

**Summary Report**

**Benthic Macroinvertebrate  
Biomonitoring Study  
Upper Colorado River**

**2023**



**Prepared for:**

**Upper Colorado River  
Wild and Scenic Stakeholder Group**

**Prepared by:**

**David E. Rees  
Timberline Aquatics, Inc.  
4219 Table Mountain Place, Suite A  
Fort Collins, Colorado 80526**

**25 March 2024**



**Summary Report**

**Benthic Macroinvertebrate  
Biomonitoring Study  
Upper Colorado River**

**2023**

**Prepared for:**

**Upper Colorado River  
Wild and Scenic Stakeholder Group**

**Prepared by:**

**David E. Rees  
Timberline Aquatics, Inc.  
4219 Table Mountain Place, Suite A  
Fort Collins, Colorado 80526**

**25 March 2024**

## Table of Contents

Introduction.....	1
Study Area .....	2
Objective.....	2
Methods.....	3
Biomonitoring Study.....	3
Multi-Metric Index (MMI v4) .....	4
Additional Metrics .....	5
Results/Discussion .....	7
Benthic Macroinvertebrate Sampling - Fall 2023.....	7
Results from the MMI v4.....	8
Results from Additional Metrics.....	11
Conclusions.....	18
Literature Cited .....	19
Appendix A.....	A-1

### List of Tables

Table 1. GPS coordinates and elevations of sample sites on the Colorado River. ....	2
Table 2. MMI v4 scores from quantitative, composited, (Hess) samples collected from the Upper Colorado River on 5 November 2023. ....	9
Table 3. Aquatic life use designations based on MMI v4 scores from five study sites on the Upper Colorado River, 5 November 2023. ....	9
Table 4. Metrics and comparative values for macroinvertebrate samples collected from the Upper Colorado River on 5 November 2023. ....	11
Table 5. Relative abundance of functional feeding groups at five study sites on the Upper Colorado River during the fall of 2023. ....	17

### List of Figures

Figure 1. Map of study sites used for macroinvertebrate sampling on the Upper Colorado River in 2023. ....	3
Figure 2. MMI v4 scores from composited quantitative (Hess) samples during the fall of 2018, 2019, 2021, and 2023 at study sites on the Upper Colorado River. ....	10
Figure 3. Sediment TIV scores from composited quantitative (Hess) samples during the fall of 2018, 2019, 2021 and 2023 at study sites on the Upper Colorado River. ....	10
Figure 4. Diversity values from quantitative samples collected during the fall of 2018, 2019, 2021 and 2023 at study sites on the Upper Colorado River. ....	12
Figure 5. HBI values from quantitative samples collected during the fall of 2018, 2019, 2021 and 2023 at study sites on the Upper Colorado River. ....	12
Figure 6. EPT Taxa values from quantitative samples collected during the fall of 2018, 2019, 2021 and 2023 at sampling sites on the Upper Colorado River. ....	13
Figure 7. Mean densities ( $\pm 1$ standard error) of <i>Pteronarcys californica</i> at five study sites on the Upper Colorado River during the fall of 2023. ....	13
Figure 8. Estimated densities of <i>Pteronarcys californica</i> collected during the fall of 2018, 2019, 2021 and 2023 at study sites on the Upper Colorado River. ....	14
Figure 9. Total number of various age classes of <i>Pteronarcys californica</i> collected during the fall of 2023 at sampling locations on the Upper Colorado River. ....	14
Figure 10. Functional feeding group composition for study sites on the Upper Colorado River in the fall of 2023. ....	18

## Introduction

The Upper Colorado River Basin is home to at least 65% of the known species of plants and animals of the western U.S., and its water supply has been credited with allowing for the economic development of the Southwest (Fradkin 1981). In the State of Colorado, this river and adjacent riparian corridor support terrestrial and aquatic ecosystem function, while also sustaining an assortment of human needs including: municipal, agricultural, and recreational opportunities. The bald eagle (*Haliaeetus leucocephalus*), American river otter (*Lontra canadensis*), brown trout (*Salmo trutta*), and the salmonfly (*Pteronarcys californica*) are among the vast group of wildlife that rely on the health of the Colorado River for survival (CPW 2015). These species all play a critical role in ecosystem function as well as providing recreational and economic value to humans.

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, this river has been irreversibly transformed into a series of regulated stream segments with water-use developments including reservoirs and diversions that remove approximately 67% of the annual flow (Carlson and Muth 1989, Colorado Parks and Wildlife 2019). In order to preserve these valuable natural resources, it is important to monitor the biotic and abiotic components that are the foundation of this river system.

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 known to be sensitive to a wide range of environmental disturbances (such as pollution, deviations from the natural flow and temperature regime, etc.), and community composition typically reflects the physical and chemical conditions that occur within a stream segment and associated watershed over time. Long-term biomonitoring studies are essential for the evaluation of aquatic life in streams with increasing water demands or changes in nearby land-use practices (Likens and Lambert 1998, Voelz et al. 2005). The results obtained from consistent sampling practices and accurate identifications should provide valuable insight regarding short-term and long-term changes in aquatic conditions.

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 5 November 2023.

## 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). Five historical sampling locations have been used for the purpose of evaluating physical habitat and the health of aquatic life in studies conducted by Colorado State University, the Eagle River Watershed Council, and Timberline Aquatics, Inc. (Beeby et al. 2014, Rees and Fenske 2022). The two most upstream sampling sites (CR-PH and CR-Rad) were located within Grand County, and the three downstream study sites (CR-SB, CR-aC, and CR-bSW) were positioned in Eagle County. The most upstream study site (CR-PH) was sampled below the Pumphouse Boat Ramp at the Pumphouse Recreation Area. Macroinvertebrate sampling for site CR-Rad was conducted in riffle habitat downstream from Radium Hot Springs. Farther downstream, site CR-SB was positioned in riffle habitat upstream from State Bridge near the intersection of New Trough Rd and Highway 131 in Eagle County. The two remaining sampling locations included CR-aC (above Elk Creek in Catamount), and site CR-bSW, which was located upstream from the confluence with the Eagle River (Table 1; Figure 1). In November of 2023, all benthic macroinvertebrate samples were collected from riffle habitat which was carefully selected to avoid disturbances associated with human activities (boating, fishing, etc.).

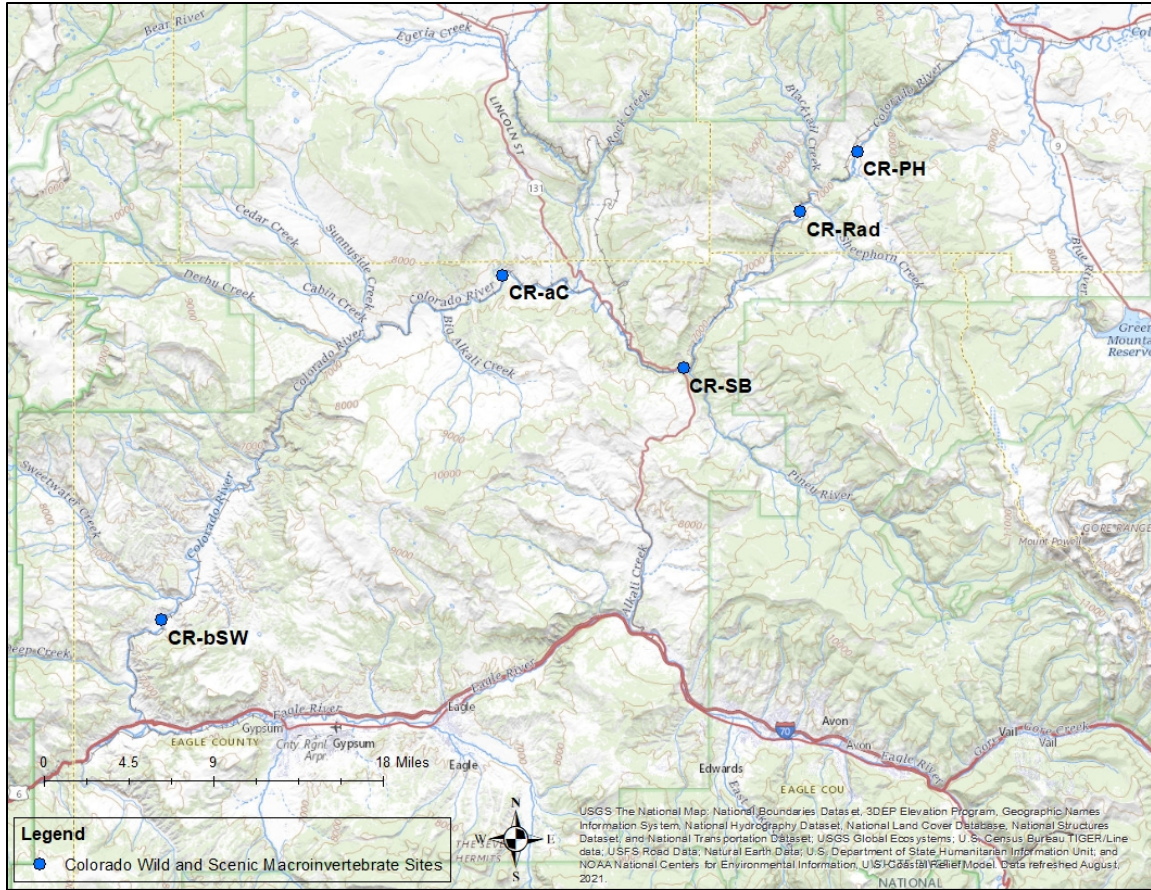
## Objective

The overall objective for the 2023 Benthic Macroinvertebrate Biomonitoring Study was to provide an assessment of the health of benthic 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)
<b>CR-PH</b>	Colorado River at Pumphouse	39.98497	-106.51365	2122
<b>CR-Rad</b>	Colorado River at Radium	39.94984	-106.55788	2100
<b>CR-SB</b>	Colorado River at State Bridge	39.85765	-106.6469	2058
<b>CR-aC</b>	Colorado River above Catamount	39.91232	-106.78523	2008
<b>CR-bSW</b>	Colorado River below Sweetwater	39.70961	-107.04671	1898





**Figure 1. Map of study sites used for macroinvertebrate sampling on the Upper Colorado River in 2023. This map was created with ArcMap.**

## Methods

### *Biomonitoring Study*

The quality and quantity of information obtained during biomonitoring studies is often dependent on the protocols used to collect benthic macroinvertebrates and procedure for processing samples in the lab. 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, changes in macroinvertebrate community parameters that may be attributed to anthropogenic stressors, and changes in the densities of various taxa throughout the study area.

Three quantitative, replicate samples were collected from each of the five study sites on 5 November 2023. All samples were taken in similar riffle 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 all specimens 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. As part of the quality control protocols at Timberline Aquatics, Inc., the residue from all sorted macroinvertebrate samples was checked by a qualified taxonomist, and approximately 10% of the identifications were checked by an additional SFS certified taxonomist. 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) of the Colorado Department of Public Health and Environment (CDPHE) developed a Multi-Metric Index (MMI v3) 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 v4 uses a rarefaction 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. This methodology followed the guidelines provided in the *Section 303(d) Listing Methodology 2020 Listing Cycle* (Colorado Department of Public Health and Environment 2019). 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:

<u><b>Biotype</b></u>	<u><b>Attainment Threshold</b></u>	<u><b>Impairment Threshold</b></u>
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:

<u><b>Biotype</b></u>	<u><b>HBI</b></u>	<u><b>Diversity</b></u>
Transitional (Biotype 1)	5.8	2.1

### ***Additional Metrics***

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):** The Diversity metric is used by the WQCD as an auxiliary metric for the MMI v4, and it was also used in this study as an independent metric to evaluate changes in 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 by the WQCD; 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 WQCD 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 perturbations. 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 found at each site, excluding the mayfly family Baetidae. The family Baetidae is considered one of the most tolerant of the mayfly families; therefore, it is excluded from this metric. A higher value from the Percent EPT (excluding Baetidae) 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 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 taxa in the Family 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 can be an important component of the aquatic and terrestrial food-web in the Upper Colorado River Basin, the mean densities (number of individuals/m<sup>2</sup>) 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. This metric 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<sup>2</sup> found at each study site. The Density value provides an opportunity to measure and compare standing crop throughout the study area, and this information can be used to monitoring changes in the macroinvertebrate portion of the food web at each sampling location.

**Functional Feeding Groups:** Most of the previously described metrics utilize macroinvertebrate information that is associated with community structure; however, macroinvertebrate taxa were also separated into functional guilds based on their method of food acquisition to provide a measurement of ecological function. Some representation of each feeding 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 a comparison of the proportions of various feeding groups among study sites in the same drainage. Changes in the proportion of functional feeding groups can provide insight into the availability or limitations of food resources at specific locations (Ward et al. 2002).

## **Results/Discussion**

### ***Benthic Macroinvertebrate Sampling - Fall 2023***

The Upper Colorado River was sampled at five study sites in the fall (5 November) of 2023 to evaluate the structure and function of benthic macroinvertebrate communities. After fieldwork was conducted, all samples were transported to the lab at Timberline Aquatics, Inc., where specimens were sorted, identified, and enumerated (Appendix A: Tables A1-A5). All macroinvertebrate data were evaluated using the previously described metrics, and results were compared both spatially (among sites) and temporally (with results from previous studies). Overall, benthic communities remained relatively healthy throughout the study area during the fall of 2023. Nevertheless, several analysis tools detected minor changes in community parameters (occurring among sites and over time) that could be interpreted as indications of slight stress. In most cases, these shifts in community parameters were relatively subtle and likely related to changes in the available habitat, rather than water quality. In general, the applied metrics suggested that all study sites supported relatively healthy benthic macroinvertebrate communities, despite some spatial and temporal changes in community structure.

## **Results from the MMI v4**

In 2023, results from the MMI v4 indicated that the five study sites in the Upper Colorado River Wild and Scenic study area supported relatively healthy benthic macroinvertebrate communities. The overall condition of these communities remained relatively stable from upstream to downstream, with MMI v4 scores ranging from a low of 61.5 at site CR-SB to a high of 73.4 at site CR-bSW (Table 2). A comparison with previous MMI v4 scores showed greater temporal stability in aquatic conditions at the upstream and downstream boundary of the study area, while recent MMI v4 scores from the middle portion of the study area demonstrated a slight decline (Figure 2).

All MMI v4 scores were derived from the same eight individual (component) metrics that are designed to measure a variety of macroinvertebrate community parameters. The individual component metrics that generated relatively high scores throughout the study area in 2023 included the EPT Taxa, Percent Non-Insect Individuals, Percent Intolerant Taxa, Percent Increasers (Mid-Elevation), and Clinger Taxa metrics (Table 2). Results from the Percent EPT Individuals (no Baetidae) and Predator/Shredder Taxa metrics were more variable among study sites, while scores from the Percent Coleoptera metric were comparatively poor (Table 2). The Percent Coleoptera metric measures the relative abundance of aquatic beetles, which can be seasonally variable. Although the Percent Coleoptera metric generated low scores for all study sites in 2023, the highest score (20.2) occurred at site CR-bSW, which was consistent with the results from the MMI v4 and several other important component metrics (Table 2).

In the fall of 2023, scores from the MMI v4 and auxiliary metrics were compared with threshold values to determine ‘attainment’ or ‘impairment’ designations within the Colorado River study area (Figure 2). MMI v4 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. Any scores between the thresholds for ‘attainment’ and ‘impairment’ would have required further evaluation using the two auxiliary metrics (Diversity and HBI) to determine a final aquatic life use designation. In 2023, the Diversity value for site CR-SB (1.95) was below the threshold (2.1) for this auxiliary metric; however, this site was still considered in ‘attainment’ because the MMI v4 score (61.5) was above the attainment threshold (Table 2; Figure 2). The low Diversity value for site CR-SB was caused by unusually high proportions of *Baetis tricaudatus* and *Simulium* sp. (Appendix A: Table A3). These two species are not necessarily an indication of environmental stress, but both have the ability to rapidly colonize stable habitats.

The MMI v4 program also provides a sediment Tolerance Indicator Value (TIV) which can be used to measure the proportion of macroinvertebrate individuals that are considered tolerant to fine sediment deposition (Table 2). If TIV values exceed the threshold of 6.3 in Sediment Region 3, it is an indication of possible stress due to sedimentation. During all biomonitoring years, the five study sites generated TIV values that remained relatively stable and well-below this threshold (Figure 3).

Overall, MMI v4 scores from 2023 suggested that all sampling locations were in ‘attainment’ for aquatic life use (Tables 2-3); however, some evidence of minor stress in the middle portion of the study area was evident when results were compared with previous biomonitoring studies (Figure 2). A detailed review of individual component metric scores from 2023 suggested that the proportion of tolerant individuals remained low throughout the study area, while the richness of the most sensitive taxa (based on the EPT Taxa and Percent Intolerant Taxa metrics) remained high (Table 2). The richness of taxa with specialized habits (Clinger Taxa) also remained relatively high among study sites. In general, these results suggested that relatively healthy aquatic conditions persisted throughout the study area in the fall of 2023.

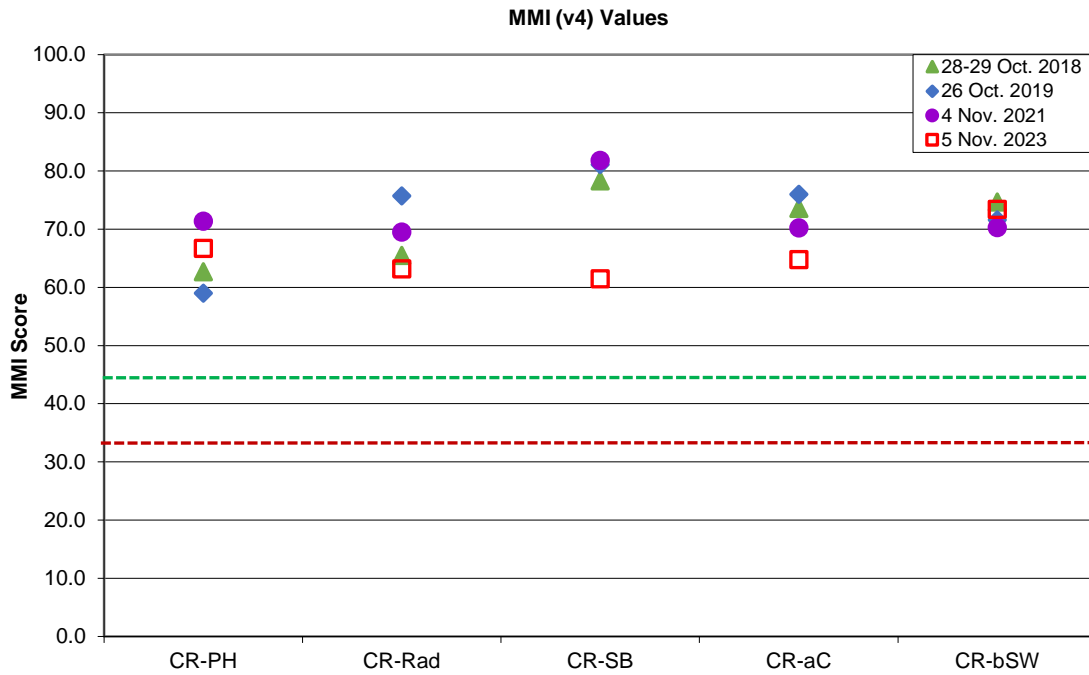
**Table 2. MMI v4 scores from quantitative, composited, (Hess) samples collected from the Upper Colorado River on 5 November 2023.**

Metric	Station ID				
	CR-PH	CR-Rad	CR-SB	CR-aC	CR-bSW
EPT Taxa	75.4	78.4	63.5	82.6	85.2
% Non-Insect Individuals	97.5	98.8	99.4	97.4	98.8
% EPT Individuals (no Baetidae)	22.2	21.6	13.6	28.0	71.9
% Coleoptera Individuals	7.9	0.8	0.7	1.6	20.2
% Intolerant Taxa	76.9	81.0	86.4	80.6	75.8
% Increasers (Mid-Elevation)	100.0	97.6	100.0	100.0	100.0
Clinger Taxa	82.2	77.4	71.5	85.5	92.7
Predator/Shredder Taxa	71.4	50.0	57.1	42.9	42.9
<b>MMI</b>	<b>66.7</b>	<b>63.2</b>	<b>61.5</b>	<b>64.8</b>	<b>73.4</b>
	Auxiliary Metrics				
Diversity	2.50	2.62	1.95	2.36	3.43
HBI	4.78	4.85	5.21	4.72	3.42
TIV (Sediment Region 3)	4.45	4.60	4.68	4.63	4.58

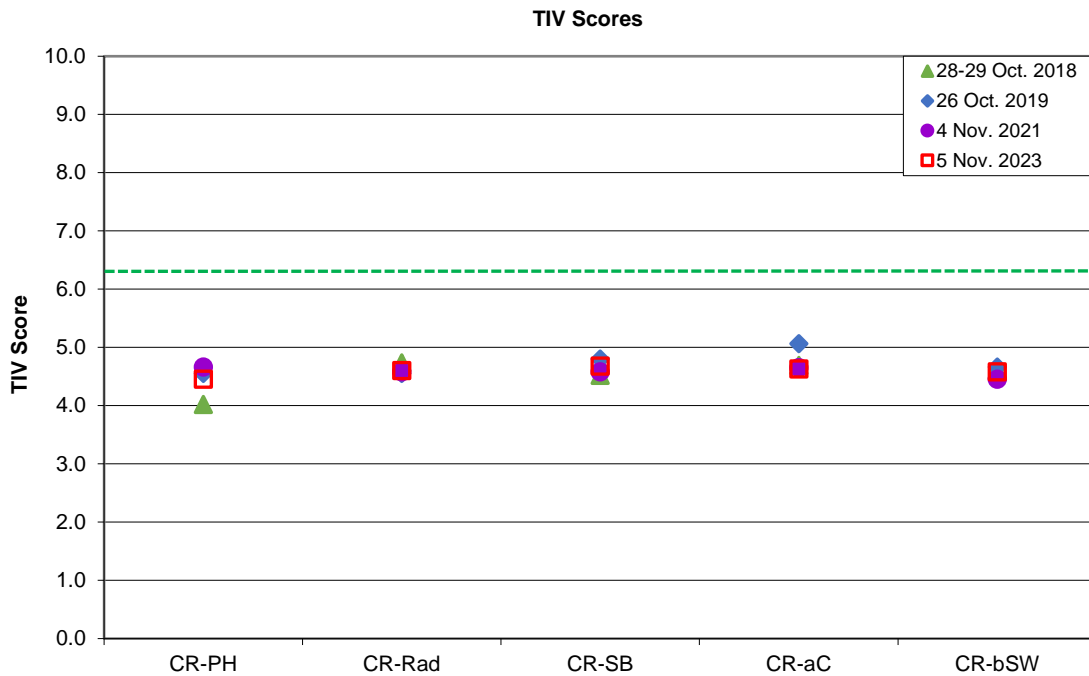
**Table 3. Aquatic life use designations based on MMI v4 scores from five study sites on the Upper Colorado River, 5 November 2023.**

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





**Figure 2. MMI v4 scores from composited quantitative (Hess) samples during the fall of 2018, 2019, 2021, and 2023 at study sites on the Upper Colorado River.**



**Figure 3. Sediment TIV scores from composited quantitative (Hess) samples during the fall of 2018, 2019, 2021 and 2023 at study sites on the Upper Colorado River.**

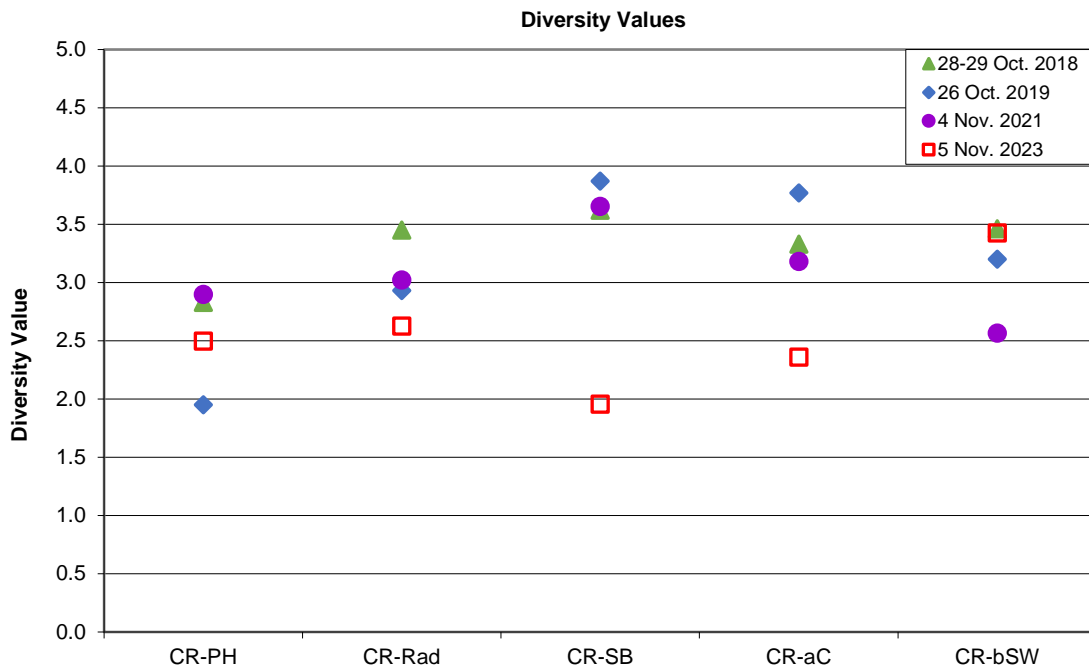
## Results from Additional Metrics

In addition to the MMI v4, eight individual metrics were used to assist in the evaluation of macroinvertebrate data collected from the Wild and Scenic (Colorado River) study area during the fall of 2023 (Table 4). Overall, results from these metrics did not show substantial impacts from human activities; however, a few individual metrics demonstrated evidence of minor stress that varied among sites (Figures 4-9). At each sampling location, benthic macroinvertebrate communities could be characterized as supporting a variety of taxa, including a relatively large number of sensitive taxa. Densities (number of macroinvertebrates/m<sup>2</sup>) were also among the highest that had been recorded in the last several years. Alternatively, metrics that measure community balance and the proportion of sensitive individuals provided evidence of minor stress that varied among sampling locations (Table 4). While the summation of individual metric results suggested that all sites maintained relatively healthy aquatic conditions, a review of specific metric results for each site was used to provide an assessment of changes in macroinvertebrate community structure occurring throughout the study area.

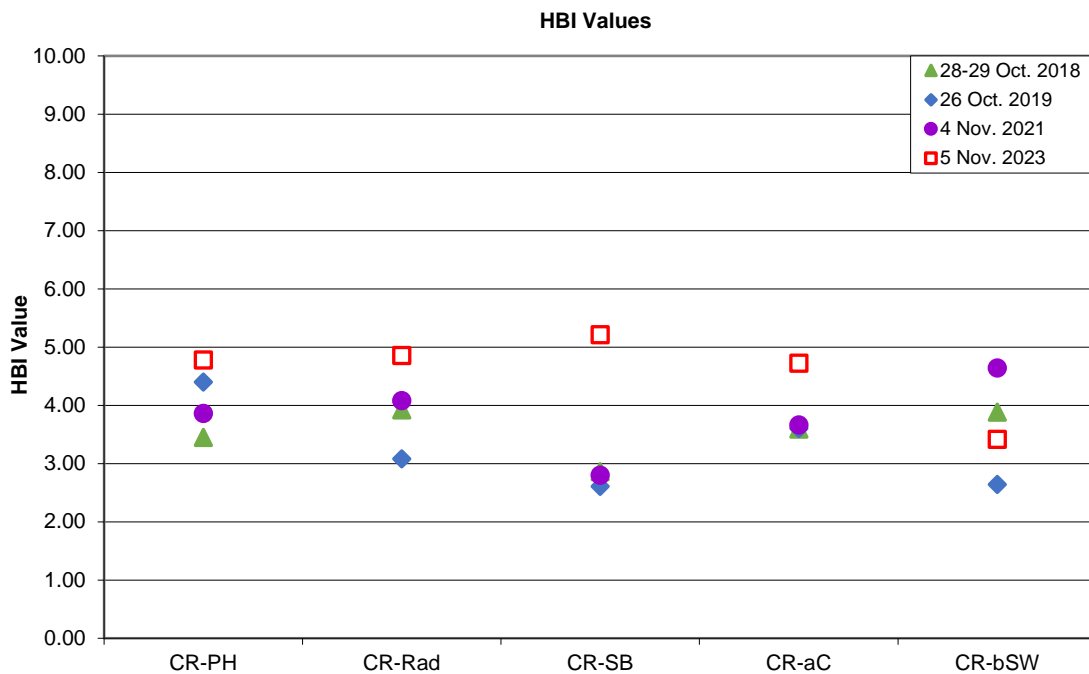
**Table 4. Metrics and comparative values for macroinvertebrate samples collected from the Upper Colorado River on 5 November 2023.**

Metric	CR-PH	CR-Rad	CR-SB	CR-aC	CR-bSW
Diversity	2.50	2.62	1.95	2.36	3.43
HBI	4.78	4.85	5.21	4.72	3.42
EPT Taxa	27	26	29	23	22
Percent EPT (excluding Baetidae)	16.70%	14.44%	10.89%	19.99%	51.41%
Percent Chironomidae	7.85%	11.40%	4.57%	2.35%	5.54%
Density of <i>Pteronarcys californica</i> (mean #/m <sup>2</sup> )	70	35	70	4	0
Taxa Richness	48	46	43	46	44
Density (mean #/m <sup>2</sup> )	20,510	13,413	25,217	22,564	13,647

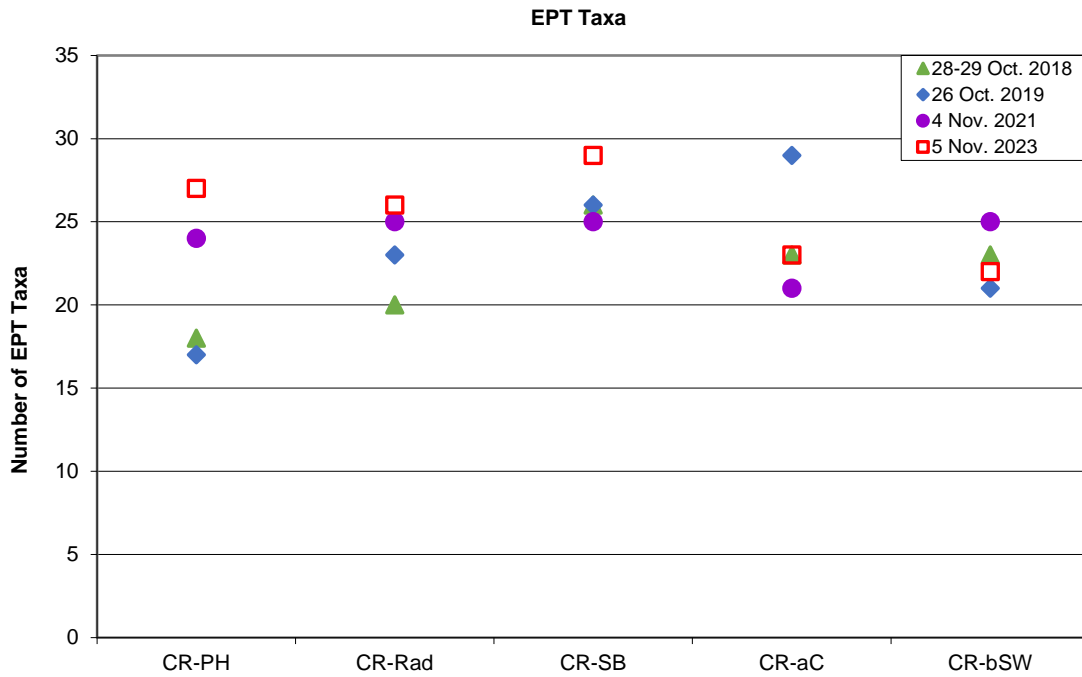
In November of 2023, the most upstream study site (CR-PH) supported an unusually high Density of benthic macroinvertebrates (20,510 individuals/m<sup>2</sup>), and the EPT Taxa and Taxa Richness values (27 and 48, respectively) indicated that this location also maintained a large assortment of taxa (including sensitive taxa) (Table 4). Alternatively, the Diversity value (2.50) suggested that community balance was less than optimal, and the Percent EPT (excluding Baetidae) value of 16.70% indicated that the proportion of sensitive individuals remained fairly low. Several variables, including increased nutrients and stable flows, can contribute to these types of inconsistencies among metric values.



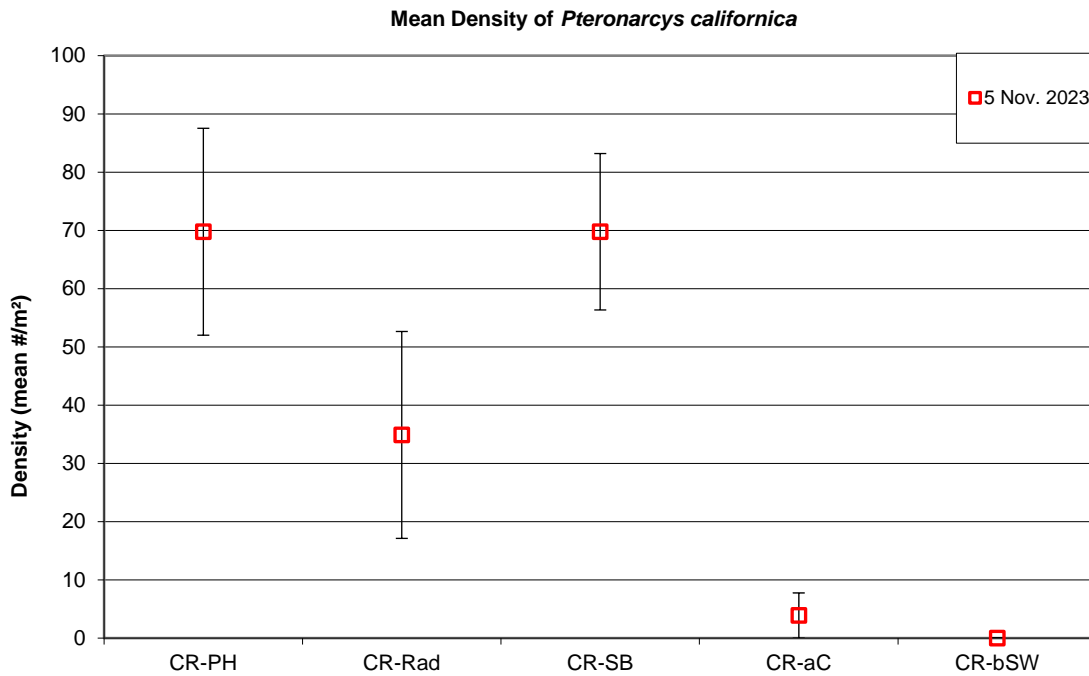
**Figure 4. Diversity values from quantitative samples collected during the fall of 2018, 2019, 2021 and 2023 at study sites on the Upper Colorado River.**



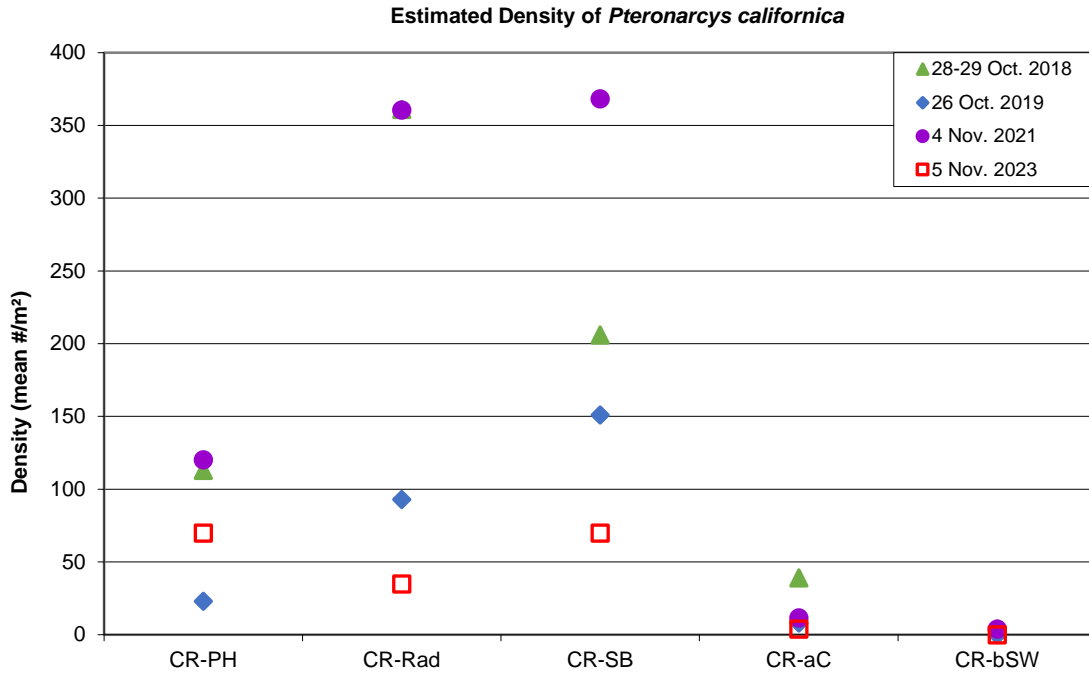
**Figure 5. HBI values from quantitative samples collected during the fall of 2018, 2019, 2021 and 2023 at study sites on the Upper Colorado River.**



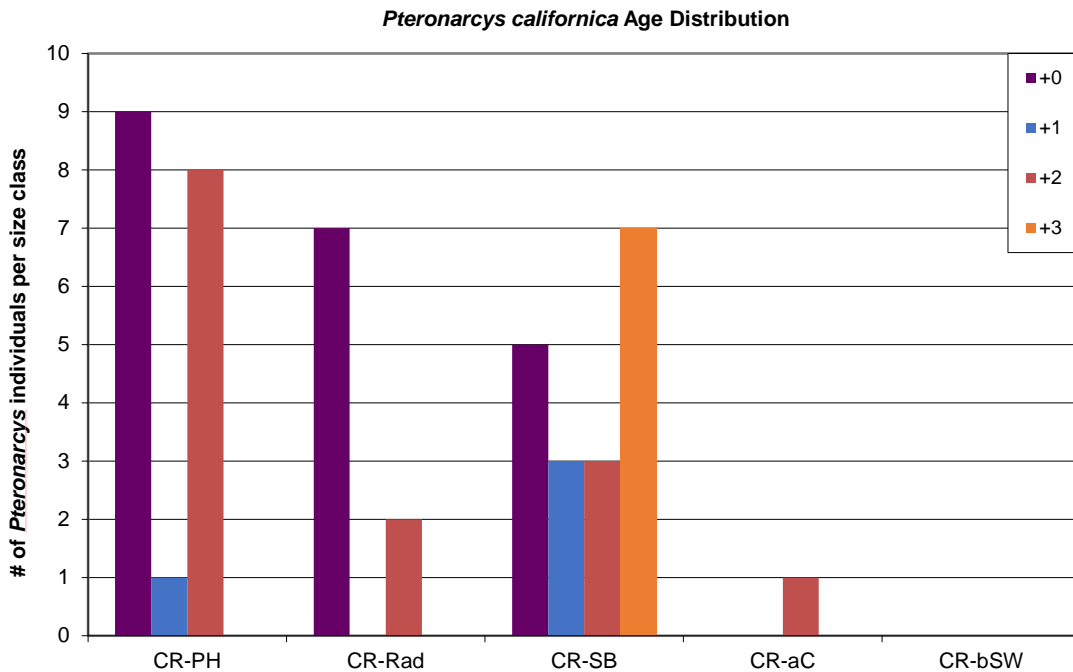
**Figure 6. EPT Taxa values from quantitative samples collected during the fall of 2018, 2019, 2021 and 2023 at sampling sites on the Upper Colorado River.**



**Figure 7. Mean densities ( $\pm 1$  standard error) of *Pteronarcys californica* at five study sites on the Upper Colorado River during the fall of 2023.**



**Figure 8. Estimated densities of *Pteronarcys californica* collected during the fall of 2018, 2019, 2021 and 2023 at study sites on the Upper Colorado River.**



**Figure 9. Total number of various age classes of *Pteronarcys californica* collected during the fall of 2023 at sampling locations on the Upper Colorado River.**



In addition to the high EPT Taxa value at site CR-PH, several other metrics provided supporting evidence of healthy aquatic conditions in the fall of 2023. These individual metrics included the Percent Chironomidae, HBI, and Density of *Pteronarcys californica* metrics (Table 4). High densities of *P. californica* are typically found in streams with healthy aquatic conditions and minimal anthropogenic influences. *P. californica* (aka the “giant stonefly” or “salmonfly”) can be an important component in the aquatic and terrestrial ecosystem, and in sufficient numbers, this aquatic insect can support a high biomass of insectivorous fish. In 2023, the estimated density for *P. californica* at site CR-PH (70 individuals/m<sup>2</sup>) was similar to density values from previous sampling events, with the strongest age classes including age 0+ and age 2+ (Figures 8-9).

Individual metric results for site CR-Rad (located approximately 6.7 km downstream of site CR-PH) suggested that most macroinvertebrate community parameters remained relatively stable, with a few minor exceptions. Overall, spatial stability in aquatic conditions was demonstrated by the Diversity, HBI, EPT Taxa, Percent EPT (excluding Baetidae), Percent Chironomidae, and Taxa Richness metrics (Table 4). When compared to the upstream study site (CR-PH), these metrics showed similar community balance and little change in the richness and relative abundance of sensitive and tolerant taxa. In contrast, the two individual metrics that detected noticeable changes in community structure at site CR-Rad included the Density and Density of *Pteronarcys californica* metrics (Table 4). Not only did these two metrics demonstrate a decline in macroinvertebrate abundance, but the low Density value for *Pteronarcys californica* (approximately 35 individuals/m<sup>2</sup>) represented a decline compared to previous sampling events (Figure 8). Overall, individual metric results were indicative of adequate community structure at site CR-Rad; however, relatively low scores from the Diversity, Percent EPT (excluding Baetidae), and Density of *Pteronarcys californica* metrics suggested that the macroinvertebrate community at this location may be responding to minor stressors.

Farther downstream, the study site near State Bridge (CR-SB) typically generates some of the most optimal individual metric values in the Colorado River study area; however, in 2023, individual metrics were less consistent in their interpretation of aquatic conditions (Table 4). For example, the EPT value for this site (29) was the highest among study sites, while the Percent EPT (excluding Baetidae) value (10.89%) indicated that site CR-SB supported a relatively low proportion of sensitive individuals (Table 4). Although the Density value for site CR-SB (25,217 individuals/m<sup>2</sup>) was the highest among study sites, the Diversity value (1.95) showed relatively poor community balance. There were also inconsistencies among metrics that measure the proportion of tolerant individuals (Table 4). The Percent Chironomidae value (4.57%) suggested that the community consisted of a low proportion of individuals that are generally tolerant to disturbances, while a relatively high HBI value (5.21) demonstrated an increase in the proportion of individuals that are tolerant to nutrient-enrichment (or excessive algal growth). Although the Density of *Pteronarcys californica* (70/m<sup>2</sup>) was among the highest in the study area, results from 2023 showed a substantial decline in abundance compared to results from previous sampling events (Figures 7-8). Fortunately, all four

age classes of *P. californica* were present at site CR-SB in the fall of 2023 (Figure 9). Overall, the individual metrics suggested that most macroinvertebrate community parameters were satisfactory at site CR-SB; however, several metrics suggested that there were low levels of stress, particularly when compared to the results from previous sampling events (Figures 4-5). It is possible that runoff from portions of the watershed burned during the East Troublesome Fire, combined with relatively stable flows in the months prior to sampling, created environmental conditions that were more conducive to the colonization of opportunistic species that have a greater tolerance to disturbances.

Individual metrics continued to demonstrate minor changes in most macroinvertebrate community parameters farther downstream at site CR-aC; however, a summary of metric results suggested that the macroinvertebrate community remained relatively healthy. Interestingly, the Density value continued to be elevated (22,564 individuals/m<sup>2</sup>) in November of 2023, while the EPT Taxa value (23) and the Density of *P. californica* value (4/m<sup>2</sup>) exhibited some decline compared to the upstream site (Table 4). The slight decline in the EPT Taxa value and reduced abundance of *P. californica* was fairly typical for site CR-aC, and was probably related to natural changes in habitat, rather than anthropogenic stressors (Figures 6 and 8). Alternatively, improvements in aquatic conditions were detected by the Diversity, HBI, Percent EPT (excluding Baetidae), Percent Chironomidae, and Taxa Richness metrics (Table 4). These metrics suggested that site CR-aC supported improved community balance, a higher proportion of sensitive individuals, and low proportions of macroinvertebrates that are tolerant to pollution or other general disturbances. Overall, most metric values remained within a range indicating that site CR-aC maintained healthy aquatic conditions during the fall of 2023.

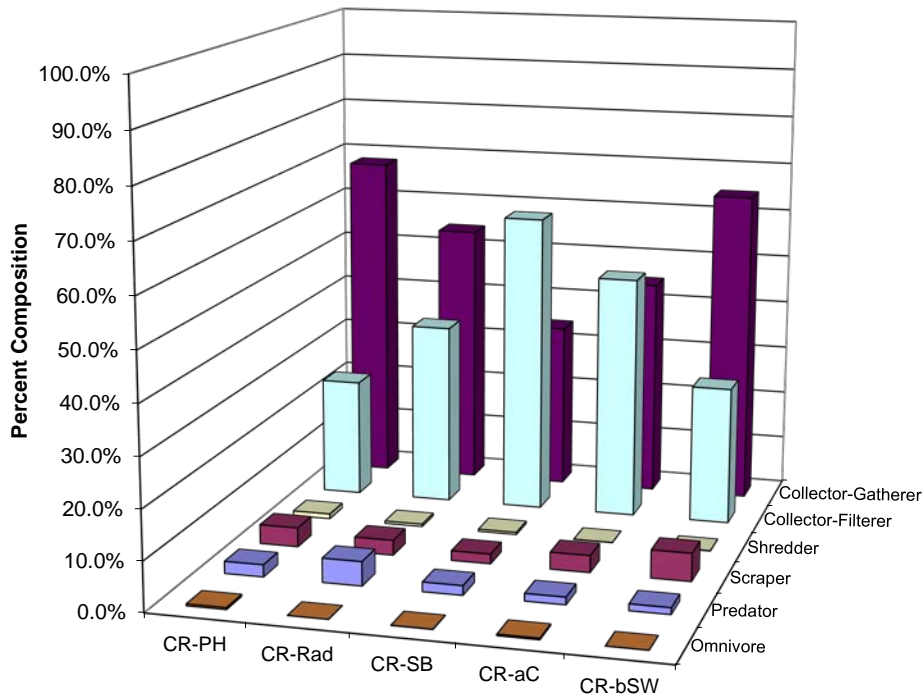
At the most downstream sampling location on the Colorado River (site CR-bSW), the applied metrics continued to detect shifts in macroinvertebrate community structure, although these shifts had little influence on the interpretation of aquatic community health. In the fall of 2023, there were noticeable improvements in community balance and the proportion of sensitive individuals at site CR-bSW; however, there was a slight decline in the richness of sensitive taxa, and a more substantial decline in the abundance of macroinvertebrates (Table 4). A comparison of EPT Taxa to Taxa Richness values (22 and 44, respectively) suggested that half of the taxa at this location could be considered sensitive to disturbances, and the Percent EPT (excluding Baetidae) value (51.41%) indicated that about half of the specimens at this location were representatives of the most sensitive taxa. The Percent EPT (excluding Baetidae) metric also showed a substantial improvement compared to other sites in the study area (Table 4). Other metrics that detected improvements in macroinvertebrate community parameters at site CR-bSW included the Diversity and HBI metrics. These metrics suggested that community balance and the proportion of nutrient-sensitive individuals (respectively) had returned to expected levels based on previous sampling events (Figures 4-5). Overall, the assortment of individual metrics suggested that detectable impacts were relatively minor throughout this study area in the fall of 2023, and detectable changes in community structure may have been related to a combination of runoff from the East Troublesome Fire, relatively stable flows, and natural changes in aquatic habitat.

As an additional means of data analysis, all benthic macroinvertebrates were reorganized according to their method of food acquisition to provide an opportunity to assess aquatic communities based on ecological function rather than taxonomic structure (Table 5; Figure 10). 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 2023, the collector-gatherer and collector-filterer groups were well-represented at all study sites, while shredders, scrapers, predators, and omnivores were under represented throughout most of the study area (Figure 10). Collector-gatherers were numerically most abundant at sites CR-PH, CR-Rad, and CR-bSW, while collector-filterers were more abundant at sites CR-SB and CR-aC (Table 5). These shifts in the abundance of the two most dominant 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.

In the fall of 2023, the combined contribution from the two most sensitive/specialized feeding groups (shredders and scrapers) was less than 6.0% throughout the study area (Table 5; Figure 10). The scraper group was found in relatively low proportions ranging from 1.98% at site CR-SB to 5.69% at site CR-bSW, while proportions of the shredder group ranged from 0.03% at site CR-aC to 1.00% at site CR-PH. Low proportions of these sensitive groups may have been an indication of increased stress, and a comparison with previous sampling events suggested that the relative abundance of both of these groups had been reduced in 2023. It is likely that the low proportions of sensitive feeding groups in 2023 resulted from same environmental conditions responsible for the slight stress detected by the MMI v4 and several individual metrics. These conditions could include runoff from portions of the watershed burned during the East Troublesome Fire and stable stream-flows in the months prior to sampling.

**Table 5. Relative abundance of functional feeding groups at five study sites on the Upper Colorado River during the fall of 2023.**

Site	Functional Feeding Group					
	Collector-Gatherer	Collector-Filterer	Shredder	Scraper	Predator	Omnivore
CR-PH	68.00%	24.25%	1.00%	3.93%	2.46%	0.36%
CR-Rad	54.22%	37.33%	0.49%	3.07%	4.89%	0.00%
CR-SB	34.16%	61.46%	0.45%	1.98%	1.92%	0.03%
CR-aC	44.83%	49.90%	0.03%	3.45%	1.51%	0.28%
CR-bSW	64.57%	28.35%	0.06%	5.69%	1.34%	0.00%



**Figure 10. Functional feeding group composition for study sites on the Upper Colorado River in the fall of 2023.**

## Conclusions

Benthic macroinvertebrate communities in the Upper Colorado River Wild and Scenic study area were found to be relatively healthy in November of 2023, despite detectable spatial changes in community structure. The MMI v4 and most individual metrics indicated that all sampling locations were able to support functioning macroinvertebrate communities that were rich in taxa (including sensitive taxa). Results from this study also demonstrated high densities of macroinvertebrates throughout the study area, with gradual changes in species composition occurring from upstream to downstream. Densities of *Pteronarcys californica* varied among study sites; although, in 2023, these densities were often lower than those reported during previous sampling events. Metrics that detected minor stress in this study area were generally influenced by a reduction in community balance and low proportions of sensitive and specialized individuals. In general, most of these community parameters improved downstream at site CR-bSW, despite the absence of *P. californica* at this location. It is possible that the recent detection of minor stress may have been associated with runoff from portions of the watershed burned during the East Troublesome Fire; however, some changes in community structure could probably be attributed to the availability of preferred habitat, food resources, competition, predation, etc. Future biomonitoring studies will help determine the persistence of recent observations and will assist in the evaluation of any new anthropogenic impacts in the Upper Colorado River Wild and Scenic study area.

## Literature Cited

Barbour, M. T., J. Gerritsen, B. D. Snyder, and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates and fish, second edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Barton, D.R. and J.L. Metcalfe-Smith. 1992. A comparison of sampling techniques and summary indices for assessment of water quality in Yamaska River, Quebec, based on benthic macroinvertebrates. *Environmental Monitoring and Assessment* 21:225-244.

Beeby, J.C., B.P. Bledsoe, and K.W. Hardie. 2014. Colorado River in Eagle County Inventory and Assessment. Report prepared for the Eagle River Watershed Council.

Carlson, C. A. and R. T. Muth. 1989. The Colorado River: Lifeline of the American Southwest, Pp. 220-229. *In*: Dodge, D. P. (ed.). Proceedings of the International Large River Symposium. Canadian Special Publication of Fisheries and Aquatic Sciences. 106.

Colorado Department of Public Health and Environment. 2010. Aquatic life use attainment: Methodology to determine use attainment for rivers and streams. Policy Statement 2010-1.

Colorado Department of Public Health and Environment. 2017. Aquatic life use attainment: Methodology to determine use attainment for rivers and streams. Policy Statement 10-1.

Colorado Department of Public Health and Environment. 2019. Section 303(d) Listing Methodology 2020 Listing Cycle.

Colorado Parks and Wildlife. 2015. State Wildlife Action Plan. 458pp.

Colorado Parks and Wildlife. 2019. Colorado River Aquatic Resource Investigations Federal Aid Project F-237-R26. Colorado Parks & Wildlife Aquatic Research Section Fort Collins, Colorado August 2019.

Christensen, N. S., A. W. Wood, N. Voisin, D. P. Lettenmaier, and R. N. Palmer. 2004. The effects of climate change on the hydrology and water resources of the Colorado River Basin. *Climate Change*. 62: 337-363.

Fore, L.S., J.R. Karr, and R.W. Wisseman. 1996. Assessing Invertebrate Responses to Human Activities: Evaluating Alternative Approaches. *Journal of the North American Benthological Society* 15: 212-231.

Fradkin, P. L. 1981. *River, No More: The Colorado River and the West*. Knopf Doubleday Publishing Group. New York, New York. 392 pp.



Hilsenhoff, W. L. 1988. Rapid field assessment of organic pollution with a family level biotic index. *Journal of the North American Benthological Society* 7(1): 65-68.

Hauer, F. R. and V. H. Resh. 2017. Pp. 297-320. Macroinvertebrates. *In: F. R. Hauer and G. A. Lamberti (eds). Methods in stream ecology (3<sup>rd</sup> edition). Volume 1. Ecosystem structure. Elsevier, Amsterdam, Holland. 494 pp.*

Huryn, A. D. and J. B. Wallace. 2019. Pp. 65-116. Habitat, life history, secondary production, and behavioral adaptations of aquatic insects. *In: Merritt, R. W., K. W. Cummins and M. B. Berg. 2019 (eds.). An Introduction to the Aquatic Insects of North America. Fifth Edition, Kendall/Hunt. Dubuque, Iowa. 1480 pp.*

Kowalski, D. A. and E. E. Richer. 2020. Quantifying the habitat preferences of the stonefly *Pteronarcys californica* in Colorado. *River Research and Applications* 36: 2043-2050.

Lenat, D.R. 1983. Chironomid Taxa Richness: Natural Variation and Use in Pollution Assessment. *Freshwater Invertebrate Biology* 2:192-198.

Lenat, D.R. 1988. Water quality assessment of streams using a qualitative collection method for benthic macroinvertebrates. *Journal of the North American Benthological Society* 7:222-33.

Likens, G. E., and K. F. Lambert. 1998. The importance of long-term data in addressing regional environmental issues. *Northeastern Naturalist* 5: 127-136.

Mandaville, S.M. 2002. Benthic Macroinvertebrates in Freshwaters-Taxa Tolerance Values, Metrics, and Protocols. Project H-1. Soil and Water Conservation Society of Metro Halifax,.xviii. 48. Pp., Appendices A-B 120pp.

Mazor, R. D., D. M. Rosenberg and V. H. Resh. 2019. Pp. 141-164. Use of aquatic insects in bioassessment. *In: Merritt, R. W., K. W. Cummins and M. B. Berg (eds.). An Introduction to the Aquatic Insects of North America. Fifth Edition, Kendall/Hunt. Dubuque, Iowa. 1480 pp.*

Merritt, R. W., K. W. Cummins and M. B. Berg. 2019. *An Introduction to the Aquatic Insects of North America. Fifth Edition, Kendall/Hunt. Dubuque, Iowa. 1480 pp.*

Miller, M. P., S. G. Buto, D. D. Susong and C. A. Rumsey. 2015. The importance of base flow in sustaining surface water flow in the Upper Colorado River Basin. *Water Resources Research*. 52: 3547-3562.

Paul, M. J., J. Gerritsen, C. Hawkins, and E. Leppo. 2005. Draft. Development of biological assessment tools for Colorado. Colorado Department of Public Health and Environment, Water Quality Control Division – Monitoring Unit. Denver, Colorado.

Plafkin, J. L., M. T. Barbour, K. D. Porter, S. K. Gross, and R. M. Hughes. 1989. Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish. EPA/444/4-89/001.

Rees, D. E., and K. R. Fenske. 2022. Benthic macroinvertebrate biomonitoring study, Upper Colorado River 2021. Report prepared for the Upper Colorado River Wild and Scenic Stakeholder Group. 36 pp.

USEPA Office of Water Recovery Potential Screening Website 09/01/2011  
<http://www.epa.gov/recoverypotential/> Recovery Potential Metrics Summary Form.

Voelz, N. J., R. E. Zuellig, S. Shieh, and J. V. Ward. 2005. The effects of urban areas on benthic macroinvertebrates in two Colorado plains rivers. *Environmental Monitoring and Assessment* 101: 175-202.

Wang, L., D. M. Robertson, and P. J. Garrison. 2007. Linkages between nutrients and assemblages of macroinvertebrates and fish in wadeable streams: implication to nutrient criteria development. *Environmental Management* 39: 194-212.

Ward, J. V., B. C. Kondratieff, and R. E. Zuellig. 2002. An illustrated guide to the mountain stream insects of Colorado. Second Edition. University Press of Colorado. Boulder, Colorado.

Weber, C.I. 1973. Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents. EPA-670/4-73-001. U.S. Environmental Protection Agency, Cincinnati, OH.

## **Appendix A**

### **Benthic Macroinvertebrate Data – Fall 2023**

**Table A1. Macroinvertebrate data collected from site CR-PH on 5 Nov 2023.**

Colorado River						
CR-PH		Sample				Estimated Mean#/m <sup>2</sup>
5 November 2023	1	2	3		Total	
<b>Ephemeroptera (mayflies)</b>						
<i>Acentrella</i> sp.	1				1	4
<i>Baetis (tricaudatus)</i>	958	986	1210		3,154	12,225
<i>Dipheter hageni</i>			2		2	8
<i>Drunella grandis</i>			2		2	8
<i>Ephemerella dorothea infrequens</i>	16	12	56		84	326
<i>Epeorus</i> sp.	11	2	10		23	90
<i>Heptagenia</i> sp.	1		5		6	24
<i>Rhithrogena</i> sp.	14	3	3		20	78
<i>Asioplax</i> sp.						
<i>Tricorythodes explicatus</i>			4		4	16
<i>Paraleptophlebia</i> sp.	4	2	12		18	70
<b>Plecoptera (stoneflies)</b>						
<i>Paracapnia</i> sp.			2		2	8
Chloroperlidae						
<i>Sweltsa</i> sp.						
<i>Claassenia sabulosa</i>						
<i>Hesperoperla pacifica</i>						
Perlodidae ( <i>Cultus</i> sp.)	6	2	6		14	55
<i>Diura knowltoni</i>						
<i>Isogenoides</i> sp.						
<i>Isoperla</i> sp.	2	2	6		10	39
<i>Isoperla fulva</i>						
<i>Pteronarcella badia</i>						
<i>Pteronarcys californica</i>	7	3	8		18	70
<b>Trichoptera (caddisflies)</b>						
<i>Brachycentrus americanus</i>	6	4	3		13	51
<i>Brachycentrus occidentalis</i>		1	3		4	16
<i>Culoptila</i> sp.			2		2	8
<i>Glossosoma</i> sp.			2		2	8
<i>Protoptila</i> sp.						
<i>Helicopsyche borealis</i>						
<i>Arctopsyche grandis</i>						
<i>Cheumatopsyche</i> sp.	4	6	10		20	78
<i>Hydropsyche cockerelli</i>	4	8	3		15	59
<i>Hydropsyche occidentalis</i>	47	62	110		219	849
<i>Hydropsyche oslari</i>	79	106	149		334	1,295
<i>Hydroptila</i> sp.	5	1	27		33	128
<i>Leucotrichia</i> sp.	2	1	2		5	20
<i>Neotrichia</i> sp.			1		1	4
<i>Lepidostoma</i> sp.	1	4	27		32	124
<i>Psychomyia flavida</i>						
<i>Rhyacophila coloradensis</i>		2			2	8

**Table A1. cont. Macroinvertebrate data collected from site CR-PH on 5 Nov 2023.**

<b>Diptera (true flies)</b>						
<b>Chironomidae (chironomids)</b>						
<i>Cardiocladius</i> sp.	19	9	5		33	128
<i>Cricotopus/Orthocladius</i> sp.	8	1	12		21	82
<i>Diamesa</i> sp.	30	9	16		55	214
<i>Eukiefferiella</i> sp.	29	54	47		130	504
<i>Lopescladius</i> sp.						
<i>Micropsectra</i> sp.	1				1	4
<i>Microtendipes</i> sp.						
<i>Pagastia</i> sp.	4	10	25		39	152
<i>Parametriocnemus</i> sp.	3		5		8	31
<i>Polypedilum</i> sp.			1		1	4
<i>Potthastia</i> sp.						
<i>Psectrotanypus</i> sp.			1		1	4
<i>Rheotanytarsus</i> sp.	1		1		2	8
<i>Synorthocladius</i> sp.						
<i>Thienemannimyia</i> group						
<i>Tvetenia</i> sp.	40	35	49		124	481
<b>Other Diptera (true flies)</b>						
<i>Atherix pachypus</i>	1		1		2	8
Ceratopogoninae						
<i>Hemerodromia</i> sp.						
<i>Simulium</i> sp.	104	390	181		675	2,617
<b>Coleoptera (beetles)</b>						
<i>Liodessus</i> sp.			1		1	4
<i>Microcylloepus pusillus</i>						
<i>Optioservus</i> sp.	25	16	75		116	450
<i>Zaitzevia parvula</i>	3		4		7	28
<b>Miscellaneous</b>						
<i>Atractides</i> sp.						
<i>Hygrobates</i> sp.						
<i>Lebertia</i> sp.						
<i>Protzia</i> sp.	2		1		3	12
<i>Sperchon</i> sp.	2		5		7	28
<i>Ferrissia</i> sp.						
<i>Physa</i> sp.						
<i>Polycelis coronata</i>	2	4	13		19	74
Lumbricidae			1		1	4
Naididae	1				1	4
Tubificidae w/out hair chaetae						
Nematoda						
<b>Totals</b>	<b>1443</b>	<b>1735</b>	<b>2109</b>		<b>5,287</b>	<b>20,510</b>

**Table A2. Macroinvertebrate data collected from site CR-Rad on 5 Nov 2023.**

Colorado River						
CR-Rad		Sample				Estimated Mean#/m <sup>2</sup>
5 November 2023	1	2	3		Total	
<b>Ephemeroptera (mayflies)</b>						
<i>Acentrella</i> sp.	7	7	1		15	59
<i>Baetis (tricaudatus)</i>	465	412	570		1,447	5,609
<i>Dipheter hageni</i>						
<i>Drunella grandis</i>						
<i>Ephemerella dorothea infrequens</i>	46	41	56		143	555
<i>Epeorus</i> sp.	3	2	1		6	24
<i>Heptagenia</i> sp.		4			4	16
<i>Rhithrogena</i> sp.	26	15	16		57	221
<i>Asioplax</i> sp.			1		1	4
<i>Tricorythodes explicatus</i>		1	1		2	8
<i>Paraleptophlebia</i> sp.	3	3	7		13	51
<b>Plecoptera (stoneflies)</b>						
<i>Paracapnia</i> sp.						
Chloroperlidae		1			1	4
<i>Sweltsa</i> sp.						
<i>Claassenia sabulosa</i>						
<i>Hesperoperla pacifica</i>						
Perlodidae ( <i>Cultus</i> sp.)	2	3			5	20
<i>Diura knowltoni</i>						
<i>Isogenoides</i> sp.						
<i>Isoperla</i> sp.	1	1	4		6	24
<i>Isoperla fulva</i>						
<i>Pteronarcella badia</i>	1				1	4
<i>Pteronarcys californica</i>		4	5		9	35
<b>Trichoptera (caddisflies)</b>						
<i>Brachycentrus americanus</i>	2	2	1		5	20
<i>Brachycentrus occidentalis</i>	1	1	1		3	12
<i>Culoptila</i> sp.	1	1	1		3	12
<i>Glossosoma</i> sp.	1				1	4
<i>Protoptila</i> sp.						
<i>Helicopsyche borealis</i>						
<i>Arctopsyche grandis</i>						
<i>Cheumatopsyche</i> sp.	1	1	2		4	16
<i>Hydropsyche cockerelli</i>	5	2	10		17	66
<i>Hydropsyche occidentalis</i>	3	3	9		15	59
<i>Hydropsyche oslari</i>	63	48	80		191	741
<i>Hydroptila</i> sp.	1		2		3	12
<i>Leucotrichia</i> sp.			1		1	4
<i>Neotrichia</i> sp.						
<i>Lepidostoma</i> sp.	1	2	4		7	28
<i>Psychomyia flavida</i>			1		1	4
<i>Rhyacophila coloradensis</i>						

**Table A2. cont. Macroinvertebrate data collected from site CR-Rad on 5 Nov 2023.**

<b>Diptera (true flies)</b>						
<b>Chironomidae (chironomids)</b>						
<i>Cardiocladius</i> sp.	3	4	5		12	47
<i>Cricotopus/Orthocladius</i> sp.	30	21	30		81	314
<i>Diamesa</i> sp.	44	25	62		131	508
<i>Eukiefferiella</i> sp.	6	7	12		25	97
<i>Lopescladius</i> sp.		1	1		2	8
<i>Micropsectra</i> sp.						
<i>Microtendipes</i> sp.						
<i>Pagastia</i> sp.	14	6	11		31	121
<i>Parametriocnemus</i> sp.						
<i>Polypedilum</i> sp.						
<i>Potthastia</i> sp.						
<i>Psectrotanypus</i> sp.						
<i>Rheotanytarsus</i> sp.						
<i>Synorthocladius</i> sp.			1		1	4
<i>Thienemannimyia</i> group		1			1	4
<i>Tvetenia</i> sp.	35	24	51		110	427
<b>Other Diptera (true flies)</b>						
<i>Atherix pachypus</i>	1	1	3		5	20
Ceratopogoninae						
<i>Hemerodromia</i> sp.			1		1	4
<i>Simulium</i> sp.	487	383	185		1,055	4,090
<b>Coleoptera (beetles)</b>						
<i>Liodessus</i> sp.						
<i>Microcylloepus pusillus</i>						
<i>Optioservus</i> sp.	10	7	12		29	113
<i>Zaitzevia parvula</i>	1				1	4
<b>Miscellaneous</b>						
<i>Atractides</i> sp.		1			1	4
<i>Hygrobates</i> sp.						
<i>Lebertia</i> sp.		1			1	4
<i>Protzia</i> sp.						
<i>Sperchon</i> sp.	3	2			5	20
<i>Ferrissia</i> sp.	1				1	4
<i>Physa</i> sp.		1			1	4
<i>Polycelis coronata</i>						
Lumbricidae						
Naididae	1				1	4
Tubificidae w/out hair chaetae						
Nematoda						
<b>Totals</b>	<b>1269</b>	<b>1039</b>	<b>1148</b>		<b>3,456</b>	<b>13,413</b>



**Table A3. Macroinvertebrate data collected from site CR-SB on 5 Nov 2023.**

Colorado River						
CR-SB		Sample				Estimated
5 November 2023	1	2	3		Total	Mean#/m <sup>2</sup>
<b>Ephemeroptera (mayflies)</b>						
<i>Acentrella</i> sp.	1	4	6		11	43
<i>Baetis (tricaudatus)</i>	637	566	482		1,685	6,531
<i>Dipheter hageni</i>			1		1	4
<i>Drunella grandis</i>						
<i>Ephemerella dorothea infrequens</i>	77	116	63		256	993
<i>Epeorus</i> sp.	4	4	5		13	51
<i>Heptagenia</i> sp.	3	1	3		7	28
<i>Rhithrogena</i> sp.	26	25	29		80	311
<i>Asiopanax</i> sp.						
<i>Tricorythodes explicatus</i>	2	2	1		5	20
<i>Paraleptophlebia</i> sp.	14	23	13		50	194
<b>Plecoptera (stoneflies)</b>						
<i>Paracapnia</i> sp.						
Chloroperlidae	2				2	8
<i>Sweltsa</i> sp.	1				1	4
<i>Claassenia sabulosa</i>						
<i>Hesperoperla pacifica</i>						
Perlodidae ( <i>Cultus</i> sp.)	3	2	3		8	31
<i>Diura knowltoni</i>	1				1	4
<i>Isogenoides</i> sp.						
<i>Isoperla</i> sp.	1		1		2	8
<i>Isoperla fulva</i>						
<i>Pteronarcella badia</i>						
<i>Pteronarcys californica</i>	4	8	6		18	70
<b>Trichoptera (caddisflies)</b>						
<i>Brachycentrus americanus</i>		1			1	4
<i>Brachycentrus occidentalis</i>	9		1		10	39
<i>Culoptila</i> sp.			1		1	4
<i>Glossosoma</i> sp.		1			1	4
<i>Protophila</i> sp.						
<i>Helicopsyche borealis</i>						
<i>Arctopsyche grandis</i>			1		1	4
<i>Cheumatopsyche</i> sp.	5	3	2		10	39
<i>Hydropsyche cockerelli</i>	22	26	16		64	249
<i>Hydropsyche occidentalis</i>		6			6	24
<i>Hydropsyche oslari</i>	50	54	47		151	586
<i>Hydroptila</i> sp.	1				1	4
<i>Leucotrichia</i> sp.			1		1	4
<i>Neotrichia</i> sp.			1		1	4
<i>Lepidostoma</i> sp.	7	2	2		11	43
<i>Psychomyia flavida</i>						
<i>Rhyacophila coloradensis</i>	1	2	3		6	24

**Table A3. cont. Macroinvertebrate data collected from site CR-SB on 5 Nov 2023.**

<b>Diptera (true flies)</b>						
<b>Chironomidae (chironomids)</b>						
<i>Cardiocladius</i> sp.	3	6	10		19	74
<i>Cricotopus/Orthocladius</i> sp.	11	7	12		30	117
<i>Diamesa</i> sp.	28	28	20		76	295
<i>Eukiefferiella</i> sp.	16	17	13		46	179
<i>Lopescladius</i> sp.						
<i>Micropsectra</i> sp.						
<i>Microtendipes</i> sp.						
<i>Pagastia</i> sp.	11	6	11		28	109
<i>Parametriocnemus</i> sp.						
<i>Polypedilum</i> sp.						
<i>Potthastia</i> sp.						
<i>Psectrotanypus</i> sp.						
<i>Rheotanytarsus</i> sp.						
<i>Synorthocladius</i> sp.						
<i>Thienemannimyia</i> group						
<i>Tvetenia</i> sp.	23	53	22		98	380
<b>Other Diptera (true flies)</b>						
<i>Atherix pachypus</i>		1			1	4
Ceratopogoninae						
<i>Hemerodromia</i> sp.						
<i>Simulium</i> sp.	1336	1234	1183		3,753	14,547
<b>Coleoptera (beetles)</b>						
<i>Liodessus</i> sp.						
<i>Microcylloepus pusillus</i>		2			2	8
<i>Optioservus</i> sp.	2	17	3		22	86
<i>Zaitzevia parvula</i>	2	7			9	35
<b>Miscellaneous</b>						
<i>Atractides</i> sp.						
<i>Hygrobates</i> sp.						
<i>Lebertia</i> sp.						
<i>Protzia</i> sp.						
<i>Sperchon</i> sp.		4	6		10	39
<i>Ferrissia</i> sp.	1				1	4
<i>Physa</i> sp.						
<i>Polycelis coronata</i>	1	1			2	8
Lumbricidae						
Naididae						
Tubificidae w/out hair chaetae						
Nematoda						
<b>Totals</b>	<b>2305</b>	<b>2229</b>	<b>1968</b>		<b>6,502</b>	<b>25,217</b>

**Table A4. Macroinvertebrate data collected from site CR-aC on 5 Nov 2023.**

Colorado River						
CR-aC		Sample				Estimated Mean#/m <sup>2</sup>
5 November 2023	1	2	3		Total	
<b>Ephemeroptera (mayflies)</b>						
<i>Acentrella</i> sp.						
<i>Baetis (tricaudatus)</i>	738	235	735		1,708	6,621
<i>Dipheter hageni</i>						
<i>Drunella grandis</i>	2				2	8
<i>Ephemerella dorothea infrequens</i>	263	134	317		714	2,768
<i>Epeorus</i> sp.	4				4	16
<i>Heptagenia</i> sp.	20				20	78
<i>Rhithrogena</i> sp.	39	24	40		103	400
<i>Asioplax</i> sp.						
<i>Tricorythodes explicatus</i>	3		21		24	93
<i>Paraleptophlebia</i> sp.	43	9	6		58	225
<b>Plecoptera (stoneflies)</b>						
<i>Paracapnia</i> sp.						
Chloroperlidae						
<i>Sweltsa</i> sp.						
<i>Claassenia sabulosa</i>	2				2	8
<i>Hesperoperla pacifica</i>						
Perlodidae ( <i>Cultus</i> sp.)	5	1	4		10	39
<i>Diura knowltoni</i>						
<i>Isogenoides</i> sp.						
<i>Isoperla</i> sp.						
<i>Isoperla fulva</i>						
<i>Pteronarcella badia</i>						
<i>Pteronarcys californica</i>	1				1	4
<b>Trichoptera (caddisflies)</b>						
<i>Brachycentrus americanus</i>						
<i>Brachycentrus occidentalis</i>	9	3	6		18	70
<i>Culoptila</i> sp.	4	2	12		18	70
<i>Glossosoma</i> sp.		1			1	4
<i>Protoptila</i> sp.	4		1		5	20
<i>Helicopsyche borealis</i>			1		1	4
<i>Arctopsyche grandis</i>						
<i>Cheumatopsyche</i> sp.	6	1	2		9	35
<i>Hydropsyche cockerelli</i>	3				3	12
<i>Hydropsyche occidentalis</i>	25	12	21		58	225
<i>Hydropsyche oslari</i>	52	31	22		105	407
<i>Hydroptila</i> sp.			4		4	16
<i>Leucotrichia</i> sp.						
<i>Neotrichia</i> sp.						
<i>Lepidostoma</i> sp.	1				1	4
<i>Psychomyia flavida</i>	1		1		2	8
<i>Rhyacophila coloradensis</i>						

**Table A4. cont. Macroinvertebrate data collected from site CR-aC on 5 Nov 2023.**

<b>Diptera (true flies)</b>						
<b>Chironomidae (chironomids)</b>						
<i>Cardiocladius</i> sp.	16	20	10		46	179
<i>Cricotopus/Orthocladius</i> sp.	4	2	4		10	39
<i>Diamesa</i> sp.	1	2	1		4	16
<i>Eukiefferiella</i> sp.	11	3	4		18	70
<i>Lopescladius</i> sp.						
<i>Micropsectra</i> sp.						
<i>Microtendipes</i> sp.			1		1	4
<i>Pagastia</i> sp.						
<i>Parametriocnemus</i> sp.						
<i>Polypedilum</i> sp.						
<i>Potthastia</i> sp.						
<i>Psectrotanypus</i> sp.						
<i>Rheotanytarsus</i> sp.	1		5		6	24
<i>Synorthocladius</i> sp.						
<i>Thienemannimyia</i> group	1				1	4
<i>Tvetenia</i> sp.	27	8	16		51	198
<b>Other Diptera (true flies)</b>						
<i>Atherix pachypus</i>	3				3	12
Ceratopogoninae			1		1	4
<i>Hemerodromia</i> sp.						
<i>Simulium</i> sp.	798	1073	832		2,703	10,477
<b>Coleoptera (beetles)</b>						
<i>Liodessus</i> sp.						
<i>Microcylloepus pusillus</i>	1				1	4
<i>Optioservus</i> sp.	15	5	10		30	117
<i>Zaitzevia parvula</i>	8	2	6		16	62
<b>Miscellaneous</b>						
<i>Atractides</i> sp.	1				1	4
<i>Hygrobates</i> sp.						
<i>Lebertia</i> sp.						
<i>Protzia</i> sp.						
<i>Sperchon</i> sp.	9	2	5		16	62
<i>Ferrissia</i> sp.		1	13		14	55
<i>Physa</i> sp.	1				1	4
<i>Polycelis coronata</i>	8	5	3		16	62
Lumbricidae	1				1	4
Naididae			1		1	4
Tubificidae w/out hair chaetae			4		4	16
Nematoda			2		2	8
<b>Totals</b>	<b>2131</b>	<b>1576</b>	<b>2111</b>		<b>5,818</b>	<b>22,564</b>

**Table A5. Macroinvertebrate data collected from site CR-bSW on 5 Nov 2023.**

Colorado River						
CR-bSW		Sample				Estimated Mean#/m <sup>2</sup>
5 November 2023	1	2	3		Total	
<b>Ephemeroptera (mayflies)</b>						
<i>Acentrella</i> sp.						
<i>Baetis (tricaudatus)</i>	206	354	206		766	2,969
<i>Dipheter hageni</i>						
<i>Drunella grandis</i>						
<i>Ephemerella dorothea infrequens</i>	340	369	203		912	3,535
<i>Epeorus</i> sp.						
<i>Heptagenia</i> sp.	28	32	12		72	280
<i>Rhithrogena</i> sp.	48	32	8		88	342
<i>Asioplax</i> sp.	1				1	4
<i>Tricorythodes explicatus</i>	8	18	12		38	148
<i>Paraleptophlebia</i> sp.	42	32	26		100	388
<b>Plecoptera (stoneflies)</b>						
<i>Paracapnia</i> sp.						
Chloroperlidae						
<i>Sweltsa</i> sp.						
<i>Claassenia sabulosa</i>	2	1	1		4	16
<i>Hesperoperla pacifica</i>	1		1		2	8
Perlodidae ( <i>Cultus</i> sp.)	2	12	5		19	74
<i>Diura knowltoni</i>						
<i>Isogenoides</i> sp.	1				1	4
<i>Isoperla</i> sp.		1			1	4
<i>Isoperla fulva</i>			1		1	4
<i>Pteronarcella badia</i>						
<i>Pteronarcys californica</i>						
<b>Trichoptera (caddisflies)</b>						
<i>Brachycentrus americanus</i>						
<i>Brachycentrus occidentalis</i>	10	15	2		27	105
<i>Culoptila</i> sp.	1	3	1		5	20
<i>Glossosoma</i> sp.						
<i>Protoptila</i> sp.						
<i>Helicopsyche borealis</i>						
<i>Arctopsyche grandis</i>	3	3	2		8	31
<i>Cheumatopsyche</i> sp.	4		2		6	24
<i>Hydropsyche cockerelli</i>	16	7	4		27	105
<i>Hydropsyche occidentalis</i>	81	187	82		350	1,357
<i>Hydropsyche oslari</i>	82	33	17		132	512
<i>Hydroptila</i> sp.	1	7	5		13	51
<i>Leucotrichia</i> sp.						
<i>Neotrichia</i> sp.						
<i>Lepidostoma</i> sp.		1			1	4
<i>Psychomyia flavida</i>						
<i>Rhyacophila coloradensis</i>						

**Table A5. cont. Macroinvertebrate data collected from site CR-bSW on 5 Nov 2023.**

<b>Diptera (true flies)</b>						
<b>Chironomidae (chironomids)</b>						
<i>Cardiocladius</i> sp.		1			1	4
<i>Cricotopus/Orthocladius</i> sp.	2	4	2		8	31
<i>Diamesa</i> sp.						
<i>Eukiefferiella</i> sp.	2	24	25		51	198
<i>Lopescladius</i> sp.	2				2	8
<i>Micropsectra</i> sp.						
<i>Microtendipes</i> sp.			2		2	8
<i>Pagastia</i> sp.						
<i>Parametriocnemus</i> sp.		1			1	4
<i>Polypedilum</i> sp.		1			1	4
<i>Potthastia</i> sp.		1			1	4
<i>Psectrotanypus</i> sp.						
<i>Rheotanytus</i> sp.		2			2	8
<i>Synorthocladius</i> sp.						
<i>Thienemannimyia</i> group	1		1		2	8
<i>Tvetenia</i> sp.	48	43	33		124	481
<b>Other Diptera (true flies)</b>						
<i>Atherix pachypus</i>	3		4		7	28
Ceratopogoninae						
<i>Hemerodromia</i> sp.	1	1			2	8
<i>Simulium</i> sp.	101	208	134		443	1,718
<b>Coleoptera (beetles)</b>						
<i>Liodessus</i> sp.						
<i>Microcylloepus pusillus</i>	66	78	101		245	950
<i>Optioservus</i> sp.	6	10	6		22	86
<i>Zaitzevia parvula</i>	4	11	5		20	78
<b>Miscellaneous</b>						
<i>Atractides</i> sp.		3			3	12
<i>Hygrobates</i> sp.		1			1	4
<i>Lebertia</i> sp.						
<i>Protzia</i> sp.						
<i>Sperchon</i> sp.		2			2	8
<i>Ferrissia</i> sp.						
<i>Physa</i> sp.						
<i>Polycelis coronata</i>						
Lumbricidae						
Naididae	1		1		2	8
Tubificidae w/out hair chaetae						
Nematoda	1				1	4
<b>Totals</b>	<b>1115</b>	<b>1498</b>	<b>904</b>		<b>3,517</b>	<b>13,647</b>



**Timberline Aquatics, Inc.**  
**4219 Table Mountain Place, Suite A**  
**Fort Collins, Colorado 80526**