

## Memorandum of Results

May 27, 2026

To: Northern Water

From: Cassidy Fenske and Erica Ricci, Spheros Environmental

Re: Results from the Windy Gap Firming Project and Habitat Project Colorado River Benthic Macroinvertebrate Biomonitoring Program, 2025

### Introduction

In fall 2025, benthic macroinvertebrates were sampled in the Upper Colorado River at (1) four locations that have been routinely sampled over the past nine years as part of the Windy Gap Firming Project (WGFP), and (2) three locations for the Upper Colorado River Habitat Project (Habitat Project). This Memorandum of Results from the 2025 Colorado River Benthic Macroinvertebrate Biomonitoring Program meets the requirements for Aquatic Life Condition 13 and 14, as described in the *Rationale for Conditional 401 Certification of the Windy Gap Firming Project* (WGFP 401). These monitoring and reporting requirements were initiated following issuance of the Section 404 Permit and will remain in effect until five years after the WGFP is fully operational. The Record of Decision (ROD) was signed on May 16, 2017, by the United States Army Corps of Engineers. The Section 404 Permit (Permit No. NWO-2003-80523-DEN) was issued on June 26, 2017, and subsequently accepted by the Municipal Subdistrict of the Northern Colorado Water Conservancy District on June 29, 2017. The biomonitoring and data analysis approach applied in this study is designed to provide site-specific information describing the health of biological communities. The results obtained from consistent sampling practices and accurate identifications provide information regarding spatial and temporal changes in aquatic conditions and potential impacts to aquatic life. The following narrative provides a brief description of the study area, methods, metric results, and key observations from the 2025 biomonitoring study.

### Key Results

The raw data and files associated with the Multi-Metric Index v5 (MMI v5) have been provided as separate documents. The following is a summary of key observations from the biomonitoring on the Colorado River during September 2025:

1. All sampling locations were in attainment for aquatic life use based off of the scores from the newly updated and implemented MMI (v5).

2. The number of Ephemeroptera, Plecoptera and Trichoptera (EPT taxa) in the WGFP study area were higher than expected during the 2025 sampling period, indicating an increase in the number of species typically considered sensitive to disturbances.
3. Community balance remained relatively stable and healthy across both study areas (based on Diversity and Evenness values), and water quality is “Excellent” for macroinvertebrate communities at all sites (based on Diversity and Taxa Index [DAT] values).
4. The giant stonefly, *Pteronarcys californica*, was collected at three sampling locations: two in WGFP (CR-WGD and CR-WFU), and one in the Habitat Project (CR-R3).
5. Hilsenhoff Biotic Index (HBI) values were higher than average at most sampling sites, indicating a higher proportion of nutrient-tolerant individuals in the community. It is possible that runoff from the 2020 East Troublesome Fire is still affecting the aquatic community.<sup>1-4</sup>

**Table 1.** Individual metrics and MMI (v5) scores from benthic macroinvertebrate samples collected from Northern Water sampling sites on the Colorado River in September 2025. **All metric scores are based on the MMI (v5) subsampling process.** Scores indicating ‘impairment’ would be provided in *red*; however, no impairments were observed.<sup>a</sup>

| Metric                                              | Station ID – Biotype 1 |        |         |        |        |       |       |
|-----------------------------------------------------|------------------------|--------|---------|--------|--------|-------|-------|
|                                                     | CR-WGU                 | CR-WGD | CR-HSPP | CR-WFU | CR-bWF | CR-R3 | CR-R4 |
| EPT Taxa                                            | 100.0                  | 100.0  | 100.0   | 100.0  | 89.5   | 94.7  | 94.7  |
| Percent Coleoptera                                  | 3.7                    | 37.1   | 11.9    | 8.1    | 6.2    | 9.7   | 1.5   |
| Percent EPT no Baetidae                             | 41.6                   | 81.9   | 52.0    | 78.1   | 77.3   | 63.3  | 44.8  |
| Percent Non-Insect                                  | 54.0                   | 93.4   | 97.4    | 88.5   | 53.9   | 93.6  | 88.3  |
| Percent Intolerant Taxa                             | 65.7                   | 73.4   | 71.2    | 81.6   | 79.3   | 72.9  | 65.4  |
| Predator/Shredder Taxa                              | 66.7                   | 86.7   | 60.0    | 93.3   | 46.7   | 60.0  | 73.3  |
| Percent Clinger Taxa                                | 72.9                   | 80.5   | 86.3    | 87.7   | 84.4   | 82.0  | 73.6  |
| Percent moderate decreaser Individuals in Biotype 1 | 37.5                   | 83.5   | 35.5    | 41.5   | 73.8   | 56.1  | 40.0  |
| MMI (v5)                                            | 55.3                   | 79.6   | 64.3    | 72.4   | 63.9   | 66.5  | 60.2  |
| Auxiliary Metrics                                   |                        |        |         |        |        |       |       |
| Diversity                                           | 4.18                   | 3.99   | 4.06    | 4.59   | 3.50   | 3.87  | 3.49  |
| HBI                                                 | 4.73                   | 2.93   | 4.03    | 3.44   | 2.74   | 3.43  | 4.04  |
| Sediment Region                                     | SR4                    | SR2    | SR4     | SR2    | SR4    | SR4   | SR4   |
| TIV                                                 | 4.83                   | 4.78   | 6.06    | 5.50   | 3.08   | 5.13  | 5.44  |

## Study Area

### Windy Gap Firing Project (WGFP)

Condition 13 of the WGFP 401 states, “The Applicant will monitor the health of aquatic communities by sampling benthic macroinvertebrates and calculating MMI scores. The macroinvertebrate sampling will be conducted using the Division’s [Water Quality Control Division] protocols, which are described in Policy Statement 10-1 Aquatic Life Use Attainment Methodology to Determine Use Attainment for Rivers and Streams. The Applicant will develop a Sampling Analysis Plan for the

<sup>a</sup> Note: Auxiliary metrics shown in red would indicate threshold exceedance for that metric only and do not necessarily signify overall site impairment.

collection and preservation of benthic macroinvertebrates that will be reviewed by the Division prior to the start of macroinvertebrate sampling.”

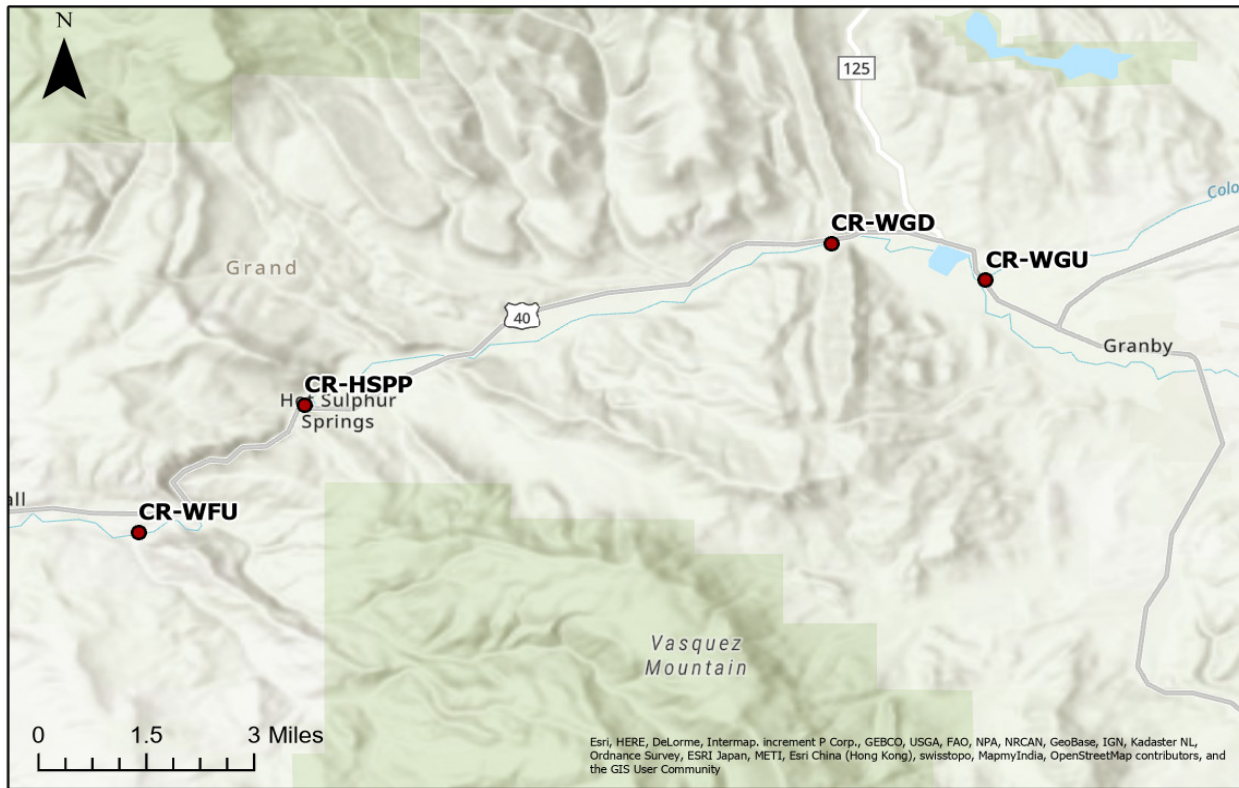
To meet these requirements, three sampling sites (CR-WGU, CR-WGD, and CR-HSPP) are monitored (shown in [Table 2](#) and [Figure 1](#)). The upstream site, CR-WGU, is located on the Colorado River immediately upstream of Windy Gap Reservoir and serves as a control location by characterizing natural variability and long-term trends. Although this site may be subject to some influence from Granby Reservoir operations, historical data (2016 – 2019) indicate that it has consistently supported relatively healthy macroinvertebrate communities. Data collected in 2020 showed a decline in MMI, which resulted in impairment and remained impaired until 2024. Downstream of CR-WGU, the Colorado River Connectivity Channel (CRCC) and associated floodplain, which was designed to reconnect the river above and below the reconfigured Windy Gap Reservoir, became operational in 2024 ([Colorado River Connectivity Channel | Northern Water](#)).

The two downstream primary sites, CR-WGD and CR-HSPP, are located below Windy Gap Reservoir and are intended to evaluate aquatic conditions potentially influenced by project operations. Site CR-WFU, located immediately upstream of the Williams Fork River confluence, is monitored annually to fully characterize conditions within this sub-segment of the Colorado River, although monitoring at this site is not required under WGFP 401. These sites will also support evaluation of benefits associated with the CRCC.

Sampling at the three primary sites has been conducted annually in the fall since 2016, continuing after issuance of the Section 404 permit and for no less than five years after the project becomes fully operational. Raw data and MMI scores from each calendar year and a report documenting any impairment of aquatic life will be submitted to the Division by June 1 following each calendar year of sampling. If conditions in a given year are considered unrepresentative, for example, following a flood or other natural disturbance, modifications to the sampling approach may be implemented upon prior approval by the Division.

**Table 2.** Site descriptions and coordinates of WGFP sampling sites on the Colorado River on September 25, 2025.

| Site Name | Description                                      | Latitude | Longitude  |
|-----------|--------------------------------------------------|----------|------------|
| CR-WGU    | Colorado River upstream of Windy Gap Reservoir   | 40.10045 | -105.97248 |
| CR-WGD    | Colorado River downstream of Windy Gap Reservoir | 40.10830 | -106.00356 |
| CR-HSPP   | Colorado River near Hot Sulphur Springs          | 40.07394 | -106.10959 |
| CR-WFU    | Colorado River upstream of Williams Fork         | 40.04689 | -106.14299 |



This map was created with ArcMap 10.8.1.

**Figure 1.** Map of study sites on the Upper Colorado River used for the WGFP Biomonitoring Study.

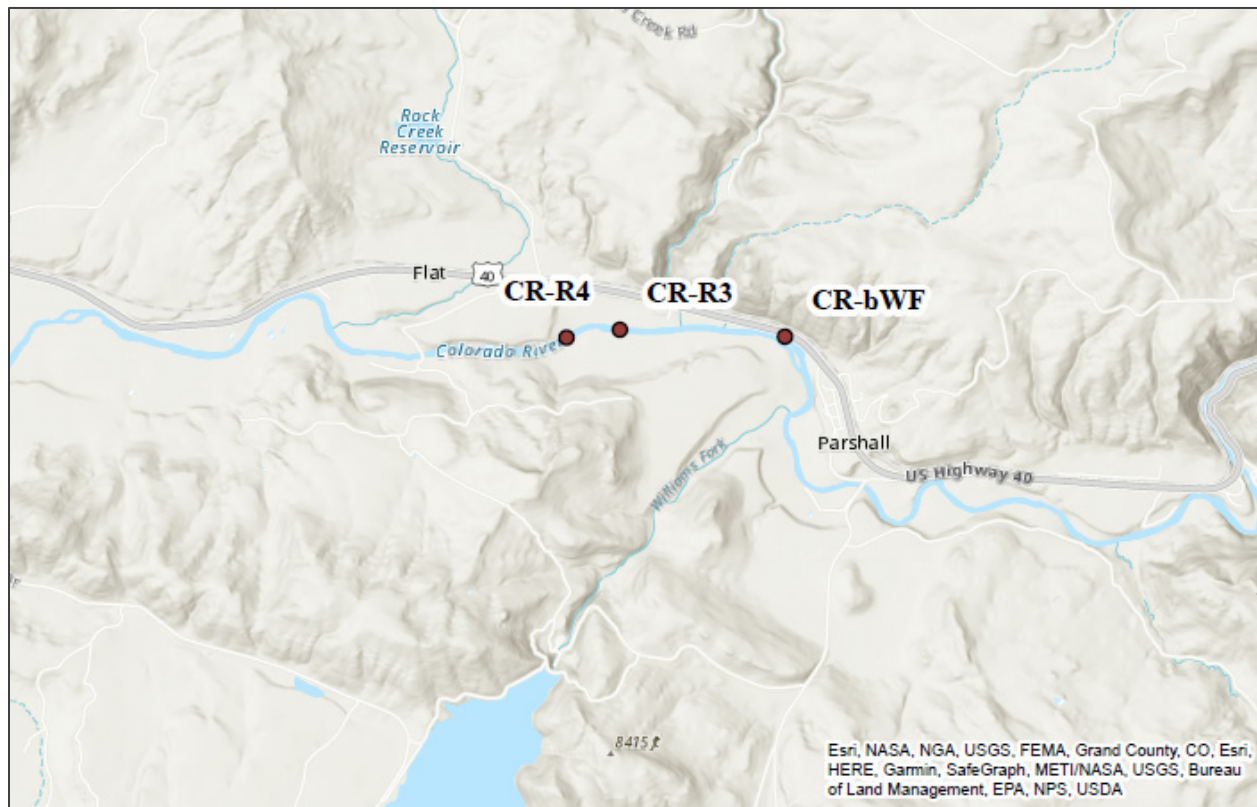
## Upper Colorado River Habitat Project (Habitat Project)

Condition 14 of the WGFP 401 states, "As soon as the plan for habitat improvements has been developed, the Applicant, in consultation with the Division, will select two additional sampling sites to be located within the reach to be modified. These sites will be sampled at least once prior to altering the habitat and will be re-sampled three and four years after the work has been completed. Sampling protocols and reporting requirements are the same as those specified for the three primary sites in Condition 13."

To meet these requirements, the Kemp-Breeze Aquatic Habitat Improvement Project (Kemp-Breeze) represents the first phase of the Upper Colorado River Habitat Project and is intended to help restore the Colorado River between Windy Gap Reservoir and the Kemp-Breeze State Wildlife area. This project focuses on approximately one mile of the Colorado River downstream of the Williams Fork River confluence and is designed to restore aquatic habitat conditions and establish a more ecologically functional river system that reflects current and anticipated future hydrological conditions. This work is being implemented in coordination with Northern Water, Denver Water, Grand County, and Trout Unlimited, in accordance with agreements associated with the Moffat and

Windy Gap Firming Projects. Work on the project began in the summer of 2022 and was completed in early 2024.

In accordance with Condition 14, Northern Water and Timberline Aquatics (now a part of Spheros Environmental), worked with the Division to establish three sampling sites: one upstream reference site and two within the Kemp-Breeze project area (shown in [Figure 2](#) and [Table 3](#)). The reference site (CR-bWF) was located downstream of the Williams Fork River confluence but upstream of any Kemp-Breeze project construction activities. The two downstream sites were established in the project area: site CR-R3, located near a wing deflector in Reach 3, and site CR-R4, located in a riffle habitat in Reach 4 (see [Figure 2](#), [Table 3](#)). Pre-project sampling was completed in 2020, with post-project sampling conducted in 2024 and 2025. Annual monitoring will continue for four years after project completion, to fulfill the WGFP 401 condition.



This map was created with ArcMap 10.8.1.

**Figure 2.** Map of the biomonitoring study sites used for the Habitat Project.

**Table 3.** Site descriptions and coordinates of the Habitat Project sites on the Colorado River on September 26, 2025.

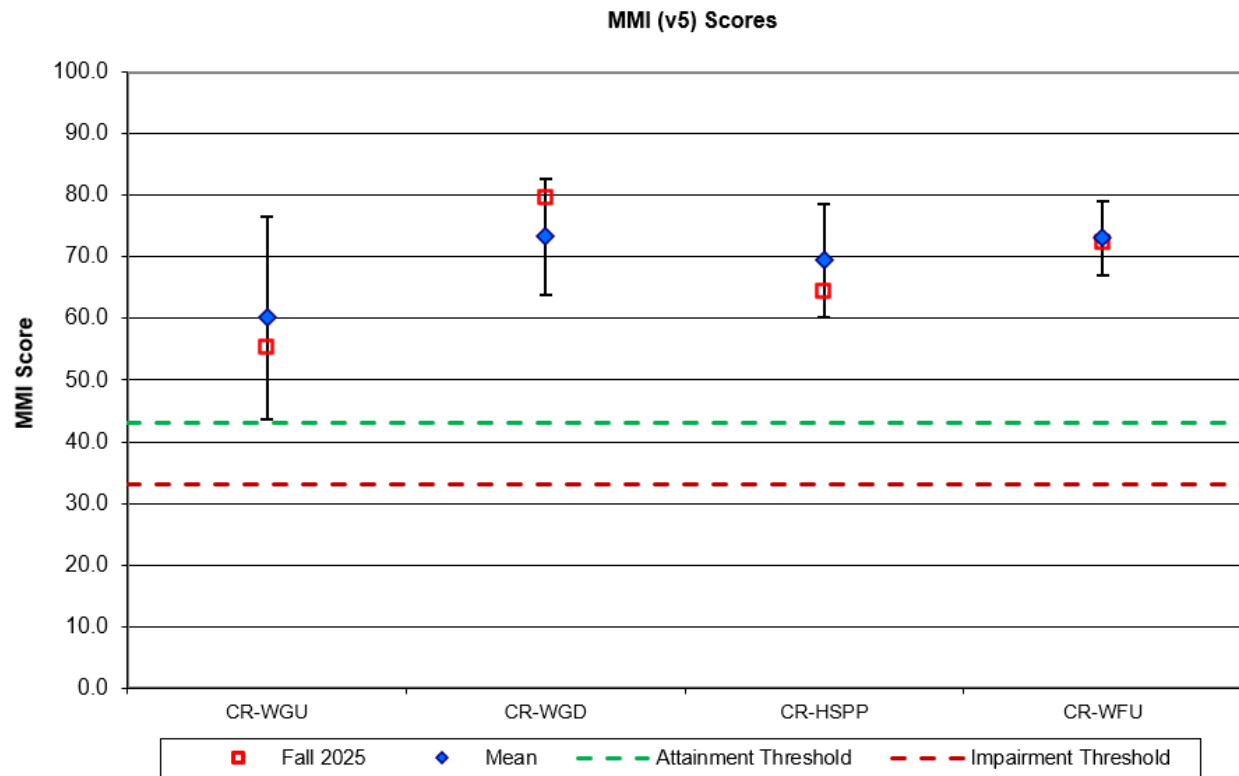
| Station ID | Description                                      | Latitude | Longitude  |
|------------|--------------------------------------------------|----------|------------|
| CR-bWF     | Colorado River below Williams Fork               | 40.06262 | -106.18113 |
| CR-R3      | Colorado River at Reach 3 downstream from bridge | 40.06300 | -106.19860 |
| CR-R4      | Colorado River at Reach 4 downstream from bridge | 40.0623  | -106.20423 |

## Sampling and Analysis Methods

All samples were collected from riffle habitat using a quantitative (Hess) sampling technique and then transported to the Spheros Environmental lab in Fort Collins, Colorado. All specimens were picked out from each sample to avoid controversies associated with sub-sampling, then identified to the lowest practical taxonomic level and enumerated. A combination of individual metrics and the newest version of Colorado's Multi-Metric Index (v5) were used to analyze the macroinvertebrate data. Descriptions of the metrics used for analysis are found in [Appendix A](#).

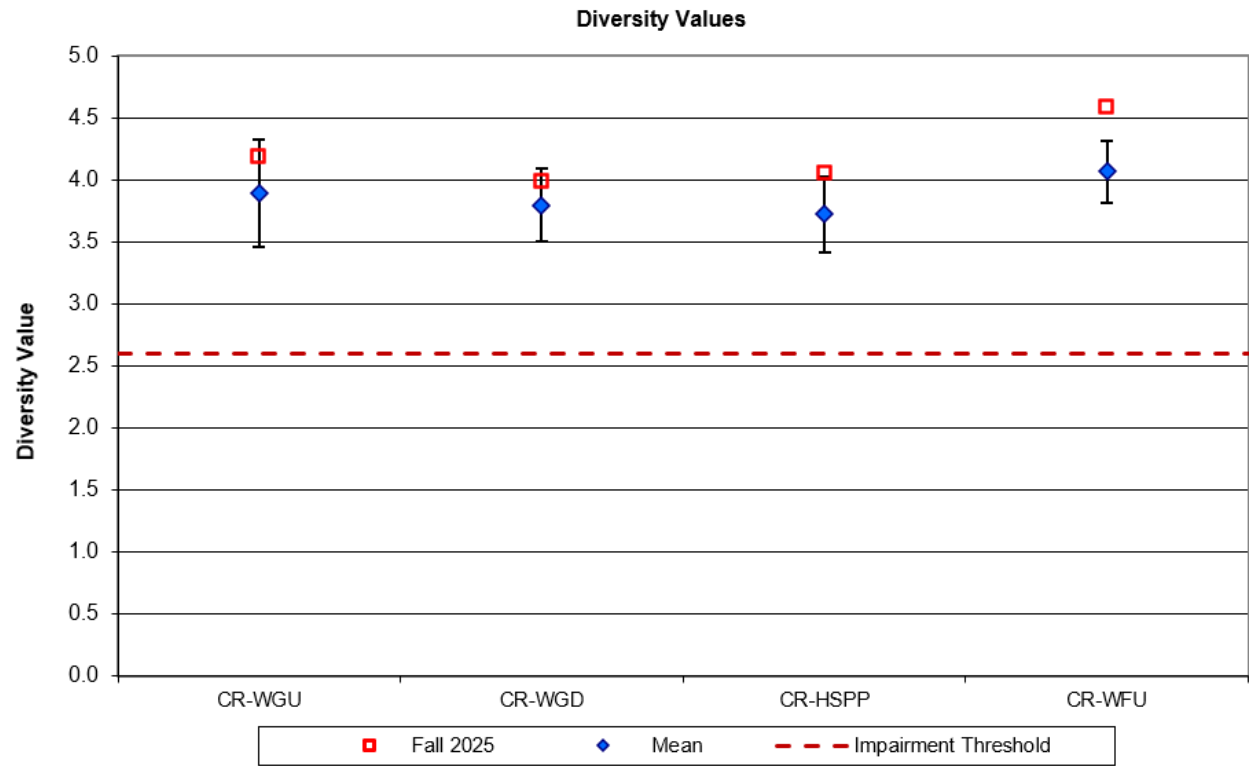
## Metric Results

### Windy Gap Firing Project

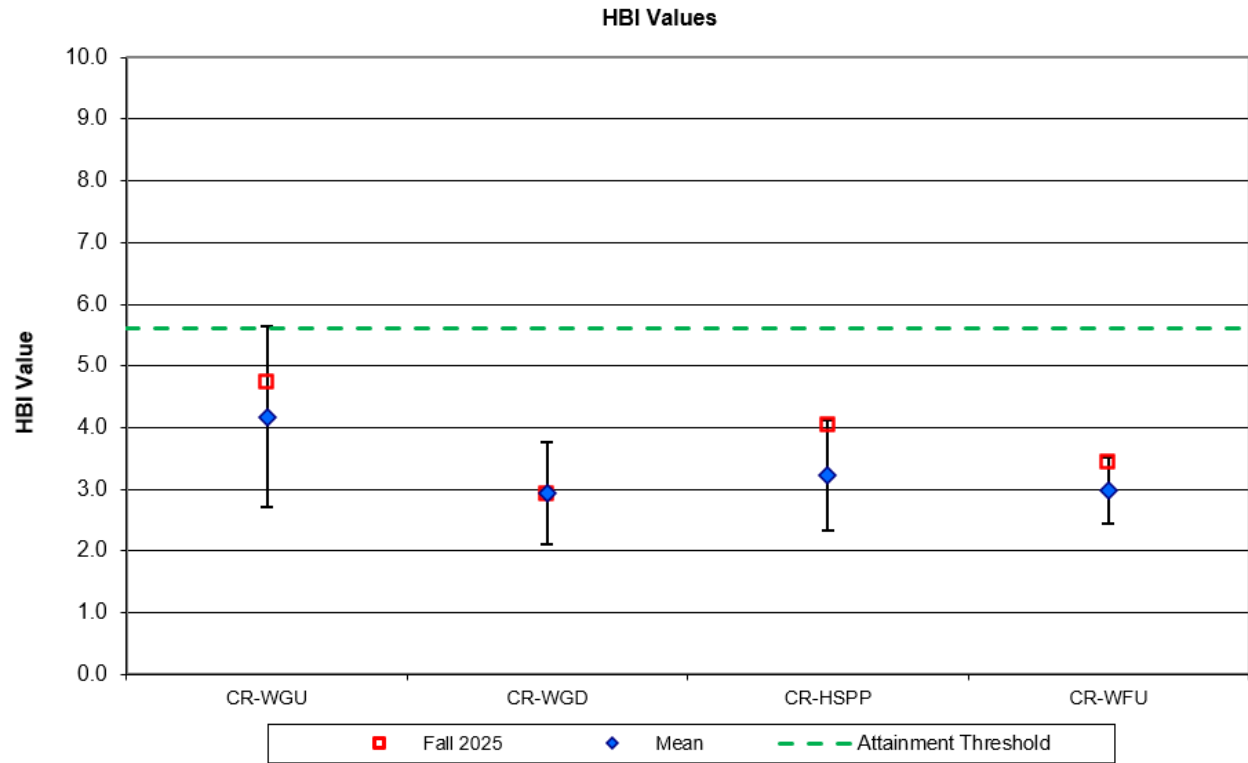


**Figure 3.** MMI (v5) scores from study sites in the WGFP study area on the Colorado River during September 2025. Mean values ( $\pm 1$  standard deviation) are also provided based on previous sampling events. All scores are based on the MMI subsampling process.





**Figure 4.** Diversity values from the WGFP study area on the Colorado River during September 2025. Mean values ( $\pm 1$  standard deviation) are also provided based on previous sampling events. All values are based on the MMI subsampling process.



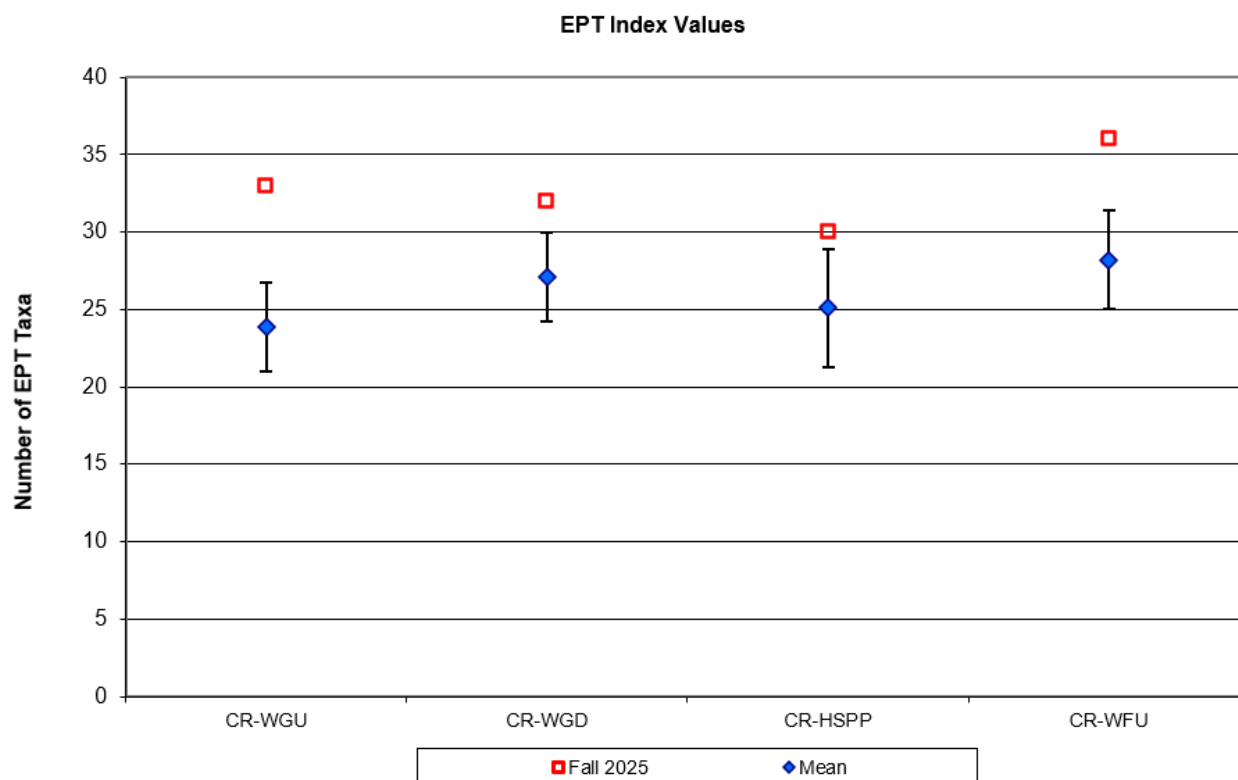
**Figure 5.** HBI values for the WGFP study area on the Colorado River during September 2025. Mean values ( $\pm 1$  standard deviation) are also provided based on previous sampling events. All values are based on the MMI subsampling process.

**Table 4.** Aquatic life use designations based on MMI scores from the WGFP study area on the Colorado River during September 2025.

| Site    | MMI (v5)   |
|---------|------------|
| CR-WGU  | Attainment |
| CR-WGD  | Attainment |
| CR-HSPP | Attainment |
| CR-WFU  | Attainment |

**Table 5.** Additional metrics and comparative values for macroinvertebrate samples collected from WGFP study area on the Colorado River in September 2025. All additional metric values are based on full count Hess samples.

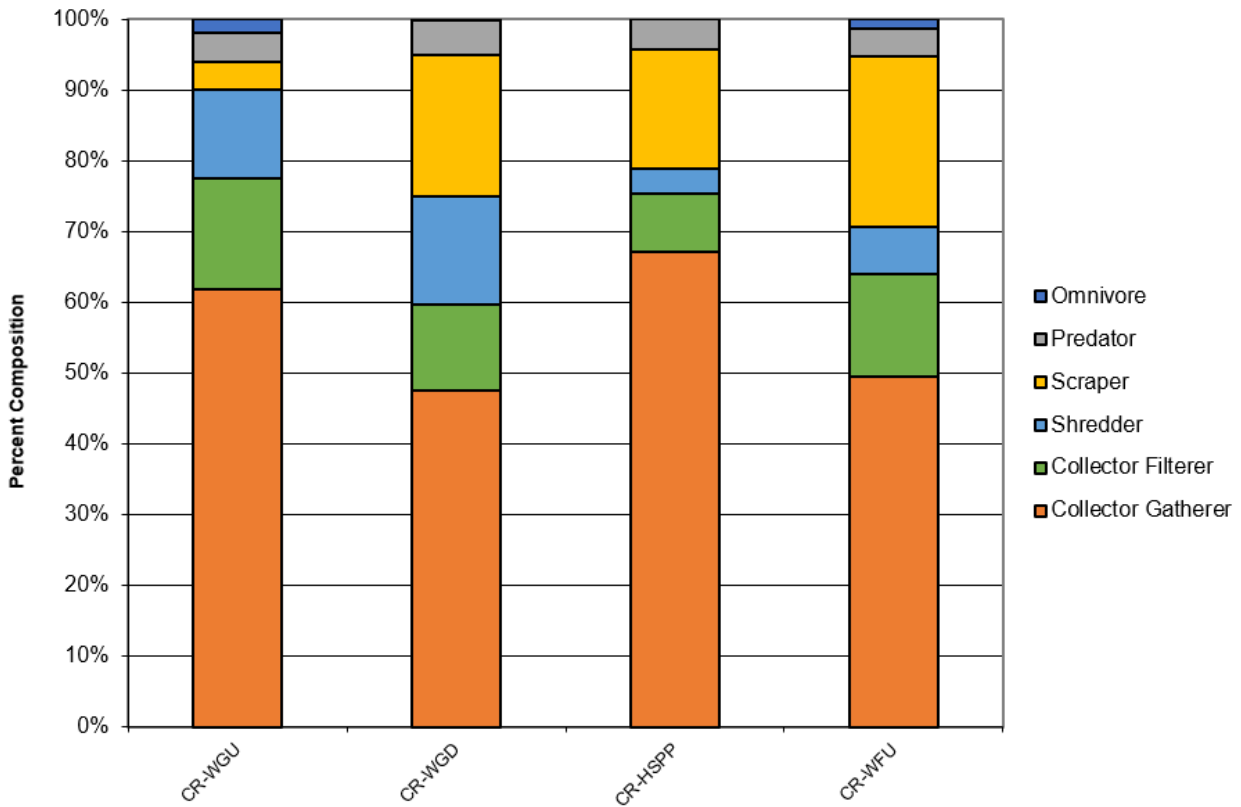
| Metric                                                        | CR-WGU | CR-WGD | CR-HSPP | CR-WFU |
|---------------------------------------------------------------|--------|--------|---------|--------|
| Diversity                                                     | 4.14   | 3.98   | 3.97    | 4.48   |
| HBI                                                           | 5.23   | 3.49   | 4.55    | 3.80   |
| EPT                                                           | 33     | 32     | 30      | 36     |
| Evenness                                                      | 0.69   | 0.66   | 0.65    | 0.73   |
| DAT                                                           | 44.4   | 44.9   | 48.8    | 48.8   |
| Insect Taxa                                                   | 53     | 55     | 58      | 61     |
| Total Taxa                                                    | 63     | 66     | 69      | 69     |
| Percent Shredders and Scrapers                                | 16.39% | 35.17% | 20.26%  | 30.85% |
| Density of <i>Pteronarcys californica</i> (#/m <sup>2</sup> ) | 0      | 8      | 0       | 24     |
| Percent EPT (excluding Baetidae)                              | 29.45% | 57.62% | 36.60%  | 55.91% |
| Density (mean #/m <sup>2</sup> )                              | 23,913 | 19,770 | 24,004  | 16,978 |
| Percent Chironomidae                                          | 39.22% | 10.55% | 50.64%  | 30.69% |
| Percent Hydropsychidae                                        | 3.50%  | 9.17%  | 4.17%   | 11.91% |
| Percent Tolerant Taxa                                         | 14.29% | 19.70% | 20.29%  | 11.59% |
| Percent Intolerant Taxa                                       | 36.51% | 37.88% | 31.88%  | 40.58% |
| Biomass (g/m <sup>2</sup> )                                   | 4.0484 | 5.1275 | 4.8465  | 6.0209 |



**Figure 6.** EPT values from quantitative sampling (full count Hess samples) collected from the WGFP study area on the Colorado River during September 2025. Mean values ( $\pm 1$  standard deviation) are also provided based on previous sampling events.

**Table 6.** Relative abundance of functional feeding groups for September 2025 sampling at WGFP study area.

| Site    | Collector-Gatherer | Collector-Filterer | Shredder | Scraper | Predator | Omnivore |
|---------|--------------------|--------------------|----------|---------|----------|----------|
| CR-WGU  | 61.92%             | 15.67%             | 12.45%   | 3.94%   | 4.14%    | 1.88%    |
| CR-WGD  | 47.64%             | 12.14%             | 15.16%   | 20.01%  | 4.93%    | 0.12%    |
| CR-HSPP | 67.18%             | 8.21%              | 3.43%    | 16.83%  | 4.35%    | 0.00%    |
| CR-WFU  | 49.58%             | 14.36%             | 6.70%    | 24.15%  | 3.98%    | 1.23%    |



**Figure 7.** Functional feeding group composition for WGFP study area on the Colorado River in September 2025.

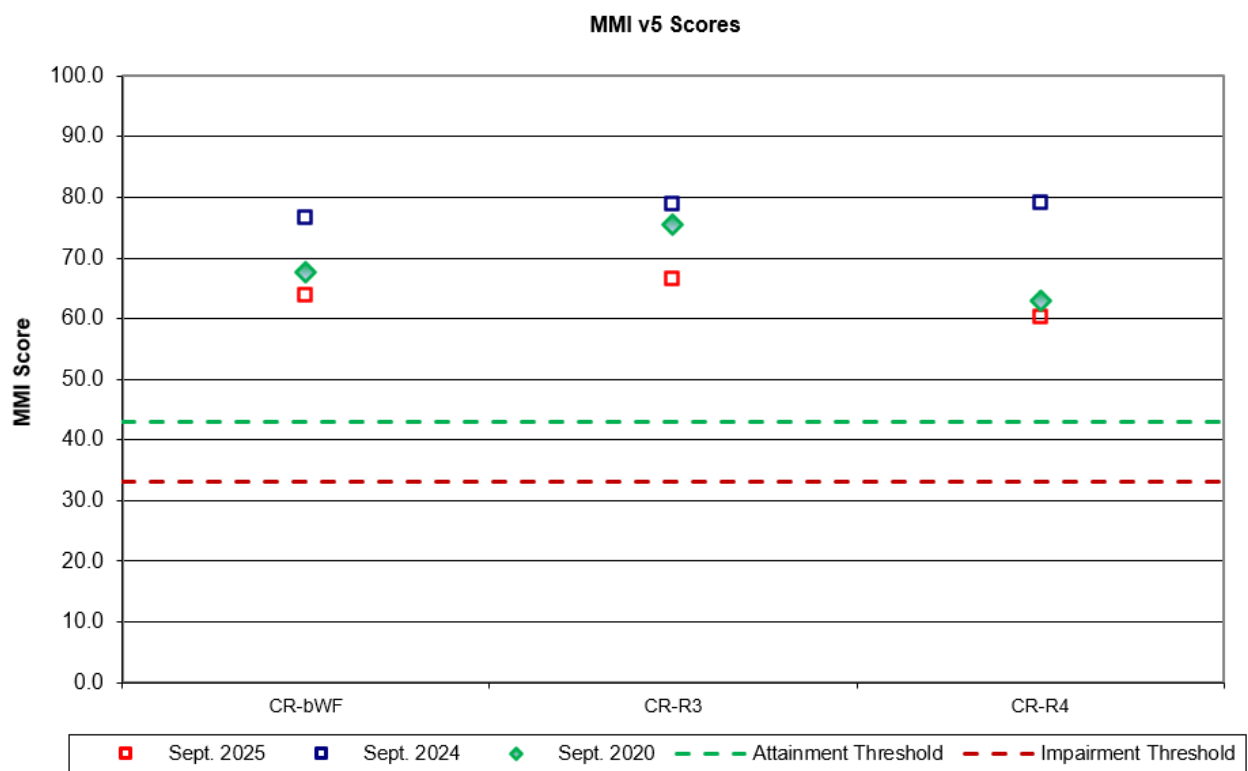
## Discussion – Windy Gap Firming Project

On September 25, 2025, benthic macroinvertebrate samples were collected from the four established sites in the WGFP study area on the Upper Colorado River to assess aquatic conditions (shown in [Tables 4 through 6](#), and [Figures 3 through 7](#)). Results from the MMI (v5) indicated that all sampling locations were in attainment for aquatic life use and all scores were within their expected range, based on historical (2016-2024) sampling events (shown in Table 4 and Figure 3). Two auxiliary metrics, Diversity and HBI, were run concurrently with the MMI, and Diversity values at all sites were above average during 2025, with site CR-WFU obtaining a higher value than anticipated (Figure 4). All HBI values were within their expected ranges and below the impairment threshold (Figure 5).

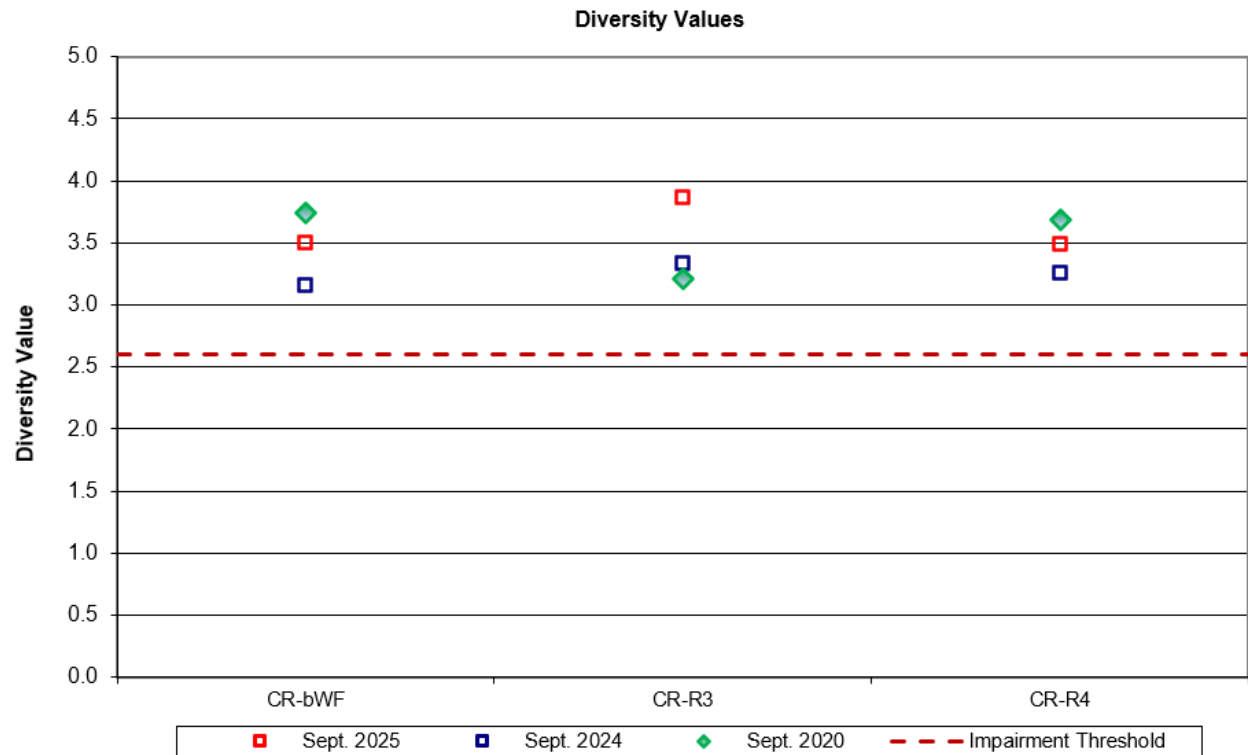
Fourteen additional metrics and a functional feeding group analysis were also used to evaluate macroinvertebrate community structure and composition at WGFP study sites (shown in Tables 5 and 6, and Figures 6 and 7). All sites were able to support a large number of different taxa (Insect Taxa and Total Taxa), including those considered sensitive to disturbances (EPT) (Table 5). In fact, EPT values were higher than expected (based on the upper bounds of the standard deviation) at all sampling locations (Figure 6). Macroinvertebrate community structure appeared to be stable and well-balanced across the study area (Evenness: 0.65-0.73). *Pteronarcys californica*, was collected at

two sites (Table 5); this species is usually a strong indicator of healthy aquatic conditions in the Upper Colorado River as it requires stable water conditions to complete its four-year life cycle. Furthermore, results from the DAT metric indicated that all sites maintained “Excellent” water quality for macroinvertebrate communities. The functional feeding group analysis supported the individual metric results, noting well-balanced communities with relatively high percentages of sensitive and specialized feeding groups (shredders and scrapers) (shown in Table 6 and Figure 7). Overall, the four sampling sites in the WGFP study area were able to support healthy macroinvertebrate community assemblages, with some improvements observed in 2025 compared to previous years.

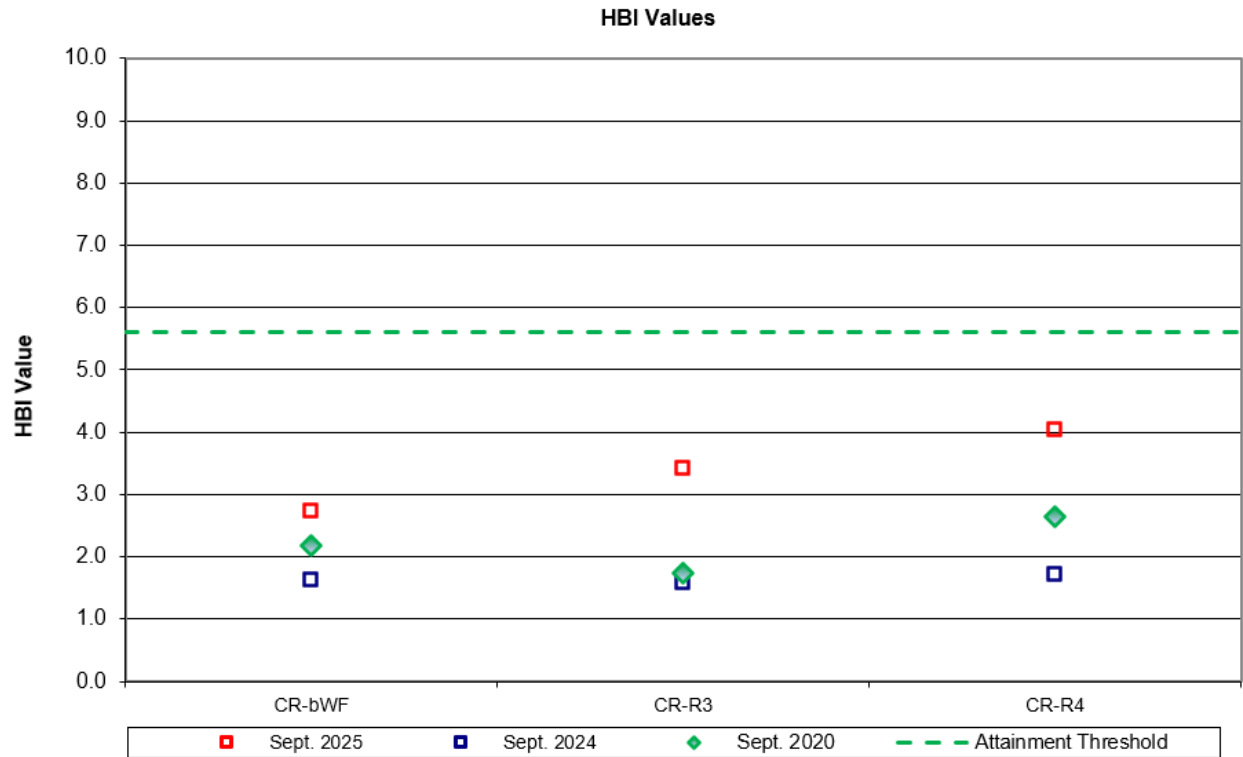
### Upper Colorado River Habitat Project (Habitat Project)



**Figure 8.** MMI (v5) scores from composited quantitative (Hess) samples collected from the Habitat Project study sites on the Colorado River during September 2020, 2024, and 2025. Data from 2020 reflect pre-restoration conditions, while 2024 and 2025 data reflect post-project conditions.



**Figure 9.** Diversity values from the Habitat Project study sites on the Colorado River during September 2020, 2024, and 2025. Data from 2020 reflect pre-restoration conditions, while 2024 and 2025 data reflect post-project conditions. All values are based on the MMI subsampling process.



**Figure 10.** HBI values for the Habitat Project study area on the Colorado River during September 2020, 2024, and 2025. Data from 2020 reflect pre-restoration conditions, while 2024 and 2025 data reflect post-project conditions. All values are based on the MMI subsampling process.

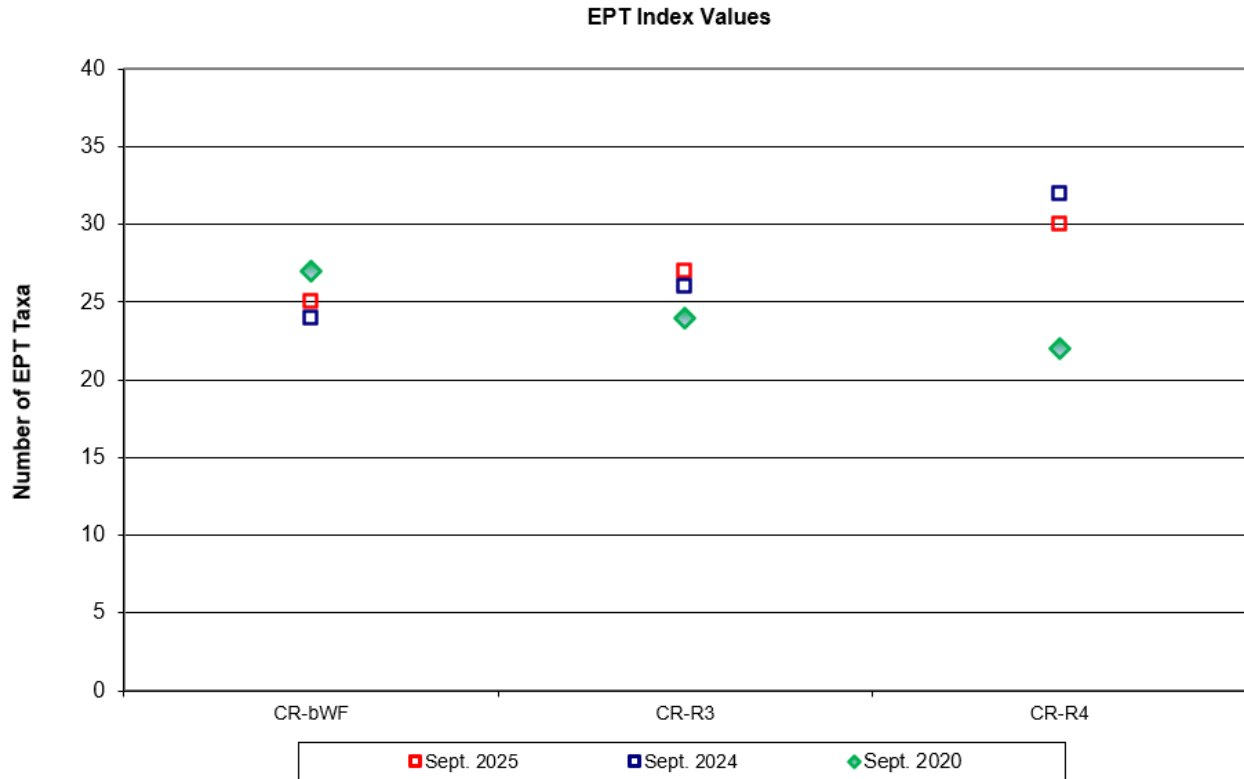
**Table 7.** Aquatic life use designations based on MMI (v5) scores from the Habitat Project study sites on the Upper Colorado River during September 2025.

| Site   | MMI (v5)   |
|--------|------------|
| CR-bWF | Attainment |
| CR-R3  | Attainment |
| CR-R4  | Attainment |



**Table 8.** Additional metrics and comparative values for macroinvertebrate samples collected from Habitat Project study sites on the Upper Colorado River during September 2025. Additional metric values are based on full count Hess samples.

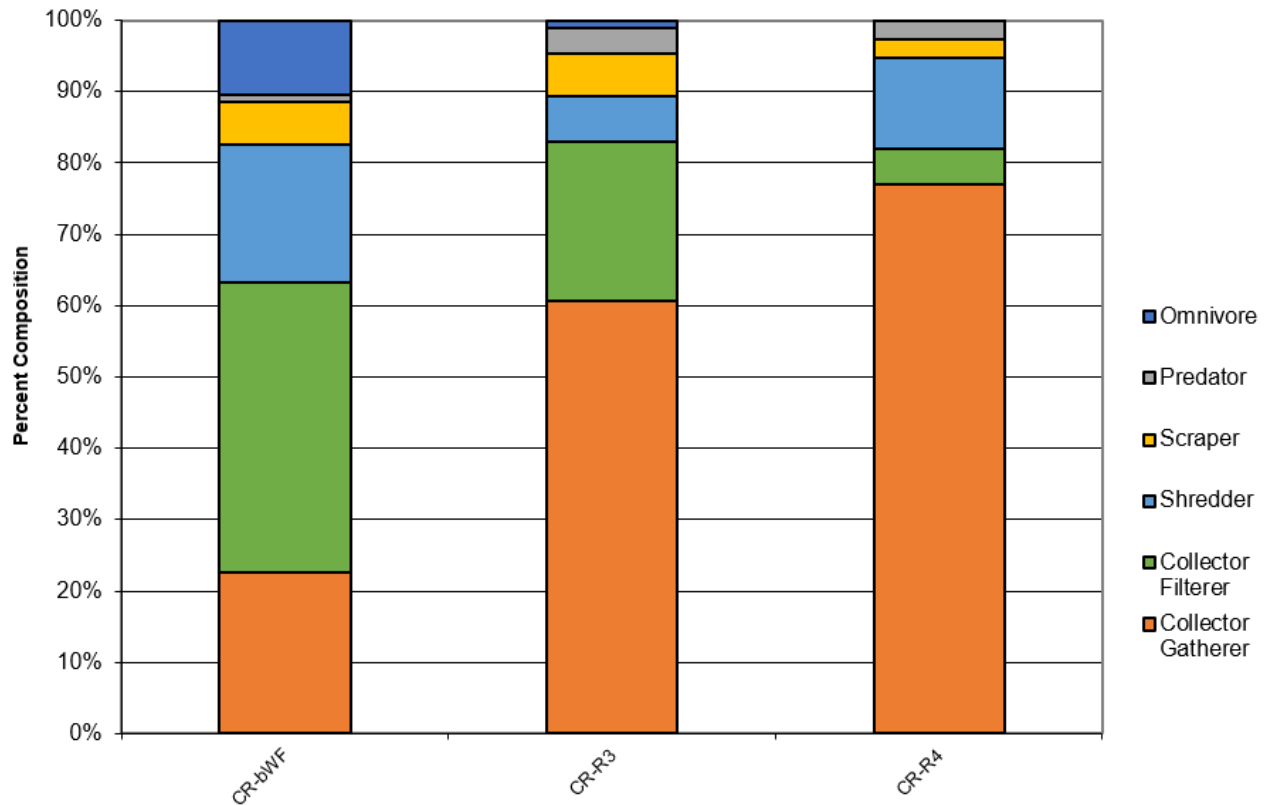
| Metric                                                        | CR-bWF | CR-R3  | CR-R4  |
|---------------------------------------------------------------|--------|--------|--------|
| Diversity                                                     | 3.43   | 3.84   | 3.43   |
| HBI                                                           | 3.50   | 3.99   | 5.03   |
| EPT                                                           | 25     | 27     | 30     |
| Evenness                                                      | 0.61   | 0.64   | 0.57   |
| DAT                                                           | 30.7   | 43.7   | 42.7   |
| Insect Taxa                                                   | 40     | 53     | 57     |
| Total Taxa                                                    | 49     | 63     | 67     |
| Percent Shredders and Scrapers                                | 25.35% | 12.38% | 15.25% |
| Density of <i>Pteronarcys californica</i> (#/m <sup>2</sup> ) | 0      | 16     | 0      |
| Percent EPT (excluding Baetidae)                              | 55.61% | 43.80% | 32.64% |
| Density (mean #/m <sup>2</sup> )                              | 23,814 | 30,136 | 36,570 |
| Percent Chironomidae                                          | 12.58% | 32.83% | 59.14% |
| Percent Hydropsychidae                                        | 4.59%  | 7.29%  | 1.36%  |
| Percent Tolerant Taxa                                         | 18.37% | 17.46% | 20.90% |
| Percent Intolerant Taxa                                       | 36.73% | 38.10% | 34.33% |
| Biomass (g/m <sup>2</sup> )                                   | 6.0926 | 9.2345 | 5.6240 |



**Figure 11.** EPT values from quantitative sampling (full count Hess samples) collected from the Habitat Project study area on the Colorado River during September 2020, 2024, and 2025. Data from 2020 reflect pre-restoration conditions, while 2024 and 2025 data reflect post-project conditions.

**Table 9.** Relative abundance of functional feeding groups in the Habitat Project study area on the Upper Colorado River during September 2025.

| Site   | Collector-Gatherer | Collector-Filterer | Shredder | Scraper | Predator | Omnivore |
|--------|--------------------|--------------------|----------|---------|----------|----------|
| CR-bWF | 22.67%             | 40.51%             | 19.32%   | 6.03%   | 0.96%    | 10.51%   |
| CR-R3  | 60.67%             | 22.21%             | 6.46%    | 5.92%   | 3.57%    | 1.17%    |
| CR-R4  | 76.92%             | 5.10%              | 12.69%   | 2.57%   | 2.65%    | 0.07%    |



**Figure 12.** Percent composition of functional feeding groups collected on the Colorado River during September of 2025.

## Discussion – Upper Colorado River Habitat Project

Three sites (CR-bWF, CR-R3, and CR-R4) that were previously sampled in 2020 and 2024 were sampled again on September 26, 2025, to evaluate aquatic conditions for the Upper Colorado River Habitat Project (shown in [Tables 7 through 9](#), and [Figures 8 through 12](#)). MMI (v5) results indicated that all sites exceeded the attainment threshold for aquatic life use; however, scores observed in 2025 were lower than both pre-project (2020) and post-project (2024) scores (shown in Table 7 and Figure 8). Community balance remained stable across the sites, based on Diversity and Evenness values, though the number of different taxa (Total Taxa and Insect Taxa), including those sensitive to disturbances (EPT), increased in a downstream direction (Table 8). Additionally, *Pteronarcys californica* was collected at one sampling location (CR-R3), and DAT values indicated that water quality “Excellent” for macroinvertebrate communities at all sites (Table 8). Notably, while all HBI values were at an acceptable level, they were elevated in 2025 compared to previous years (Figure 10), indicating that the sites had a higher proportion of nutrient-tolerant individuals comprising the macroinvertebrate communities. A review of the functional feeding group analysis reinforced the individual metric results, showing that all feeding groups were represented at each site, including adequate representation of the two specialized and more sensitive groups—shredders and scrapers

(shown in Table 9 and Figure 12). Overall, macroinvertebrate community assemblages appeared to be relatively healthy in the Habitat Project study area during 2025.

## Literature Cited

1. Malison Rachel L. & Baxter Colden V. Effects of wildfire of varying severity on benthic stream insect assemblages and emergence. *J. North Am. Benthol. Soc.* 29, 1324–1338 (2010).
2. Minshall, W. Responses of Stream Benthic Macroinvertebrates to Fire. (In press).
3. Preston, D. L. *et al.* Short-term effects of wildfire on high elevation stream-riparian food webs. *Oikos* 2023, e09828 (2023).
4. Vieira, N. Impacts of wildfire on benthic insects in burned streams: community and population responses at multiple scales. (2003).
5. Colorado Department of Public Health and Environment. Policy 10-1. *Aquatic life use attainment: Methodology to determine use attainment for rivers and streams* [https://drive.google.com/file/d/1Hj\\_ytnBJS2kF7T-e2uY6P1dfEvkEbmVS/view](https://drive.google.com/file/d/1Hj_ytnBJS2kF7T-e2uY6P1dfEvkEbmVS/view) (2025).
6. *Section 303(d) Listing Methodology 2026 Listing Cycle.*
7. Barbour, M., Gerritsen, J., Snyder, B. & Stribling, J. B. Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates, and fish, 2nd edition. *EPA 841-B-99-002 US Environ. Prot. Agency Off. Water* (1999).
8. Hilsenhoff, W. L. Rapid Field Assessment of Organic Pollution with a Family-Level Biotic Index. *J. North Am. Benthol. Soc.* 7, 65–68 (1988).
9. Lenat, D. R. Water Quality Assessment of Streams Using a Qualitative Collection Method for Benthic Macroinvertebrates. *J. North Am. Benthol. Soc.* 7, 222–233 (1988).
10. Wang, L., Robertson, D. M. & Garrison, P. J. Linkages between nutrients and assemblages of macroinvertebrates and fish in wadeable streams: implication to nutrient criteria development. *Environ. Manage.* 39, 194–212 (2007).
11. Ward, J. V., Kondratieff, B. C. & Zuellig, R. E. *An Illustrated Guide to the Mountain Streams Insects of Colorado.* (University Press of Colorado, 2002).
12. Mangum, F. A. Macroinvertebrates. in *Inventory and Monitoring of Wildlife Habitat* 661–675 (US Department of the Interior, Bureau of Land Management, Denver, Co, 1986).
13. Weber, C. I. *Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents. Program Element 1BA027.* <https://eric.ed.gov/?id=ED159028> (1973).

14. Courtemanch, D. L. Commentary on the Subsampling Procedures Used for Rapid Bioassessments. *J. North Am. Benthol. Soc.* 15, 381–385 (1996).
15. Resh, V. & Jackson, J. Rapid Assessment Approaches to Biomonitoring Using Benthic Macroinvertebrates. in 195–233 (1993).
16. Kowalski, D. A. & Richer, E. E. Quantifying the habitat preferences of the stonefly *Pteronarcys californica* in Colorado. *River Res. Appl.* 36, 2043–2050 (2020).
17. Plafkin, J. L., Barbour, M. T., Porter, K. D., Gross, S. K. & Hughes, R. M. *Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish*. <https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB95194759.xhtml> (1989).
18. Lenat, D. R. Chironomid Taxa Richness: Natural Variation and Use in Pollution Assessment. *Freshw. Invertebr. Biol.* 2, 192–198 (1983).
19. Mandaville, S. M. Benthic Macroinvertebrates in Freshwater Taxa Tolerance Values, Metrics, and Protocols. *Soil Water Conserv. Soc. Metro Halifax Project H-1*, 48 (2002).
20. Barton, D. R. & Metcalfe-Smith, J. L. A comparison of sampling techniques and summary indices for assessment of water quality in the Yamaska River, Québec, based on benthic macroinvertebrates. *Environ. Monit. Assess.* 21, 225–244 (1992).

## Appendix A. Benthic Macroinvertebrate Sampling Methods and Metric Descriptions – Fall 2025

### Methods

#### *The Multi-Metric Index (v5)*

In 2010, the Colorado Department of Public Health and Environment (CDPHE) published guidelines for benthic macroinvertebrate sampling and analysis, introducing the Multi-Metric Index (MMI v3) for evaluating aquatic life.<sup>5</sup> The MMI was updated to v4 in 2017, and again to the current MMI (v5) in 2025.<sup>5</sup> Data collected from WGFP and Habitat Project sampling sites was processed using the latest MMI (v5) program for use in this study. A detailed description of the metrics and methods used to calculate MMI (v5) scores can be found in the Aquatic Life Use Attainment: Methodology to Determine Use Attainment for Rivers and Streams, Policy 10-1 and Appendix D in Section 303(d) Listing Methodology 2026 Listing Cycle.<sup>5,6</sup> Each of the metrics used in the MMI (v5) produces a score that is adjusted to a scale from 1 to 100 based on “reference sites” in the State of Colorado. Metrics used for Biotype 1 are listed in [Table A-1](#).

**Table A-1.** Metrics used for Biotype 1.

| EPTTax        | Number of EPT Taxa                                              |
|---------------|-----------------------------------------------------------------|
| ColeoPct      | % Coleoptera individuals of all individuals                     |
| EPTnoBPct     | Number of EPT individuals excluding Baetidae of all individuals |
| NonInPct      | Non-insect individuals as a percentage of all individuals       |
| IntolIPT      | Intolerant taxa as a percentage of all taxa                     |
| PredShrTaxFAC | Number of Facultative Predator or Shredder Taxa                 |
| CIngrPctTax   | % Clinger taxa of all taxa                                      |
| ModDecrTrans  | % moderate decreaser individuals in Biotype 1                   |

Thresholds for the MMI (v5) in Biotype 1 are as follows:

| <u>Biotype</u>           | <u>Attainment Threshold</u> | <u>Impairment Threshold</u> |
|--------------------------|-----------------------------|-----------------------------|
| Transitional (Biotype 1) | 43                          | 33                          |

MMI scores that fall between the thresholds for ‘attainment’ and ‘impairment’ (the ‘Gray Zone’) require further evaluation using additional metrics to determine an aquatic life use designation. The additional metrics include the Diversity and Hilsenhoff Biotic Index (HBI). The specific thresholds for the auxiliary metrics in Biotype 1 are listed below, followed by descriptions of each metric:

| <u>Biotype</u>           | <u>HBI</u> | <u>Diversity</u> |
|--------------------------|------------|------------------|
| Transitional (Biotype 1) | 5.6        | 2.6              |

### *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. 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. The Diversity metric provides a measure of macroinvertebrate community balance.

### *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.<sup>7</sup> 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.<sup>8</sup> 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 aquatic disturbances. Values for the HBI range from 0.0 to 10.0 and increase as water quality decreases.

An additional means of determining 'attainment' or 'impairment' designations using the MMI involves the rapid decline of scores from high scoring waters. When MMI scores are available from multiple years for the same sampling location, and a large decline in scores occurs over the span of at least 12 months, that site will automatically be considered 'impaired' for aquatic life use. The requirements for an allowable decline in the MMI in Biotype 1 are as follows:

| <u>Biotype</u>           | <u>High Scoring Water (MMI score)</u> | <u>Allowable MMI Decline</u> |
|--------------------------|---------------------------------------|------------------------------|
| Transitional (Biotype 1) | >53                                   | -20                          |

### *Additional Study Metrics*

In addition to the MMI and associated auxiliary metrics, several other individual metrics were applied in the analysis of macroinvertebrate data from the Colorado River to provide a more thorough evaluation of macroinvertebrate community structure and function. A description of these additional metrics is provided below.

### *Ephemeroptera/Plecoptera/Trichoptera Taxa (EPT Taxa)*

The design of this metric assumes that the insect orders of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) are generally more sensitive to pollution than other benthic macroinvertebrate orders.<sup>9</sup> This metric is currently an important and widely used tool in many regions of the United States.<sup>7</sup> 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.<sup>10</sup>

### *Shannon Evenness (Evenness)*

Evenness values were used at all sites in this study to detect changes in macroinvertebrate community structure and balance. Evenness values range between 0.0 and 1.0, and lower values indicate greater stress. Values lower than 0.3 are considered indicative of organic pollution or other substantial perturbations.<sup>11</sup>

### *Diversity and Taxa Index (DAT)*

The DAT index was used in this study to evaluate water quality based on benthic community structure and diversity. This metric is unique because it incorporates components of community balance along with taxa richness. Calculated DAT values fall within a range of numbers that are correlated to a scale describing stream condition.<sup>12</sup> The DAT scale (adjusted for chironomids identified to the genus level) is as follows:

| DAT Value | Scale     |
|-----------|-----------|
| 24-35     | Excellent |
| 17-23     | Good      |
| 11-16     | Fair      |
| 0-10      | Poor      |

### *Insect Taxa*

Insect Taxa is an example of a metric that relies on community richness for detection of disturbances. The Insect Taxa value is reported as a total count of distinguishable insect taxa at each site. This metric is similar to the Total Taxa metric; however, the Insect Taxa metric measures the number of taxa in insect families exclusively, rather than the summation of aquatic insects and non-insects. In general, the Insect Taxa value is expected to decrease as water quality becomes more degraded.<sup>13</sup>



### *Taxa Richness (Total Taxa)*

The Total Taxa metric is reported as the total number of identifiable taxa collected from each sampling location. Total Taxa has become one of the most widely used metrics to evaluate stream health, as it provides a general indication of community health and stability.<sup>14</sup> Total Taxa values are expected to decrease with increased perturbations in the aquatic environment.<sup>15</sup>

### *Percent Shredders and Scrapers*

Shredders and scrapers are often considered sensitive to disturbance because they are specialized feeders.<sup>7</sup> Consequently, these sensitive groups are expected to be well-represented in healthy streams. Much of the value in this type of analysis comes from a comparison among sites within a specific study area.

### *Density of *Pteronarcys californica**

This metric utilizes the abundance of *Pteronarcys californica* from three replicate quantitative samples to provide a mean number of individuals per square meter. *Pteronarcys californica* is a large species of stonefly that requires specific aquatic conditions for survival, and it is known to be sensitive to a variety of anthropogenic disturbances.<sup>16</sup> Additionally, this species can be an important part of the aquatic food-web that typically requires leaf material from a healthy riparian corridor.

### *Percent EPT (Excluding Baetidae)*

As previously stated, most taxa in the orders Ephemeroptera, Plecoptera, and Trichoptera are expected to be sensitive to environmental perturbations or pollution. However, members of the family Baetidae (Order: Ephemeroptera) tend to be more tolerant to disturbances than other EPT taxa. Therefore, the Percent EPT (excluding Baetidae) metric provides a measure of the percent composition of benthic macroinvertebrates (at each sampling location) that are expected to be highly sensitive to anthropogenic stressors or pollution. A decrease in this metric value suggests that the benthic macroinvertebrate community consists of a higher proportion of tolerant individuals.

### *Density*

Macroinvertebrate abundance (Density) was reported as the mean number of macroinvertebrates per square meter found at each study site. Density values provide a means of measuring and comparing standing crop at each site. This information can be helpful when assessing the macroinvertebrate portion of the aquatic food-web.

### *Percent Chironomidae*

The insect family Chironomidae (Order: Diptera) are considered fairly tolerant to environmental disturbances when compared to other aquatic insect families.<sup>17</sup> Most species of Chironomidae also

tend to have a relatively short life cycle, which enables them to continually recolonize unstable or polluted habitats.<sup>18</sup> The Percent Chironomidae metric relies on the assumption that the relative proportion of Chironomidae will increase with decreasing water quality. Streams that are undisturbed often have a relatively even distribution of Ephemeroptera, Plecoptera, Trichoptera, and Chironomidae,<sup>19</sup> while study sites degraded by metals or other pollutants are often dominated by members of the Chironomidae family.<sup>20</sup>

### *Percent Hydropsychidae*

The Percent Hydropsychidae metric was reported for each study site as the proportion of caddisflies that are in the family Hydropsychidae (Order: Trichoptera). Members of this family provide some insight into macroinvertebrate community structure and function because they are considered collector-filterers, and their large body size makes them an important food source for fish. These caddisflies are known to be moderately sensitive to a variety of stressors, particularly ammonia and fine sediment.

### *Percent Tolerant Taxa*

The Percent Tolerant Taxa value is reported as the percentage of taxa that are considered tolerant to a variety of environmental disturbances and stressors. This metric measures the relative abundance of all taxa that have tolerance values of 7 or greater.

### *Percent Intolerant Taxa*

This metric value is expressed as the percentage of taxa that are expected to be sensitive to a variety of anthropogenic disturbances and environmental stressors. Intolerant taxa include all taxa with a tolerance value of 3 or lower.

### *Functional Feeding Groups*

Most of the previously described metrics use macroinvertebrate information that is related to community structure; however, macroinvertebrate taxa were also separated into functional guilds based on their method of food acquisition to provide a measurement of community function. Aquatic macroinvertebrates were categorized according to feeding strategy to determine the relative proportion of various groups. Some representation from each group usually indicates healthy aquatic conditions; however, it is normal for certain groups (collector-gatherers) to be more abundant than others.<sup>11</sup> The major functional feeding groups are listed in [Table A-2](#).

**Table A-2.** Major functional feeding groups.

| Functional Feeding Group | Role                                                                                            |
|--------------------------|-------------------------------------------------------------------------------------------------|
| Scrapers/grazers         | Consume algae and associated material                                                           |
| Shredders                | Consume leaf litter or other coarse particulate organic matter (CPOM), including wood           |
| Collector-gatherers      | Collect fine particulate organic matter (FPOM) from the stream bottom                           |
| Collector-filterers      | Collect FPOM from the water column using a variety of filters                                   |
| Predators                | Feed on other consumers                                                                         |
| Omnivores                | Feeds on both plant/algal material and animal prey. Do not fit easily into the other categories |