity of interactions between *Cladophora* growths and macroinvertebrate assemblages. A review of the literature by Ward and Ricciardi (2010) found that *Cladophora* was in some cases associated with increased density and taxonomic richness within the macroinvertebrate assemblage, but that some true fly taxa such as *Simulium* were negatively affected, likely through competition for attachment space on substrates. This contrasted with Ellsworth (2000), as discussed above, which found positive relationships between *Simulium* and *Cladophora* densities, suggesting that *Simulium* may have utilized the epiphytic growths within *Cladophora* as a food resource. The differing results in this study reviewed in Ward and Ricciardi (2010) may have resulted from more limited substrates ideal for attachment of

either taxa. *Simulium* is a filter-feeder that generally attaches to bare substrates, with high numbers often observed in patchy distributions on the same type of hard substrates that *Cladophora* colonizes.

## 8.2 Metric Selection

As with the previous analyses, the basic metrics and the ones describing the composition and tolerance of the macroinvertebrate communities (Table 1) were included in the analysis of the potential effects of *Cladophora* on macroinvertebrate communities. In addition, based on the review above, dense growths of *Cladophora* would be expected to cause shifts in macroinvertebrate assemblages with respect to the FFG and Habit metrics, so these sets of metrics were also included (Table 1). Two of the FFGs consume algae as a significant portion of their diet. Scraper taxa are those that graze on algae (largely diatoms) attached to hard substrates or other surfaces, and collector-filterer taxa are suspension feeders that can also target diatoms or other algae in the water column (Cummins et al. 2008). Not all taxa within these groups would directly feed upon *Cladophora* (Jansen 2018), but likely they could utilize the epiphytic diatoms associated with it as a food resource. Chironomid midges were noted to be the most common grazers when *Cladophora* growth was at its peak in Jansen (2018); many chironomid midges are categorized as scrapers or collector-filterers as their primary or facultative FFG (Ferrington et al. 2008). Filamentous algae such as *Cladophora* may also retain organic debris that also supports other groups of taxa such as shredders (Tonkin et al 2014).

While only a single study was located that associated *Cladophora* with positive or negative associations with any of the habit groups, logically we would expect that clingers, sprawlers, or climbers could be positively associated with *Cladophora* growths. Clingers build fixed retreats and/or have other adaptations such as tarsal claws that would allow them to “cling” to surfaces, including potentially filamentous algae. Clinger abundance was positively correlated with decaying *Cladophora* in a study by Grandinetti (2016). Sprawlers, which include many of the chironomid midges, inhabit the surface of leaves or hydrophytes, and climbers are adapted to live on overhanging branches, roots, vegetation, and submerged brush. The Grandinetti (2016) study also evaluated whether increases in sprawlers were associated with *Cladophora* growths, but no relationship was detected. This study also cited a second study (Highsmith 1985) that indicated that climbers colonized *Cladophora* quicker than sprawlers, likely because sprawlers more often colonize flatter surfaces rather than filamentous strands.

## 8.3 Analysis

In each year of sampling, the presence of the filamentous green algae, *Cladophora*, was noted at each site sampled. Dense growths of *Cladophora* were not observed at any of the SFWR sites in any year and were also not observed at the three upstream NFWR sites, including the sites below Trappers Lake, below Missouri Creek, and below Lost Creek (M.

May, CPW, personal communication, September 2020). The three downstream NFWR sites, including the sites at County Road 14, above Buford, and Bel Aire, all had *Cladophora* observed, as did all four of the WRM sites. Based on this, two of the nine sites sampled in 2017, five of the ten sites sampled in 2018, and six of the 11 sites sampled in 2019 did not have dense growths of *Cladophora*. The following analyses compare macroinvertebrate communities and metrics between sites with and without *Cladophora* growths.

Based on our literature review, our approach to these analyses was to first determine if any broad differences in taxonomic composition were detected between sites with and without *Cladophora* growths. Based on this, the dominant taxon and group at these sites were compared to determine if any patterns were apparent; the results of the statistical analyses discussed below also provided information on other taxonomic shifts that could be related to *Cladophora*. The literature review also identified some specific taxa that occur in the White River drainage that were influenced by *Cladophora* growths; the presence/absence and relative abundance of these taxa were evaluated as well to determine if any of these could be considered indicator taxa that were or were not present at the *Cladophora* sites. Following our initial evaluation of any differences in taxonomic composition, the extensive list of metrics in Table 1 were compared statistically utilizing t-tests or the Mann-Whitney U-test between the two groups of sites, with select comparisons presented graphically. Of note, most graphs are presenting the same data as were included in the earlier figures, but the sites have been rearranged so that differences between the *Cladophora* and non-*Cladophora* sites could more easily be discerned. The PCT and BCDI values (Appendix B) were also evaluated to determine if sites with or without *Cladophora* growth were more similar within each group.

The initial evaluation of the taxonomic composition of the sites with and without *Cladophora* indicated that 70% of the sites (14 of 20 sites) without *Cladophora* growths were dominated by caddisflies, with various caddisfly species, most commonly *Lepidostoma*, being the most abundant taxon at most of these sites. In contrast, true flies were more commonly the dominant group at the sites with *Cladophora*, with 61% of these sites (14 of 23 sites) dominated by this group. The most abundant taxon at the sites with *Cladophora* varied extensively, although the mayfly *Ephemerella* was most commonly the dominant taxon, particularly at the WRM sites.

The literature review that was conducted listed several taxa that other studies indicated had positive or negative relationships to *Cladophora* growths. While the taxa mentioned were each present at least one site within the White River drainage, several of these taxa were observed infrequently and at low abundances, so no relationship with or without *Cladophora* growths could be investigated. Of the taxa more frequently present, no difference in relative abundances between the two groups of sites was observed with *Brachycentrus*, *Hydropsyche*, or *Antocha*. The taxa that differed more between the two groups of sites were the mayfly *Baetis*, the chironomid *Eukiefferiella*, and the black fly *Simulium*, all three of which were

found at higher relative abundances at sites with *Cladophora* growths. The differences were moderate. For example, *Baetis*, *Eukiefferiella*, and *Simulium* on average comprised 5.3, 8.0, and 3.3% of the total abundance at the sites with *Cladophora*, respectively, compared to 0.9, 3.4, and 0.6% of the total abundance at the sites without *Cladophora*. All three of these taxa were noted in one or more studies to be grazers on *Cladophora* or its epiphytes, so increased abundances might be expected although *Simulium* was also observed to be negatively affected by *Cladophora* growths based on competition for attachment sites.

Statistical analyses of metric values when data for the kick and Hess samples for all sites were combined highlighted some key differences between sites with (n = 23) and without (n = 20) *Cladophora* growths (Appendix C). While the data set for this analysis is larger than for the previous analyses, as mentioned earlier, statistical analyses of smaller datasets carry the risk of finding “significant” differences that do not actually exist. In addition, these tests do not directly factor in the results of the duplicate analysis that indicated that high variability in some metrics existed. Based on this, the results of the statistical analysis should be viewed in the context of the other analyses and observations as only one part of the approach to data evaluation:

- Of the basic metrics, MMI and the total number of taxa did not differ significantly between the two groups, while diversity index values were higher at the sites with dense *Cladophora* growths (Figure 11).
- More favorable values for metrics associated with EPT and intolerant taxa occurred at the sites without *Cladophora* growths, including the number of EPT taxa, % EPT individuals of all individuals, and HBI metrics (Figure 11, Figure 12).
  - An exception to this occurred with the % Ephemeroptera metric, which was significantly higher at sites with *Cladophora* than without *Cladophora* growths.
  - The difference among the two groups of sites in the % Ephemeroptera metric was reversed when baetid mayflies were excluded. Baetid mayflies are a common mayfly family that is abundant throughout Colorado and are moderately tolerant of disturbance and pollution.
- Following the trends in intolerant taxa metrics, multiple metrics describing the number and percentage of tolerant taxa were significantly higher at the sites with *Cladophora* growths.
  - Chironomids (Figure 13) and other true flies were more abundant at the sites with *Cladophora* growths.
- Statistical analysis of the FFG metrics (Appendix C) also indicated that differences existed between the sites with and without *Cladophora* growths.
  - Sites without *Cladophora* consisted of higher percentages of collectors, scrapers, and shredders. These differences did not necessarily align with the literature review.

- Collectors and scrapers both utilize diatoms as a primary food source. The epiphytic growths within *Cladophora* mats are often diatoms; however, *Cladophora* growths may also limit diatoms to an extent by the extensive coverage on cobbles and boulders that diatoms also colonize.
- Shredders were predicted to potentially benefit from *Cladophora* growths under the assumption that detritus might be trapped within these growths. However, the caddisfly *Lepidostoma* is an obligate shredder (Wiggins and Curry 2008) that more commonly dominated the macroinvertebrate assemblages at the sites without *Cladophora*; this taxon likely heavily influenced this metric, although it is unknown if the differences in the abundances of this taxon at the two groups of sites was related to food availability or some other factor.
- With the habit metrics, the percent climbers and number of clinger taxa were greater at the sites without *Cladophora*, while the percentage of sprawlers and number of sprawler taxa were greater at the sites with *Cladophora*.
  - While the literature review indicated that climbers also might be expected to be greater at sites with *Cladophora* since they have specialized adaptations that would appear to be adept at attaching to filamentous algae (Highsmith 1985 as cited in Grandinetti 2016); other factors may be influencing these metrics more.
  - Many of the caddisflies present within the White River drainage are categorized as clingers, and caddisflies were statistically less abundant on average at the sites with *Cladophora* as well; likely these relationships are tied together. If caddisfly taxa are less common at sites with *Cladophora* compared to those without *Cladophora*, then there would likely be a corresponding decrease in the number of clinger taxa, as this group represented a substantial proportion of the clingers present throughout the White River drainage. The decrease in caddisflies and scraper taxa may be related to the FFG but could also be related to some other habit or habitat preference common in caddisflies.

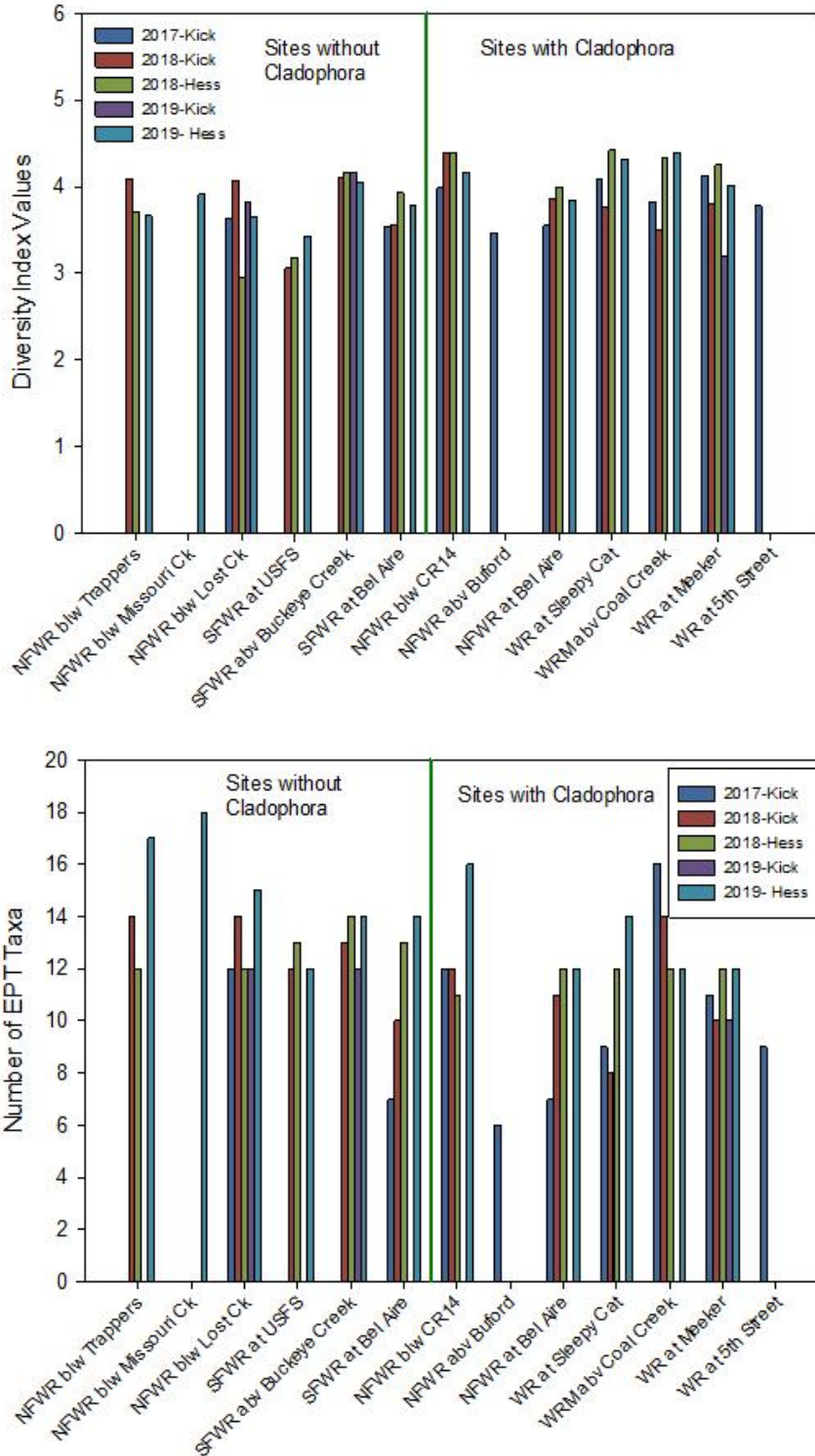


Figure 11: Diversity index values (above) and number of EPT taxa (below) for samples collected at sites on the North Fork White River (NFWR), South Fork White River (SFWR), and White River Mainstem (WRM) with and without *Cladophora* growths sampled in 2017 through 2019.

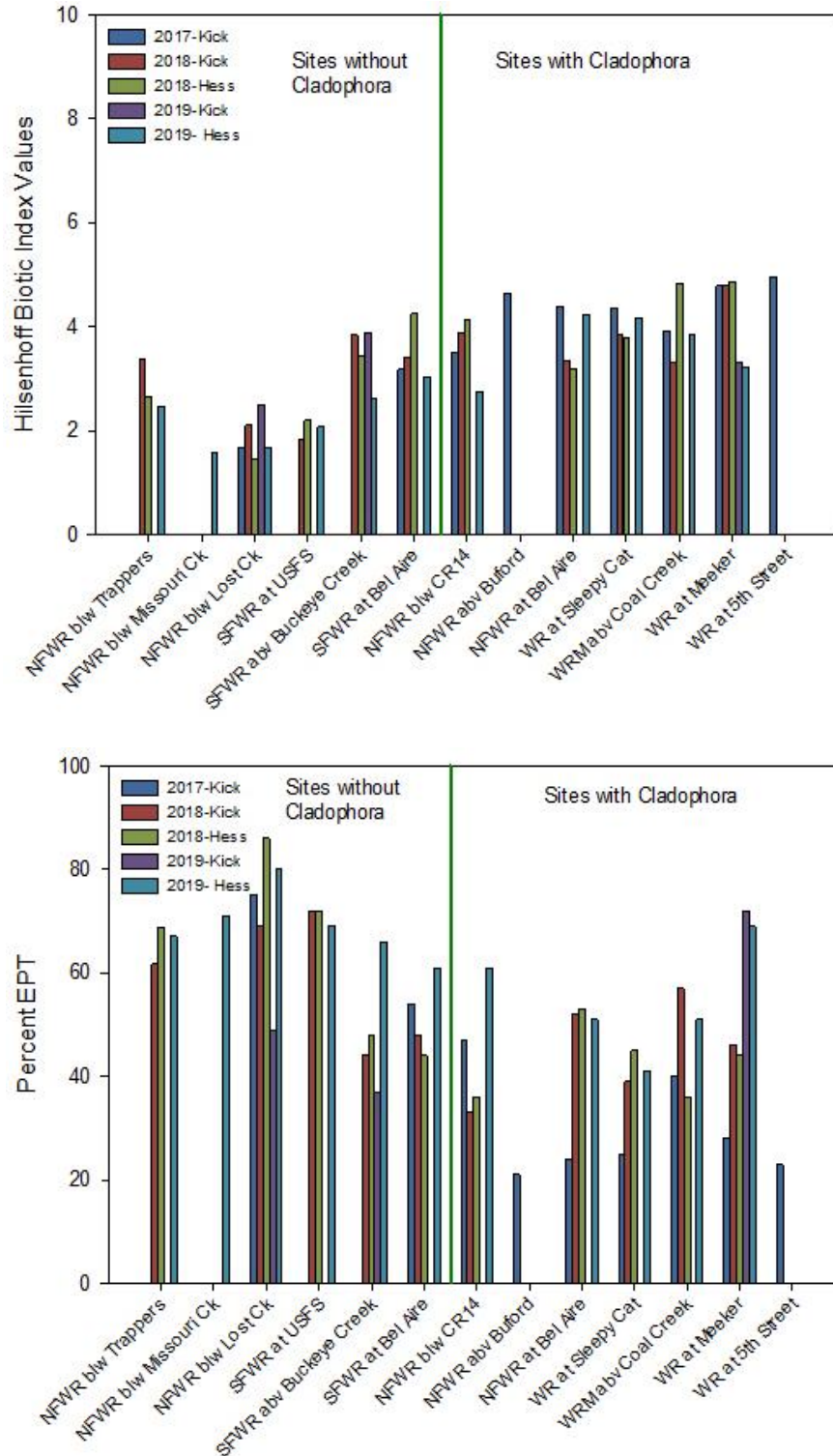
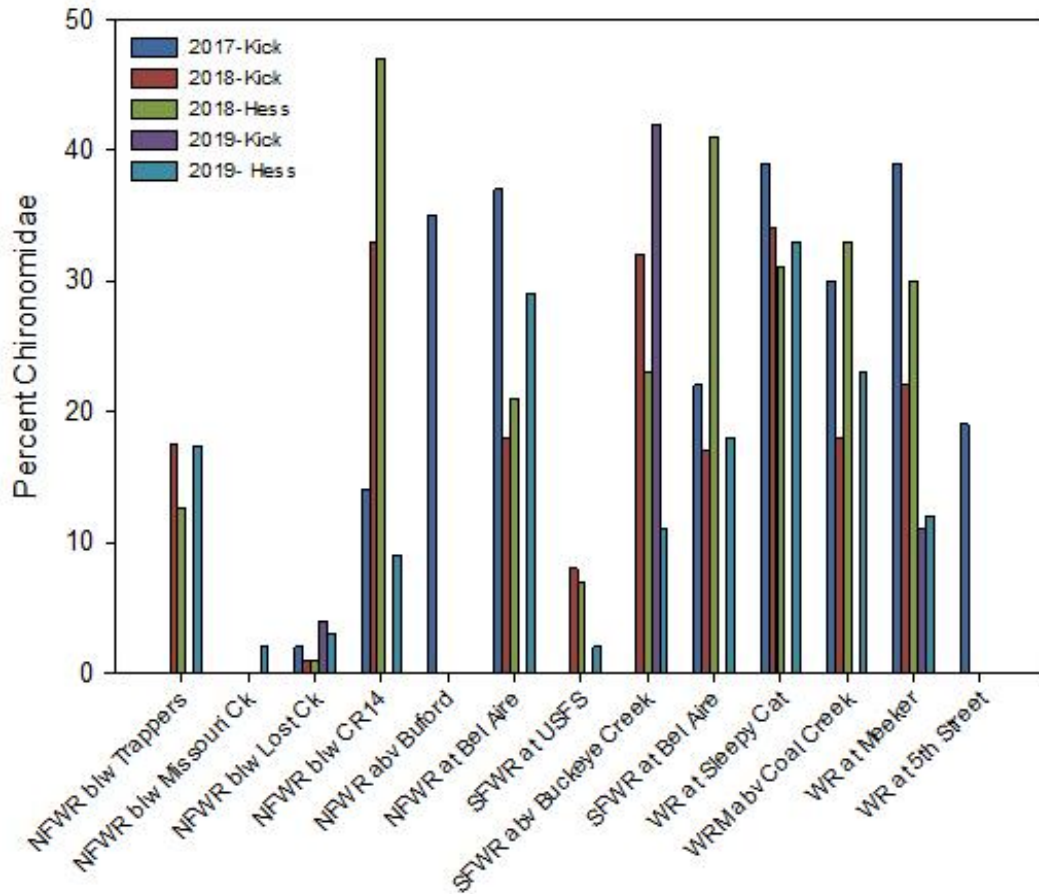


Figure 12: Hilsenhoff Biotic Index (HBI) values (above) and Percent Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa (below) and for samples collected at sites on the North Fork White River (NFWR), South Fork White River (SFWR), and White River Mainstem (WRM) with and without *Cladophora* growths sampled in 2017 through 2019.





**Figure 13: Percent Chironomidae of all individuals for all samples collected at sites on the North Fork White River (NFWR), South Fork White River (SFWR), and White River Mainstem (WRM) with and without *Cladophora* growths sampled in 2017 through 2019.**

Of note, as referenced with the previous analyses, the high variability in some metric values that was observed in the duplicate sample analyses suggests these results should be viewed with some caution. Further data collection may be necessary to verify if these differences continue to exist or are weakened by natural variability over time. However, almost all of the significant differences in mean metric values with and without *Cladophora* were of a magnitude that was greater than the mean differences observed in the duplicate sample analysis. Exceptions to this occurred with the diversity index values, %Ephemeroptera excluding Baetidae, and number of clinger taxa.

As noted in Section 11, the continuation of this study in upcoming years with some adaptations in the sampling methods would strengthen our confidence that the differences detected are associated with actual differences in the macroinvertebrate communities rather than resulting from spatial or annual variability. However, the number of differences detected in the macroinvertebrate assemblages between sites with and without *Cladophora* in this

initial analysis suggest that *Cladophora* blooms may likely be influencing the macroinvertebrate communities.

While not presented specifically here, statistical analysis was also conducted on a more limited dataset that only included the NFWR sites. This was done to determine if differences between the tributaries and mainstem themselves were unduly influencing the comparison between sites with and without *Cladophora*, as the SFWR sites were all grouped as without *Cladophora*, while the WRM sites were all grouped with *Cladophora*. Comparison between these two groups differed somewhat, with some relationships that were significant in the larger dataset no longer being so, particularly for the FFG and habit metrics (Appendix C); however, overall, limiting the dataset in this manner did not change the overall conclusion that the sites with *Cladophora* supported lower taxa richness and abundances of intolerant taxa and most of the EPT groups compared to sites without *Cladophora*. Of note, with the smaller dataset utilized for this analysis, the strength of the statistical analysis is decreased further from that of the analyses of the full dataset, particularly in light of the high variability in some metric values observed in the duplicate analyses.

Utilizing the PCT and BCDI values did not conclusively indicate that benthic assemblages were more similar between sites within the same group; i.e., paired sites with *Cladophora* growths were not more similar than a pair of sites where one had *Cladophora* growths apparent and one did not (Appendix B). For the PCT, often the most similar site pair included one with and one without *Cladophora* growths while the least similar pair occurred within the same group. For example, in 2017, the NFWR at Bel Aire and SFWR at Bel Aire scored the highest PCT value, while the NFWR above Buford and the WRM at 5<sup>th</sup> were the least similar, despite both being potentially influenced by *Cladophora*. The same lack of a consistent pattern was observed in the BCDI values.

To further this analysis, the average PCT and BCT values were used for paired comparisons of sites which both had *Cladophora* growths, both did not have *Cladophora* growths, and had one site with and one site without *Cladophora* growths (Table 4). For the PCT, the average value for the paired sites with one with and one without *Cladophora* was within the range observed between the sites that both had or both did not have *Cladophora* in both datasets for 2018, was slightly above this range in the 2017 dataset, and was slightly below this range in the 2019 dataset. For the BCDI, the paired sites that differed in having *Cladophora* present were slightly more dissimilar than the range observed at the same sites in 2017 and 2019 but was within the range observed in both of the 2018 datasets. Based on these results, there was no consistent pattern of sites being more or less similar taxonomically based on the presence or absence of *Cladophora*. Of note, the BCDI indicated that sites with *Cladophora* were less similar to one another based on the higher BCDI scores than sites without *Cladophora* (Table 4).

**Table 4: Average Percent Common Taxa (PCT) and Bray-Curtis Dissimilarity Index (BCDI) values for each dataset from 2017 to 2019 for paired sites that both had *Cladophora* present, paired sites that both did not have *Cladophora* present, and paired sites with one site that did and one site that did not have *Cladophora* present.**

Metric	Dataset	Average Values		
		Paired Sites with <i>Cladophora</i>	Paired Sites without <i>Cladophora</i>	Paired Sites with one with and one without <i>Cladophora</i>
Percent Common Taxa	2017 (Kick)	37.0	34.2	37.1
	2018 (Hess)	27.5	39.4	31.9
	2018 (Kick)	25.8	42.2	29.3
	2019 (Hess)	38.7	37.2	35.4
Bray-Curtis Dissimilarity Index	2017 (Kick)	0.56	0.44	0.60
	2018 (Hess)	0.72	0.62	0.72
	2018 (Kick)	0.77	0.62	0.71
	2019 (H)	0.65	0.62	0.69

**In summary:**

- Interactions between macroinvertebrate assemblages and dense growths of *Cladophora* are complex, with some taxa potentially benefitting from the food resources, habitat niches, and protection from flows and predation that *Cladophora* provides, while others are adversely affected through competition for attachment spaces, shifts in food resources, reduced availability of hard substrate, diel fluctuations in dissolved oxygen, and increased competition with taxa that are benefitting from *Cladophora*.
- Evaluation of the taxonomic composition of the macroinvertebrate assemblages with and without *Cladophora* growths was not conclusive, although caddisflies tended to be more dominant at the sites without *Cladophora* growths while true flies were more frequently the most abundant group at the sites with *Cladophora* growths.
- Statistical analysis indicated that some metric values differed between sites with and without *Cladophora* growths, suggesting that macroinvertebrate community composition varied between the two groups of sites in some respects. The high variability observed in the duplicate sample analysis should be considered when evaluating these differences; in addition, further data collection would be beneficial to determine if these differences persisted if the dataset was increased over the long-term. Other unidentified factors other than the presence or absence of *Cladophora* growths could also be influencing the macroinvertebrate populations that are not apparent without a larger dataset and more years of data collection..
- Generally, sites with *Cladophora* growths were comprised of more abundant tolerant taxa, specifically chironomid and other true fly taxa. They also exhibited to reduced numbers and abundance of EPT and other intolerant taxa.

- *Cladophora* growths may be impacting sensitive species such as the EPTs through diel variations in dissolved oxygen, decreased appropriate habitat niches, shifts in food resources, or competition with other macroinvertebrates that are favored more by *Cladophora* growths (Dodds and Gudder 1992; Ellsworth 2000, Tonkin et al. 2014).
- Many EPT taxa inhabit riffle habitat with cobble and boulder substrate upon which *Cladophora* preferentially attaches, and thus these groups may be disproportionately affected.
- Chironomids have been noted to increase in reaches with *Cladophora* growths (Dodds and Gudder 1992; Ellsworth 2000; Tonkin et al. 2014). Many chironomids graze on *Cladophora* in the early stages of growth, and also utilize the epiphytes as a food resource (Ellsworth 2000, Jansen 2018).
- FFG and habit metrics were also affected, but not always in the predicted direction, with collectors, scrapers, shredders, and climbers being more abundant at sites without *Cladophora*, although the limited amount of literature indicated that these groups might be expected to benefit from *Cladophora* growths.
- Similarity indices such as the PCT and BCDI did not indicate that pairs of sites with or without *Cladophora* growths were consistently more similar than paired sites in which one was potentially affected by *Cladophora* growths and one was not.

## 9. Insecticide Effect on Macroinvertebrates

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Our investigation of whether aerial spraying of insecticides resulted in adverse and detectable effects on benthic macroinvertebrate communities focused on samples collected from NFWR and SFWR sites in June, July, and August of 2018. Prior to the aerial spraying, two SFWR and two NFWR sites were sampled on June 15: the SFWR above Bel Aire, SFWR at Bel Aire, NFWR below Lost Creek, and NFWR at Bel Aire sites. Of note, at this time, no major *Cladophora* growths were observed, potentially because of the timing of the sampling event.

The first spraying event occurred upstream of CR 14 on the NFWR on June 29<sup>th</sup>. Following this event, the same two sites on each stream were resampled on July 2<sup>nd</sup> or 3<sup>rd</sup>. The SFWR sites did not receive aerial spraying, and the NFWR site below Lost Creek was upstream of the spraying, so only the NFWR site at Bel Aire would be considered potentially impacted by this event. Also, both NFWR sites had dense *Cladophora* growth at the time of this second sampling event, while the two SFWR sites did not. A second spraying event occurred downstream of CR 14 on the NFWR on July 23<sup>rd</sup>. Following this second event, three NFWR sites were sampled. As in the June and July events, the NFWR below Lost Creek and the NFWR at Bel Aire were sampled to provide information on one site upstream of the spraying event and one site downstream of the spraying event, respectively. In addition, the NFWR at County Road 14 site was sampled to provide information for a second site potentially impacted by the spraying events. Of note, this site was only downstream of the first spraying event in June, as this site is upstream of where the second spraying event occurred. As in July, all three NFWR sites had *Cladophora* growths during sampling in August.

### 9.1 Literature Review

If insecticides are sprayed aerially or on riparian areas where they can be washed into streams, true flies, including chironomid midges, could potentially be the most susceptible to adverse effects, as insecticides are often designed to eradicate mosquitoes, which are true flies (Family Culicidae). Muir et al. (1985) documented that the chironomid midge larvae, *Chironomus tentans.*, accumulated synthetic pyrethroid insecticides, with bioconcentration factors varying widely based on the type of substrate the midges inhabited. Sand substrates contributed to a higher bioavailability of insecticides compared to silt and clay. Chironomid midges in this study had no observed effects on behavior after 24 hours of exposure to the lower concentrations tested, but midges were immobilized at the higher concentrations. Many survived after they were transferred to a clean system at the end of the study. Other studies cited within this study also noted that populations recovered quickly, possibly due to the short life cycle of midges and the availability of nearby untreated ponds that could act as a source for recolonization.

Antwi and Reddy (2015) summarized research on pyrethroid insecticides and indicated that aquatic insects were highly sensitive to them, even if exposed to low concentrations. They cited a study by Mian and Mulla (1992) showing that multiple other macroinvertebrate species were as sensitive to the effects of insecticides as mosquito larvae were. These species included the mayfly taxa *Baetis* spp., *Cloeon dipterum*, *Ephemerella* sp., and *Hexagenia* spp.; the stonefly *Pteronarcys dorsata*, the caddisflies *Brachycentrus americanus* and *Hydropsyche* spp; and the true fly *Antherix*. Several of these taxa were present in the White River in 2017 through 2019. Antwi and Reddy (2015) further stated that some field studies indicated that recovery occurred within six months, while other suggested that the effects were more minor and transient. The differences in recovery time likely depends on the severity of effects initially, the presence of source populations nearby that can aerially disperse, and the generation times of the organisms affected. For example, taxa with short life cycles such as chironomid midges would be expected to recover more quickly than a stonefly with a much longer life cycle such as *Pteronarcys*.

Lima Fernandez et al. (2019) examined effects of pesticides on macroinvertebrate assemblages in terms of potential changes in FFG composition. This study focused on the introduction of pesticides through contaminated plant matter, and found that a stonefly shredder had decreased survival, body length, and biomass following insecticide exposure compared to uncontaminated conditions. These stoneflies were in turn provided as prey to a second stonefly, *Isoperla*, that is categorized as a predator. Biomass and length of this stonefly were also reduced. Based on this study, effects of insecticides can be transferred up the food web within the macroinvertebrate communities.

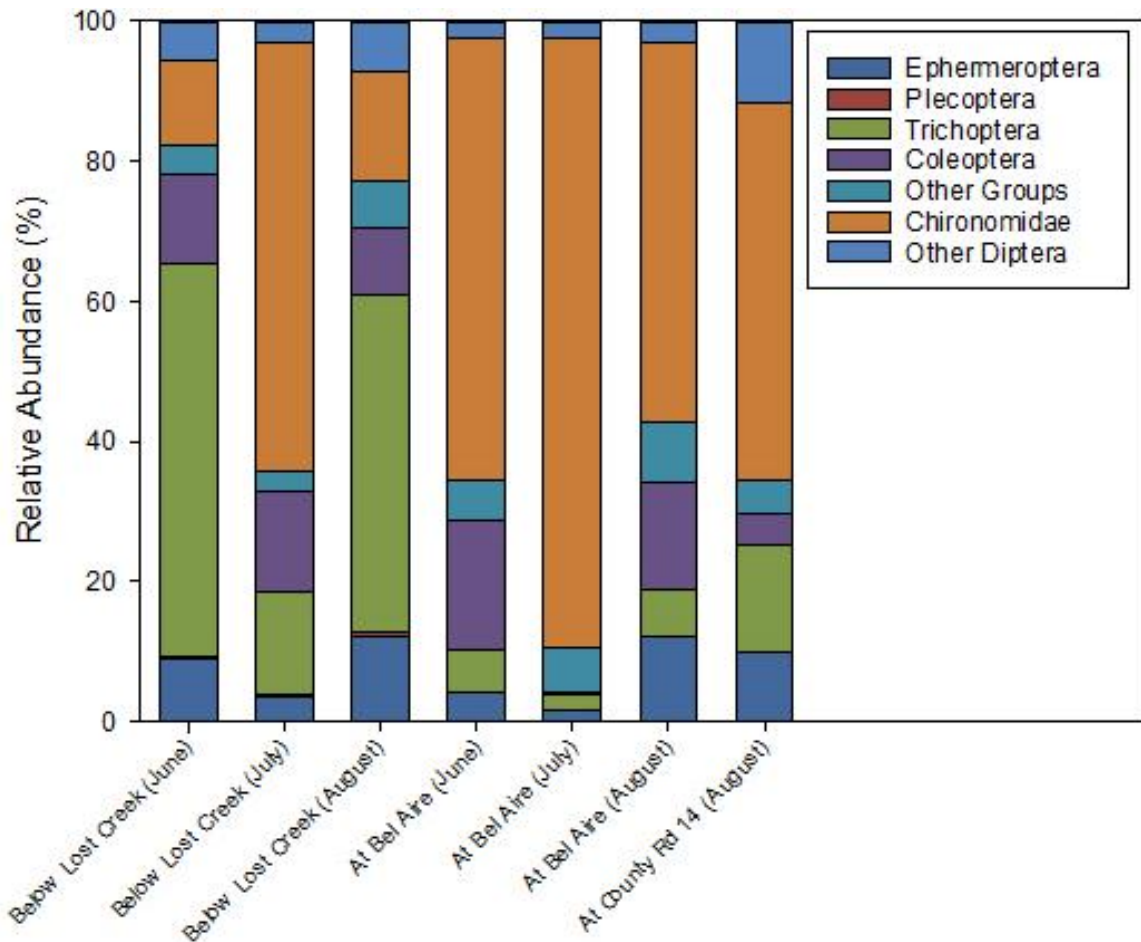
Due to time constraints, this literature review does not cover all of the complexities of interactions with and toxicity to macroinvertebrate assemblages as a result of insecticide use in riparian areas or aerial spraying. However, clearly the effects of insecticide use are not limited to target organism such as true fly taxa, but instead have the potential to affect EPT taxa as well as other groups. In addition, insecticide use has the potential to impact the FFG attributes of macroinvertebrate communities.

## 9.2 Metric Selection and Analysis

Based on the literature review, our approach to determining if the aerial application of insecticide affected populations in the NFWR first evaluated whether shifts in the dominant taxa and orders were apparent when comparing sites upstream of or unaffected by the insecticide application (control sites) with sites sampled after and downstream of the insecticide application (impact sites). In addition, the two similarity metrics, the PCT and BCDI, were calculated for the NFWR sites to determine if any patterns could be detected. Considering the high variability within the duplicate sample analysis and the low number of samples collected for this part of the project, particularly from control sites, we determined that statistical analyses would not be appropriate on the limited data set. However, the

metrics listed in Table 1 were visually assessed to determine if differences were apparent that could be investigated further if this study were repeated.

In general, almost all sites were dominated by chironomid midges, with the exception of the NFWR site below Lost Creek in the June sampling event, which had a macroinvertebrate community in which over half the organisms present were caddisflies, largely from two genera, *Lepidostoma* sp. and *Glossosoma* sp. (Figure 14). The caddisfly *Lepidostoma* sp. was also abundant at almost all other sites. For this insecticide study, chironomid midges were only identified at the subfamily level, but Orthocladiinae chironomid midges were the most abundant group at all other sites. Chironominae midges were also abundant at these sites.



**Figure 14: Relative abundances of the major orders of macroinvertebrates at the North Fork White River and South Fork White River sites sampled for the insecticide study in June, July, and August 2018.**

The NFWR at Bel Aire site was downstream of both the first and second spraying event. When assessing the general composition of the macroinvertebrate composition from June to July to August, a shift in the abundance of some macroinvertebrate groups occurred between the June sampling event prior to the aerial spraying and the July sampling event after the

spraying, but abundances in these groups were more similar between the June sampling event and the August sampling events that occurred after the second spraying. For example, the percent abundance of mayflies, caddisflies, and beetles decreased between the June and July sampling event but rebounded in August (Figure 14). In contrast, chironomid midges increased in relative abundance from June to July and decreased again in August. Similar patterns in some of these groups were observed between the sampling events at the NFWR site that was upstream of the spraying event, with mayflies and caddisflies also decreasing between the June and July event before increasing again in August (Figure 14). This suggests that the pattern likely was not related to the insecticide spraying. In contrast, chironomid midges did not exhibit the same pattern at the upstream site.

Of note, dense *Cladophora* growths were not observed at any of the sites in June but were observed in July and August (M. May, CPW, personal communication, September 2020). Based on multiple differences in taxonomic composition that could have been associated with *Cladophora* growths as described in Section 8.0, these growths could potentially also explain the taxonomic changes between June and July, but not the changes between July and August. The NFWR site at County Road 14 that was only affected by the first spraying event but was not sampled until August had a macroinvertebrate community that was similar in some respects to the communities at the NFWR at Bel Aire site in August in terms of a similar percentage of mayflies and chironomids (Figure 14).

Based on these PCT and BCDI metrics (Appendix B), the macroinvertebrate assemblages at the upstream NFWR site were more similar across the three sampling events than this site was to the two downstream impact sites in any of the three sampling events. Within the pairwise comparisons including upstream control and downstream impact sites, the macroinvertebrate assemblage at the upstream control site in July 2018 was also relatively similar to the downstream NFWR at Bel Aire site in August 2018 based on the PCT, although it was less similar based on the BCDI. BCDI values instead indicated that the most similar control and impact sites were the upstream site and the downstream site at County Road 14 site during the August sampling event.

Comparisons between the downstream NFWR at Bel Aire site prior to and after the first and second aerial spraying events suggested the macroinvertebrate assemblages at this site were more similar in June and July than in August based on the BCDI. However, the PCT metric for this site varied little between June (prior to the spraying) and the subsequent sampling events that occurred at this site and the site at County Road 15.

In previous analyses of spatial differences (Section 7), similarity was noted in the macroinvertebrate communities between the SFWR and NFWR at Bel Aire sites. Based on the abundances of multiple groups, taxonomic composition between these two sites in June was similar in terms of relative abundances of mayflies, stoneflies, beetles, chironomids, and other true flies. However, the macroinvertebrate composition at the SFWR changed little between June and July, unlike at the NFWR site at Bel Aire. Most notably, caddisflies and

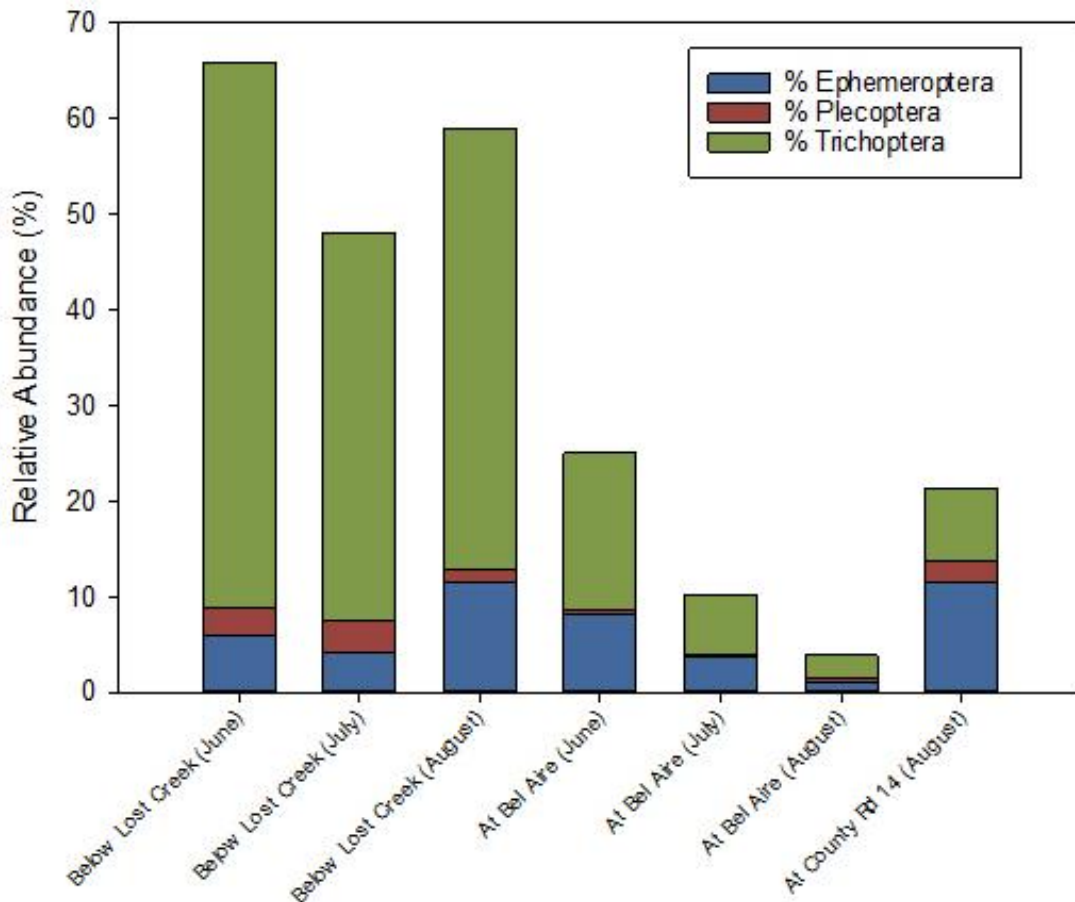


beetles comprised a lower percentage of the total abundance at the NFWR site while chironomids comprised a higher percentage in comparison to the SFWR. The SFWR site was not sampled in August, so further comparisons could not be made. As discussed previously, the changes in taxonomic composition that occurred in the NFWR sites but not the SFWR sites could have been attributed to the first spraying event or to the appearance of *Cladophora* growths; however, the rebound that was observed in the macroinvertebrate populations at the NFWR site in August following the second spraying event indicates that likely the differences between June and July were related to other factors. Dense blooms did not develop at the SFWR sites, so comparison of changes at the NFWR and SFWR sites could not be used to separate the effects of *Cladophora* blooms and aerial insecticide spraying.

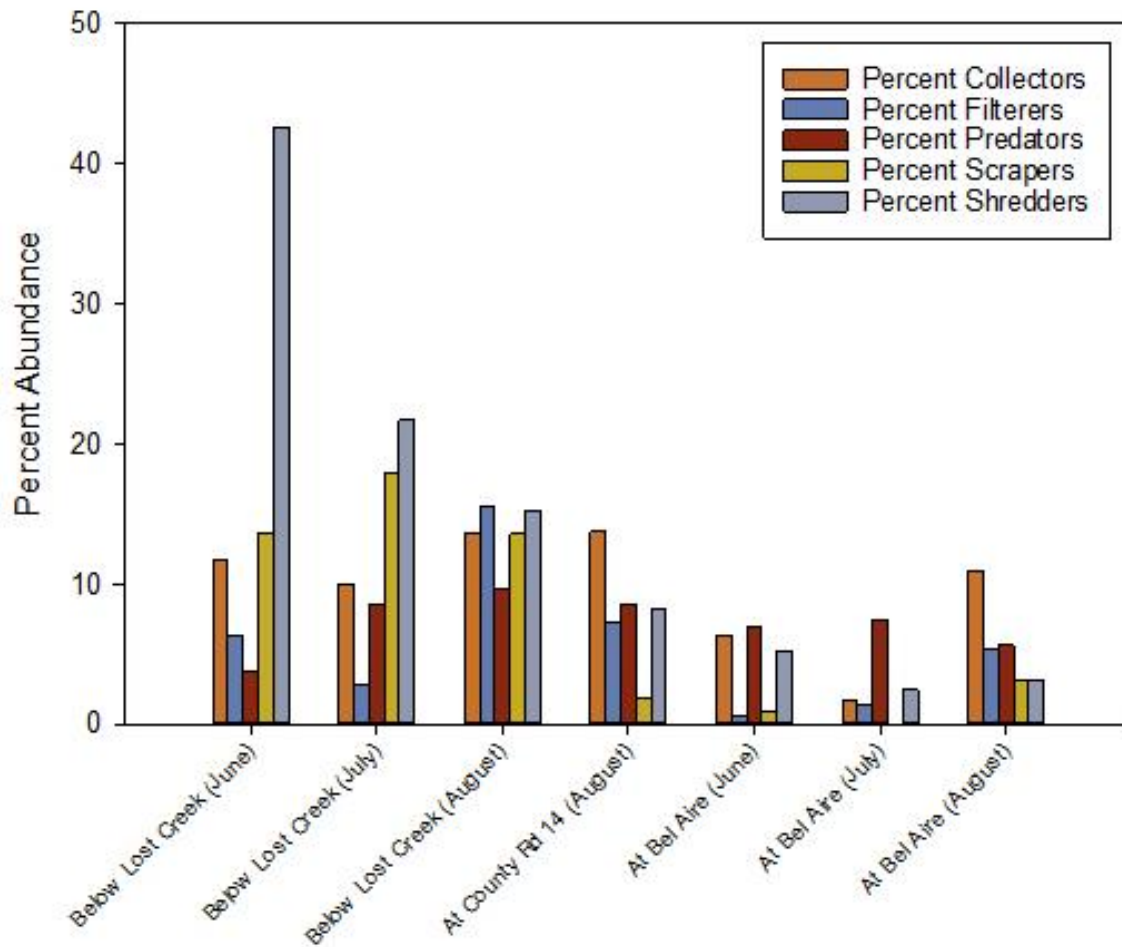
Visual assessment of the metrics indicated that multiple metrics were less favorable at the impact sites compared to the control sites. While some of these trends could be related to the aerial spraying of insecticides, attributing the observed trends to that with confidence would be difficult considering that only a single year of data were available with a limited number of control sites. In addition, the presence of *Cladophora* at the sites downstream of the spraying events confounds the interpretation of any trends, as does the high variability in the duplicate sample analysis. Despite the inability to definitively relate these differences to insecticide use, we presented them here, as they would be relevant if this study is repeated in the future:

- The average relative abundance of chironomid midges was substantially greater at the impact sites (67%) compared to the averages for the NFWR control sites (29%) and the combined NFWR and SFWR control sites (43 percent).
- Mean % dominant taxon metric being higher at the impact sites (50%) compared to the NFWR and combined control sites (34 and 38%, respectively). This was also reflected in the diversity index scores, which averaged 2.66 at the impact sites and 3.64 and 3.33 at the NFWR and combined control sites, respectively.
- Most of the EPT metrics were reduced at the impact sites compared to the control sites.
  - The %EPT individuals of all individuals averaged 17% at the impact sites compared to 46 and 34% at the NFWR and combined control sites, respectively (Figure 15).
  - The number of EPT taxa was also lower on average at the impact sites (4 taxa) than at the control sites (7 and 6 taxa, respectively), largely as a result of fewer caddisfly taxa being present at the impact sites.
- The average % Chironomidae at the impact sites (67%) was much higher than at the control sites above or before the insecticide application (29%); the same pattern was observed when all Diptera were combined.

- HBI values were on average higher, indicating a higher percentage of more tolerant organisms, at the impact sites (5.24) compared to the NFWR and combined control sites (3.42 and 4.05, respectively). This trend was also observed in the other metrics that related to the tolerance of the macroinvertebrate assemblages.
- Within the FFG metrics, some groups were relatively similar between the impact and control sites, such as collectors, filterers, and predators, while others were not (Figure 16).
  - Scrapers comprised on average 2 percent of the total abundance at impact sites compared to 12 and 7% at the NFWR and combined control sites, respectively.
  - The shredder percentages were significantly different, with shredders comprising on average 5% at the impact sites compared to 21 and 17%, respectively, at the NFWR and combined control sites.



**Figure 15: Relative abundance of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa at the North Fork White River sites sampled for the insecticide study in June, July, and August 2018.**



**Figure 16: Relative abundances of Functional Feeding groups at the North Fork White River sites sampled for the insecticide study in June, July, and August 2018. Of note, percentages do not equal 100% based on uncategorized taxa, such as the Chironomidae subfamilies.**

Overall, these differences between the control and impact sites indicate that the macroinvertebrate assemblages were somewhat less balanced and diverse than at the control sites, based on the % Dominant taxon and diversity index values. In addition, the assemblages at the impact sites were comprised of more abundant tolerant taxa and less abundant sensitive taxa such as the EPT groups. However, as noted above, these differences could be related to the aerial pesticide use, but also could be attributed to *Cladophora* growths or other factors. There was no indication from this analysis that the insecticide application impacted true fly taxa, even though mosquitoes in the true fly family were likely the target of the insecticide application. Of note, no mosquito larvae (Culicidae) were observed in any of the samples collected for the insecticide study or for any of the samples collected for the *Cladophora* study; these taxa would be expected to be more prevalent in lentic habitats than in the White River or White River tributaries. As organisms within the true fly family Chironomidae were only identified at the subfamily level (i.e., Chironominae,

Orthoclaadiinae, Tanypodinae, etc.), we were not able to determine if the numbers of chironomid taxa differed similarly or if this trend only occurred in midge abundances.

### **In Summary:**

- While few statistically significant differences were observed, this could have been an artifact of the low sample sizes in the analysis, particularly in the case of the impact sites (n=3).
- There were notable shifts in the taxonomic composition at the impact site on the NFWR at Bel Aire site between June and July, although the assemblages sampled in August more closely resembled those in June prior to the spraying..
  - Similar if less pronounced shifts also occurred at the site upstream of the spraying.
  - As *Cladophora* growths were not observed at any of the North Fork sites in June but were observed at all of these sites in July and August, the shifts in composition could also have been related to the presence of *Cladophora*, particularly because no similar shifts were observed at the SFWR sites, which did not develop *Cladophora* blooms.
- While our literature review suggested insecticide spraying could result in shifts in the FFG composition of the macroinvertebrate assemblages, the FFG composition was relatively similar between control and impact sites.
  - Further differences in FFG composition may have been clearer if chironomids were identified past the subfamily level rather than being grouped together, as FFG classifications differ for the various taxa included under chironomid subfamilies.
- Our literature review also suggested that chironomid and other true fly taxa might be affected most by insecticide application, but there were no indications of that, as these groups comprised higher percentages in the impact sites compared to the control sites. These groups also comprised higher percentages of the total abundance at the sites with *Cladophora* growths compared to those without.
- Overall, while there were multiple metrics that differed between the control sites and the impact sites, the small sample size, low number of control samples, and presence of *Cladophora* growths at the same sites that were potentially affected by the insecticide spraying confounded the results. Similar differences in some metrics were observed between the sites with and without *Cladophora*.

## 10. Summary

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The MMI and other metrics suggest that the macroinvertebrate assemblages in the NFWR, SFWR, and WRM are generally healthy and not impaired based on the data collected in 2017 – 2019. All MMI scores, including those collected following different protocols from those described by the WQCD, were above the threshold indicating attainment of the Aquatic Life Use. Often, a caddisfly or mayfly taxon was the most abundant organisms at these sites. While a few sites and sampling events had low percentages of mayflies and somewhat high percentages of chironomids, most samples contained numerous sensitive species, as evidenced in the number and percent of EPT taxa, low HBI scores, and high numbers of intolerant taxa. In addition, the diversity values were above 2.50 at all sites, indicating balanced communities were present.

For these studies, duplicate samples were collected from one or more sites during each sampling event, and two different types of samples (WQCD kicks and Hesses) were collected in 2018 and 2019. In addition, samples were processed by two different laboratories over the course of the study. The duplicate sample analysis indicated that even when samples are taken at the same site on the same day from presumably similar locations, moderate to high variability in taxonomic composition and some metric values occurred. This variability was considered when evaluating the differences observed in the remaining analyses. The analyses conducted to determine the effect of the different sampling methods indicated that 82% of the metric values were similar between the two samples after the organism counts were standardized through EDAS via random resampling. After reviewing the data, the differences observed between the processing and analysis completed by the two laboratories would also not be expected to impact the remaining analyses.

Macroinvertebrate assemblages at the NFWR, SFWR, and WRM sites were evaluated to determine if spatial or temporal differences were occurring in the populations. Overall, while some metric values were more favorable at one of the upstream sites on the NFWR compared to downstream sites, there were no spatial trends that were consistent across all years or metrics in the NFWR, SFWR, or WRM. Similarity indices indicated that in general the taxonomic composition at the tributary sites were more similar to one another and less similar to the WRM sites. Geographic location also appeared to influence the composition of the macroinvertebrate assemblages, as the two sites on the NFWR and SFWR that were both near the confluence with the White River and geographically close (via aerial distance) had relatively similar macroinvertebrate communities.

Trends over time were difficult to detect as only three years of data exist for some of the sites, and other sites were only sampled in one or two of these years. The trends that were detected were isolated to a single site and did not extend to tributary or mainstem-wide trends, suggesting that the factors influencing the macroinvertebrate populations were likely

not broad-scale factors relating to climate or stream flows. In addition, the moderate to high variability observed in some metric values in the duplicate sample analyses suggests that the collection of data in additional years could be helpful in determining if the initial trends that were detected persist or if annual variability in the populations negates the presence of these trends in the future.

Dense growths of *Cladophora* have been observed at sites on the NFWR and WRM over the course of this study from 2017 to 2019, and one of the main objectives of the macroinvertebrate sample collection was to determine if these filamentous algal growths are affecting the macroinvertebrate assemblages within this watershed. Interactions between macroinvertebrate assemblages and dense growths of *Cladophora* are complex, with some taxa potentially benefitting from the food resources, habitat niches, and protection from flows and predation that *Cladophora* provides, while other are adversely affected through competition for attachment spaces, shifts in food resources, reduced availability of preferred habitat, diel fluctuations in dissolved oxygen, and increased competition with taxa that are benefitting from *Cladophora*.

Multiple metrics differed significantly between sites with and without *Cladophora* growths, suggesting that these growths may be impacting the macroinvertebrate assemblages. Considering the variability observed in the duplicate sample analysis in addition to natural variability that exists between sites and years, further data analysis would be useful to more confidently attribute these differences to *Cladophora* rather than other confounding factors. However, sites with *Cladophora* growths were generally comprised of more abundant tolerant taxa, specifically chironomid and other true fly taxa, in comparison to reduced numbers and abundance of EPT and other intolerant taxa. Functional feeding group and habitat metrics also differed between sites with and without *Cladophora* growths, but not always in the predicted direction, with collectors, scrapers, shredders, climbers, and clingers being more abundant or taxonomically rich at sites without *Cladophora*, although the limited amount of literature available indicated some of these groups might be expected to benefit from *Cladophora* growths.

A study of potential effects of aerial insecticide spraying on the NFWR was conducted separately from the other studies in summer 2018. This study compared control sites upstream of (and prior to) the spraying on the NFWR and those unaffected by the spraying on the SFWR to potentially impacted sites on the NFWR downstream of the one or both spraying events. Multiple metrics differed substantially between the control and impact sites, with fewer and less abundant EPT and intolerant taxa at the impact sites and more abundant chironomids and other tolerant taxa. In addition, there were notable shifts in the taxonomic composition at the impact site on the NFWR at Bel Aire site downstream of the spraying events between June and July; however, the populations in August were more similar to those in June, contrary to what would be expected following the second spraying event if the population shifts in July were related to the first spraying event. In addition, similar if less

pronounced shifts also occurred at the site upstream of the spraying. Overall, there were some indications that the insecticide application could have affected the composition of the macroinvertebrate assemblages; however, the small sample size, low number of control sites, high variability in the duplicate sample analysis, and presence of *Cladophora* growths at the same sites that were potentially affected by the insecticide spraying confounds the results. Of note, many of the patterns noted between the control and impact sites were similar to those observed at the sites with and without *Cladophora*. Based on this, further data collection is necessary to determine if any of the differences observed could in fact be related to the spraying events or to the *Cladophora* growths. Initially, a spraying event on the SFWR was planned for summer 2018 as well, but, in the end, only the NFWR spraying events occurred. Data from control and impact sites on the SFWR, had the spraying event occurred, may have been informative in differentiating potential effects of the spraying event from possible effects of *Cladophora* growths, as *Cladophora* growths were not observed at the SFWR sites.

## 10.1 Recommendations

We have not discussed with the District, CPW, or TU if continuation of these studies is planned. The studies from 2017 to 2019 provided data suggesting that *Cladophora* is affecting the macroinvertebrate assemblages and that insecticides may have also impacted populations. Continuing these studies for additional years would be instrumental in verifying the patterns identified in this report. If these studies are planned to continue in 2021 or later years, we have the following recommendations. Of note, we are aware that budgetary constraints do influence sampling design and that limited resources may make at least some of these suggested recommendations difficult or infeasible.

- Overall, these studies would benefit from focusing on collecting a single sample type. Few metric values were significantly different between the WQCD kick and Hess samples; however, limiting sample collection to one method would eliminate a possible source of variation and could also reduce costs. As MMI scores have almost all been in attainment of the aquatic life use, we would recommend discontinuing collection of the WQCD kick samples unless there is further regulatory pressure to provide sample data determining attainment and instead continue to collect the Hess composite samples. While few differences were noted, collection of composite Hess samples of a defined and contained area would likely provide more consistent data and be less susceptible to sampling variation. Statistically speaking, the most robust option would be to continue to collect the Hess replicate samples and process them individually prior to compositing them. The concern over the effect of the variability observed in taxonomic composition and metric values observed in the duplicate sample analysis would be addressed in this manner, as routine statistical analyses could be conducted that would account for this variability when determining if differences between sites and groups of samples were significant. However, this would increase processing effort (and therefore cost). While this would not allow for complete consistency with the Hess sample composites

collected in 2018 and 2019, the number of replicates collected at each site could be reduced to three to five at each site to offset the costs to a degree.

- If the composite Hess samples continue to be collected, we would recommend also continuing to target a 600-count of organisms when subsampling. While both laboratories used appeared to have qualified taxonomists, we would recommend using Timberline for future analyses based on a more straightforward presentation and format for the data output.
- We would recommend that data collection to detect trends over time or geographic locations should continue for an additional two years or more if possible, to provide a total of five years of data. Sampling should also focus on a consistent set of sites from year to year, including those sites that already have three years of data collected. Natural variability in macroinvertebrate communities is often high (note that some variability was observed even within the duplicate samples), making trends between sites and years difficult to detect. Additional years of data would be informative in terms of determining whether detected differences truly related to habitat, water quality, stream flows or other limiting factors or if differences were just an artifact of high natural variability. As an example, peak stream flows were much higher in 2019 than in the other two years of the study, but the potential effect of this on the macroinvertebrate communities was difficult to assess based on three years of data.
- For the *Cladophora* study, GEI recommends using a rating scale that describes the density and thickness of filamentous algae at a site (standard methods are available for this). If all sites have similar *Cladophora* coverage, this may not be informative, but if sites differed in the extent of the coverage and/or the thickness of the growths at each site, this would provide additional information to evaluate and requires little time to incorporate into the sampling protocols.
- As noted for the insecticide study, *Cladophora* was not present at the NFWR sites downstream of the Lost Creek site in the early summer sampling event in June but were present in July. If possible, additional sampling events at sites before and after *Cladophora* blooms occur could also provide valuable information.
- Based on the USGS presentation (Day et al. 2020) and older data provided with the data we received from the CDPHE, water quality sampling has been conducted at these sites as well. This could also be incorporated into our interpretation of differences between sites, particularly between the NFWR and SFWR sites.
- If repeating the insecticide spraying study is possible, we would recommend that additional sites upstream and downstream of the insecticide spraying locations be added to the study. Alternatively, if spraying events in the future are planned for both the NFWR and SFWR, sampling control and impact sites on both streams would be informative in differentiating potential effects of the spraying events and the *Cladophora* growths, assuming that *Cladophora* growths continue to not occur on the



SFWR sites and to occur on the NFWR sites. Identification of chironomids to the lowest practical level (generally genus or species) for this study would also add to the information available to detect differences. There was no apparent adverse effect observed on chironomid or true fly taxa at sites downstream of the spraying based on the 2018 study, but potentially chironomid richness could have been affected or certain chironomid taxa could have been reduced or eliminated at the downstream sites.

## 11. References

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- Antwi, F.B. and G.V.P. Reddy. 2015. Toxicological effects of pyrethroids on non-target aquatic insects. *Environmental Toxicology and Pharmacology* 40: 915-923.
- Cummins, K.W., R.W. Merritt, and M.B. Berg. 2008. Ecology and Distribution of Aquatic Insects. In Merritt, R.W., K.W. Cummins, and M.B. Berg (eds). *An Introduction to Aquatic Insects of North America*. 4<sup>th</sup> Edition. Dubuque, IA.
- Day, N., C.A. Williams, and K. Leib. 2020. White River Benthic Algae Study. United States Geological Survey presentation at the 2020 White River Conservation District Annual Meeting and Water Expo. January 17, 2020. Accessed at: [http://www.whiterivercd.com/uploads/2/6/0/6/26068836/whiteriverpresentation\\_usgs\\_approved.pdf](http://www.whiterivercd.com/uploads/2/6/0/6/26068836/whiteriverpresentation_usgs_approved.pdf).
- Dodds, W.K. and D.A. Gudder. 1992. Review: The Ecology of *Cladophora*. *Journal of Phycology* 28: 415-427. Accessed at: <https://www.k-state.edu/doddslab/journalarts/dodds%20and%20gudder%20j%20phycol%201992.pdf>
- Ellsworth, S.D. 2000. Influence of substrate size, *Cladophora*, and caddisfly pupal cases on colonization of macroinvertebrates in Sagehen Creek, California. *Western North American Naturalist* 60: 311-319.
- Feminella, J.W. and V.H. Resh. 1991. Herbivorous caddisflies, macroalgae, and epilithic microalgae: dynamic interactions in a stream grazing system. *Oecologia* 87(2): 247-256.
- Grandinetti, M.E. 2016. Decay of Macroalgae and Leaves and Their Relation to Detrital Food Webs. Western Kentucky University. Masters Thesis and Specialist Projects, Paper 1601.
- Highsmith, R.C. 1985. Floating and algal rafting as potential dispersal mechanisms in brooding invertebrates. *Marine Ecology Progress Series* 25: 169-179 (As cited in Grandinetti 2016)
- Hilsenhoff, W. L. 1987. An improved biotic index of organic stream pollution. *Great Lakes Entomologist* 20(1): 31-39.
- Jansen, L.S. 2018. A Comparison of Community Structure in Regulated and Unregulated Reaches in the Upper Eel River, California. Thesis presented to faculty of Humboldt State University. Accessed at: <https://digitalcommons.humboldt.edu/cgi/viewcontent.cgi?article=1204&context=etd>.

- Liess, M. and P. C. Vond Der Ohe. 2005. Analyzing effects of pesticides on invertebrate communities in streams. *Environmental Toxicology and Chemistry* 34(4): 954-965.
- Lima-Fernandes, E., M. Bundschuh, N. Bakanov, D. Englert, R. Shculz and R. B. Shafer. 2019. Effects of systemic pesticide along an aquatic tri-trophic food chain. *Bulletin of Environmental Contamination and Toxicology* 103: 507-514.
- Mian, L., and M. Mulla. 1992. Effects of pyrethroid insecticides on nontarget invertebrates in aquatic ecosystems. *Journal of Agricultural Entomology* 9: 73-98.
- Muir, D.C.G., G.P. Rawn, B.E. Townsend, W.L. Lockhart, and R. Greenhalgh. 1985. Bioconcentration of cypermethrin, dletmehtrin, fenvalerate, and permethrin by *Chironomus tentans* larvae in sediment and water. *Environmental Toxicology and Chemistry* 4: 51-61.
- Patrick, R., C.F. Rhyne, R. W. Richardson, R. A. Larson, T. L. Bott, and K. Rogenmuser. 1983. *The Potential for Biological Controls of Cladophora glomerata*. United States Environmental Protection Agency. EPA-600/53-83-066. Duluth, MN.
- Tinsley, B.E., S. A. Grubbs, J. M. Yates, and A. J. Meier. 2016. Notes on the ecological roles of *Podostemum ceratophyllum* Michx., 1803 and *Cldaophora glomerata* (L.) Kutz., 1843 in the habitat and diet of riverine hydropyschid caddisflies (Trichoptera). *Aquatic Insects*: 37(3): 225-239.
- Tonkin, J.D., R.G. Death, and J. Barquin. 2014. Periphyton control on stream invertebrate diversity: is periphyton architecture more important than biomass? *Marine and Freshwater Research* 65: 818-829.
- Ward, J. and A. Ricciardi. 2010. Community-level effects of co-occurring native and exotic ecosystem engineers. *Freshwater Biology* 55: 1803- 1817.
- Water Quality Control Commission (WQCD). 2017. Policy Statement 10-1: Aquatic Life Use Attainment, Methodology to Determine Use Attainment for Rivers and Streams. Colorado Department of Public Health and Environment. Denver, CO.
- Wiggins, G.B. and D.C. Currie. 2008. Trichoptera Families. In Merritt, R.W., K.W. Cummins, and M.B. Berg (eds). *An Introduction to Aquatic Insects of North America*. 4<sup>th</sup> Edition. Dubuque, IA.

# Appendix A Equations for Calculations

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The Percent Common Taxa Metric (PCT) is calculated as follows:

$$PCT = \frac{\# \text{ Taxa Common to Both Samples}}{\text{Total \# of Taxa in Both Samples}} \times 100$$

The Bray-Curtis Dissimilarity Index (BCDI) is calculated as:

$$BCDI_d = \frac{\sum |x_i - x_j|}{\sum (x_i + x_j)}$$

$x_i$  = abundance of taxon 1 at Site 1

$x_j$  = abundance of taxon 1 at Site 2

The Percent Difference Metric utilized in the duplicate sample analysis is calculated as follows:

$$\% \text{ Difference} = \frac{\frac{\text{Metric}_{\text{Sample 1}} - \text{Metric}_{\text{Sample 2}}}{\text{Average}_{\text{Sample 1, Sample 2}}}}{100}$$

## **Appendix B Percent Common Taxa and Bray-Curtis Dissimilarity Index Data, 2017 – 2019**

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**Table B-1: Percent similarity and Bray-Curtis dissimilarity values for the macroinvertebrate sampled collected in 2018 for the comparison of sampling methods study. Values indicate the comparison between the WQCD kick and Hess samples collected at each site. NFWR = North Fork White River, SFWR = SFWR, WRM = White River Mainstem.**

Sites	NFWR Below Trappers Lake (3079)	NFWR Below Lost Creek (6111)	NFWR at CR15 (6110)	NFWR at Bel Aire (6107)	SFWR at USFS (3077)	SFWR above Buckeye Creek (3078)	SFWR at Bel Aire (6106)	WRM at Sleepy Cat (6105)	WRM above Coal Creek (6104)	WRM Below Meeker Pasture (6103)
<b>Percent Common Taxa</b>										
NFWR Below Trappers Lake (3079)	44.7		--	--	--	--	--	--	--	--
NFWR Below Lost Creek (6111)	--	58.5	--	--	--	--	--	--	--	--
NFWR at CR15 (6110)	--	--	54.0	--	--	--	--	--	--	--
NFWR at Bel Aire (6107)	--	--	--	52.2	--	--	--	--	--	--
SFWR at USFS (3077)	--	--	--	--	52.8	--	--	--	--	--
SFWR above Buckeye Creek (3078)	--	--	--	--	--	17.7	--	--	--	--
SFWR at Bel Aire (6106)	--	--	--	--	--	--	61.0	--	--	--
WRM at Sleepy Cat (6105)	--	--	--	--	--	--	--	40.0	--	--
WRM above Coal Creek (6104)	--	--	--	--	--	--	--	--	44.4	--
WRM Below Meeker Pasture (6103)	--	--	--	--	--	--	--	--	--	48.9
<b>Bray-Curtis Dissimilarity Index</b>										
NFWR Below Trappers Lake (3079)	0.30	--	--	--	--	--	--	--	--	--
NFWR Below Lost Creek (6111)	--	0.40	--	--	--	--	--	--	--	--
NFWR at CR15 (6110)	--	--	0.44	--	--	--	--	--	--	--
NFWR at Bel Aire (6107)	--	--	--	0.34	--	--	--	--	--	--
SFWR at USFS (3077)	--	--	--	--	0.24	--	--	--	--	--
SFWR above Buckeye Creek (3078)	--	--	--	--	--	0.31	--	--	--	--
SFWR at Bel Aire (6106)	--	--	--	--	--	--	0.31	--	--	--
WRM at Sleepy Cat (6105)	--	--	--	--	--	--	--	0.53	--	--
WRM above Coal Creek (6104)	--	--	--	--	--	--	--	--	0.45	--
WRM Below Meeker Pasture (6103)	--	--	--	--	--	--	--	--	--	0.28

**Table B-2: Percent similarity and Bray-Curtis dissimilarity values for the macroinvertebrate sampled collected in 2019 for the comparison of sampling methods study. Values indicate the comparison between the WQCD kick and Hess samples collected at each site. NFWR = North Fork White River, SFWR = SFWR, WRM = White River Mainstem.**

Sites	NFWR Below Lost Creek (6111)	SFWR above Buckeye Creek (3078) Kick	WRM at Meeker Pasture (6103)
<b>Percent Common Taxa</b>			
North Fork White River Below Lost Creek (6111)	59.5	--	--
South Fork above Buckeye Creek (3078)	--	44.4	--
White River at Meeker Pasture (6103)	--	--	34.5
<b>Bray-Curtis Dissimilarity Index</b>			
NFWR Below Lost Creek (6111)	0.44	--	--
SFWR above Buckeye Creek (3078)	--	0.5	--
WRM at Meeker Pasture (6103)	--	--	0.44



**Table B-3: Percent similarity and Bray-Curtis dissimilarity values for the macroinvertebrate sampled collected in 2017 using the WQCD kick method for the *Cladophora* study. NFWR = North Fork WRM, SFWR = SFWR, WRM = WRM Mainstem.**

Sites	NFWR Below Lost Creek (6111)	NFWR at County Rd 14 (6110)	NFWR above Buford (6108)	NFWR at Bel Aire (6107)	SFWR at Bel Aire (6106)	WRM at Sleepy Cat (6105)	WRM above Coal Creek (6104)	WRM at Meeker Pasture (6103)	WRM at 5th Street Bridge (531)
<b>Percent Common Taxa</b>									
NFWR Below Lost Creek (6111)	--	47.6	41.7	24.4	34.1	32.7	31.3	27.7	21.4
NFWR at County Rd 14 (6110)	47.6	--	50.0	47.6	45.2	51.1	35.3	32.0	18.8
NFWR above Buford (6108)	41.7	50.0	--	54.5	56.3	42.9	31.8	27.9	17.9
NFWR at Bel Aire (6107)	24.4	47.6	54.5	--	57.1	44.4	34.0	27.7	18.6
SFWR at Bel Aire (6106)	34.1	45.2	56.3	57.1	--	48.8	31.9	28.3	25.0
WRM at Sleepy Cat (6105)	32.7	51.1	42.9	44.4	48.8	--	44.0	43.8	25.0
WRM above Coal Creek (6104)	31.3	35.3	31.8	34.0	31.9	44.0	--	45.7	38.1
WRM at Meeker Pasture (6103)	27.7	32.0	27.9	27.7	28.3	43.8	45.7	--	44.7
WRM at 5th Street Bridge (531)	21.4	18.8	17.9	18.6	25.0	25.0	38.1	44.7	--
<b>Bray-Curtis Dissimilarity Index</b>									
NFWR Below Lost Creek (6111)	--	0.52	0.66	0.72	0.44	0.62	0.67	0.67	0.78
NFWR at County Rd 14 (6110)	0.52	--	0.33	0.48	0.42	0.49	0.63	0.68	0.76
NFWR above Buford (6108)	0.66	0.33	--	0.36	0.46	0.43	0.62	0.68	0.76
NFWR at Bel Aire (6107)	0.72	0.48	0.36	--	0.43	0.30	0.63	0.64	0.82
SFWR at Bel Aire (6106)	0.44	0.42	0.46	0.43	--	0.39	0.60	0.67	0.79
WRM at Sleepy Cat (6105)	0.62	0.49	0.43	0.30	0.39	--	0.53	0.54	0.75
WRM above Coal Creek (6104)	0.67	0.63	0.62	0.63	0.60	0.53	--	0.37	0.50
WRM at Meeker Pasture (6103)	0.67	0.68	0.68	0.64	0.67	0.54	0.37	--	0.40
WRM at 5th Street Bridge (531)	0.78	0.76	0.76	0.82	0.79	0.75	0.50	0.40	--

**Table B-4: Percent similarity and Bray-Curtis dissimilarity values for the macroinvertebrate sampled collected in 2018 using the WQCD kick method for the *Cladophora* study. NFWR = North Fork WRM, SFWR = SFWR, WRM = WRM Mainstem.**

Sites	NFWR Below Trappers Lake (3079)	NFWR Below Lost Creek (6111)	NFWR at CR15 (6110)	NFWR at Bel Aire (6107)	SFWR at USFS (3077)	SFWR above Buckeye Creek (3078)	SFWR at Bel Aire (6106)	WRM at Sleepy Cat (6105)	WRM above Coal Creek (6104)	WRM Below Meeker Pasture (6103)
<b>Percent Common Taxa</b>										
NFWR Below Trappers Lake (3079)	--	35.8	23.3	22.4	24.0	26.3	22.6	16.1	18.3	15.3
WRM Below Lost Creek (6111)	35.8	--	29.8	36.5	37.8	33.3	30.0	20.4	20.3	17.2
NFWR at CR15 (6110)	23.3	29.8	--	35.2	45.5	51.0	42.6	31.4	32.7	20.7
NFWR at Bel Aire (6107)	22.4	36.5	35.2	--	29.8	47.9	56.1	36.2	34.6	26.4
SFWR at USFS (3077)	24.0	37.8	45.5	29.8	--	40.9	25.0	14.6	19.6	11.5
SFWR above Buckeye Creek (3078)	26.3	33.3	51.0	47.9	40.9	--	47.7	38.3	29.1	21.4
SFWR at Bel Aire (6106)	22.6	30.0	42.6	56.1	25.0	47.7	--	48.7	39.1	29.8
WRM at Sleepy Cat (6105)	16.1	20.4	31.4	36.2	14.6	38.3	48.7	--	36.2	27.1
WRM above Coal Creek (6104)	18.3	20.3	32.7	34.6	19.6	29.1	39.1	36.2	--	52.3
WRM Below Meeker Pasture (6103)	15.3	17.2	20.7	26.4	11.5	21.4	29.8	27.1	52.3	--
<b>Bray-Curtis Dissimilarity Index</b>										
NFWR Blw Trappers Lake (3079)	--	0.83	0.85	0.86	0.87	0.84	0.93	0.93	0.89	0.90
NFWR Blw Lost Creek (6111)	0.83	--	0.69	0.62	0.64	0.59	0.46	0.78	0.82	0.78
NFWR at CR15 (6110)	0.85	0.69	--	0.65	0.63	0.42	0.57	0.58	0.81	0.82
NFWR at Bel Aire (6107)	0.86	0.62	0.65	--	0.82	0.59	0.42	0.60	0.52	0.51
SFWR at USFS (3077)	0.87	0.64	0.63	0.82	--	0.69	0.75	0.90	0.93	0.98
SFWR above Buckeye Creek (3078)	0.84	0.59	0.42	0.59	0.69	--	0.50	0.61	0.81	0.80
SFWR at Bel Aire (6106)	0.93	0.46	0.57	0.42	0.75	0.50	--	0.59	0.73	0.69
WRM at Sleepy Cat (6105)	0.93	0.78	0.58	0.60	0.90	0.61	0.59	--	0.71	0.74
WRM above Coal Creek (6104)	0.89	0.82	0.81	0.52	0.93	0.81	0.73	0.71	--	0.44
WRM Below Meeker Pasture (6103)	0.90	0.78	0.82	0.51	0.98	0.80	0.69	0.74	0.44	--

**Table B-5: Percent similarity and Bray-Curtis dissimilarity values for the macroinvertebrate sampled collected in 2018 using the Hess sampler for the *Cladophora* study. NFWR = North Fork WRM, SFWR = SFWR, WRM = WRM Mainstem.**

Sites	NFWR Below Trappers Lake (3079)	NFWR Below Lost Creek (6111)	NFWR at CR15 (6110)	NFWR at Bel Aire (6107)	SFWR at USFS (3077)	SFWR above Buckeye Creek (3078)	SFWR at Bel Aire (6106)	WRM at Sleepy Cat (6105)	WRM above Coal Creek (6104)	WRM Below Meeker Pasture (6103)
<b>Percent Common Taxa</b>										
NFWR Below Trappers Lake (3079)	--	24.5	22.4	19.6	29.8	25.0	23.2	23.1	21.0	20.7
NFWR Below Lost Creek (6111)	24.5	--	30.8	33.3	52.6	42.6	29.4	24.2	10.8	17.5
NFWR at CR15 (6110)	22.4	30.8	--	42.3	33.3	54.0	46.2	42.6	28.1	28.3
NFWR at Bel Aire (6107)	19.6	33.3	42.3	--	36.2	52.1	50.0	48.2	30.0	25.9
SFWR at USFS (3077)	29.8	52.6	33.3	36.2	--	34.0	22.2	22.2	16.1	15.5
SFWR above Buckeye Creek (3078)	25.0	42.6	54.0	52.1	34.0	--	41.5	41.0	28.6	28.8
SFWR at Bel Aire (6106)	23.2	29.4	46.2	50.0	22.2	41.5	--	46.6	29.0	25.0
WRM at Sleepy Cat (6105)	23.1	24.2	42.6	48.2	22.2	41.0	46.6	--	46.8	45.8
WRM above Coal Creek (6104)	21.0	10.8	28.1	30.0	16.1	28.6	29.0	46.8	--	42.1
WRM Below Meeker Pasture (6103)	20.7	17.5	28.3	25.9	15.5	28.8	25.0	45.8	42.1	--
<b>Bray-Curtis Dissimilarity Index</b>										
NFWR Below Trappers Lake (3079)	--	0.86	0.77	0.77	0.84	0.85	0.86	0.81	0.88	0.87
NFWR Below Lost Creek (6111)	0.86	--	0.74	0.68	0.66	0.65	0.59	0.80	0.89	0.89
NFWR at CR15 (6110)	0.77	0.74	--	0.60	0.73	0.56	0.51	0.55	0.70	0.66
NFWR at Bel Aire (6107)	0.77	0.68	0.60	--	0.68	0.43	0.53	0.47	0.67	0.71
SFWR at USFS (3077)	0.84	0.66	0.73	0.68	--	0.65	0.74	0.82	0.91	0.92
SFWR above Buckeye Creek (3078)	0.85	0.65	0.56	0.43	0.65	--	0.48	0.61	0.78	0.80
SFWR at Bel Aire (6106)	0.86	0.59	0.51	0.53	0.74	0.48	--	0.53	0.69	0.72
WRM at Sleepy Cat (6105)	0.81	0.80	0.55	0.47	0.82	0.61	0.53	--	0.46	0.48
WRM above Coal Creek (6104)	0.88	0.89	0.70	0.67	0.91	0.78	0.69	0.46	--	0.35
WRM Below Meeker Pasture (6103)	0.87	0.89	0.66	0.71	0.92	0.80	0.72	0.48	0.35	--

**Table B-6: Percent similarity and Bray-Curtis dissimilarity values for the macroinvertebrate sampled collected in 2019 using the WQCD kick method for the *Cladophora* study. NFWR = North Fork WRM, SFWR = SFWR, WRM = WRM Mainstem.**

Sites	North Fork WRM Below Lost Creek (6111)	South Fork above Buckeye Creek (3078) Kick	WRM at Meeker Pasture (6103)
<b>Percent Common Taxa</b>			
NFWR Below Lost Creek (6111)	--	38.0	25.0
SFWR above Buckeye Creek (3078)	38.0	--	32.1
WR at Meeker Pasture (6103)	25.0	32.1	--
<b>Bray-Curtis Dissimilarity Index</b>			
NFWR Below Lost Creek (6111)	--	0.61	0.87
SFWR above Buckeye Creek (3078)	0.61	--	0.79
WR at Meeker Pasture (6103)	0.87	0.79	--

**Table B-7: Percent similarity and Bray-Curtis dissimilarity values for the macroinvertebrate sampled collected in 2019 using the Hess sampler for the *Cladophora* study. NFWR = North Fork WRM, SFWR = SFWR, WRM = WRM Mainstem.**

Sites	NFWR Below Trappers Lake (3079)	NFWR Below Missouri Creek (445)	NFWR Below Lost Creek (6111)	NFWR at CR15 (6110)	NFWR at Bel Aire (6107)	SFWR at USFS Camp- ground (3077)	SFWR above Bucke- ye Creek (3078)	SFWR at Bel Aire (6106)	WRM at Sleepy Cat (6105)	WRM above Coal Creek (6104)	WRM at Meeker Pasture (6103)
<b>Percent Common Taxa</b>											
NFWR Below Trappers Lake (3079)	--	32.20	29.3	37.5	26.2	32.1	30.6	25.4	30.6	28.8	30.8
NFWR Below Missouri Creek (445)	32.2	--	55.3	44.2	33.9	46.9	43.6	33.3	33.9	23.9	25.8
NFWR Below Lost Creek (6111)	29.3	55.32	--	50.0	30.9	40.8	40.7	35.3	40.7	21.2	19.4
NFWR at CR15 (6110)	37.5	44.23	50.0	--	39.6	39.2	50.0	47.9	44.4	32.3	24.2
NFWR at Bel Aire (6107)	26.2	33.93	30.9	39.6	--	31.5	39.3	39.2	44.4	46.4	28.1
SFWR at USFS Campground (3077)	32.1	46.94	40.8	39.2	31.5	--	50.0	38.8	38.9	25.4	23.4
SFWR above Buckeye Creek (3078)	30.6	43.64	40.7	50.0	39.3	50.0	--	44.2	49.1	28.4	30.3
SFWR at Bel Aire (6106)	25.4	33.33	35.3	47.9	39.2	38.8	44.2	--	53.1	38.6	23.4
WRM at Sleepy Cat (6105)	30.6	33.90	40.7	44.4	44.4	38.9	49.1	53.1	--	41.0	36.5
WRM above Coal Creek (6104)	28.8	23.88	21.2	32.3	46.4	25.4	28.4	38.6	41.0	--	50.0
WRM at Meeker Pasture (6103)	30.8	25.76	19.4	24.2	28.1	23.4	30.3	23.4	36.5	50.0	--
<b>Bray-Curtis Dissimilarity Index</b>											
NFWR Below Trappers Lake (3079)	--	0.82	0.85	0.82	0.84	0.86	0.84	0.85	0.73	0.80	0.80
NFWR Below Missouri Creek (445)	0.82	--	0.25	0.52	0.77	0.51	0.52	0.57	0.76	0.83	0.83
NFWR Below Lost Creek (6111)	0.85	0.25	--	0.47	0.75	0.52	0.46	0.39	0.70	0.79	0.53
NFWR at CR15 (6110)	0.82	0.52	0.47	--	0.54	0.72	0.43	0.41	0.52	0.73	0.84
NFWR at Bel Aire (6107)	0.84	0.77	0.75	0.54	--	0.84	0.65	0.52	0.44	0.69	0.80
SFWR at USFS Campground (3077)	0.86	0.51	0.52	0.72	0.84	--	0.63	0.59	0.79	0.92	0.93
SFWR above Buckeye Creek (3078)	0.84	0.52	0.46	0.43	0.65	0.63	--	0.39	0.54	0.79	0.81
SFWR at Bel Aire (6106)	0.85	0.57	0.39	0.41	0.52	0.59	0.39	--	0.48	0.71	0.82
WRM at Sleepy Cat (6105)	0.73	0.76	0.70	0.52	0.44	0.79	0.54	0.48	--	0.66	0.70

<b>Sites</b>	<b>NFWR Below Trappers Lake (3079)</b>	<b>NFWR Below Missouri Creek (445)</b>	<b>NFWR Below Lost Creek (6111)</b>	<b>NFWR at CR15 (6110)</b>	<b>NFWR at Bel Aire (6107)</b>	<b>SFWR at USFS Camp- ground (3077)</b>	<b>SFWR above Bucke ye Creek (3078)</b>	<b>SFWR at Bel Aire (6106)</b>	<b>WRM at Sleepy Cat (6105)</b>	<b>WRM above Coal Creek (6104)</b>	<b>WRM at Meeker Pasture (6103)</b>
WRM at above Coal Creek (6104)	0.80	0.83	0.79	0.73	0.69	0.92	0.79	0.71	0.66	--	0.56
WRM at Meeker Pasture (6103)	0.80	0.83	0.53	0.84	0.80	0.93	0.81	0.82	0.70	0.56	--

**Table B-8: Percent similarity and Bray-Curtis dissimilarity values for the macroinvertebrate sampled collected in 2018 using the Hess sampler for the Pesticide Study. NFWR = North Fork WRM, SFWR = SFWR, WRM = WRM Mainstem.**

Sites	NFWR below Lost Creek June 2018	NFWR below Lost Creek July 2018	NFWR below Lost Creek August 2018	NFWR at Bel Aire June 2018	NFWR at Bel Aire July 2018	NFWR at Bel Aire August 2018	NFWR at County Line Road 15 August 2018
<b>Percent Common Taxa</b>							
NFWR below Lost Creek June 2018	--	49.1	46.6	36.2	27.7	34.6	37.3
NFWR below Lost Creek July 2018	49.1	--	50.9	32.7	32.6	47.9	42
NFWR below Lost Creek August 2018	46.6	50.9	--	32.7	27.1	42	44.9
NFWR at Bel Aire June 2018	36.2	32.7	32.7	--	37.9	38.9	38.9
NFWR at Bel Aire July 2018	27.7	32.6	27.1	37.9	--	43.8	31.4
NFWR at Bel Aire August 2018	34.6	47.9	42	38.9	43.8	--	55.6
NFWR at County Line Road 15 August 2018	37.3	42	44.9	38.9	31.4	55.6	--
<b>Bray-Curtis Dissimilarity Index</b>							
NFWR below Lost Creek June 2018	--	0.41	0.5	0.74	0.81	0.73	0.69
NFWR below Lost Creek July 2018	0.41	--	0.35	0.62	0.7	0.59	0.6
NFWR below Lost Creek August 2018	0.5	0.35	--	0.62	0.72	0.58	0.52
NFWR at Bel Aire June 2018	0.74	0.62	0.62	--	0.26	0.4	0.32
NFWR at Bel Aire July 2018	0.81	0.7	0.72	0.26	--	0.47	0.34
NFWR at Bel Aire August 2018	0.73	0.59	0.58	0.4	0.47	--	0.36
NFWR at County Line Road 15 August 2018	0.69	0.6	0.52	0.32	0.34	0.36	--

## **Appendix C Results of Statistical Analysis for *Cladophora* Study**

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**Table C-1: Results of statistical analyses of metrics to determine if sites with *Cladophora* growths differed significantly from those without. C = sites with *Cladophora* (n = 23), NC = sites without *Cladophora* (n = 20). Metrics not shown were not significantly different ( $p \geq 0.063$ ). Metrics that are italicized were also significantly different when the dataset was narrowed to only the NFWR sites.**

Metric	Comparison	p-value	Metric	Comparison	p-value
<b>Basic Metrics</b>			<b>Tolerance Metrics</b>		
<i>HBI</i>	C > NC	<0.001	% Intolerant	C < NC	<0.001
Diversity Index	C > NC	0.023	# of Intolerant Taxa	C < NC	0.001
# of EPT taxa	C < NC	0.023	% Intolerant Taxa	C < NC	<0.001
<b>Composition Metrics</b>			% Tolerant	C > NC	0.002
% Dominant Taxon	C < NC	0.003	# of Tolerant Taxa	C > NC	0.001
% EPT	C < NC	0.001	<b>FFG Metrics</b>		
% EPT excluding Baetidae	C < NC	<0.001	% Collectors	C < NC	<0.001
% Coleoptera	C < NC	0.026	% Scrapers	C < NC	0.011
% Chironomidae	C > NC	0.001	# of Scraper Taxa	C < NC	0.014
# of Chironomid Taxa	C > NC	0.003	% Shredders	C < NC	<0.001
% Diptera	C > NC	0.002	<b>Habit Metrics</b>		
# of Diptera Taxa	C > NC	0.005	% Climbers	C < NC	0.005
% Ephemeroptera	C > NC	0.001	# of Clinger Taxa	C < NC	0.047
% Ephemeroptera excluding Baetidae	C < NC	<0.001	% Sprawlers	C > NC	<0.001
# of Plecoptera taxa	C < NC	0.015	# of Sprawler Taxa	C > NC	0.001
% Trichoptera	C < NC	<0.001			