2021 Blue River Periphyton Sampling Report

Blue River Integrated Water Management Plan



Submitted to:



Submitted by:



June 2022

Section 1.0 – Background

Algal biomonitoring is a vital field of study as primary productivity forms the baseline of most aquatic ecosystems, comprised of photosynthetic organisms that drive energy exchange in most streams (Hauer and Lamberti, 2007). Because of this, benthic algae are commonly used to indicate the health of streams, as they are susceptible to changes in environmental conditions (Rosenberg 1998; Oertel and Salanki 2003). Moreover, it was initially proposed to the Blue River Enhancement Workgroup (BREW), that one of the root causes for declines in the Blue River fishery and ecological function was the lack of primary productivity. The result would be a decreased forage capacity for target macroinvertebrates and juvenile trout and a cause for reduced annual growth rates in various age classes of trout.

While the debate may continue about whether the Blue River ever supports a wild population of mature trout per mile, TU set out to determine whether lack of primary productivity was a limiting factor in the main stem of the Blue River. In coordination with the Blue River Integrated Water Management Plan (BRIWMP or IWMP, hereafter) managed by the Blue River Watershed Group (BRWG), Trout Unlimited (TU) completed seasonal benthic algae biomonitoring at ten monitoring sites along the main stem of the Blue River. According to the IWMP, biomonitoring targets the Upper and Middle Reaches of the Blue River. Two IWMP monitoring sites are in the Upper Blue: one immediately upstream of Swan Mountain Road and the other upstream of the Blue River's confluence with French Gulch. The remaining eight sites span roughly 14 miles of the 17-mile reach of the Middle Blue. Through the 2021 field season and with the support of Blue Valley Ranch staff, TU collected benthic algae samples on April 21st, August 12th, and October 28th. This report summarizes the results from that sampling event.

TU did not complete sampling at the IWMP monitoring site, Blue Above French (BAF), as it was not outlined in the scope for 2021. For 2022, TU set out to sample benthic algae at this monitoring site, but the channel was void of water on April 21st and could not be sampled. As a follow-up on this discovery, TU discussed this finding with the Town of Breckenridge water resources staff. It was explained that a large portion of the Blue River flows subsurface during the winter and early spring months. This stretch is roughly thought to be from south of Ski Hill Road to the inlet of French Gulch. Based on this discovery, TU would suggest omitting BAF as a biomonitoring location. Additionally, as part of the IWMP, an investigation of the root cause may be warranted, and the feasibility of restoring annual surface water flow to this section of the Blue River may be.

In addition to the 2021 algae biomonitoring, TU maintained a series of Onset Hobo TidBit and Pendant temperature loggers on behalf of the Blue River IWMP. Dataloggers have been deployed year-round and will remain until 2026, when the USFS null effects research permit expires. Data captured from the series of instruments was collated and disseminated to KSQRD Fisheries for analysis and reporting, arranged by BRWG for the BRIWMP. This summary sampling report presents data from those instruments to explain impacts on benthic algae.

Section 2.0 – Sampling

To coalesce more data on periphyton communities throughout the Blue River, TU and Blue Valley Ranch (BVR) coordinated field sampling methods. In 2020, TU adopted sampling methods proposed by BVR and their Blue River Nutrient Remediation Project. As stated, this step would allow for more comparisons between the three Blue River Reaches identified in the IWMP. Regardless of the long-term fate of the BVR nutrient injections study plan, the data collected by BVR since 2019 provide valuable background information, and BVR's contribution to TU's field sampling events was valuable. BVR contributed time

and resources to the periphyton study, which resulted in cohesive methods deployed throughout this study.

This benthic algae field sampling did not adhere to Water Quality Control Division (WQCD) stream chlorophyll sampling protocols. Instead, a modified sampling approach was used to coincide with the methods set forth by BVR. This alternative sampling methodology is comparable to the WQCD approach but is tailored to larger standing crop scrapes and a decreased number of replicates at each IWMP site.

Ultimately, benthic algae samples will identify differences and similarities between the two Blue River reaches that can be used to inform future management decisions. Data collation in conjunction with the BVR nutrient study will provide quantified data for whether nutrient enhancement could be useful on the Middle Blue (Reach 2) and, if so, whether it would be an effective management tool for restoring ecological function. This periphyton sampling was also intended to serve as continued foundational data to be used in determining root causes for the decline of Blue River's ecological function. TU encourages data overlap and collaboration with BVR and the SWQC to further strengthen an understanding of the potential impacts of water quality and water quantity.

2.1 – Methods

At each Site, a total of eight small to large cobbles with an estimated range of 60 - 180 mm are collected from a single riffle/run segment. Each specimen is collected from a targeted depth of less than 1-foot downstream to upstream orientation until four substrate samples are collected. Sub-sample rocks are placed in a site-specific bin to ensure upstream, and downstream transect specimens are not mixed.

Based on the low number of replicated per Site, top rock scrapes from a relatively uniform depth decrease the probability of variability from exposure to increased flows and seasonal scour. Once all top rock samples are collected, TU and BVR staff expand a stream-side sampling station with various smaller bins, rinse bottles, and scrub brushes. The top margin of the rock is then scraped and delineated to decipher the surface area scraped. The section of the rock exposed to surface water and to be scraped for benthic algae is referred to as the "standing crop". The standing crop is collected over a small plastic tub, and the standing crop margin is scraped and brushed to dislodge benthic algae and organic matter. Algae-laden water is then funneled into a 16-ounce/500 mL lab-quality polypropylene Nalgene bottle and labeled for lab analysis. Following the scrapes, aluminum foil was placed over the top of each rock and cut to fit the total area scraped; the foil is used to determine the surface area to quantify the mass per unit area of each subsample. Following the delineation of scraped surface area from the four samples that comprise a transect, instruments are scrubbed and rinsed with river water to reduce pollution of the next set of samples.

Each sample and respective sub-sample corresponds with a Chain of Custody (CoC) form completed and submitted to the lab with the cooler of samples. Coolers contain ice packs placed on the bottom and top of sample bottles to keep specimens cool; the internal target temperature of the cooler is 43°F. The benthic algae samples are then sent EnviroScience, Inc., a biological lab based in Stow, Ohio, since 1989. EnviroScience was chosen to process algae samples due to affordability and willingness to provide expertise to the project. EnviroScience processes samples and preserves each composite sample the same day it's received.

For 2021, lab analysis focused on three main parameters: Chlorophyll *a* (Chl-*a*), Ash free dry weight (AFDW), and genus-level algal taxonomy. The concentration of Chl-*a* is the measure of pigment-producing plant matter, identified using spectrophotometry. AFDW is a general quantification of the total organic mass using oxidation methods for the total organic mass of a sample; AFDW does not

differentiate the type of organics (Steinman et al. 2006). The advantage of a pigment analysis compared to AFDW is its ability to differentiate algal biomass from organics such as detritus or fungi (Steinman et al. 2006). Genus-level taxonomy was accomplished by identifying major algae groups (i.e., diatoms, green algae, blue-green algae, and cryophytes) from each IWMP monitoring site.

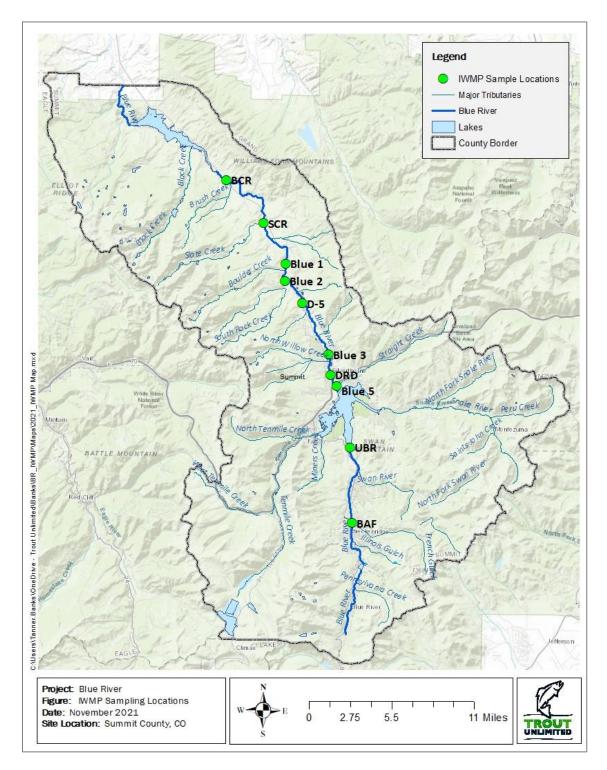


Figure 1. 2021-22 site locations according the Blue River IWMP. (*BAF is not was not sampled for benthic algae)

Section 3.0 – Results

A challenge that remains for the Blue River biomonitoring study is the lack of proper reference conditions. The lack of a reference site may have a more significant impact on the benthic algae study in comparison to the benthic macroinvertebrate study, as multi-metric indices developed specifically for Colorado's various ecoregions permit comparisons of similar stream types. In the case of benthic algae, multi-metric indices are still being developed, and the spatial variability of periphyton requires an increased number of sample replicates for statistical comparisons. Despite this, the benthic algae study on the Blue River is accomplishing what it set out to answer: are food web dynamics to inform whether macroinvertebrate productivity is limited by primary productivity?

After several seasons of biological stream assessments, it is apparent that Blue 5 supports an unnaturally high concentration of Chl-*a* and AFDW. The state surface water threshold for Chl-*a* is 150 mg/m², which Blue 5 exceeded in the Fall 2021 sample. However, this exceedance is not reportable because the methods employed for algal monitoring do not adhere to Colorado's WQCD monitoring methods. Importantly, TU does not see a value in reporting a single documented Chl-*a* exceedance.

Despite high concentrations of Chl-*a* and AFDW at Site Blue 5, observed concentrations of algal biomass diminish quickly with the addition of Straight Creek and other inputs that may diffuse higher concentrations of nitrogen and phosphorus immediately downstream of Dillon Reservoir, which is most likely leading to increased algal productivity. River impoundments drive major changes in downstream algal communities by altering thermal, flow, nutrient, and sediment regimes, and leads to an unnaturally coarse streambed, all of which can have lasting effects on stream biota (Blinn et al. 1998, Stevens et al. 1997, Blinn and Cole 1999). Blinn et al. (1998) explain that changes in the timing and occurrence of discharge patterns drive the most considerable changes in biological stream communities (Resh et al. 1988). The physical manipulation of flow and temperature regimes is most extreme at Blue 5, and for this reason, the conditions at this Site are telling, but may not be as relevant as we initially thought.

Table 1. Shows all sites according to the IWMP nomenclature with lab results by Site and subsample. Chlorophyll *a* concentrations are reported as milligrams per square meter (mg/m^2); ash free dry weight (AFDW) is reported as grams per square meter (g/m^2).

			Fall 2020			Spring 2021			Summer 2021			Fall 2021		
IWMP Site Name	Site Notes	Lat , Long	Surface Area Scraped (cm ²)	Chl-a (mg/m²)		Surface Area Scraped (cm ²)	Chl-a (mg/m²)	AFDW (g/m²)	Surface Area Scraped (cm ²)	Chl-a (mg/m²)	AFDW (g/m²)	Surface Area Scraped (cm ²)	Chl-a (mg/m²)	AFDW (g/m²)
UBR	Historic FS Site - Above Swan Mtn Rd	39.56627, - 106.04929	584.52 550.99	6.79 11.50	2.63 3.20	612.43 448.47	12.32 4.66	2.06 3.85	417.71 454.43	32.34 23.89	9.02 4.99	433.55 554.83	8.50 2.05	
Blue 5	Historic FS Site - Above Straight Cr	39.62604, - 106.06712	602.93 178.32	109.13 54.40	39.29 17.08	622.47 693.01	28.35 48.68	7.39 17.94	448.70 338.39		31.11 29.69	471.91 291.45	63.29 180.07	29.96
DRD	Dillon Ranger Station	39.63626, - 106.07526	515.47 613.28	0.94 1.14	1.04 1.17	414.21 318.86	2.44 5.54		789.93 667.59	5.82 1.47	3.54 0.93	546.46 498.97	8.71 1.34	
Blue 3	Historic FS Site - Below Willow Cr	39.65606, - 106.07747	637.67 303.22	1.94 9.23	3.06 3.20		10.24 20.67	2.69 7.25	532.35 603.31	10.90 16.06	4.58 5.19	569.47 453.50	6.42 14.88	
D5	Historic FS Site - Pioneer Cr	39.70523, - 106.11146	387.94 409.83	8.80 59.18	5.70 9.54	281.77 430.53	101.06 76.48		544.48 553.28	8.02 5.70	4.20 4.43	468.58 299.56	49.43 89.80	
Blue 2	Historic FS Site - Campground	39.72716, - 106.13264	454.00 327.44	22.26 11.69	7.78 5.09	258.78 304.86	40.11 20.50		800.31 672.61	9.01 5.98	5.10 4.74	334.63 316.67		21.92 12.34
Blue 1	Historic FS Site - Below Boulder Cr	39.74358, - 106.13282	415.33 351.06	43.66 14.40	12.67 3.88	259.74 446.54	27.38 51.71	10.38 11.27	530.02 625.41	10.06 7.15	3.81 3.21	472.42 441.97	48.11 42.49	21.05 14.49
SCR	Above Slate Cr	39.78226, - 106.16085	380.82 427.01	23.40 8.13	6.37 7.96	258.01 361.65	17.96 24.68	6.36 9.33	634.15 774.65	6.19 11.33	3.90 5.02	394.15 492.73	25.55 9.96	
BCR	Below Brush Cr	39.82165, - 106.20679	406.35 527.27	0.65 1.30	0.91 0.81	357.13 327.69	5.79 6.78	2.17 2.48	477.62 622.46	3.89 0.85	2.06 0.85	485.71 388.78	0.43 1.02	

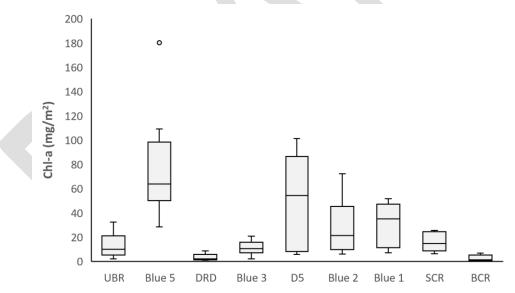


Figure 2. Illustrates the concentration of Chlorophyll *a* at each IWMP sample location across all sample seasons (Fall 2020 through Fall 2021).

According to the limited number of relevant studies on periphyton abundance, algal abundance in high elevation streams is driven more by temperature and length of the growing season than nutrient availability (Lewis and McCutchan 2010). The 2010 study by Lewis and McCutchan explains that based on their 74-site study, the growing season decreases with increased elevation, which leads to diminishing primary productivity, measured in mg/m² of Chlorophyll *a*. The IWMP monitoring sites range from 9250 feet (2819 m) to 8105 feet (2470 m) in elevation above mean sea level (AMSL). According to the 2010 study, sites with the elevation ranges of the BRWIMP sample sites, mean Chl-*a* concentrations

roughly range from 20 to 100 mg/m². Therefore, comparing Chl-*a* concentrations discovered through IWMP sampling to those presented by Lewis and McCutchan (2010), site UBR, DRD, Blue 3, and BCR may not attain the mean suggested in the case study. However, the data range presented by Lewis and McCutchan (2010) is highly variable, with observed concentrations as low as 4 mg/m² and as high as 300 mg/m².

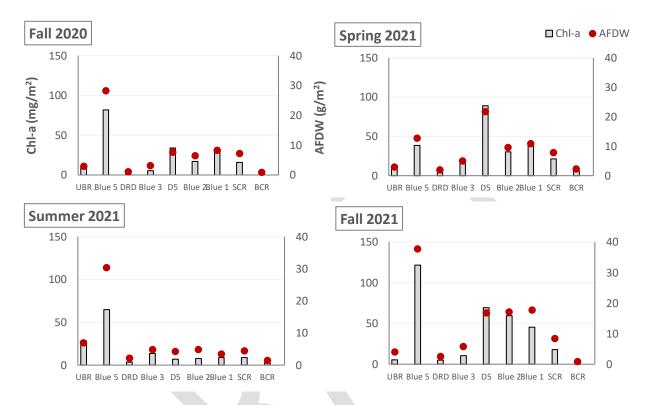


Figure 3. Seasonal representation of Chl-a concentrations (gray bars) by IWMP biomonitoring site, compared to the concentration of ash free dry weight (AFDW, red circles). The Y-axes are normalized for Chl-a and AFDW; Chlorophyll *a* surface water standard is 150 mg/m².

Figure 3 presents Chl-a and AFDW concentrations for each sampling season. Chlorophyll *a* is represented by gray bars and the primary y-axis, measured up to 150 mg/m², whereas AFDW is defined as the red circles and the secondary y-axis, measured up to 40 g/m². Presenting each sampling season independently reveals that the fall sample is most representative of the standing crop of algae. Our summer sample had lower Chl-*a* concentrations below Dillon Reservoir, with higher concentrations above the reservoir at UBR.

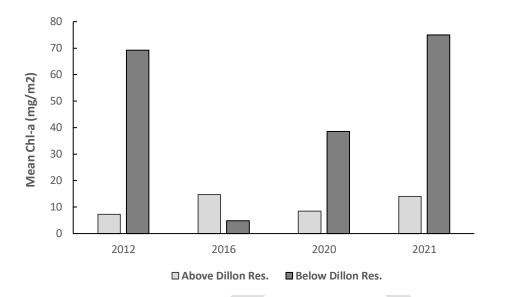


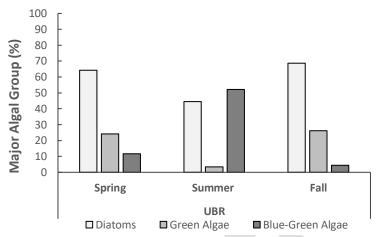
Figure 4. Compares mean Chlorophyll a concentration from 2012 and 2016, collected by Lewis and McCutchan, as well as 2020 and 2021, collected by Trout Unlimited staff.

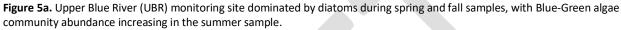
The 2016 and 2020 sampling events carried out by Lewis et al., and this Trout Unlimited study reveal that Chl-a concentrations immediately downstream of Dillon Dam are significantly higher than those observed in the Upper Blue River before the inlet into Dillon Reservoir (Figure 3). The 2020 results also reveal that Chl-a concentrations at sites north of Silverthorne down to SCR have reasonable concentrations of Chl-a. Figure 4 confirms the concentration of pigment-producing algae is higher below Dillon Dam than above Dillon Reservoir during most years.

3.1 – Taxonomy

Algal groups appear to be responding normally to seasonal changes where diatoms dominate in the winter; spring run-off occurs, allowing for excess nutrients to become bio-available, shifting to a diatomgreen algae community. In summer, nutrients typically become limiting, but seasonal low flows may reentrain "slugs" of concentrated nutrients/ions downstream with rain events. This, paired with higher temperatures, typically shift the community to fewer green algae and more blue-green algae (cyanobacteria) at the peak of the summer (EnviroScience, personal communication).

Figure 5(a-i) Illustrates periphyton abundance as Chlorophyll a in mg/m² as the primary y-axis compared to the average ash-free dry weight (AFDW) in g/m² as the secondary axis. The x-axis is represented by the sample number, starting at the most southerly Site (UBR) and ending at the most northerly Site in Reach 2 (BCR).





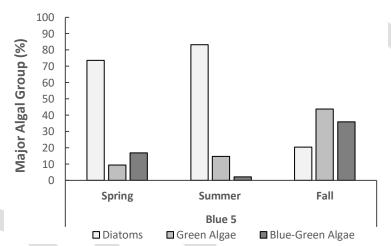


Figure 5b. Site Blue 5 (immediately downstream of Dillon Reservoir) dominated by diatoms in spring and summer, with a signicant increase in green algae and blue-green algae abundance in the fall sample.

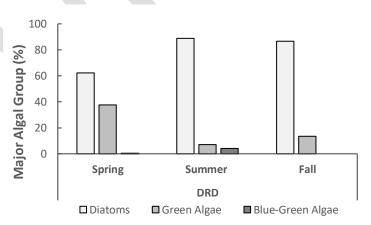


Figure 5c. Site DRD (Dillon Ranger District) dominated by diatoms alagel group across all growing seasons.

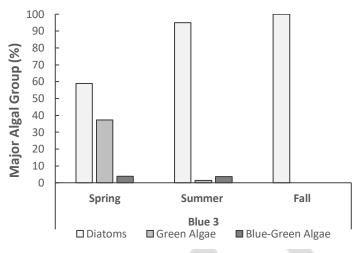


Figure 5d. Site Blue 3, below Antler Creek bridge, is dominated by diatoms during all sample seasons.

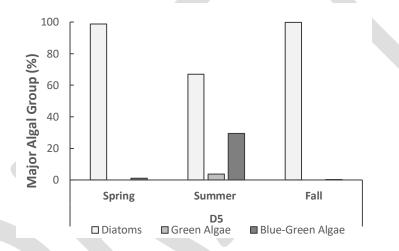


Figure 5e. Site D5, upstream of the private Pioneer Creek bridge, is dominated by diatoms in all seasonal samples, with an increase in blue-green algae during the summer sample.

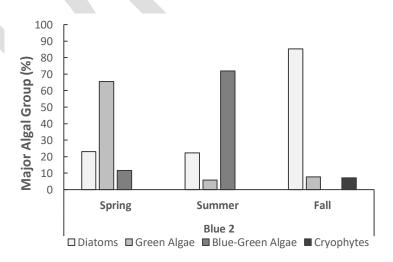


Figure 5f. Blue 2, downstream of the Blue River Campground and the confluence of Rock Creek, shifts algal group abundance with each growing season. This includes the only monitoring site with detected cryophyte species in the fall sample, with a complete loss of blue-green algae. The relative inconsistency is not necessarily unexpected in high mountain streams but does indicate that there may be non-point source input(s) upstream of this Site influencing taxa diversity.

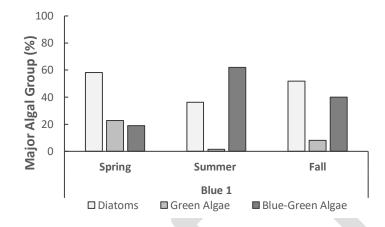


Figure 5g. Site Blue 1, downstream of the confluence of Boulder Creek. Spring and fall samples are dominated by diatoms, with the summer sample being dominated by blue-green algae. The shift in the proportion of blue-green algal taxa during the growing season could be partly attributed to input from Boulder Creek, a prominent tributary that is a undeveloped and largely unimpacted source water drainage.

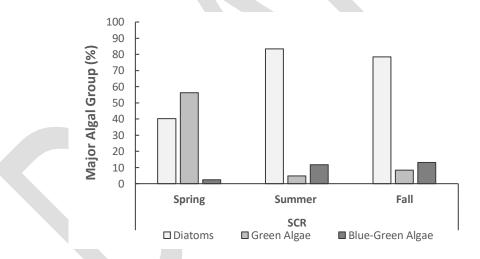


Figure 5h. Site SCR (Slate Creek Ranch) is our only monitoring site located on private lands, owned and operated by the Mosier-Connolly family. SCR samples shift from a blue-green algae community in spring to being dominated by diatoms through the growing season.

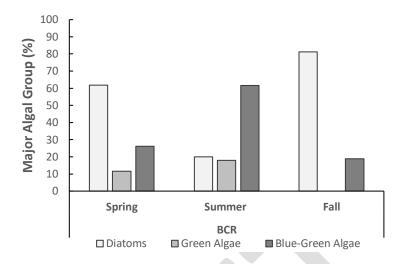


Figure 5i. Site BCR (downstream of Brush Creek) is accessed through the CPW State Wildlife Area and, much like Blue 2 and Blue 1, shifts from Diatoms to soft algae during the summer season, then back to diatoms in the fall.

Algal taxonomy was an important step for this study as it allowed for the comparison between sites and reaches of the Blue River. Again, the hypothesis for the Middle Reach of the Blue River was target nutrient concentrations were depleted to the degree that could limit primary productivity and, subsequently, the fishery. An original hypothesis of this study was that as agricultural practices increased as the valley margins widened, so too would the amount of algal diversity and abundance. This is not always the case, and this study supports the notion that non-point source contributions of agricultural bioproducts such as nitrogen and phosphorus may only the detectable at a prominent input such as a return ditch. Even so, nutrient levels in the Blue River may be low enough that it is absorbed rapidly and has little impacts on the overall function of the Blue River.

Based on the data presented in figure 5a-i, the only sites that undergo seasonal shifts in the major algal groups worth noting are Blue 2, Blue 1, and BCR. Blue 2 may shift to a summer green algae community for several reasons, but its proximity to Rock Creek and the Blue River Campground may be a source of nutrients and, subsequently a shift in the algal community. Rock Creek has seen a significant increase in user traffic' the NFS lands for which it runs through support large amounts of day-use hiking and dispersed overnight camping. Much of the same can be said for IWMP site Blue 1, which is situated below Boulder Creek and the Sierra Boss neighborhood, of which most homes rely on septic systems. Boulder Creek, however, has not been subject to the same extent of anthropogenic impacts. Brush Creek (BCR, hereafter) originates from the eastern face of Peak M in the Gore Range and flows adjacent to the Mount Powell Ranch and various other private landholdings. For the most part, impacts to Brush Creek appear minimal. The taxonomy presented in Figures 5a-I, indicates that the IWMP sites in the Town of Silverthorne may not have the same natural shift in major algal groups through the growing season due to anthropogenic impacts and reduced concentrations of nutrients due to Dillon Reservoir and a general lack of tributary influence. Chemical influence of Straight Creek and stormwater infrastructure may also be a limiting factor in algal community diversity and abundance.

An auxiliary goal of the taxonomic work on the Blue River was to determine the extent of Didymo colonization in the Upper and Middle Blue. The data that EnviroScience provided to TU suggests that Didymo is present in both reaches but in very low concentrations. Didymo was only detected in the Fall

2021 samples at a concentration of less than 1%. Didymo presence may be more abundant in the Upper Reaches of the Blue, but it suggests that this diatom that thrives in nutrient-deficient streams has not yet taken hold. Based on communication with BVR, Didymo blooms below Green Mountain Reservoir are abundant, and based on this, TU would encourage BRIWMP collaboration with State and Federal IWMP partners to inform and encourage users to clean and fully dry waders and footwear after visits to the Blue River below Green Mountain Reservoir, among other water bodies of the state.

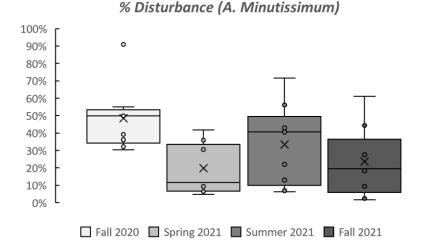
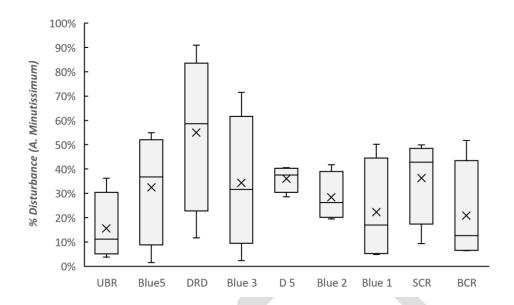
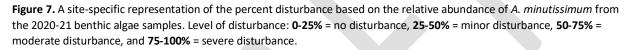


Figure 6. Level of disturbance is presented in the box and whisker plots according to each periphyton sampling season. The mean for each sampling season is represented in Figure 10 as X.

According to several studies, *A. minutissimum* is a widely distributed diatom that takes advantage of highly disturbed habitats, typically reaching higher numbers after periods of flooding (Stenger-Kovács et. al. 2006). According to Stevenson and Bahls (1999), the percent *A. minutissimum* "has been found to be directly proportional to the time that has elapsed since the last scouring flow..." or chemical insult. They recommend quartiles of the metric to be interpreted as follows: 0-25% = no disturbance, 25-50% = minor disturbance, 50-75% = moderate disturbance, and 75-100% = severe disturbance. Percent *A. minutissimum* was determined from the relative abundance of the species in each sample. Using the mean for percent disturbance, fall of 2020 displayed the highest level of disturbance. This dataset was skewed by the outlying datapoint at 91-percent in the fall 2020 sample. This datapoint represents Site DRD and is designated as a site with severe disturbance.

Of note, variability of *A. minutissimum* is less apparent in the Fall 2020 sample, which represents genus level taxonomy for only the diatom algal group. Recalling back to 2021 when TU received funding by the IWMP to investigate periphyton communities in the Blue more closely, the IWMP managers approved additional funding to complete taxonomic work on the Fall 2020 sample. The 2021 data presented in Figures 6 and 7 represent genus level taxonomy for all of the major algal groups captured in the samples. Diatoms were the only algal group identifiable in the Fall 2020 backlogged samples between diatoms are single-walled cells, comprised of silica. These silica cell walls are class-like and do not deteriorate over time like soft algae's such as green algae and blue-green algae (Kumar and Singh, 1973, Hauer and Lamberti 2007). The variability in the relative abundance of *A. minutissimum* in the 2021 samples is partly because these samples account for soft algae that are not present in the 2020 sample.





Site-specific percent disturbance is illustrated in Figure 6, depicting the high level of variability between sites and seasons according to this metric. What is worthy of note is that across all sampling events, the highest abundance of *A. minutissimum* were observed at DRD and Blue 3. Moreover, Blue 5, commonly associated with a degraded site, also received disturbance scores between 1.56% (Fall 2021) and 54.99% (Fall 2020). According to the percent *A. minutissimum* metric, the Fall 2021 sample had the lowest level of disturbance among all sites. Presumably, this may be driven mainly because Blue 5 site does not readily observe scouring events and is subject to highly altered thermal regimes (Figure 8 & 9). Directly downstream of Dillon Reservoir, there is a significant uptick in algal density paired with an insignificant change in diversity. The increase in diversity of algal groups may be linked to available nutrients from the bottom-fed portion of the reservoir. For 2022, taxonomy will target a more thorough analysis of diatoms and omit the investigation of the soft algae groups.

3.2 – Temperature

Trout Unlimited installed Onset Hobo Pendant and TidBit temperature dataloggers at six of the nine IWMP monitoring sites. Temperature loggers were deployed in an enclosed PVC coupler with a plastic-coated braided cable affixed to the logger/coupler and then to immobile stream substrate or riparian vegetation. Loggers are semi-permanent and can be easily removed. The methods used to build and deploy temperature loggers/housing adhere to USFS protocols (Heck et al., 2018). In most instances, loggers have remained affixed to the original deployment location. Site DRD, adjacent to the Dillon Ranger District Office, has been the main outlier, with several instances of finding the logger on the stream bank. Figure 8 shows these temporary "outages" at DRD as large periods of time with large daily temperature spikes.

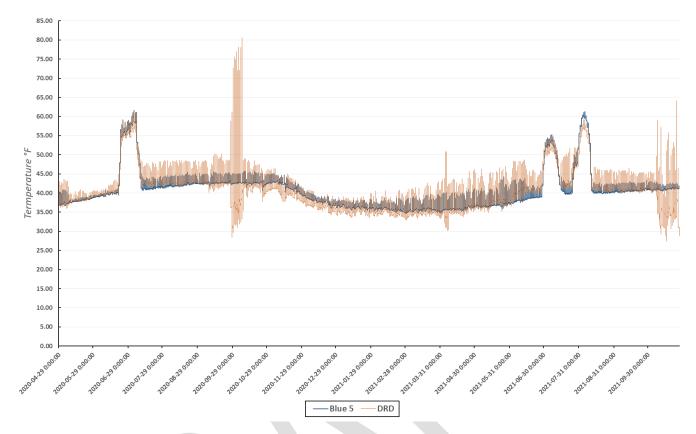


Figure 8. A temperature profile for sites Blue 5 and DRD, from April 2020 through October 2021.

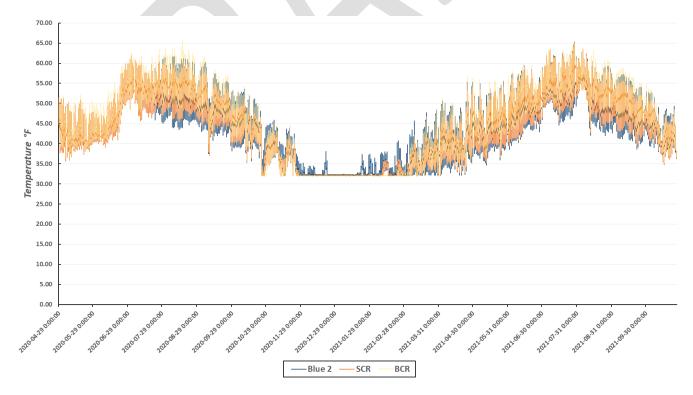


Figure 9. A temperature profile for sites Blue 2, SCR, and BCR, from April 2020 through October 2021.

Figures 8 and 9 depict the annual temperature regime and the longitudinal variation in daily and seasonal stream temperature changes. Figure 8 presents water temperatures in the Town of Silverthorne (Blue 5 and DRD), illustrating the uniform thermal regime and rapid thermal changes when Dillon Reservoir spills over the glory hole. Sites Blue 5 and DRD experience unnaturally rapid thermal shifts in warming and cooling stream temperatures resulting from surface water spills from Dillon Reservoir. Figure 9 illustrates the seasonal influence of tributaries and warming through solar radiation yet still clearly identifies surface spills longitudinal impacts on Reach 2.

In a natural system, surface waters during the winter and spring months should be coldest, with exceptions surrounding the influence of groundwater (Hauer and Lamberti 2007). In the instance of the main stem of the Blue River, cold winter and spring flow should be followed by a slow and steady incline in water temperature as days get longer and ambient air temperatures increase. For the sites illustrated in Figure 8, the thermal regime is essentially inverted and driven by relatively rapid and significant changes in water temperature. The thermograph depicted in Figure 9 suggests that the Blue River is more resilient to these unnatural temperature shifts, driven in part by the influence of freestone tributaries, connectivity to wetlands, loss of riparian vegetation, increasing solar radiation and to a lesser degree, return flows from irrigation diversions.

The winter water temperature extremes where loggers plateau at 32°F are observed from Site D5 and continue as the Blue River flows northward. Loggers at D5, Blue 2, and SCR are situated at depths of over two feet, yet the winter temperatures at all three of these sites indicate there may be ice formation on substrate for several months each year at unexpected surface water depths. Regardless, this ecotype expects low winter flows and anchor ice formation, even under natural conditions. The effects of anchor ice development are less well known but based on the diminished algal and macroinvertebrate abundance at IWMP sites SCR and BCR; seasonal biological productivity may be limited by anchor ice and spring scour.

Section 4.0 – Conclusions and Recommendations

Low concentrations of Chl-*a* during the Summer (August) sampling may be partially attributed to the increased regional precipitation received throughout the watershed during monsoonal months. Monsoonal rains led to the unexpected two-pronged surface spill, leading to prolonged flows and increased water temperatures in the Middle Reach of the Blue River. The temperatures observed from this spill more accurately resemble target summer thermal regimes for this ecoregion. The sustained 400 CFS in late-July through August led TU to sample stream substrate that in recent years, is not within the wetted channel area. TU followed State and Federal sampling protocols and completed sampling after several weeks of stabilized flow, allowing for the colonization of the sample areas. The summer benthic macroinvertebrate samples collected on the sample day as periphyton also indicate impairment. Based on the 2021 biological data, the two-pronged spill from Dillon Reservoir may have altered flow and temperature regimes in the Middle Reach that stunted biological life cycles. To address this conclusion, TU suggests a summer sample during a non-spill year to draw comparisons to 2021.

As stated in the 2020 benthic algae report, the results of this study should be combined with chemical and biological data collected before and as part of the IWMP. While this has been a target goal of the IWMP, synthesizing all the datasets collected as part of the Blue River IWMP is not part of the scope of work for this summary report. TU encourages with the 2020 report that Site Blue 5 should be resampled

according to the sampling protocols set forth in 2020 to determine why this Site consistently supports increased primary productivity. The algal community at this is driven by consistent manipulation of temperature and flow regimes, the unlikeliness of an active bedload and subsequent scour, and what is most likely to be nutrient uptake by the algal community that persists year-round. As mentioned in the body of this report, 2021 data suggests this Site may exceed state water quality standards, measured by Chlorophyll *a* mass per unit area. If IWMP stakeholders are concerned about this exceedance, TU suggests a fall sampling event that follows Water Quality Control Division (WCQD) sampling protocols. Albeit, TU does not feel this investigation is necessary given the various impacts at this Site.

Based on what we learned from the *Algae Test 2*, performed by EnviroScience, the 2021 fall samples and all 2022 samples will be analyzed for only diatoms. By completing a more comprehensive diatom analysis of our target fall samples, TU and EnviroScience can measure IWMP site health and overall stream health more easily, using diatom-specific ecological guilds and diversity indices, and similarity matrices. This method will not show seasonal changes in algal groups, which does present value, but TU and EnviroScience agree that there is more value in a more thorough diatom analysis for a better understanding of longitudinal changes in diversity and community assemblages of diatoms.

The data presented in this sampling report indicate that the unnatural abundance of aquatic algae at Blue 5 is most likely due to the input of nutrients from Dillon Reservoir, in conjunction with altered thermal regimes and lack of scouring flows. Blue Valley Ranch is collecting monthly surface water samples from three locations along the Middle Blue to inform upon seasonal concentrations of nitrogen and phosphorus. Based on existing data, the standing crop of algae is most likely a byproduct of Dillon Reservoir outflows. The rapid decline in algal biomass suggests rapid uptake of bioavailable nutrients by persistent plant communities beneath the reservoir, dilution, and the potential chemical influence of Straight Creek.

Finally, benthic algae data and respective metrics should by collated to a closer degree with benthic macroinvertebrate data metrics, where applicable. While Trout Unlimited and Timberline Aquatics staff have coordinated all biomonitoring sample events, it's been challenging to coalesce reports for each respective monitoring method. Additionally, for the 2023 Phase 3 2020-23 benthic algae monitoring report, TU will coordinate with BVR to present comparative data to relevant top rock scrapes from Reach 3.

Section 5.0 - Literature Cited

- Blinn, D.W., Shannon, J.P., Benenati, P.L., Wilson, K.P., (1998) Algal Ecology in Tailwater Stream Communities: The Colorado River Below Glenn Canyon Dam, Arizona. Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona. Academia, Journal of Phycology.
- Blinn, D. W. & Cole, G. A. (1991) Algal and invertebrate biota in the Colorado River: comparison of preand post-dam conditions. In Committee on Glen Canyon Environmental Studies [Eds.] Colorado River Ecology and Dam Management. National Academy Press, Washington, DC, pp. 85–104.
- Colorado Water Quality Control Division (WQCD), Standard Operating Procedures for the Collection of Streams Periphyton Samples.
- Hauer, R.F. & Lamberti, G.A., (2007) Methods in Stream Ecology, Second Edition. Academic Press, Elsevier., Burlington, MA.
- Heck, M.P., Schultz, L.D., Hockman-Wert, D., Dinger, E.C., and Dunham, J.B. 2018. Monitoring stream temperatures—A guide for non-specialists: US Geological Survey Techniques and Methods, book 3, chap. A25, 76 p.
- Kumar, H.D & Singh, H.N. (1979) A Textbook on Algae. Macmillan International College Edition.
- Lewis, W.M., and McCutchan, J.H. (2010) Ecological Responses to Nutrients in Streams and Rivers of the Colorado Mountains and Foothills, Freshwater Biology, Center for Limnology, Cooperative Institute for Research in Environmental Sciences, Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO,
- Lewis, W.M., and McCutchan, J.H. (2012) Evaluation and Interpretation of Multi-Metric Index (MMI) Information on Invertebrate Communities of the Blur River below the Dillon Reservoir Dam, Summit County, Colorado, Rpt. 337.
- Lewis, W.M., and McCutchan, J.H. (2013) Results of a Field Survey of Benthic Chlorophyll Abundance and its Possible Relationship to Nutrient Concentrations for Streams within and Just Below the Lake Dillon Watershed, Rpt. 328.
- Lewis, W.M., and McCutchan, J.H., Roberson, J. (2016) Chlorophyll and Nutrient Concentrations for Selected Sites in the Lake Dillon Watershed, Rpt. 378.
- Oertel, N. and Salánki, J. (2003) Biomonitoring and Bioindicators in Aquatic Ecosystems. In: Ambasht RS, Ambasht NK (Eds. Modern trends in applied aquatic ecology. Kluwer Academic/Plenum Publishers, New York, pp. 219-246.
- Rosenberg, D.M. 1998. A National Aquatic Ecosystem Health Program for Canada: We should go against the flow. Bull. Entomol. Soc. Can., 30(4):144-152.
- Rost, A.L., & Fritsen, C.H. (2014) Influence of a tributary stream on benthic communities in Didymosphenia geminata impacted stream in the Sierra Nevada, USA, Diatom Research, 29:3, 249-257, DOI: 10.1080/0269249X.2014.929029.

- Steinman et al., (2006) Biomass and Pigments of Benthic Algae. Methods of Stream Ecology: Second Edition, Edited by F. Richard Hauer and Gary A. Lamberti, Ch. 17, 357-368.
- Stevens, L. E., Shannon, J. P. & Blinn, D. W. (1997) Colorado River benthic ecology in Grand Canyon, Arizona, USA: dam, tributary and geomorphological influences. Regul. Rivers 13:129–49.
- Plafkin J.L., Barbour M.T., Porter K.D., Gross S.K. & Hughes R.M. (1989) Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish. US Environmental Protection Agency, EPA/ 444 / 4-89-001. Office of Water, Washington, DC.
- Puccinelli, C.M., Marcheggiani, (2019) A Patented Rapid Method for Identification of Italian Diatom Species. International Journal of Environmental Restoration and Public Health.
- Resh, V. H., Brown, A. V., Covich, A. P., Gurtz, M. E., Li, H. W., Minshall, G. W., Reice, S. R., Sheldon, A. L., Wallace, J. B. & Wissmar, R. C. 1988. The role of disturbance in stream ecology. J. North Am. Benthol. Soc. 7:433–55.
- Stevenson R.J., Bothwell M.L. & Lowe R.L. (1996) Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, NY.

Section 6.0 – Appendices (to be included in Final Draft)