Summary Report

Benthic Macroinvertebrate Biomonitoring Study, Upper Colorado River

2019

Prepared for:

Upper Colorado River
Wild and Scenic Stakeholder Group

Prepared by:

David E. Rees and Elise S. Grape
Timberline Aquatics, Inc.
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Introduction

The Colorado River originates in the Rocky Mountains of Colorado and eventually provides water for approximately 40 million people (USBR 2012). This freshwater resource sustains a variety of aquatic and terrestrial species (including rare and sensitive taxa), while also supporting municipal, agricultural, and recreational opportunities (Beeby et al. 2014). Specifically, the Upper Colorado River flows through a series of water-use and allocation developments such as diversions and recreational areas to support a wide-range of human needs and activities. Water diversions remove approximately 67% of the annual flow from the upper Colorado River, and future projects are likely to continue to impact the natural flow regime (CPW 2019). The variety of anthropogenic influences (including urban and agricultural runoff) have the ability to alter the hydrology (stream flow), biology (species diversity and interactions), chemistry (nutrient cycling), and physical habitat (substrate composition) both locally and farther downstream (WQCC 2014, CPW 2019, EPA 2011). Therefore, biomonitoring studies that assess the health of aquatic life provide a valuable tool that can be used to evaluate the influence of anthropogenic activities in the Upper Colorado River. Benthic macroinvertebrate communities have been used more than any other biological group to assess anthropogenic impacts on aquatic life in streams (Bonada et al. 2005, Chang et al. 2013).

An analysis of benthic macroinvertebrate community structure and function at specific sampling locations is expected to provide insight into the effects of anthropogenic influences on aquatic life (Alvarez-Cabria et al. 2009). Benthic macroinvertebrates are particularly effective as bioindicators because they are common in aquatic habitats, species-rich, and well described. Additionally, they exhibit a variety of tolerances to disturbances, and they are an important trophic link between primary production and top predators in aquatic ecosystems (Barbour 1999, Bonada et al. 2006, Alvarez-Cabria et al. 2009). In the Colorado River, for example, the salmonfly (*Pteronarcys californica*) processes leaf material, then eventually provides an important food source for brown trout and other aquatic (and terrestrial) species (CPW 2015). Species such as the salmonfly are highly sensitive to changes in the aquatic environment (like sedimentation, water chemistry, and temperature) and their presence (along with other sensitive taxa) in the river system can be used to assess the health of aquatic conditions (Bryce et al. 2010, Anderson et al. 2019).

This biomonitoring study included a stream section of the Upper Colorado River where recreational use (rafting, fishing, etc.) has been historically high and upstream diversions may be altering the natural flow regime. The wide range of stressors and potential interaction among disturbances can make identification of the predominant sources of stress difficult (Johnson et al. 2013). However, results from this study should provide a reliable measurement of the health of benthic macroinvertebrate communities at specific locations within the study area. The results from benthic macroinvertebrate sampling on 26 October 2019 are the focus of this report.

Study Area

The Upper Colorado River study area included approximately 83 km of the Colorado River within Grand and Eagle Counties (Table 1, Figure 1). The five (5) sampling locations were previously established for the purpose of evaluating physical habitat and the health of aquatic life in assessments conducted by Colorado State University and the Eagle River Watershed Council (Beeby et al. 2014). The two most upstream study sites (CR-PH and CR-Rad) were located within Grand County, and the three downstream sites (CR-SB, CR-aC, and CR-bRD) were located in Eagle County. This benthic macroinvertebrate monitoring study was conducted during October of 2019 using the same coordinates used during 2018 (Rees and Musto 2019). The most upstream site (CR-PH) was sampled below the Pumphouse Boat Ramp at Pumphouse Recreation Area, while site CR-Rad was located approximately 6.7 km downstream in riffle habitat below Radium Hot Springs. Farther downstream, site CR-SB was specifically located in riffle habitat upstream from State Bridge near the intersection of New Trough Rd and Highway 131 in Eagle County. The two remaining study sites included CR-aC (above Elk Creek in Catamount), and the farthest downstream site (CR-bRD), which was located upstream from the confluence with the Eagle River (Figure 1). A comparison of metric values was used to assess macroinvertebrate community health at each sampling location.

Objectives

The overall objective for the Benthic Macroinvertebrate Sampling and Analysis portion of this study was to provide an assessment of the health of macroinvertebrate communities in the Upper Colorado River and identify areas with potential anthropogenic impacts.

Table 1. GPS coordinates and elevations of sample sites on the Colorado River.

	Location	Latitude	Longitude	Elevation (m)
CR-PH	Colorado River at Pumphouse	39.98497	-106.51365	2122
CR-Rad	Colorado River at Radium	39.94984	-106.55788	2100
CR-SB	Colorado River at State Bridge	39.85765	-106.6469	2058
CR-aC	Colorado River above Catamount	39.91232	-106.78523	2008
CR-bRD	Colorado River below Red Dirt	39.70961	-107.04671	1898

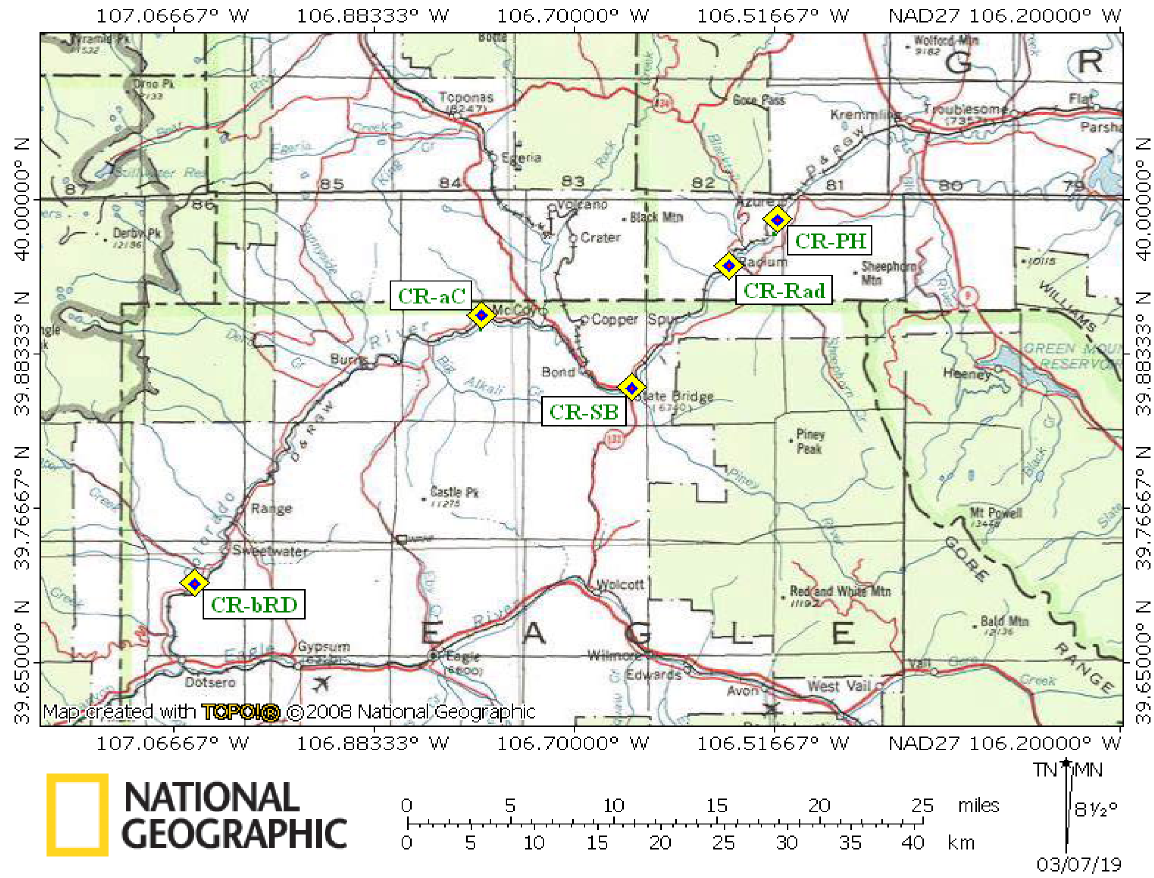

Figure 1. Map of study sites used for macroinvertebrate studies on the Upper Colorado River in 2019. This map was created with TOPO! © National Geographic Maps.

Methods

Biomonitoring Study

The effort that is used during benthic macroinvertebrate sampling (and processing of samples) is often proportional to the quality and quantity of information obtained in the investigation. The objective of this particular study required that three (3) replicate, quantitative Hess samples were taken from similar habitat at each study site. The Multi-Metric Index (MMI v4) and several individual biotic indices (metrics) were included in the data analysis to evaluate different aspects of macroinvertebrate community health, and account for different responses to various types of disturbances. The biomonitoring and analysis approach used for this project was intended to provide information describing local aquatic conditions, level of potential disturbances, and densities of various taxa.

Three quantitative, replicate samples were collected from each of the five sites in the study area on 26 October 2019. All samples were taken in similar habitat at each sampling location using a Hess Sampler to provide quantitative benthic macroinvertebrate data. Substrate within each sample was thoroughly agitated and individual rocks were scrubbed by hand to dislodge benthic organisms. All macroinvertebrates were rinsed into sample jars and preserved in 80% ethanol solution. Each sample jar was labeled (with date, location, and sample ID number) on the outside and inside of each container. Samples were transported to the lab at Timberline Aquatics, Inc. where they were sorted, identified, and enumerated. The sorting and identification process was conducted for each entire sample to avoid potential problems or controversy associated with subsampling.

The sorting and identification process used in this study required that all macroinvertebrates be removed from each sample and placed into vials containing major taxonomic groups. As part of the quality control protocols at Timberline Aquatics, Inc., all sorted macroinvertebrate samples were checked by a qualified taxonomist, and approximately 10% of the identifications were checked by Dr. Boris Kondratieff (Professor of Entomology at Colorado State University). As an additional means of QA/QC, Dr. Kondratieff confirmed identifications in all cases where the classification of a species was difficult or questionable. Macroinvertebrates were identified using a variety of taxonomic keys including Ward et al. (2002) and Merritt et al. (2008).

Multi-Metric Index (MMI v4)

In the fall of 2010, the Water Quality Control Division (WQCD) for the Colorado Department of Public Health and Environment (CDPHE) developed a Multi-Metric Index (MMI) to assist in the evaluation of benthic macroinvertebrate data from across the State of Colorado (Colorado Department of Public Health and Environment 2010). In 2017, the MMI was recalibrated and updated to produce a new analysis tool - the MMI v4 - that relies on specific methods and protocols for sample processing and analysis (Colorado Department of Public Health and Environment 2017).

The MMI v4 was applied to quantitative macroinvertebrate data collected from the Colorado River using the guidelines established in the WQCD Listing Methodology, 2018 Listing Cycle. Macroinvertebrates collected from the Upper Colorado River were identified to a taxonomic level consistent with the Operational Taxonomic Unit (OTU) established by the CDPHE. This level of identification is typically genus or species for mayflies, stoneflies, caddisflies, and many dipterans. Members of the family Chironomidae were also identified to the genus level. The MMI tool uses a rarefication process in the calculation of scores; however, any taxa that were both large and rare were included in the data used to generate final scores. The inclusion of rare taxa may provide important biological information because many rare taxa are considered sensitive to disturbances (Fore et al. 1996).

The group of metrics used in MMI v4 calculations depends on the sampling location and corresponding Biotype (Mountains, Transitional, or Plains). All sampling locations for the Upper Colorado River Study were located within Biotype 1 (the Transition Zone) which includes lower mountain areas in the State of Colorado. Each of the individual metrics used in the analysis produces a score that is adjusted to a scale from 1 to 100 based on the range of metric scores found at "reference sites". In Biotype 1, these metrics include: EPT Taxa, Percent Non-Insect Individuals, Percent EPT Individuals (excluding Baetidae), Percent Coleoptera Individuals, Percent Intolerant Taxa, Percent Increaser Individuals (Mid-Elevation), Clinger Taxa, and Predator/Shredder Taxa. A detailed description of individual metrics and the development of the MMI v4 can be found in the "Aquatic Life Use Attainment: Methodology to Determine Use Attainment for Rivers and Streams, Policy 10-1" (Colorado Department of Public Health and Environment 2017). Thresholds for the MMI v4 in Biotype 1 are as follows:

Biotype	Attainment Threshold	Impairment Threshold
Transitional (Biotype 1)	45.2	33.7

MMI v4 scores that fall between the thresholds for 'attainment' and 'impairment' are in the 'grey zone' and require further evaluation using two auxiliary metrics (Diversity and HBI). The following thresholds for the Diversity and HBI metrics have been adjusted specifically for the MMI v4 by the WQCD:

Biotype	HBI	Diversity
Transitional (Biotype 1)	5.8	2.1

Additional metrics used in the study:

Population densities and species lists were developed for each sampling location in the study area, and data were used in a variety of individual metrics to provide additional information regarding aquatic conditions. The following section provides a description of each individual metric used in this study:

Shannon Diversity (Diversity): Diversity was used as an auxiliary metric for the MMI and as an independent metric in this study to evaluate changes in macroinvertebrate community structure. The Diversity metric provides a measure of macroinvertebrate community balance. In unpolluted waters, Diversity values typically range from near 3.0 to 4.0. In polluted waters, this value is generally less than 1.0.

Hilsenhoff Biotic Index (HBI): The HBI is another auxiliary metric used for the MMI; however, it is also valuable as an independent metric and has been widely used and/or recommended in numerous regional biomonitoring studies (Paul et al. 2005). Most of its value lies in the detection of organic pollution, but it is also used to evaluate aquatic conditions in a variety of other circumstances. The HBI was originally developed using macroinvertebrate taxa from streams in Wisconsin; therefore, it may require regional modifications (Hilsenhoff 1988). Tolerance values for taxa occurring in this study area were taken from a list provided by the CDPHE which was derived from a variety of regional sources. Although HBI values may naturally vary among regions, a comparison of the values produced within the same river system should provide information regarding locations impacted by nutrients and/or other disturbances. Values for the HBI range from 0.0 to 10.0, and increase as water quality decreases.

Ephemeroptera Plecoptera Trichoptera Taxa (EPT Taxa): The design of this metric is based on the assumption that the benthic macroinvertebrate orders of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) are generally more sensitive to pollution than other orders (Lenat 1988). The EPT Taxa metric is currently an important and widely used metric in many regions of the United States (Barbour et al. 1999). The EPT Taxa value is simply given as the total number of distinguishable taxa in the orders Ephemeroptera, Plecoptera, and Trichoptera found at each station. This number will naturally vary among river systems, but it can be an excellent indicator of disturbance within a specific drainage. The EPT Taxa value is expected to decrease in response to a variety of stressors including nutrients (Wang et al. 2007).

Percent EPT (excluding Baetidae): This metric value is expressed as the percent composition of mayflies, stoneflies, and caddisflies in the sample, excluding the mayfly family Baetidae. The family Baetidae is considered one of the more tolerant families that is included among EPT taxa. A higher percentage from this metric is expected to indicate lower levels of stress in the aquatic environment. This metric is also included as a component of the MMI v4, where the metric value is transformed into a score (based on a scale from 0 to 100).

Percent Chironomidae: Chironomidae taxa are generally considered to be fairly tolerant of environmental stress when compared to other aquatic insect families (Plafkin et al. 1989). The Percent Chironomidae metric relies on the assumption that Chironomidae density will increase with decreasing water quality. Streams that are undisturbed often have a relatively even distribution of Ephemeroptera, Plecoptera, Trichoptera, and Chironomidae (Mandaville 2002); while the Chironomidae family often dominates (75% or more of the macroinvertebrate density) at sites degraded by metals or other pollutants (Barton and Metcalf-Smith 1992). Most species of Chironomidae tend to have a relatively short life cycle which enables them to continually re-colonize unstable or polluted habitats, making their abundance a relatively reliable indicator of environmental stress (Lenat 1983).

Density of *Pteronarcys californica*: *Pteronarcys californica* (aka the salmonfly or giant stonefly) is one of the largest stoneflies occurring in the western U.S. Since this species provides a major food source for fish and other aquatic and terrestrial species in the Upper Colorado River, the mean densities (number/m^2) of *Pteronarcys californica* were provided (based on quantitative replicate samples) for each study site.

Taxa Richness: Taxa Richness is often used to provide an indication of habitat adequacy and general water quality. Taxa Richness, or the total spectrum of taxa groups present at a given site, will generally decrease in response to decreasing water quality or habitat degradation (Weber 1973). The Taxa Richness measurement is reported as the total number of identifiable taxa collected from each sampling location. It is similar to the EPT Taxa metric, except that it includes all aquatic macroinvertebrate taxa (including those thought to be tolerant to disturbance).

Density: Macroinvertebrate abundance (Density) was reported as the mean number of macroinvertebrates per m^2 found at each study site. The Density value offers a means of measuring and comparing standing crop at each site, and this information can be used as part of the evaluation for the macroinvertebrate portion of the food web at each sampling location.

Functional Feeding Groups: Most of the previously described metrics use macroinvertebrate information that relies on community structure; however, macroinvertebrate taxa were also separated into functional guilds based on food acquisition to provide a measurement of ecological function. Some representation of each group usually indicates good aquatic conditions, although it is normal for certain groups (such as collector-gatherers) to be more abundant than others (Ward et al. 2002). Scrapers and shredders are often considered sensitive to disturbance because they are specialized feeders (Barbour et al. 1999). Consequently, these sensitive groups are expected to be well-represented in healthy streams. Much of the value from this type of analysis comes from comparison among sites within a specific study area. Changes in the proportion of functional feeding groups can provide insight into various types of stress in river systems (Ward et al. 2002).

Results/Discussion

Benthic Macroinvertebrate Sampling - Fall 2019

Five study sites on the Upper Colorado River were sampled for benthic macroinvertebrates in the fall (26 October) of 2019 in order to evaluate the health of aquatic life. Following the collection of macroinvertebrates in the field, samples were transported to the lab at Timberline Aquatics, Inc. where all specimens were sorted, identified, and enumerated (Appendix A: Tables A1-A5). The previously described metrics were applied to the macroinvertebrate data, and results were compared among sites to evaluate potential changes in the structure and function of benthic communities. Overall, macroinvertebrate communities remained relatively healthy throughout the study area; however, several metrics detected changes in community composition and structure. These changes were often subtle and most likely related to the available habitat, rather than a consequence of impacts to water quality. Results from select metrics were also compared to results from 2018 to assess any annual changes or similarities in benthic macroinvertebrate community structure.

Results from the MMI v4

In 2019, MMI v4 scores ranged from 59.0 (site CR-PH) to 81.1 (site CR-SB) at sampling locations within the Upper Colorado River Wild and Scenic study area. While all of these scores suggested that study sites were able to support relatively healthy macroinvertebrate communities, the difference between the lowest and highest score in the study area (>20.0% on the MMI v4 scale) was indicative of changes in community structure that were occurring among sites (Table 2, Figure 2). All MMI v4 scores were derived from individual (component) metrics that appeared to be somewhat inconsistent in their evaluation of aquatic conditions at each study site (Table 2). The EPT Taxa and Clinger Taxa metrics attained their highest scores in the middle portion of the study area, while the Percent Non-Insect Individuals and Percent Increasers (Mid-Elevation) scores remained relatively stable among sites. The Percent Coleoptera metric performed poorly throughout the study area; however, this metric was supportive of other component metrics that produced their most optimum score at site CR-SB (Table 2). The distribution and relative abundance of aquatic beetles is often seasonally variable, which may have contributed to the unusually low scores from this metric.

Overall, the combination of scores from the component metrics suggested that the proportion of tolerant individuals (macroinvertebrates that are expected to be tolerant of pollution and other stressors) remained low throughout the study area. This was an indication of good water quality at all study sites. Alternatively, the richness and abundance of the most sensitive taxa (EPT Taxa and % EPT Individuals (no Baetidae), respectively) and richness of taxa with specialized habitat and food requirements (Clinger Taxa and Predator/Shredder Taxa, respectively) was comparatively low at site CR-PH, and generally improved in the middle portion of the study area (Table 2). These component metrics were likely influenced by changes in habitat that were occurring throughout the study area.

The MMI v4 scores from 2019 (and 2018) were compared with threshold values to determine 'attainment' or 'impairment' within the study area (Figure 2). MMI scores greater than 45.2 (the green line in Figure 2) are considered in attainment for aquatic life use, while MMI scores below 33.7 (the red line in Figure 2) would have indicated impaired aquatic conditions. Although MMI scores exhibited some variability among sites (and between years), all sampling locations produced scores that were in attainment for aquatic life use (Figure 2, Table 3). Most study sites also demonstrated some consistency in MMI v4 scores between years, although site CR-Rad showed evidence of recent improvements in 2019 (Figure 2).

The MMI v4 program also provides a sediment Tolerance Indicator Value (TIV) which can be used to measure the proportion of the macroinvertebrate community that is considered tolerant to fine sediment (Table 2). TIV values exceeding the threshold of 6.3 in sediment region 3 often indicate stress due to sedimentation. During 2019 (and 2018) all sites generated values well-below this threshold, and the similarity in TIV scores between years suggested there was little change in substrate size composition (Figure 3).

Table 2. MMI v4 scores from quantitative, composited, (Hess) samples collected from the Upper Colorado River in October 2019.

Metric	Station ID				
	CR-PH	**CR-Rad**	**CR-SB**	**CR-aC**	**CR-bRD**
EPT Taxa	54.5	87.6	100.0	100.0	75.2
% Non-Insect Individuals	96.9	98.2	94.1	95.5	96.6
% EPT Individuals (no Baetidae)	33.0	75.1	90.0	72.4	95.0
% Coleoptera Individuals	2.9	13.5	24.1	8.0	14.3
% Intolerant Taxa	71.7	81.4	82.1	64.6	61.0
% Increasers (Mid-Elevation)	100.0	100.0	94.7	96.1	98.6
Clinger Taxa	62.9	92.8	100.0	100.0	81.8
Predator/Shredder Taxa	50.0	57.1	64.3	71.4	50.0
MMI	**59.0**	**75.7**	**81.1**	**76.0**	**71.6**
	Auxiliary Metrics				
Diversity	1.95	2.93	3.87	3.77	3.20
HBI	4.40	3.08	2.61	3.60	2.64
TIV (Sediment Region 3)	4.55	4.55	4.80	5.06	4.66

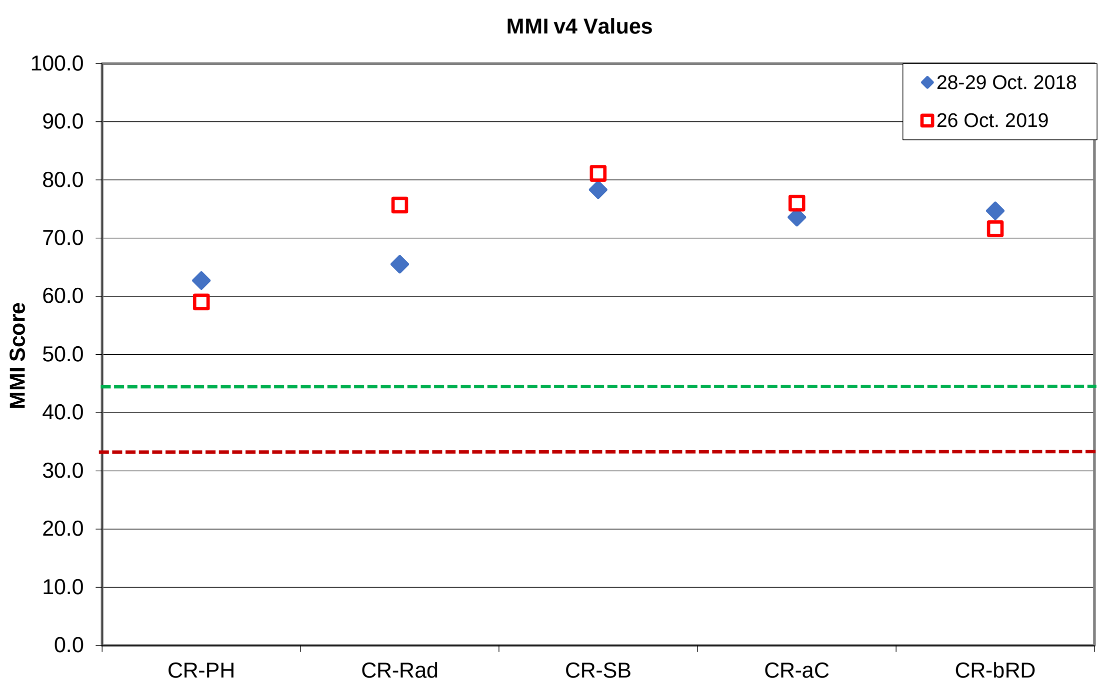

Figure 2. MMI v4 scores from composited quantitative (Hess) samples during the fall of 2018 and 2019 at sampling sites on the Upper Colorado River.

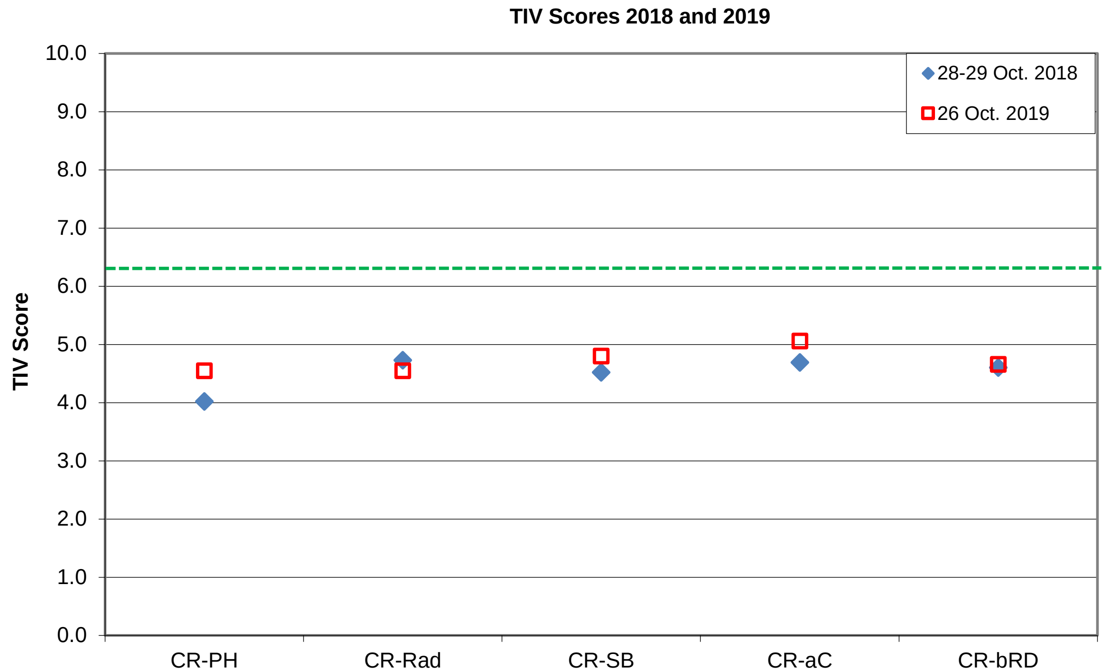

Figure 3. Sediment TIV scores from composited quantitative (Hess) samples during the fall of 2018 and 2019 at sampling sites on the Upper Colorado River.

Table 3. Aquatic life designations based on MMI v4 scores for five sample sites on the Upper Colorado River, 26 October 2019.

Aquatic Life Designations	
Site	Quantitative (Hess) Samples
CR-PH	Attainment
CR-Rad	Attainment
CR-SB	Attainment
CR-aC	Attainment
CR-bRD	Attainment

Results from Additional Metrics

In addition to the MMI v4, eight individual metrics were applied to the macroinvertebrate data from the Wild and Scenic study area to further analyze aquatic community health during the fall of 2019 (Table 4). Although the individual metrics were able to detect subtle changes in macroinvertebrate community structure among sites, most of these results were not indicative of stressed aquatic conditions. Overall, the five study sites could be characterized as supporting a variety of taxa in ecologically functioning communities. The stonefly *Pteronarcys californica* was not collected at site CR-bRD; however, a variety of sensitive taxa were found at all study sites (Table 4). The following comparison of individual metric values among sites provides a more detailed description of changes in aquatic communities occurring throughout the study area.

The most upstream site in this study area (site CR-PH) produced individual metric values that generally fell within a range indicating healthy aquatic conditions; however, many of these metric values also showed signs of increased stress when compared with other sites in the study area (Table 4). The Diversity value (1.95) suggested that the macroinvertebrate community was not optimally balanced at site CR-PH, and the EPT Taxa, Percent EPT (excluding Baetidae), and Taxa Richness values were the lowest among study sites. Although the combination of these results suggested that the benthic macroinvertebrate community may have been slightly stressed at site CR-PH, the ratio of sensitive taxa (EPT Taxa = 17) to total taxa (Taxa Richness = 28) remained high. Both tolerant and intolerant taxa were somewhat under-represented at this site, which is often a sign of habitat limitations rather than water quality impairment. The relatively low Density value (5,220 individuals/m^2) may have been another indication of habitat limitations, while the slightly elevated HBI value suggested that this site supported an elevated proportion of nutrient-tolerant taxa (Table 4). Historically, recreational use (fishing, rafting, etc.) at site CR-PH has been fairly high, and it is likely that wadable habitat is frequently disturbed. This could account for some of the stress detected by individual metrics at site CR-PH. While evidence of some stress was perceived at site CR-PH (based on a comparison of metric results with other sites in the study area), most of the individual metrics suggested that this location maintained an adequately functioning benthic macroinvertebrate community.

Individual metric results for site CR-Rad (located approximately 6.7 km downstream of site CR-PH) generally detected improvements in macroinvertebrate community structure. These observations were supported by metrics measuring community balance (Diversity), the total number of taxa (Taxa Richness), and the number of sensitive taxa (EPT Taxa), while the ratio of sensitive taxa to total taxa remained similar to site CR-PH (Table 4). Site CR-Rad also supported a high proportion of individuals representing sensitive taxa, (Percent EPT excluding Baetidae value = 54.08%), and lower proportions of tolerant taxa (Percent Chironomidae value = 1.41%). Several of these individual metrics may have been positively influenced by the increased abundance of *Pteronarcys californica* (Figure 4). *Pteronarcys californica* (the giant stonefly or salmonfly) is considered highly sensitive to stress, and the relatively high density (93/m^2) of this stonefly likely had a positive influence on certain individual metrics. Additionally, the Density metric suggested that aquatic conditions at site CR-Rad were able to support the highest abundance of macroinvertebrates (11,560 individuals/m^2) in the study area (Table 4). The combination of individual metric results indicated that site CR-Rad supported a high number of benthic macroinvertebrates (with relatively high proportions of sensitive individuals) during the fall of 2019.

Table 4. Metrics and comparative values for macroinvertebrate samples collected from the Upper Colorado River in October 2019.

Metric	CR-PH	CR-Rad	CR-SB	CR-aC	CR-bRD
Diversity	1.95	2.93	3.87	3.77	3.20
HBI	4.40	3.08	2.61	3.60	2.64
EPT Taxa	17	23	26	29	21
Percent EPT (excluding Baetidae)	23.14%	54.08%	65.94%	49.75%	71.50%
Percent Chironomidae	4.76%	1.41%	4.80%	11.76%	4.51%
Density of *Pteronarcys californica* (mean #/m^2)	23	93	151	8	0
Taxa Richness	28	39	54	52	41
Density (mean #/m^2)	5,220	11,560	6,563	8,621	11,358

Improvements in most metrics continued in a downstream direction to the study site established immediately upstream from State Bridge (site CR-SB). The Diversity and Taxa Richness values were the highest in the study area at site CR-SB, while the HBI value (2.61) indicated that this site supported the lowest proportion of nutrient-tolerant taxa (Table 4). The Percent EPT (excluding Baetidae) value of 65.94% suggested that the macroinvertebrate community was numerically dominated by sensitive individuals, and the Percent Chironomidae metric showed a relatively low proportion of tolerant taxa at this study site. Additionally, site CR-SB supported the highest Density of the stonefly *Pteronarcys californica* (151/m^2), indicating that aquatic conditions and habitat were favorable for this species (Table 4, Figure 4). *P. californica* also typically requires a healthy riparian corridor upstream of the sampling location to provide a preferred food source (willow leaves). Site CR-SB was the only site in the study area that produced all four of the available age classes of *P. californica* (Figure 5). Overall, results from the individual metrics suggested that the aquatic conditions at site CR-SB supported a well-balanced and taxa-rich macroinvertebrate community during October of 2019.

Farther downstream at site CR-aC, individual metrics detected similar overall aquatic community health; however, some metrics displayed minor changes in macroinvertebrate community structure. The EPT Taxa metric produced the most optimal value in the study area (29) at site CR-aC (Figure 6), and the Diversity and Taxa Richness values remained comparatively high among sites (Table 4). The HBI metric provided evidence of a slight increase in the proportion of nutrient-tolerant individuals at this site, and this was supported by a minor decrease in the Percent EPT (excluding Baetidae) value (Table 4). Additionally, there were fewer *Pteronarcys californica* (8/m^2) collected at site CR-aC in the fall of 2019. In general, the individual metrics suggested that some changes in habitat may have caused a shift in benthic community structure at site CR-aC; however, the overall health of the aquatic community remined similar to the adjacent upstream study site (Table 4).

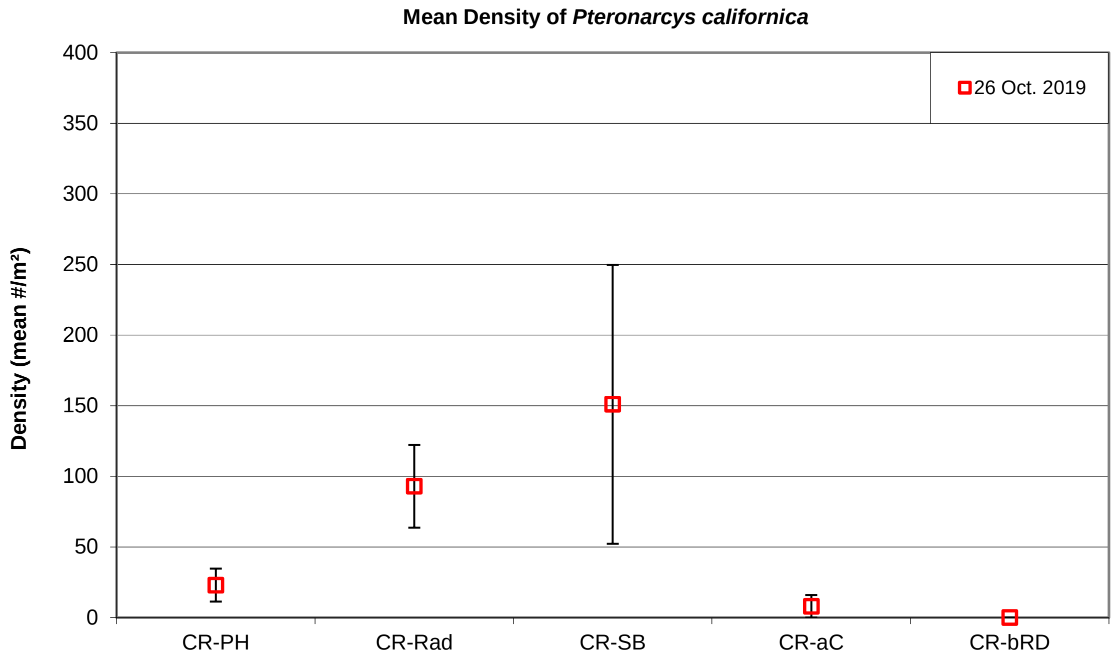

Figure 4. Mean densities (± 1 standard error) of *Pteronarcys californica* collected during the fall of 2019 at sampling sites on the Upper Colorado River.

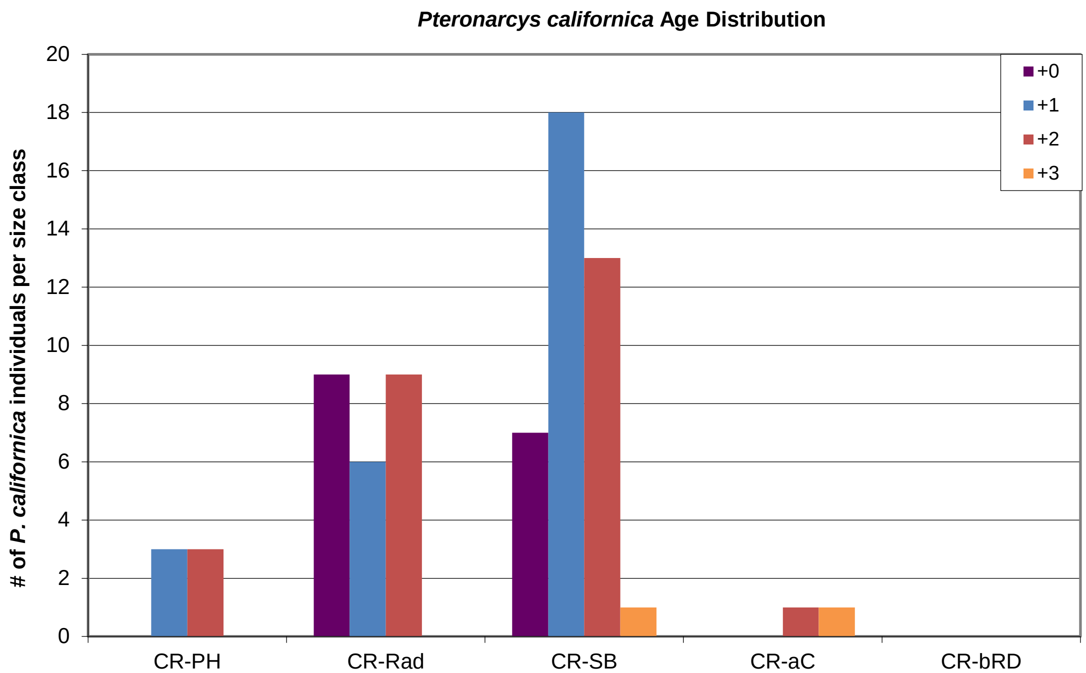

Figure 5. Densities (number/sample) of various age classes of *Pteronarcys californica* collected during the fall of 2019 at sampling locations on the Upper Colorado River.

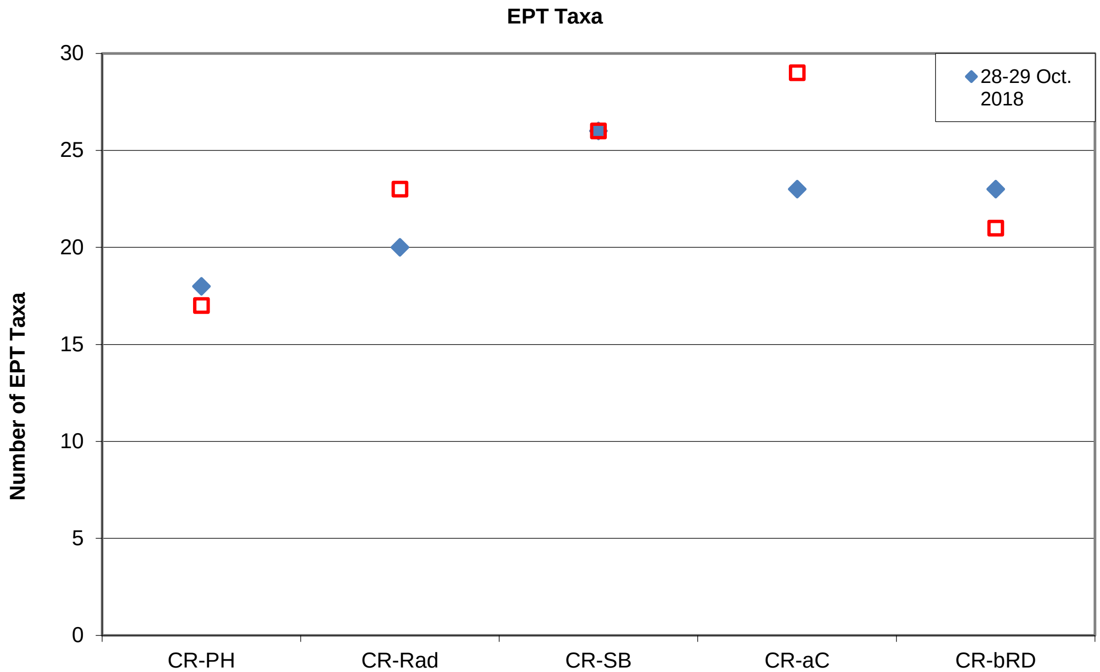

Figure 6. EPT Taxa collected during the fall of 2018 and 2019 at sampling sites on the Upper Colorado River.

The aquatic community at the farthest downstream site in the study area (CR-bRD) showed signs of continued changes in community structure, although most metrics continued to detect healthy aquatic conditions. While site CR-bRD produced a lower EPT Taxa value (21) and Taxa Richness value (41) than site CR-aC, the ratio of EPT Taxa to Taxa Richness remained similar to other sites in the study area (Table 4). The Density and Percent EPT (excluding Baetidae) values were among the highest in the study area, suggesting that site CR-bRD supported an abundance of macroinvertebrates (11,358 individuals/m^2) with a high proportion (71.50 %) of sensitive individuals. Additionally, the HBI metric decreased to one of the lowest (most optimal) values (2.64) within the study area. Although *Pteronarcys californica* was not collected in the fall of 2019 at this site, this species appeared to be replaced by an increased abundance of other sensitive taxa (Table 4, Figure 4). The absence of *Pteronarcys californica* and several of the observed shifts in metric values could likely be attributed to natural changes in the aquatic habitat.

A reorganization of benthic macroinvertebrates based on their method of food acquisition (ecological function) was conducted to assist in the interpretation of macroinvertebrate data collected during the fall of 2019 (Table 5, Figure 7). Healthy aquatic ecosystems typically support some representation from most feeding groups; however, it is common for certain groups (such as collector-gatherers) to be proportionally dominant. During the fall of 2019, the collector-gatherer group was dominant throughout the study area;

however, other feeding groups that are considered sensitive and/or specialized (collector-filterers, shredders, and scrapers) were also well-represented (Figure 7). Predators were found in consistently low proportions, and omnivores were rarely encountered within the study area. The most optimal distribution of functional feeding groups occurred at site CR-SB where collector-filterers made up 32.29% of the benthic macroinvertebrate community, and the most sensitive/specialized groups (shredders and scrapers) were present in their highest proportions (4.83% and 19.49%, respectively). Farther downstream at sites CR-aC and CR-bRD, there was some decline in the proportions of the most sensitive feeding groups, and the relative abundance of collector-gatherers increased (Table 5, Figure 7). These minor shifts in the relative abundance of various feeding groups may have been caused (in part) by changes in the availability of fine particulate organic material (FPOM) and coarse particulate organic material (CPOM) within the study area. An increase in leaf material (CPOM) entering the river from the riparian corridor upstream from site CR-SB may have been a factor contributing to the increase in *Pteronarcys californica* (a shredder) at that location. Overall, the results generated from the evaluation of functional feeding groups supported the results from the MMI v4 and individual metrics by suggesting that relatively healthy aquatic conditions existed at all study sites, with the most optimal ecological balance occurring in the middle portion of the study area (Figure 7).

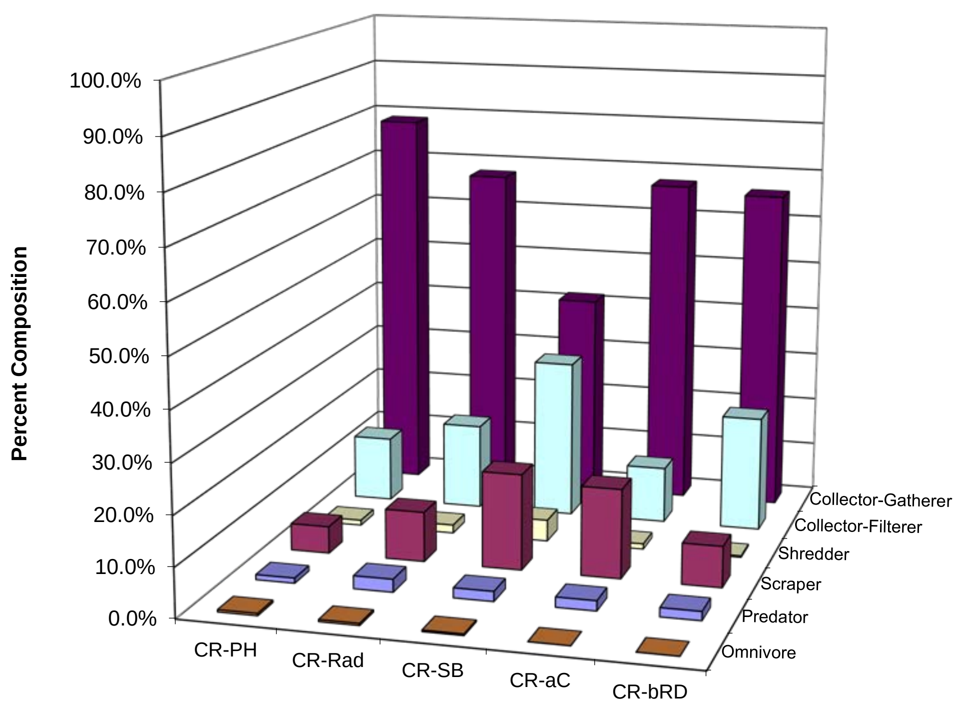

Figure 7. Functional feeding group composition for study sites on the Upper Colorado River in fall of 2019.

Table 5. Relative abundance of functional feeding groups during fall 2019 sampling on the Upper Colorado River.

Site	Functional Feeding Group					
	Collector-Gatherer	Collector-Filterer	Shredder	Scraper	Predator	Omnivore
CR-PH	78.65%	13.32%	1.04%	5.51%	1.04%	0.45%
CR-Rad	67.61%	17.52%	1.68%	10.17%	2.62%	0.40%
CR-SB	41.53%	32.29%	4.38%	19.49%	2.01%	0.30%
CR-aC	67.33%	11.45%	1.08%	18.12%	2.03%	0.00%
CR-bRD	66.10%	23.44%	0.31%	8.34%	1.81%	0.00%

Conclusions

Overall, the benthic macroinvertebrate communities in the Wild and Scenic study area demonstrated minor changes in structure and function while remaining relatively healthy. The MMI v4 and most individual metrics indicated that all sampling locations were able to support functioning macroinvertebrate communities with high proportions of sensitive taxa. A comparison of metric results among study sites suggested that site CR-PH may have been slightly more stressed than other sites in the study area. The applied metrics showed possible impacts to taxa richness (including sensitive and tolerant taxa) which could potentially be associated with disruptions in aquatic habitat. It is possible that higher recreational use at site CR-PH may have had some minor impacts on the macroinvertebrate community; however, the HBI value was also slightly elevated at this location suggesting an increase in nutrient-tolerant taxa. Overall, most of the changes in macroinvertebrate community structure (including densities of individual species) appeared to be related to changes in the availability of preferred habitat, food resources, competition, predation, etc. Future biomonitoring efforts will be helpful in the validation of recent observations and will assist in the monitoring of stressors that continue to threaten aquatic life in the Upper Colorado River.

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Appendix A

Benthic Macroinvertebrate Data – Fall 2019

Table A1. Macroinvertebrate data collected from site CR-PH on 26 Oct 2019.

Colorado River						
CR-PH		Sample				
26 October 2019	1	2	3		Totals	Mean#/m²
Ephemeroptera (mayflies)						
Ameletus sp.						
Acentrella sp.	1	1			2	8
Baetis (*tricaudatus*)	235	458	209		902	3497
Diphetor hageni						
Drunella grandis						
Ephemerella dorothea infrequens	29	13	46		88	342
Serratella micheneri						
Epeorus sp.	4	4	1		9	35
Heptagenia sp.	2				2	8
Rhithrogena sp.	2	10	3		15	59
Asioplax sp.						
Tricorythodes explicatus						
Paraleptophlebia sp.		1			1	4
Plecoptera (stoneflies)						
Paracapnia angulata						
Sweltsa sp.						
Triznaka sp.						
Claassenia sabulosa						
Perlodidae (*Cultus* sp.)		2	2		4	16
Isoperla sp.		2	1		3	12
Pteronarcys californica	4	1	1		6	24
Trichoptera (caddisflies)						
Brachycentrus americanus	1	1			2	8
Brachycentrus occidentalis						
Culoptila sp.						
Glossosoma sp.		3			3	12
Protoptila sp.						
Helicopsyche borealis						
Arctopsyche grandis						
Cheumatopsyche sp.						
Hydropsyche cockerelli			1		1	4
Hydropsyche occidentalis	8	6	10		24	93
Hydropsyche oslari	34	27	69		130	504
Hydroptila sp.	3	1	11		15	59
Leucotrichia pictipes						
Lepidostoma sp.	6	1	1		8	31
Oecetis sp.						
Psychomyia flavida						
Rhyacophila coloradensis						
Oligophlebodes sp.						

Table A1. cont. Macroinvertebrate data collected from site CR-PH on 26 Oct 2019.

Diptera (true flies)						
Chironomidae (chironomids)						
Cardiocladius sp.						
Cricotopus/Orthocladius sp.	6	8	36		50	194
Diamesa sp.	1	3	5		9	35
Eukiefferiella sp.		1	2		3	12
Lopescladius sp.						
Micropsectra/Tanytarsus sp.						
Microtendipes sp.						
Pagastia sp.						
Parametriocnemus sp.			2		2	8
Polypedilum sp.						
Potthastia sp.						
Thienemannimyia group						
Tvetenia sp.						
Other Diptera (true flies)						
Atherix pachypus			1		1	4
Hemerodromia sp.						
Simulium sp.		13	8		21	82
Antocha sp.						
Coleoptera (beetles)						
Helichus striatus						
Dubiraphia sp.						
Microcylloepus sp.						
Optioservus sp.	11		19		30	117
Zaitzevia parvula						
Odonata (dragonflies & damselflies)						
Ophiogomphus sp.						
Miscellaneous						
Atractides sp.						
Hygrobates sp.						
Protzia sp.						
Sperchon sp.	1		3		4	16
Ferrissia sp.						
Lymnaeidae						
Physa sp.						
Gyraulus sp.						
Pisidium sp.			1		1	4
Sphaerium sp.						
Dugesia sp.						
Polycelis coronata	1	2	3		6	24
Naididae						
Tubificidae w/out hair chaetae						
Nematoda	1		1		2	8
Totals	**350**	**558**	**436**		**1344**	**5,220**

Table A2. Macroinvertebrate data collected from site CR-Rad on 26 Oct 2019.

Colorado River						
CR-Rad		Sample				
26 October 2019	1	2	3		Totals	Mean#/m²
Ephemeroptera (mayflies)						
Ameletus sp.						
Acentrella sp.						
Baetis (*tricaudatus*)	143	433	379		955	3702
Diphetor hageni						
Drunella grandis						
Ephemerella dorothea infrequens	176	327	465		968	3752
Serratella micheneri						
Epeorus sp.	5	8	9		22	86
Heptagenia sp.	1		5		6	24
Rhithrogena sp.	25	50	40		115	446
Asioplax sp.						
Tricorythodes explicatus	1		1		2	8
Paraleptophlebia sp.	8	9	26		43	167
Plecoptera (stoneflies)						
Paracapnia angulata		1			1	4
Sweltsa sp.		1			1	4
Triznaka sp.	1				1	4
Claassenia sabulosa			2		2	8
Perlodidae (*Cultus* sp.)	1	9	7		17	66
Isoperla sp.	1	4	3		8	31
Pteronarcys californica	6	5	13		24	93
Trichoptera (caddisflies)						
Brachycentrus americanus		1	2		3	12
Brachycentrus occidentalis						
Culoptila sp.	1	5	11		17	66
Glossosoma sp.	2	2	8		12	47
Protoptila sp.	2	2			4	16
Helicopsyche borealis						
Arctopsyche grandis						
Cheumatopsyche sp.						
Hydropsyche cockerelli		2	2		4	16
Hydropsyche occidentalis	11	19	7		37	144
Hydropsyche oslari	68	108	113		289	1121
Hydroptila sp.		1	9		10	39
Leucotrichia pictipes						
Lepidostoma sp.	7	9	9		25	97
Oecetis sp.						
Psychomyia flavida						
Rhyacophila coloradensis						
Oligophlebodes sp.						

Table A2. cont. Macroinvertebrate data collected from site CR-Rad on 26 Oct 2019.

Diptera (true flies)						
Chironomidae (chironomids)						
Cardiocladius sp.	1	2	1		4	16
Cricotopus/Orthocladius sp.			6		6	24
Diamesa sp.		1	1		2	8
Eukiefferiella sp.	1		1		2	8
Lopescladius sp.			1		1	4
Micropsectra/Tanytarsus sp.						
Microtendipes sp.			1		1	4
Pagastia sp.						
Parametriocnemus sp.	1				1	4
Polypedilum sp.						
Potthastia sp.						
Thienemannimyia group			4		4	16
Tvetenia sp.	4	9	8		21	82
Other Diptera (true flies)						
Atherix pachypus		2	7		9	35
Hemerodromia sp.						
Simulium sp.	13	155	20		188	729
Antocha sp.						
Coleoptera (beetles)						
Helichus striatus						
Dubiraphia sp.						
Microcylloepus sp.						
Optioservus sp.	21	27	69		117	454
Zaitzevia parvula	1	3	9		13	51
Odonata (dragonflies & damselflies)						
Ophiogomphus sp.						
Miscellaneous						
Atractides sp.						
Hygrobates sp.						
Protzia sp.						
Sperchon sp.	3	3	17		23	90
Ferrissia sp.						
Lymnaeidae						
Physa sp.						
Gyraulus sp.						
Pisidium sp.						
Sphaerium sp.						
Dugesia sp.						
Polycelis coronata	2	6	4		12	47
Naididae						
Tubificidae w/out hair chaetae						
Nematoda	1	3	5		9	35
Totals	**507**	**1207**	**1265**		**2979**	**11,560**

Table A3. Macroinvertebrate data collected from site CR-SB on 26 Oct 2019.

Colorado River						
CR-SB		Sample				
26 October 2019	1	2	3		Totals	Mean#/m²
Ephemeroptera (mayflies)						
Ameletus sp.						
Acentrella sp.	1	3	2		6	24
Baetis (*tricaudatus*)	118	120	19		257	997
Diphetor hageni	1				1	4
Drunella grandis		2			2	8
Ephemerella dorothea infrequens	89	84	82		255	989
Serratella micheneri						
Epeorus sp.	2	5	7		14	55
Heptagenia sp.		5	3		8	31
Rhithrogena sp.	13	7	4		24	93
Asioplax sp.						
Tricorythodes explicatus	10	33	12		55	214
Paraleptophlebia sp.	11	9	7		27	105
Plecoptera (stoneflies)						
Paracapnia angulata						
Sweltsa sp.						
Triznaka sp.						
Claassenia sabulosa						
Perlodidae (*Cultus* sp.)	1		2		3	12
Isoperla sp.	1		1		2	8
Pteronarcys californica	30	5	4		39	152
Trichoptera (caddisflies)						
Brachycentrus americanus						
Brachycentrus occidentalis	48	63	284		395	1531
Culoptila sp.	4	13	38		55	214
Glossosoma sp.	15	19	11		45	175
Protoptila sp.	1	4			5	20
Helicopsyche borealis		1			1	4
Arctopsyche grandis						
Cheumatopsyche sp.	1	1			2	8
Hydropsyche cockerelli	26	16	6		48	186
Hydropsyche occidentalis	11	5	5		21	82
Hydropsyche oslari	32	14	10		56	218
Hydroptila sp.		13	2		15	59
Leucotrichia pictipes						
Lepidostoma sp.	8	15	11		34	132
Oecetis sp.						
Psychomyia flavida						
Rhyacophila coloradensis	1				1	4
Oligophlebodes sp.		6			6	24

Table A3. cont. Macroinvertebrate data collected from site CR-SB on 26 Oct 2019.

Diptera (true flies)						
Chironomidae (chironomids)						
Cardiocladius sp.						
Cricotopus/Orthocladius sp.		7	5		12	47
Diamesa sp.		1			1	4
Eukiefferiella sp.	2		3		5	20
Lopescladius sp.	1				1	4
Micropsectra/Tanytarsus sp.						
Microtendipes sp.	1	1			2	8
Pagastia sp.	1	1	1		3	12
Parametriocnemus sp.	1				1	4
Polypedilum sp.	1				1	4
Potthastia sp.						
Thienemannimyia group			1		1	4
Tvetenia sp.	37	10	7		54	210
Other Diptera (true flies)						
Atherix pachypus		1			1	4
Hemerodromia sp.	1				1	4
Simulium sp.	17		2		19	74
Antocha sp.			1		1	4
Coleoptera (beetles)						
Helichus striatus						
Dubiraphia sp.						
Microcylloepus sp.						
Optioservus sp.	68	50	24		142	551
Zaitzevia parvula	8	5	3		16	62
Odonata (dragonflies & damselflies)						
Ophiogomphus sp.		1			1	4
Miscellaneous						
Atractides sp.		2	1		3	12
Hygrobates sp.						
Protzia sp.	1				1	4
Sperchon sp.	9	1	1		11	43
Ferrissia sp.			6		6	24
Lymnaeidae		1			1	4
Physa sp.	1	4			5	20
Gyraulus sp.						
Pisidium sp.		2			2	8
Sphaerium sp.						
Dugesia sp.						
Polycelis coronata	3	2			5	20
Naididae	4	1			5	20
Tubificidae w/out hair chaetae	1				1	4
Nematoda	8	1			9	35
Totals	**589**	**534**	**565**		**1688**	**6,563**

Table A4. Macroinvertebrate data collected from site CR-aC on 26 Oct 2019.

Colorado River						
CR-aC		Sample				
26 October 2019	1	2	3		Totals	Mean#/m²
Ephemeroptera (mayflies)						
Ameletus sp.		1			1	4
Acentrella sp.		3	3		6	24
Baetis (*tricaudatus*)	151	324	189		664	2574
Diphetor hageni						
Drunella grandis						
Ephemerella dorothea infrequens	119	112	244		475	1842
Serratella micheneri		1			1	4
Epeorus sp.	2	2	5		9	35
Heptagenia sp.	1	3	1		5	20
Rhithrogena sp.	14	16	31		61	237
Asioplax sp.		6	5		11	43
Tricorythodes explicatus	14	15	20		49	190
Paraleptophlebia sp.	1	8	8		17	66
Plecoptera (stoneflies)						
Paracapnia angulata						
Sweltsa sp.						
Triznaka sp.						
Claassenia sabulosa	1				1	4
Perlodidae (*Cultus* sp.)	2	3	6		11	43
Isoperla sp.		2	1		3	12
Pteronarcys californica	2				2	8
Trichoptera (caddisflies)						
Brachycentrus americanus						
Brachycentrus occidentalis	3	7	6		16	62
Culoptila sp.	56	27	64		147	570
Glossosoma sp.	2	2	1		5	20
Protoptila sp.	2				2	8
Helicopsyche borealis						
Arctopsyche grandis	1				1	4
Cheumatopsyche sp.	4	14	4		22	86
Hydropsyche cockerelli	6	4	5		15	59
Hydropsyche occidentalis	22	14	19		55	214
Hydropsyche oslari	23	18	19		60	233
Hydroptila sp.	47	58	7		112	435
Leucotrichia pictipes		1			1	4
Lepidostoma sp.	6	9	4		19	74
Oecetis sp.		2			2	8
Psychomyia flavida	1				1	4
Rhyacophila coloradensis						
Oligophlebodes sp.						

Table A4. cont. Macroinvertebrate data collected from site CR-aC on 26 Oct 2019.

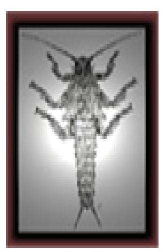
Diptera (true flies)						
Chironomidae (chironomids)						
Cardiocladius sp.	2	4			6	24
Cricotopus/Orthocladius sp.	23	37	8		68	264
Diamesa sp.	13	54	2		69	268
Eukiefferiella sp.	13	13	1		27	105
Lopescladius sp.						
Micropsectra/Tanytarsus sp.		1			1	4
Microtendipes sp.	4	8	1		13	51
Pagastia sp.						
Parametriocnemus sp.						
Polypedilum sp.	2	1			3	12
Potthastia sp.	4	1	1		6	24
Thienemannimyia group						
Tvetenia sp.	33	20	15		68	264
Other Diptera (true flies)						
Atherix pachypus	1		4		5	20
Hemerodromia sp.						
Simulium sp.	7	58	7		72	280
Antocha sp.						
Coleoptera (beetles)						
Helichus striatus						
Dubiraphia sp.		1			1	4
Microcylloepus sp.		1			1	4
Optioservus sp.	12	17	12		41	159
Zaitzevia parvula	1	6	4		11	43
Odonata (dragonflies & damselflies)						
Ophiogomphus sp.						
Miscellaneous						
Atractides sp.	1				1	4
Hygrobates sp.	1	2			3	12
Protzia sp.						
Sperchon sp.	4	4	5		13	51
Ferrissia sp.	3	2	1		6	24
Lymnaeidae	1	1			2	8
Physa sp.	2	6			8	31
Gyraulus sp.	1	2			3	12
Pisidium sp.						
Sphaerium sp.						
Dugesia sp.						
Polycelis coronata						
Naididae		17			17	66
Tubificidae w/out hair chaetae						
Nematoda						
Totals	**608**	**908**	**703**		**2219**	**8,621**

Table A5. Macroinvertebrate data collected from site CR-bRD on 26 Oct 2019.

Colorado River						
CR-bRD		Sample				
26 October 2019	1	2	3		Totals	Mean#/m²
Ephemeroptera (mayflies)						
Ameletus sp.						
Acentrella sp.						
Baetis (*tricaudatus*)	71	158	245		474	1838
Diphetor hageni						
Drunella grandis						
Ephemerella dorothea infrequens	366	405	408		1179	4570
Serratella micheneri						
Epeorus sp.	1	2			3	12
Heptagenia sp.	2	6	10		18	70
Rhithrogena sp.	13	28	34		75	291
Asioplax sp.	1	1			2	8
Tricorythodes explicatus	11	7	13		31	121
Paraleptophlebia sp.	8	22	27		57	221
Plecoptera (stoneflies)						
Paracapnia angulata						
Sweltsa sp.						
Triznaka sp.						
Claassenia sabulosa	4	5	7		16	62
Perlodidae (*Cultus* sp.)	6	2	3		11	43
Isoperla sp.	3	1			4	16
Pteronarcys californica						
Trichoptera (caddisflies)						
Brachycentrus americanus						
Brachycentrus occidentalis	49	119	60		228	884
Culoptila sp.	11	14	31		56	218
Glossosoma sp.						
Protoptila sp.		2	1		3	12
Helicopsyche borealis						
Arctopsyche grandis	4	11	7		22	86
Cheumatopsyche sp.	19	11	24		54	210
Hydropsyche cockerelli	12	25	8		45	175
Hydropsyche occidentalis	2	3			5	20
Hydropsyche oslari	82	134	59		275	1066
Hydroptila sp.	1	1			2	8
Leucotrichia pictipes						
Lepidostoma sp.	2	1	3		6	24
Oecetis sp.						
Psychomyia flavida						
Rhyacophila coloradensis						
Oligophlebodes sp.						

Table A5. cont. Macroinvertebrate data collected from site CR-bRD on 26 Oct 2019.

Diptera (true flies)						
Chironomidae (chironomids)						
Cardiocladius sp.						
Cricotopus/Orthocladius sp.	5	4	5		14	55
Diamesa sp.						
Eukiefferiella sp.	2	2	4		8	31
Lopescladius sp.						
Micropsectra/Tanytarsus sp.	1				1	4
Microtendipes sp.		3	2		5	20
Pagastia sp.						
Parametriocnemus sp.	1	1	3		5	20
Polypedilum sp.	2				2	8
Potthastia sp.			1		1	4
Thienemannimyia group	3	2	1		6	24
Tvetenia sp.	33	35	22		90	349
Other Diptera (true flies)						
Atherix pachypus						
Hemerodromia sp.		1			1	4
Simulium sp.	5	40	6		51	198
Antocha sp.						
Coleoptera (beetles)						
Helichus striatus			1		1	4
Dubiraphia sp.						
Microcylloepus sp.	6	14	2		22	86
Optioservus sp.	31	35	21		87	338
Zaitzevia parvula	13	23	13		49	190
Odonata (dragonflies & damselflies)						
Ophiogomphus sp.						
Miscellaneous						
Atractides sp.						
Hygrobates sp.						
Protzia sp.						
Sperchon sp.	3		3		6	24
Ferrissia sp.						
Lymnaeidae						
Physa sp.						
Gyraulus sp.						
Pisidium sp.						
Sphaerium sp.	1				1	4
Dugesia sp.	1		1		2	8
Polycelis coronata						
Naididae						
Tubificidae w/out hair chaetae			1		1	4
Nematoda	5	2			7	28
Totals	**780**	**1120**	**1026**		**2926**	**11,358**



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