Summary Report

Benthic Macroinvertebrate Biomonitoring Study Upper Colorado River

2021

Prepared for:

Upper Colorado River
Wild and Scenic Stakeholder Group

Prepared by:

David E. Rees and Kassidy R. Fenske
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4219 Table Mountain Place, Suite A
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Introduction

The Colorado River flows some 2,092 km from its headwaters in Wyoming and Colorado to its mouth in Baja California, Mexico (Meyers 1966). The Colorado River is fed by snow-melt, draining 1/12th of the United States (Bishop and Porcella 1980, Stanford and Ward 1986), and this water supply has been credited with allowing for the economic development of the Southwest (Fradkin 1981). Unfortunately, the Colorado River has also been identified as the most overallocated river in the world (Christensen et al. 2004, Miller et al. 2015). In less than a century, the Colorado River has been irreversibly transformed into a tame, man-made system of regulated segments (Carlson and Muth 1989). The upper Colorado River flows through a series of water-use and allocation developments including reservoirs and diversions, removing about 67% of the annual flow (Colorado Parks and Wildlife 2019).

The Upper Colorado River Basin is home to at least 65% of the known species of plants and animals of the western U.S., even though this basin comprises only 5% of the actual land area (Triedman 2012). These taxa also include benthic macroinvertebrates that inhabit the mainstem of the Upper Colorado River. For example, the well-known stonefly *Pteronarcys californica* (or "Salmonfly") is a large insect species that requires specific aquatic conditions to complete its relatively long four-year life cycle (Kowalski and Richer 2020). Therefore, this species is known to be sensitive to a variety of anthropogenic disturbances and it is an integral component of the Upper Colorado River food-web, processing leaf material from a healthy riparian corridor as a food source.

Biomonitoring (or bioassessment) studies that utilize benthic macroinvertebrates are often recommended for the evaluation of aquatic environments (Plafkin et al. 1989, Barbour et al. 1999, Paul et al. 2005, USEPA 2011, Hauer and Lamberti 2017, Merritt et al. 2019). The biomonitoring of aquatic life in streams allows for a scientific assessment of aquatic conditions that cannot be achieved through other types (chemical, physical, etc.) of monitoring programs (Ward et al. 2002, Hauer and Resh 2017, Mazor et al. 2019). Evolution and ecological processes have resulted in benthic macroinvertebrate communities with specific adaptations and sensitivities to their surrounding environment (Huryn and Wallace 2019). Therefore, aquatic macroinvertebrates are sensitive to a wide range of environmental disturbances (such as pollution, deviations from the natural flow and temperature regime, etc.) and community composition reflects the physical and chemical conditions that occur within a stream segment and associated watershed over time. Consequently, benthic macroinvertebrate communities can be monitored using specific sampling methodologies to assess the ecological integrity of aquatic systems.

This biomonitoring study included a 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. Results from this study were expected to provide a reliable assessment of the health of benthic macroinvertebrate communities at specific locations within the Wild and Scenic study area on 4 November 2021.

Study Area

The Upper Colorado River study area includes 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 November of 2021 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 established 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

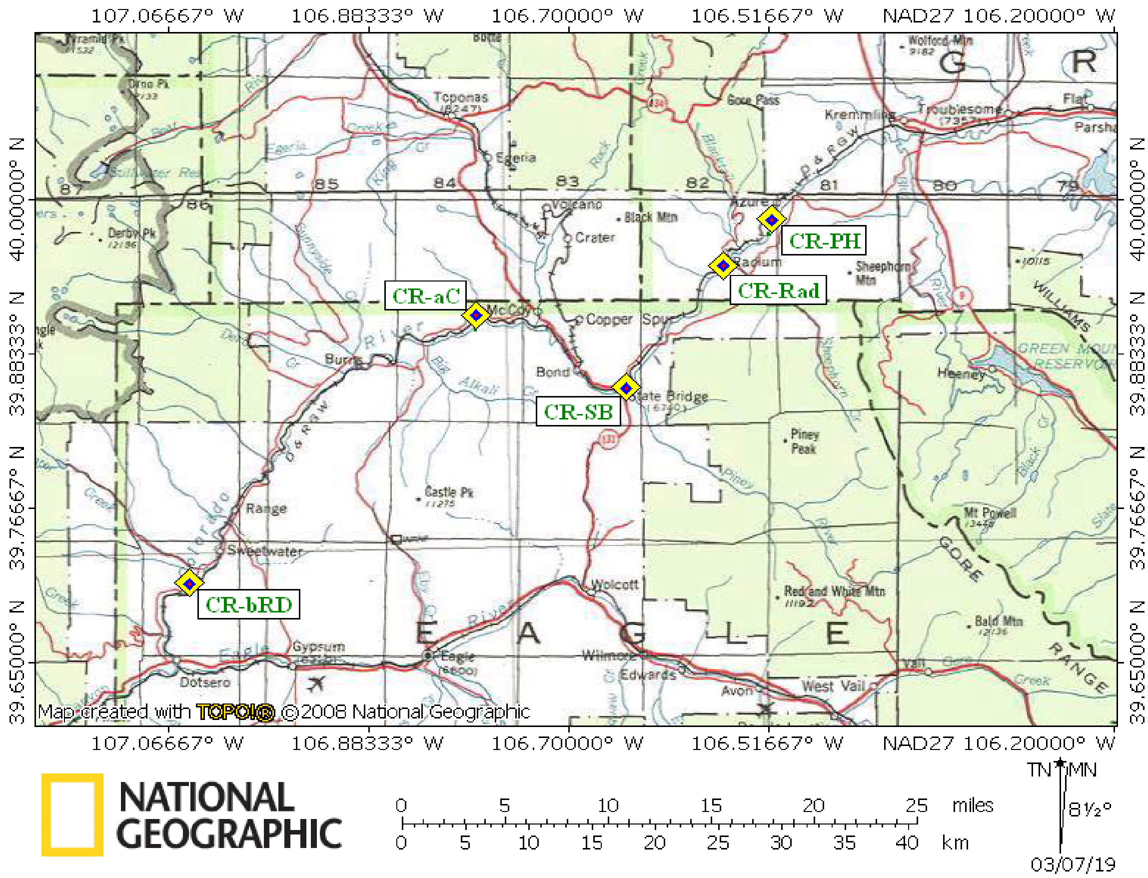

Figure 1. Map of study sites used for macroinvertebrate studies on the Upper Colorado River in 2021. This map was created with TOPO! © National Geographic Maps.

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 4 November 2021. 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., <u>all</u> sorted macroinvertebrate samples were checked by a qualified taxonomist, and approximately 10% of the identifications were checked by a professor of Entomology at Colorado State University. Macroinvertebrates were identified using a variety of taxonomic keys including Ward et al. (2002) and Merritt et al. (2019).

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 updated and recalibrated 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 Upper Colorado River study area using the guidelines established in Appendix D of the *Section 303(d) Listing Methodology 2020 Listing Cycle* (Colorado Department of Public Health and Environment 2019). 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 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 Basin, the mean densities (number of individuals/m^2) of *P. 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 provides an opportunity to measure and compare 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 food resources or stress in river systems (Ward et al. 2002).

Results/Discussion

Benthic Macroinvertebrate Sampling - Fall 2021

Five study sites on the Upper Colorado River were sampled for aquatic macroinvertebrates in the fall (4 November) of 2021 in order to evaluate the health of benthic communities. 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, benthic macroinvertebrate communities remained relatively healthy throughout the study area in the fall of 2021; however, several metrics detected changes in community structure and function occurring from upstream to downstream. In most cases these changes were often subtle and most likely related to changes in the available habitat, rather than a consequence of impacts to water quality. Results from select metrics used in 2021 were also compared to results from 2018 and 2019 to assess any annual changes or similarities in benthic macroinvertebrate community structure that may have been occurring over time.

Results from the MMI v4

In 2021, the MMI v4 indicated that all study sites in the Upper Colorado River Wild and Scenic study area supported healthy macroinvertebrate communities and the overall health of these communities remained relatively stable from upstream to downstream. The range in MMI v4 scores in the study area was less than 13.0% (on the MMI v4 scale) with the lowest score (69.5) occurring at site CR-Rad and the highest score (81.8) occurring at site CR-SB (Table 2). Additionally, all study sites produced MMI v4 scores that were similar to those observed during 2018 and 2019 (Figure 2).

All MMI v4 scores were derived from eight individual (component) metrics that measure different characteristics of the macroinvertebrate community (Table 2). The component metrics that generated relatively high scores throughout the study area included the EPT Taxa, Percent Non-Insect Individuals, Percent Increasers (Mid-Elevation), and Clinger Taxa metrics. The Percent EPT Individuals (no Baetidae) and Predator/Shredder Taxa metrics were more variable among sites, while the Percent Coleoptera metric performed rather poorly throughout much of the study area (Table 2). The Percent Coleoptera metric measures the relative abundance of aquatic beetles, which is often seasonally variable; however, this metric, like most others, indicated that site CR-SB supported the healthiest macroinvertebrate community among study sites (Table 2).

Overall, results from the individual 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. Alternatively, the richness and abundance of the most sensitive taxa (EPT Taxa and Percent Intolerant Taxa, respectively) and richness of taxa with specialized habitats (Clinger Taxa) remained high (Table 2). This was an indication of good water quality and healthy aquatic conditions at all study sites.

The MMI v4 scores from 2021 (and 2018-2019) 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 minor 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 consistency in MMI v4 scores among years, with some evidence of recent improvements at site CR-PH in 2021 (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 deposition (Table 2). TIV values exceeding the threshold of 6.3 in sediment region 3 would be an indication of stress due to sedimentation. During all monitoring years (2018-2019, and 2021) the five sites in this study area generated stable TIV values that were well-below this threshold (Figure 3).

Table 2. MMI v4 scores from quantitative, composited, (Hess) samples collected from the Upper Colorado River on 4 November 2021.

Metric	Station ID				
	CR-PH	**CR-Rad**	**CR-SB**	**CR-aC**	**CR-bRD**
EPT Taxa	79.6	78.4	90.8	82.6	95.3
% Non-Insect Individuals	96.2	97.6	96.9	97.0	96.7
% EPT Individuals (no Baetidae)	53.2	47.5	84.0	64.8	26.5
% Coleoptera Individuals	5.5	3.1	22.3	10.7	18.1
% Intolerant Taxa	87.5	93.4	89.1	73.3	71.9
% Increasers (Mid-Elevation)	97.5	98.8	100.0	100.0	98.7
Clinger Taxa	87.0	87.7	100.0	90.8	98.1
Predator/Shredder Taxa	64.3	50.0	71.4	42.9	57.1
MMI	**71.4**	**69.5**	**81.8**	**70.2**	**70.3**
	Auxiliary Metrics				
Diversity	2.90	3.02	3.65	3.18	2.56
HBI	3.86	4.08	2.80	3.66	4.64
TIV (Sediment Region 3)	4.66	4.58	4.58	4.65	4.45

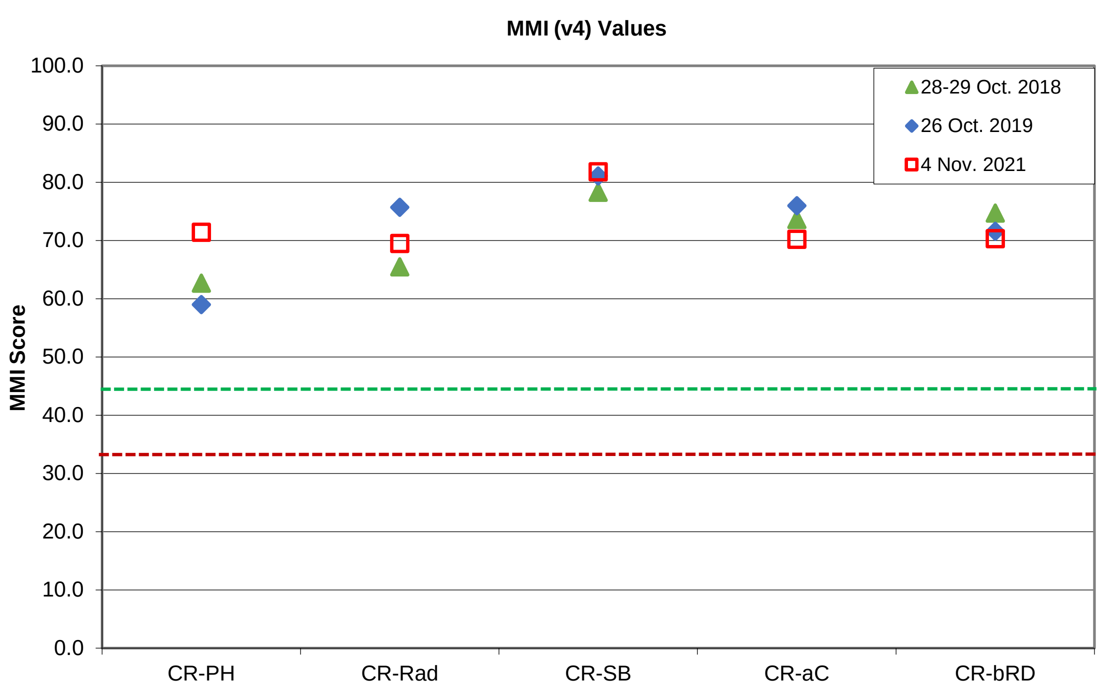

Figure 2. MMI v4 scores from composited quantitative (Hess) samples during the fall of 2018, 2019, and 2021 at sampling sites on the Upper Colorado River.

Table 3. Aquatic life use designations based on MMI v4 scores for five sample sites on the Upper Colorado River, 4 November 2021.

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

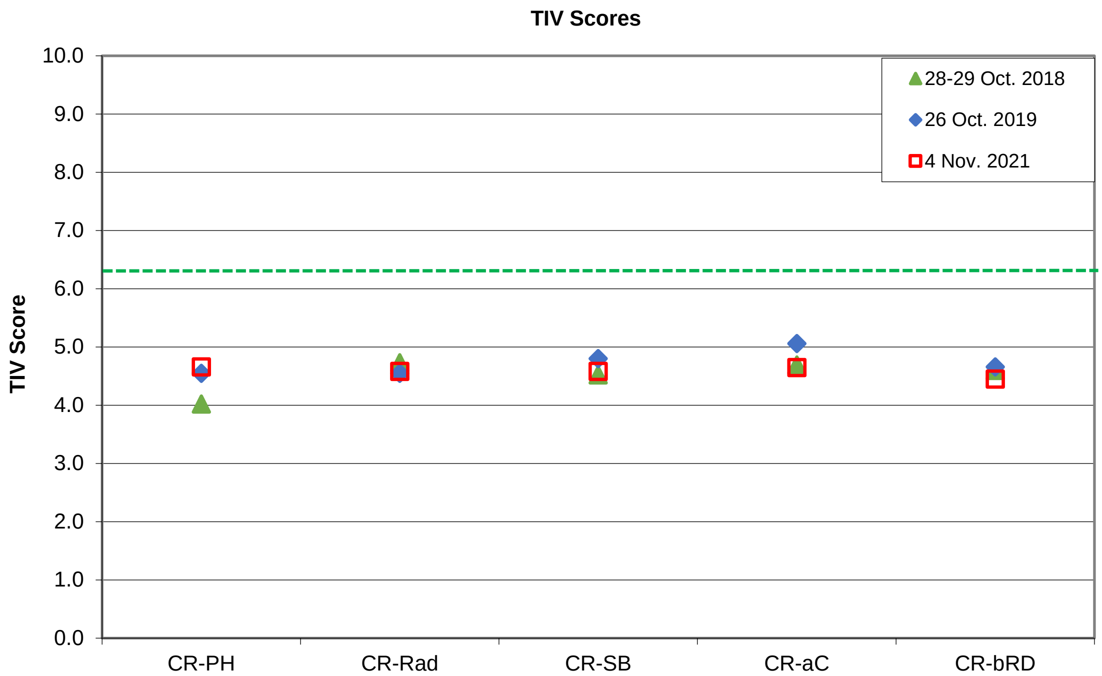

Figure 3. Sediment TIV scores from composited quantitative (Hess) samples during the fall of 2018, 2019, and 2021 at sampling sites on the Upper Colorado River.

Results from Additional Metrics

In addition to the MMI v4, eight individual metrics were used to assist in the evaluation of macroinvertebrate data collected from the Upper Colorado River study area during the fall of 2021 (Table 4). While several of the individual metrics were able to detect subtle changes in macroinvertebrate community structure among sites, most metric results suggested that all study sites maintained healthy aquatic conditions. Overall, macroinvertebrate communities could be characterized as supporting relatively high densities of sensitive individuals and a variety of sensitive taxa (including the "Giant Stonefly", *Pteronarcys californica*). In 2021, there was some variability in community balance and macroinvertebrate abundance throughout the study area; however, these community parameters had little influence on the overall health of benthic macroinvertebrate communities. A detailed evaluation of the individual metric values found at each sampling location has been provided below.

Individual metric values from the most upstream study site, CR-PH, were fairly consistent in detecting healthy aquatic conditions during the fall of 2021 (Table 4). The Diversity value (2.90) suggested that the macroinvertebrate community was adequately balanced, and the relatively low HBI value (3.86) was an indication that this location supported a low proportion of nutrient-tolerant individuals. A comparison of the EPT Taxa value (24) to the Taxa Richness value (45) confirmed that more than half of the identifiable taxa at site CR-PH could be considered 'sensitive' to disturbances. The EPT Taxa value from 2021 also showed an improvement compared to previous sampling events (Figure 4). Other individual metrics, including the Percent EPT (excluding Baetidae) (39.77%) and Percent Chironomidae (2.99%), provided additional evidence that this site supported high proportions of sensitive individuals (Table 4). With a macroinvertebrate density value of 16,589/m^2, and a relatively high density of *P. californica* (120/m^2) (Figure 5), it is likely that site CR-PH had the capacity to support a high biomass of insectivorous fish. Although the density of *P. californica* was similar to the density found in the fall of 2018 (Figure 6), most of the individuals collected in 2021 were relatively small (age 0+) (Figure 7). 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 variability in individual metric values that was observed among years at site CR-PH.

Table 4. Metrics and comparative values for macroinvertebrate samples collected from the Upper Colorado River on 4 November 2021.

Metric	CR-PH	CR-Rad	CR-SB	CR-aC	CR-bRD
Diversity	2.90	3.02	3.65	3.18	2.56
HBI	3.86	4.08	2.80	3.66	4.64
EPT Taxa	24	25	25	21	25
Percent EPT (excluding Baetidae)	39.77%	34.35%	61.38%	46.34%	21.02%
Percent Chironomidae	2.99%	1.80%	2.67%	1.82%	4.10%
Density of *Pteronarcys californica* (mean #/m^2)	120	360	368	12	4
Taxa Richness	45	42	45	35	43
Density (mean #/m^2)	16,589	16,623	10,324	8,124	13,993

Site CR-Rad was located approximately 6.7 km downstream from site CR-PH. Individual metric results from this location demonstrated slight changes in community structure while still showing evidence of a healthy aquatic conditions (Table 4). Minor improvements in the Diversity, EPT Taxa, and Percent Chironomidae values were observed at site CR-Rad; although, there was also some decline in Taxa Richness and Percent EPT (excluding Baetidae) (Table 4). Additionally, site CR-Rad produced the highest Density of benthic macroinvertebrates (16,623/m^2) in the study area. When compared to the upstream site (CR-PH), there was a noticeable increase in the abundance of *P. californica* at site CR-Rad (Figures 5-6), with all four age classes being represented in the quantitative replicate samples (Figure 7). Since *P. californica* is considered highly sensitive to disturbances, the high density of this stonefly (360 individuals/m^2) provided additional evidence of healthy aquatic conditions at site CR-Rad in the fall of 2021. Overall, the combination of individual metric results indicated that site CR-Rad supported a high abundance of benthic macroinvertebrates (including a large number of sensitive taxa) during the fall of 2021.

Farther downstream, most of the individual metrics (and the MMI v4) generated their most optimal values in the study area at site CR-SB (Tables 2 and 4). The Diversity value (3.65) was the highest among study sites, indicating that site CR-SB maintained optimal community balance, while the EPT and Taxa Richness metrics demonstrated the ability of this sampling location to support a variety of aquatic taxa (including a large number of sensitive taxa). The relatively low Percent Chironomidae and HBI values suggested that site CR-SB maintained low proportions of tolerant taxa (including nutrient-tolerant taxa), while the Percent EPT (excluding Baetidae) metric indicated that more than half (61.38%) of the aquatic community was sensitive to anthropogenic disturbances (Table 4). The Percent EPT (excluding Baetidae) may have also been influenced by the high density of *P. californica* (368/m^2) at site CR-SB, which was the highest in the study area and represented a substantial increase compared to previous sampling events at this site (Figure 6). Site CR-SB was also one of only two study sites where all four age classes of *P. californica* were collected in 2021 (Figure 7). High densities of *P. californica*, along with the other sensitive taxa, provided evidence of healthy aquatic conditions at site CR-SB, while also suggesting that this stream segment was able to sustain a robust fish population.

Several detectable changes in macroinvertebrate community structure were observed downstream at site CR-aC during November of 2021. The EPT Taxa value (21) and Density value (8,124 individuals/m^2) were the lowest among sampling sites, and the density of *P. californica* (12/m^2) also exhibited a substantial decline compared to upstream study sites (Table 4). Alternatively, the macroinvertebrate community at site CR-aC was well-balanced (Diversity = 3.18) and aquatic conditions were able to support a high proportion of sensitive taxa (Percent EPT {excluding Baetidae} = 46.34%). While these metrics detected changes in macroinvertebrate community structure compared to upstream study sites, these changes were likely related to natural changes in habitat, rather than anthropogenic stressors. Most metric values remained within a range indicating that site CR-aC maintained healthy aquatic conditions during the fall of 2021.

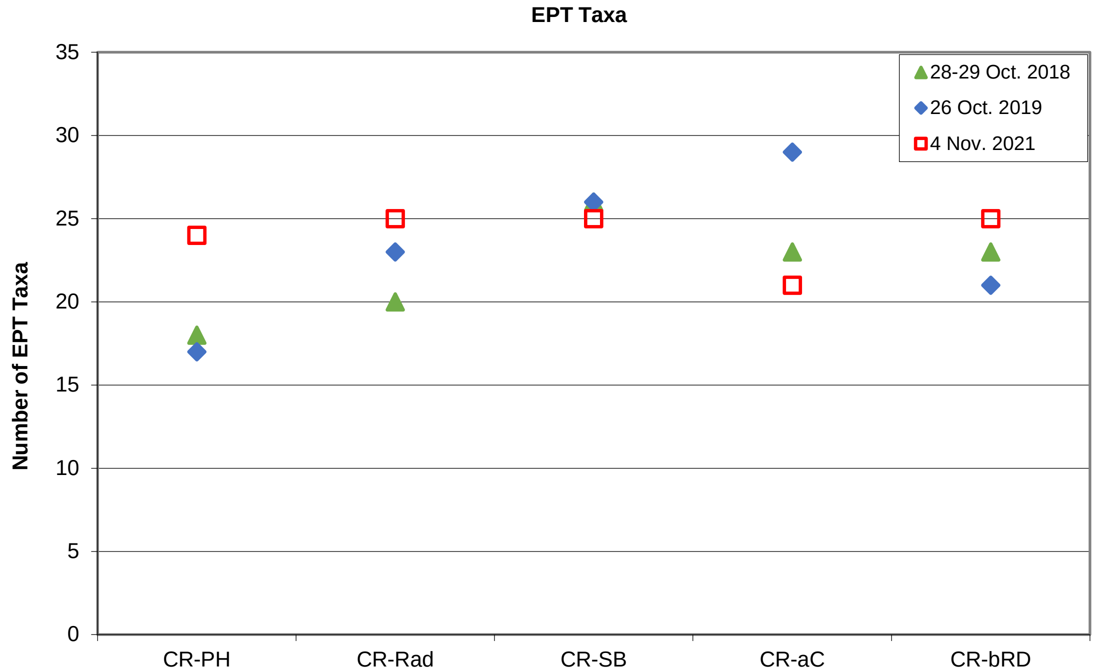

Figure 4. EPT Taxa collected during the fall of 2018, 2019, and 2021 at sampling sites on the Upper Colorado River.

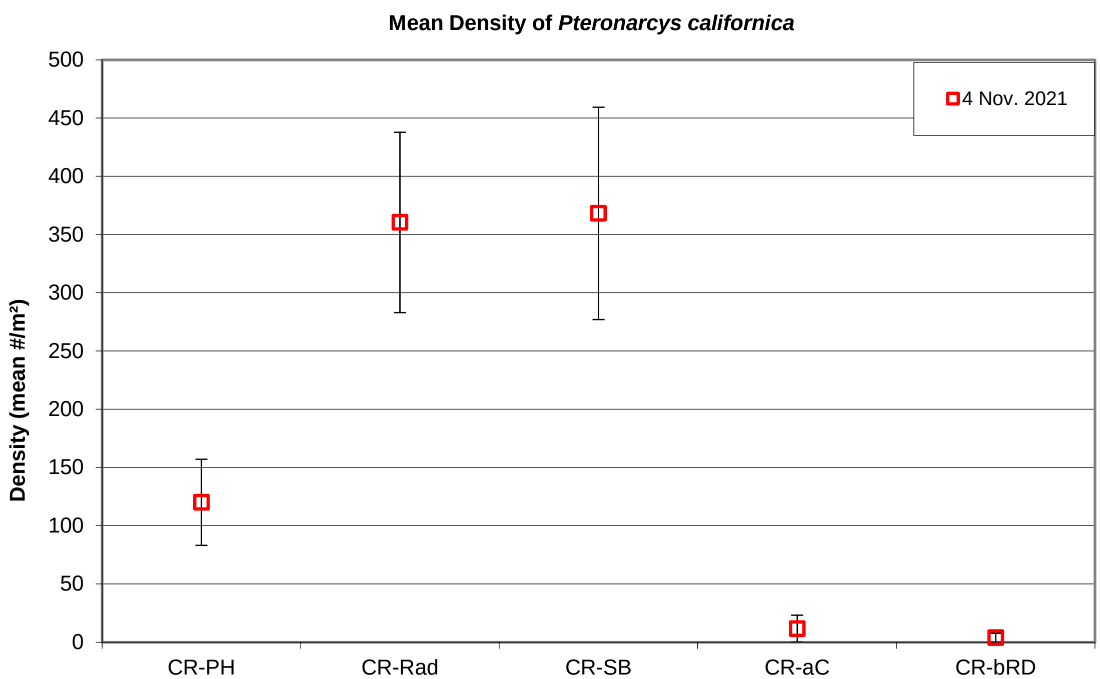

Figure 5. Mean densities (± 1 standard error) of *Pteronarcys californica* collected during the fall of 2021 at sampling sites on the Upper Colorado River.

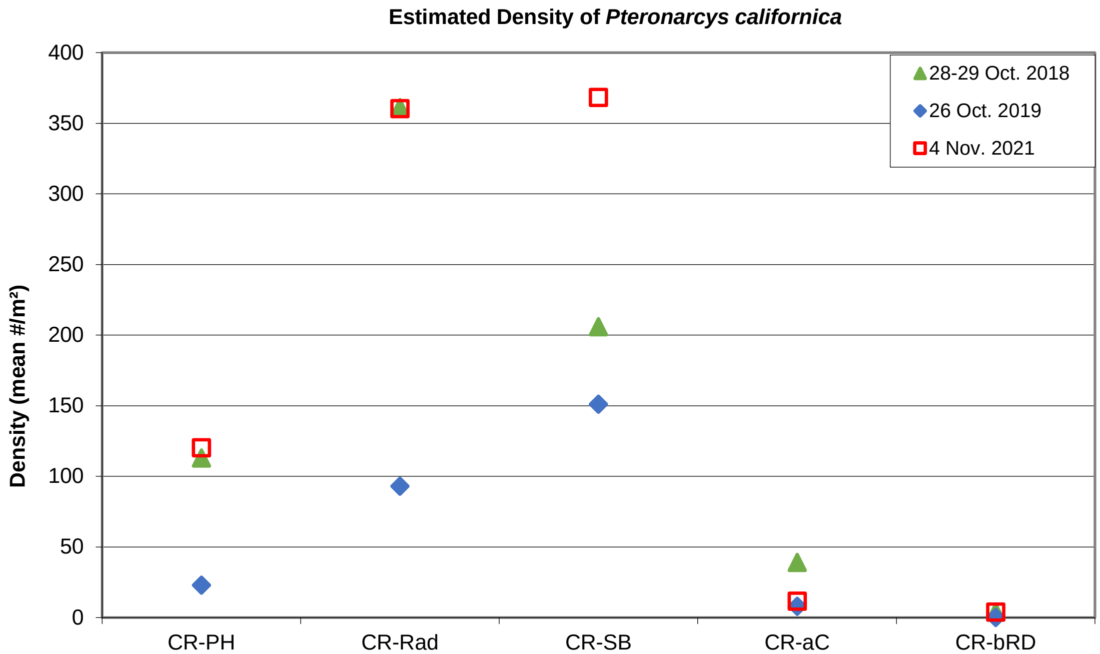

Figure 6. Estimated densities of *Pteronarcys californica* collected during the fall of 2018, 2019, and 2021 at sampling sites on the Upper Colorado River.

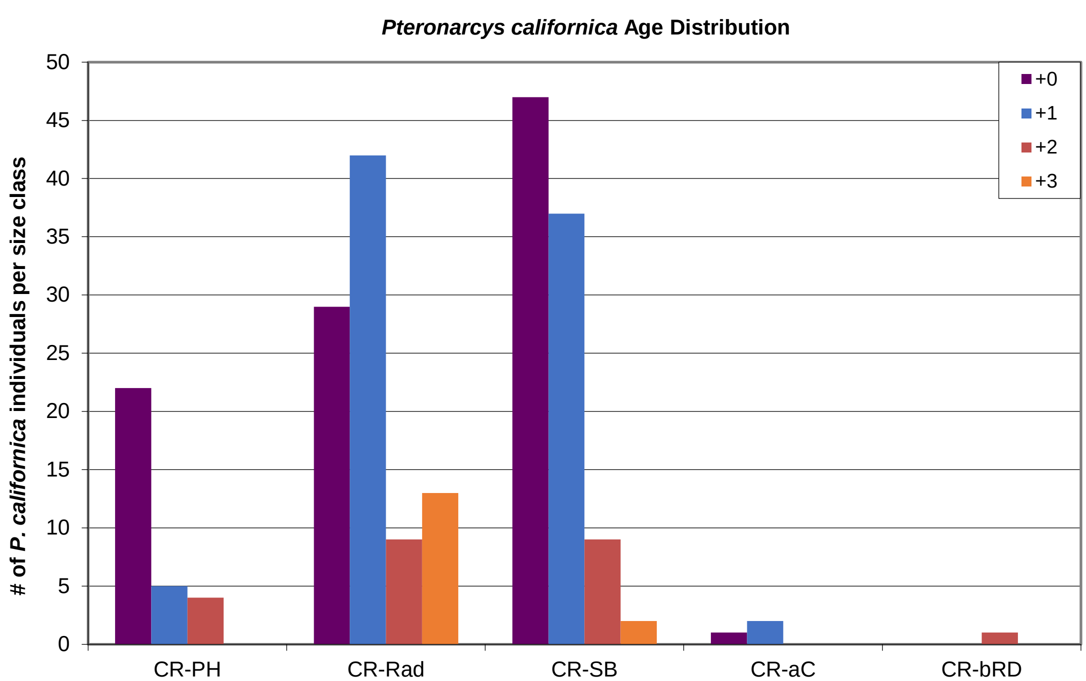

Figure 7. Total number of various age classes of *Pteronarcys californica* collected during the fall of 2021 at sampling locations on the Upper Colorado River.

At the most downstream sampling location in the study area (site CR-bRD), the applied metrics continued to detect relatively healthy macroinvertebrate community parameters along with minor changes in community structure. Individual metrics that showed improvements compared to the adjacent upstream site included the EPT Taxa, Taxa Richness, and Density metrics (Table 4). A comparison of EPT Taxa to Taxa Richness values (25 and 43, respectively) suggested that the majority of taxa at this location could be considered sensitive to disturbances; however, the Percent EPT (excluding Baetidae) metric value (21.02%) detected a decline in the proportion of individuals representing the most sensitive taxa. Other individual metrics that demonstrated a slight decline in macroinvertebrate community health at site CR-bRD included the Diversity, HBI, and Percent Chironomidae metrics, which generally suggested that the community was less balanced and consisted of a slightly higher proportion of nutrient-tolerant individuals. Overall, these changes in metric values were relatively minor and may have been related to changes in habitat. Gradual changes in the aquatic and riparian habitat may have also been responsible for the observed reduction in the density of *Pteronarcys californica* at site CR-bRD (Figures 5-7).

The reorganization of benthic macroinvertebrate specimens according to their method of food acquisition provided an opportunity to evaluate aquatic communities based on ecological function rather than taxonomic structure (Table 5, Figure 8). Healthy ecosystems typically support adequate representation from several feeding groups; however, it is common for certain feeding groups (such as collector-gatherers) to be proportionally dominant. During the fall of 2021, the collector-gatherer, collector-filterer, and scraper groups were well-represented at all study sites (Figure 8). Collector-gatherers were numerically most abundant at sites CR-PH, CR-Rad, CR-SB, and CR-bRD, while collector-filterers were slightly more abundant at site CR-aC (Table 5). These minor shifts in the dominance of feeding groups may have been caused (in part) by changes in the availability of fine particulate organic material (FPOM) within the study area. FPOM is a preferred food resource for collector-filterers.

The two most sensitive/specialized groups (shredders and scrapers) were also present in the study area (Table 5); however, the scraper group was found in relatively high proportions (10.29% - 22.65%) while the shredder group was found in lower proportions (0.11% - 3.84%). These results may have been somewhat deceptive because they are reported as a numerical percentage and do not account for biomass. Since *Pteronarcys californica* is a comparatively large sized shredder, it is likely that the proportional biomass of shredders is much greater at most sites, particularly at the three most upstream sampling locations. The availability of leaf material or coarse particulate organic material (CPOM) entering the river from the riparian corridor may have been a factor contributing to the high densities of *P. californica* in the upstream portion of the study area.

The remaining feeding groups (predators and omnivores) were consistently found in low proportions throughout the study area; however, this was not necessarily an indication of stress as these groups are often poorly represented in Colorado mountain streams.

Overall, results from the evaluation of functional feeding groups in the fall of 2021 supported the results from the MMI v4 and other 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 (Table 5, Figure 8).

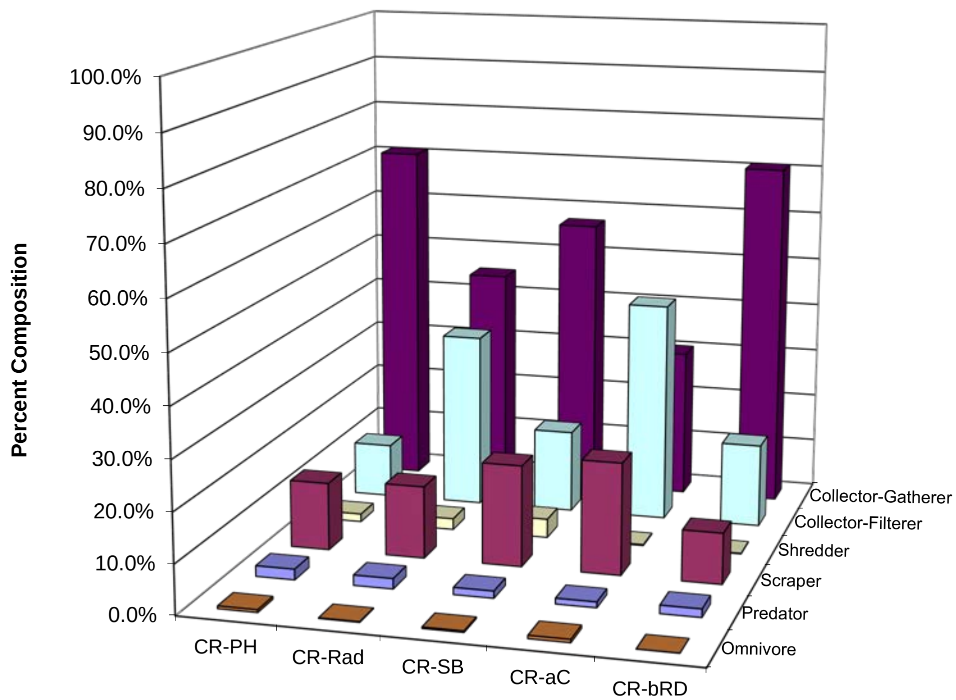

Figure 8. Functional feeding group composition for study sites on the Upper Colorado River in fall of 2021.

Table 5. Relative abundance of functional feeding groups during fall 2021 sampling on the Upper Colorado River.

Site	Functional Feeding Group					
	Collector-Gatherer	Collector-Filterer	Shredder	Scraper	Predator	Omnivore
CR-PH	70.88%	11.04%	1.61%	13.78%	2.15%	0.54%
CR-Rad	45.04%	35.82%	2.26%	14.68%	2.08%	0.12%
CR-SB	57.05%	16.89%	3.84%	20.53%	1.43%	0.26%
CR-aC	30.43%	44.96%	0.19%	22.65%	1.15%	0.62%
CR-bRD	70.94%	17.00%	0.11%	10.29%	1.66%	0.00%

Conclusions

In general, benthic macroinvertebrate communities in the Upper Colorado River Wild and Scenic study area demonstrated minor changes in structure and function while remaining relatively healthy in November of 2021. 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. It is possible that high recreational use at certain locations may have had minor impacts on aquatic communities; however, the metrics used in this biomonitoring study were unable to detect any substantial anthropogenic stressors during the fall of 2021.

Results from this study showed changes in the densities of most aquatic insect species (including *Pteronarcys californica*) from upstream to downstream that could likely be attributed to changes in the availability of preferred habitat, food resources, competition, predation, etc. While all sites maintained healthy benthic macroinvertebrate communities, most metrics were in agreement that site CR-SB supported the healthiest community in the study area. The optimum metric values that were produced by this sampling location were strongly influenced by a species-rich and diverse community that supported high proportions of sensitive taxa and high densities of *P. californica*. The observed changes or shifts in in metric values throughout the remainder of the study area could mostly be attributed to changes in habitat, temperature, riparian input, etc., rather than anthropogenic stress or pollution. Future biomonitoring efforts will be helpful in the validation of recent observations and will assist in the monitoring of any potential anthropogenic impacts that may occur in the Upper Colorado River Wild and Scenic study area.

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Appendix A

Benthic Macroinvertebrate Data – Fall 2021

Table A1. Macroinvertebrate data collected from site CR-PH on 4 Nov 2021.

Colorado River						
CR-PH		Sample				Estimated
4 November 2021	1	2	3		Total	Mean#/m²
Ephemeroptera (mayflies)						
Acentrella sp.			1		1	4
Baetis (*tricaudatus*)	501	991	772		2,264	8,776
Drunella grandis						
Ephemerella dorothea infrequens	64	188	334		586	2,272
Epeorus sp.	2	7	14		23	90
Heptagenia sp.						
Rhithrogena sp.	25	40	34		99	384
Tricorythodes explicatus		1	2		3	12
Paraleptophlebia sp.	6	31	18		55	214
Plecoptera (stoneflies)						
Chloroperlidae						
Claassenia sabulosa						
Perlodidae (*Cultus* sp.)	3	7	4		14	55
Isoperla sp.	2	16	16		34	132
Isoperla fulva		1			1	4
Skwala americana						
Pteronarcella badia						
Pteronarcys californica	4	13	14		31	121
Trichoptera (caddisflies)						
Brachycentrus americanus		13	32		45	175
Brachycentrus occidentalis	6	7	3		16	62
Culoptila sp.	39	109	15		163	632
Glossosoma sp.	5	12			17	66
Protoptila sp.	2	20	19		41	159
Helicopsyche borealis						
Arctopsyche grandis						
Cheumatopsyche sp.	1	6			7	28
Hydropsyche cockerelli			1		1	4
Hydropsyche occidentalis	30	145	98		273	1,059
Hydropsyche oslari	9	28	33		70	272
Hydroptila sp.	19	66	96		181	702
Lepidostoma sp.		6	32		38	148
Oecetis sp.		1			1	4
Polycentropus sp.						
Psychomyia flavida	1				1	4
Rhyacophila coloradensis						
Diptera (true flies)						
Chironomidae (chironomids)						
Cardiocladius sp.		10	3		13	51
Cricotopus/Orthocladius sp.	3	14			17	66
Diamesa sp.	4	7	1		12	47
Eukiefferiella sp.	15	23	15		53	206
Lopescladius sp.						
Microtendipes sp.						
Pagastia sp.	1	11	1		13	51
Polypedilum sp.						
Rheotanytarsus sp.						
Thienemannimyia group		1			1	4
Tvetenia sp.		5	14		19	74

Table A1. cont. Macroinvertebrate data collected from site CR-PH on 4 Nov 2021.

Other Diptera (true flies)						
Atherix pachypus	1	1	1		3	12
Hemerodromia sp.	1		2		3	12
Simulium sp.	13	23	24		60	233
Antocha sp.		1			1	4
Coleoptera (beetles)						
Dubiraphia sp.						
Optioservus sp.	8	18	26		52	202
Zaitzevia parvula	1	1	1		3	12
Odonata (dragonflies & damselflies)						
Ophiogomphus sp.						
Miscellaneous						
Lebertia sp.			2		2	8
Sperchon sp.	2	8	10		20	78
Ferrissia sp.			1		1	4
Lymnaeidae			2		2	8
Physa sp.	1		6		7	28
Gyraulus sp.			3		3	12
Caecidotea sp.			2		2	8
Dugesia sp.						
Polycelis coronata		8	15		23	90
Lumbricidae						
Naididae						
Tubificidae w/out hair chaetae						
Nematoda						
Totals	**769**	**1839**	**1667**		**4,275**	**16,589**

Table A2. Macroinvertebrate data collected from site CR-Rad on 4 Nov 2021.

Colorado River						
CR-Rad		Sample				Estimated
4 November 2021	1	2	3		Total	Mean#/m²
Ephemeroptera (mayflies)						
Acentrella sp.						
Baetis (*tricaudatus*)	479	485	568		1,532	5,938
Drunella grandis						
Ephemerella dorothea infrequens	78	138	96		312	1,210
Epeorus sp.	1	6	6		13	51
Heptagenia sp.		3			3	12
Rhithrogena sp.	53	73	70		196	760
Tricorythodes explicatus						
Paraleptophlebia sp.	2	4	4		10	39
Plecoptera (stoneflies)						
Chloroperlidae		1			1	4
Claassenia sabulosa						
Perlodidae (*Cultus* sp.)	2	5	10		17	66
Isoperla sp.	4	17	6		27	105
Isoperla fulva		3			3	12
Skwala americana						
Pteronarcella badia						
Pteronarcys californica	20	30	43		93	361
Trichoptera (caddisflies)						
Brachycentrus americanus	4	2	2		8	31
Brachycentrus occidentalis	2	8	2		12	47
Culoptila sp.	82	108	66		256	993
Glossosoma sp.	22	5	8		35	136
Protoptila sp.	5	1	4		10	39
Helicopsyche borealis						
Arctopsyche grandis		1			1	4
Cheumatopsyche sp.		1	2		3	12
Hydropsyche cockerelli	6	12	16		34	132
Hydropsyche occidentalis	17	25	41		83	322
Hydropsyche oslari	71	79	142		292	1,132
Hydroptila sp.	31	22	6		59	229
Lepidostoma sp.		2			2	8
Oecetis sp.	1				1	4
Polycentropus sp.						
Psychomyia flavida						
Rhyacophila coloradensis			1		1	4
Diptera (true flies)						
Chironomidae (chironomids)						
Cardiocladius sp.	3	2	2		7	28
Cricotopus/Orthocladius sp.		1	1		2	8
Diamesa sp.						
Eukiefferiella sp.	7	4	8		19	74
Lopescladius sp.	1	3	4		8	31
Microtendipes sp.						
Pagastia sp.	2	1	3		6	24
Polypedilum sp.	1	1			2	8
Rheotanytarsus sp.						
Thienemannimyia group						
Tvetenia sp.	10	10	13		33	128

Table A2. cont. Macroinvertebrate data collected from site CR-Rad on 4 Nov 2021.

Other Diptera (true flies)						
Atherix pachypus	3	3	10		16	62
Hemerodromia sp.						
Simulium sp.	701	134	267		1,102	4,272
Antocha sp.		1			1	4
Coleoptera (beetles)						
Dubiraphia sp.						
Optioservus sp.	17	16	22		55	214
Zaitzevia parvula	1	1	5		7	28
Odonata (dragonflies & damselflies)						
Ophiogomphus sp.						
Miscellaneous						
Lebertia sp.						
Sperchon sp.	5	9	1		15	59
Ferrissia sp.						
Lymnaeidae						
Physa sp.			1		1	4
Gyraulus sp.	1				1	4
Caecidotea sp.						
Dugesia sp.						
Polycelis coronata	3	1	1		5	20
Lumbricidae						
Naididae						
Tubificidae w/out hair chaetae						
Nematoda	1				1	4
Totals	**1636**	**1218**	**1431**		**4,285**	**16,623**

Table A3. Macroinvertebrate data collected from site CR-SB on 4 Nov 2021.

Colorado River						
CR-SB		Sample				Estimated
4 November 2021	1	2	3		Total	Mean#/m²
Ephemeroptera (mayflies)						
Acentrella sp.						
Baetis (*tricaudatus*)	238	198	191		627	2,431
Drunella grandis	1	2			3	12
Ephemerella dorothea infrequens	193	225	196		614	2,380
Epeorus sp.	9	5	3		17	66
Heptagenia sp.	4	2	9		15	59
Rhithrogena sp.	19	13	13		45	175
Tricorythodes explicatus	1	1	1		3	12
Paraleptophlebia sp.	75	34	37		146	566
Plecoptera (stoneflies)						
Chloroperlidae		1			1	4
Claassenia sabulosa	1				1	4
Perlodidae (*Cultus* sp.)	1	5	2		8	31
Isoperla sp.		6	3		9	35
Isoperla fulva		1	1		2	8
Skwala americana						
Pteronarcella badia						
Pteronarcys californica	40	16	39		95	369
Trichoptera (caddisflies)						
Brachycentrus americanus						
Brachycentrus occidentalis	24	8	7		39	152
Culoptila sp.	129	110	60		299	1,159
Glossosoma sp.			2		2	8
Protoptila sp.	2				2	8
Helicopsyche borealis						
Arctopsyche grandis			1		1	4
Cheumatopsyche sp.	13	5	7		25	97
Hydropsyche cockerelli	21	40	27		88	342
Hydropsyche occidentalis	35	26	18		79	307
Hydropsyche oslari	32	54	40		126	489
Hydroptila sp.	4	1			5	20
Lepidostoma sp.	3		4		7	28
Oecetis sp.						
Polycentropus sp.						
Psychomyia flavida						
Rhyacophila coloradensis						
Diptera (true flies)						
Chironomidae (chironomids)						
Cardiocladius sp.						
Cricotopus/Orthocladius sp.	6				6	24
Diamesa sp.	5	1			6	24
Eukiefferiella sp.	14	9	2		25	97
Lopescladius sp.						
Microtendipes sp.						
Pagastia sp.	3	1			4	16
Polypedilum sp.						
Rheotanytarsus sp.						
Thienemannimyia group		3	1		4	16
Tvetenia sp.	8	15	3		26	101

Table A3. cont. Macroinvertebrate data collected from site CR-SB on 4 Nov 2021.

Other Diptera (true flies)						
Atherix pachypus		1	3		4	16
Hemerodromia sp.		2	1		3	12
Simulium sp.	31	40	20		91	353
Antocha sp.						
Coleoptera (beetles)						
Dubiraphia sp.						
Optioservus sp.	31	61	54		146	566
Zaitzevia parvula	20	16	19		55	214
Odonata (dragonflies & damselflies)						
Ophiogomphus sp.	1				1	4
Miscellaneous						
Lebertia sp.						
Sperchon sp.		3	1		4	16
Ferrissia sp.	1				1	4
Lymnaeidae		1			1	4
Physa sp.						
Gyraulus sp.	2	4	4		10	39
Caecidotea sp.						
Dugesia sp.						
Polycelis coronata	2		5		7	28
Lumbricidae		4			4	16
Naididae						
Tubificidae w/out hair chaetae	1				1	4
Nematoda			1		1	4
Totals	**970**	**914**	**775**		**2,659**	**10,324**

Table A4. Macroinvertebrate data collected from site CR-aC on 4 Nov 2021.

Colorado River						
CR-aC		Sample				Estimated
4 November 2021	1	2	3		Total	Mean#/m²
Ephemeroptera (mayflies)						
Acentrella sp.						
Baetis (*tricaudatus*)	111	66	99		276	1,070
Drunella grandis			1		1	4
Ephemerella dorothea infrequens	75	42	54		171	663
Epeorus sp.	1		4		5	20
Heptagenia sp.	2	1	1		4	16
Rhithrogena sp.	11	10	15		36	140
Tricorythodes explicatus	5	3			8	31
Paraleptophlebia sp.	35	16	40		91	353
Plecoptera (stoneflies)						
Chloroperlidae						
Claassenia sabulosa		1	1		2	8
Perlodidae (*Cultus* sp.)	1	2	1		4	16
Isoperla sp.						
Isoperla fulva						
Skwala americana						
Pteronarcella badia						
Pteronarcys californica			3		3	12
Trichoptera (caddisflies)						
Brachycentrus americanus						
Brachycentrus occidentalis	2	4	11		17	66
Culoptila sp.	137	81	171		389	1,508
Glossosoma sp.	1		1		2	8
Protoptila sp.	2		3		5	20
Helicopsyche borealis						
Arctopsyche grandis	1				1	4
Cheumatopsyche sp.	35	15	51		101	392
Hydropsyche cockerelli	2	4	5		11	43
Hydropsyche occidentalis	44	26	40		110	427
Hydropsyche oslari	1		7		8	31
Hydroptila sp.						
Lepidostoma sp.						
Oecetis sp.			1		1	4
Polycentropus sp.						
Psychomyia flavida						
Rhyacophila coloradensis						
Diptera (true flies)						
Chironomidae (chironomids)						
Cardiocladius sp.						
Cricotopus/Orthocladius sp.						
Diamesa sp.						
Eukiefferiella sp.	8	4	7		19	74
Lopescladius sp.		1			1	4
Microtendipes sp.						
Pagastia sp.						
Polypedilum sp.	1				1	4
Rheotanytarsus sp.		1			1	4
Thienemannimyia group	2	3	2		7	28
Tvetenia sp.	2	1	6		9	35

Table A4. cont. Macroinvertebrate data collected from site CR-aC on 4 Nov 2021.

Other Diptera (true flies)						
Atherix pachypus						
Hemerodromia sp.						
Simulium sp.	225	86	381		692	2,683
Antocha sp.						
Coleoptera (beetles)						
Dubiraphia sp.						
Optioservus sp.	7	12	10		29	113
Zaitzevia parvula	7	28	27		62	241
Odonata (dragonflies & damselflies)						
Ophiogomphus sp.						
Miscellaneous						
Lebertia sp.						
Sperchon sp.	2	3	3		8	31
Ferrissia sp.	1		1		2	8
Lymnaeidae	1				1	4
Physa sp.						
Gyraulus sp.						
Caecidotea sp.						
Dugesia sp.						
Polycelis coronata	6	3	4		13	51
Lumbricidae						
Naididae						
Tubificidae w/out hair chaetae						
Nematoda	1		1		2	8
Totals	**729**	**413**	**951**		**2,093**	**8,124**

Table A5. Macroinvertebrate data collected from site CR-bRD on 4 Nov 2021.

Colorado River						
CR-bRD		Sample				Estimated
4 November 2021	1	2	3		Total	Mean#/m²
Ephemeroptera (mayflies)						
Acentrella sp.						
Baetis (*tricaudatus*)	814	485	776		2,075	8,043
Drunella grandis						
Ephemerella dorothea infrequens	15	15	16		46	179
Epeorus sp.			1		1	4
Heptagenia sp.	23	8	2		33	128
Rhithrogena sp.	2	4	5		11	43
Tricorythodes explicatus	53	17	10		80	311
Paraleptophlebia sp.	14	6	7		27	105
Plecoptera (stoneflies)						
Chloroperlidae						
Claassenia sabulosa	4	1			5	20
Perlodidae (*Cultus* sp.)	3				3	12
Isoperla sp.			1		1	4
Isoperla fulva						
Skwala americana	1				1	4
Pteronarcella badia		2	1		3	12
Pteronarcys californica		1			1	4
Trichoptera (caddisflies)						
Brachycentrus americanus						
Brachycentrus occidentalis	33	23	34		90	349
Culoptila sp.	29	72	75		176	683
Glossosoma sp.						
Protoptila sp.	34	5			39	152
Helicopsyche borealis	5				5	20
Arctopsyche grandis	2	2			4	16
Cheumatopsyche sp.	12	4	5		21	82
Hydropsyche cockerelli	2				2	8
Hydropsyche occidentalis	53	57	70		180	698
Hydropsyche oslari	5	1	1		7	28
Hydroptila sp.	6	1	2		9	35
Lepidostoma sp.						
Oecetis sp.	9	3			12	47
Polycentropus sp.	1				1	4
Psychomyia flavida						
Rhyacophila coloradensis						
Diptera (true flies)						
Chironomidae (chironomids)						
Cardiocladius sp.						
Cricotopus/Orthocladius sp.	5		2		7	28
Diamesa sp.						
Eukiefferiella sp.	6	6	90		102	396
Lopescladius sp.						
Microtendipes sp.	7	3			10	39
Pagastia sp.			1		1	4
Polypedilum sp.						
Rheotanytarsus sp.	2		5		7	28
Thienemannimyia group	3				3	12
Tvetenia sp.	6	4	8		18	70

Table A5. cont. Macroinvertebrate data collected from site CR-bRD on 4 Nov 2021.

Other Diptera (true flies)						
Atherix pachypus						
Hemerodromia sp.	2		1		3	12
Simulium sp.	35	49	208		292	1,132
Antocha sp.						
Coleoptera (beetles)						
Dubiraphia sp.	2				2	8
Optioservus sp.	37	44	16		97	376
Zaitzevia parvula	108	40	40		188	729
Odonata (dragonflies & damselflies)						
Ophiogomphus sp.	1				1	4
Miscellaneous						
Lebertia sp.						
Sperchon sp.	1	3			4	16
Ferrissia sp.						
Lymnaeidae						
Physa sp.						
Gyraulus sp.						
Caecidotea sp.						
Dugesia sp.	15	8	3		26	101
Polycelis coronata						
Lumbricidae		1			1	4
Naididae			2		2	8
Tubificidae w/out hair chaetae	9				9	35
Nematoda						
Totals	**1359**	**865**	**1382**		**3,606**	**13,993**

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