Benthic Macroinvertebrate and Water Quality Characterization of the Yampa–Green Rivers

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Introduction

The Yampa and Green Rivers are two perennial rivers that run through Dinosaur National Monument in the state of Utah. The Yampa is an unregulated tributary of the heavily regulated Green. Flaming Gorge Dam, on the Green, sits about 60 miles north-west of the Green-Yampa confluence and alters the natural flow regime of the river system.

Benthic macroinvertebrates (BMIs) are often studied when assessing aquatic habitat because of their widespread occurrence, diversity, ease of sampling, and long generation times. BMI indices serve as indicators of stream biotic health (Resh, 2008). Many indices exist for evaluating water quality levels in freshwater communities. One of these indices, EPT, is a measure of the richness of three macroinvertebrate species orders: Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). The index is useful for measuring the overall health of the freshwater stream because all three orders in EPT are considered sensitive species to various aquatic stressors. Their abundance indicates a generally healthy stream, whereas their lack of abundance could indicate poor water quality conditions (NRCS National Water and Climate Center, n.d.).

Our study looked at BMI counts from samples taken along the Yampa and Green rivers, and related these numbers to relative location along the river, water quality data, and substrate type. Previous studies in other systems have demonstrated that invertebrate density and taxonomic richness increases as substrate size increases from sand to cobbles (Hickey & Quinn, 1990). Cobble are a much larger grain than sand and when compiled have gaps between grains of much greater size than gaps between sand sized grains. These gaps between cobble-sized grains trap and retain more coarse particulate organic matter and thus supply a greater food source to BMI than small sand-sized grain substrate (Rounick & Winterboum, 1983; Webster et al, 1987). Larger cobbles also have increased boundary layers for macroinvertebrates to seek refuge in (Smith, 1975; Davis, 1986). Furthermore, the large size of cobbles, relative to sand-sized grains, creates a larger boundary layer that both enhances the exchange of dissolved gases and nutrients and maintains a greater area protected from the fast flow of the river (Statzner, 1981). These benefits led Quinn & Hickey to postulate that Ephemeroptera, Plecoptera, and Trichoptera are more likely to be found in coarser substrates as EPT have high oxygen requirements (Hickey and Quinn, 1990; Nebeker 1972; Wiley & Kohler 1980; Williams et al., 1987). For these reasons, we expected to find more invertebrates in substrate of larger sediment sizes than substrates of smaller sediment sizes, as cobble dominated substrate should create a more suitable habitat for BMIs than cobble-sand mixed or sand dominated substrates.

Vegetated substrata would also be expected to increase the abundance of BMI, as vascular hydrophytes greatly enhance invertebrate abundance in silty and sandy rivers (Hickey & Quinn, 1990). Vegetation provides structure, enlarges the boundary layer, and produces a high amount of organic matter. In a vegetated substrate, benthic macroinvertebrates have a large food source, optimal gas exchange, and less physical stress from flow (Barmuta et al., 2011; Berg, 2008). Thus, we expected to see a large abundance of BMI in vegetated substrate.

Temperature is an important physiological parameter for aquatic macroinvertebrates. Temperature greatly influences BMI metabolism, growth, development, reproduction, and food availability (Sweeney, 1978; Sweeney & Vannote, 1986; Rempel & Carter, 1987). Plecoptera and Ephemeroptera have been experimentally shown to be relatively sensitive to extreme stream temperatures, generally preferring lower water temperatures (Quinn et al, 1994). Therefore, we expected to see higher abundances or percentages of EPT in colder waters.

Natural flows of the Yampa River peak with snowmelt in late spring, while the Green River hydrograph is more homogenous throughout the year due to flow controlled through Flaming Gorge Dam. Hydropeaking created from the cyclical release of water from the dam creates abnormally high water levels on the Green River during primary egg laying hours. Subsequently, water levels drop when hydropeaking ceases for the day. Eggs laid just below the surface during high water level from hydropeaking become subject to desiccation when water levels return to normal. This pattern of egg desiccation has been shown to severely reduce the abundance of insects in the Colorado River downstream of Glen Canyon Dam (Kennedy et al., 2016). We predicted this potential hydropeaking-egg desiccation phenomena was present along the Green River downstream of Flaming Gorge Dam as well and decreased BMI abundance.

In this paper, we compared the results of EPT abundance, as well as total BMI abundance, to flow regimes, measures of water quality (temperature and electroconductivity), and substrate type longitudinally along the Yampa and Green Rivers.

Study Purpose

The purpose of this study was to find systematic patterns or trends in species assemblage and diversity related to substrate, water quality conditions, and longitudinal location on the Yampa and Green rivers..

Methods

Study Location

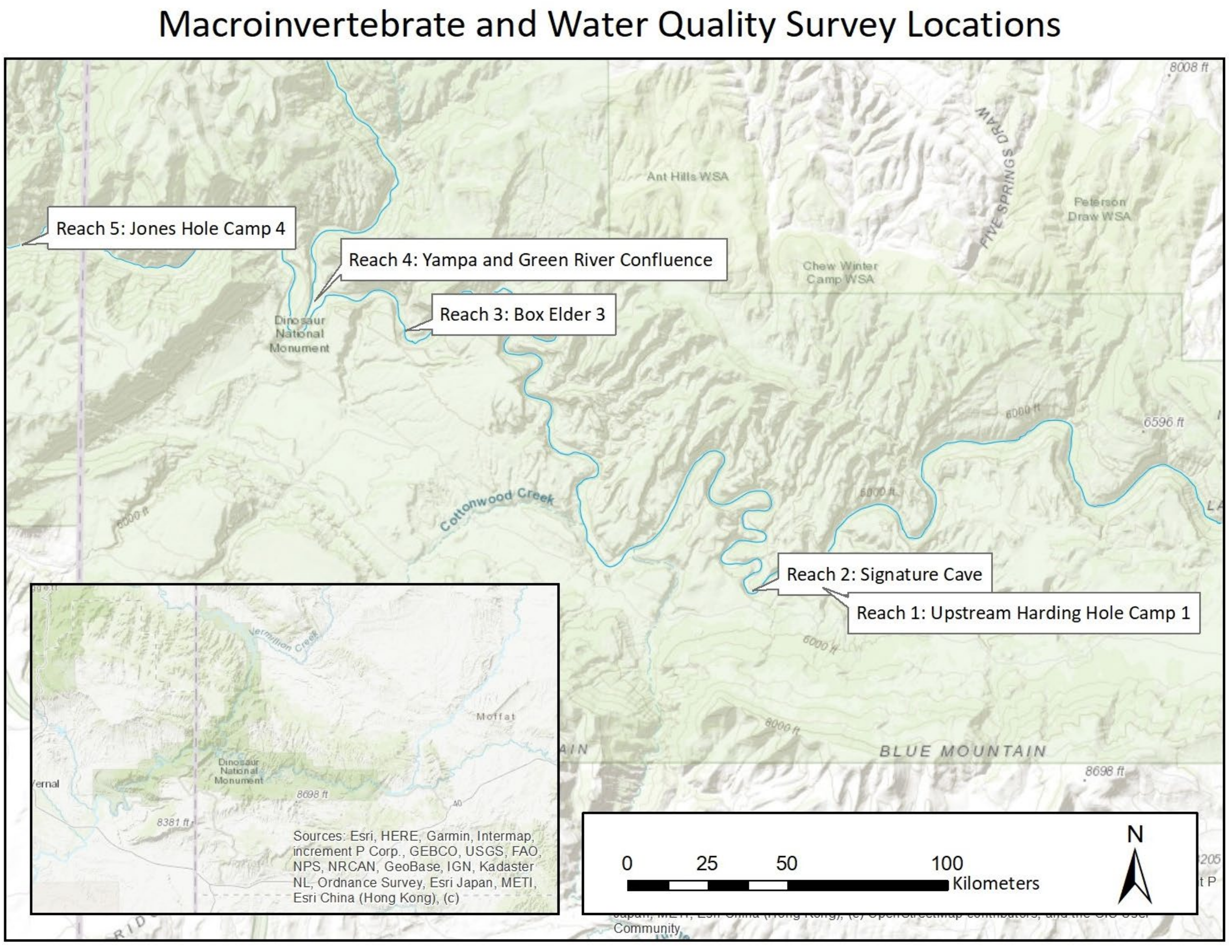
This macroinvertebrate stream health assessment took place while rafting down the Yampa and Green Rivers within Dinosaur National Monument. Reach sites chosen for each survey were not predetermined because flows in the Yampa River were extremely high during the week this assessment took place. High flows submerged many rocks and sandbars and altered the hydraulic conditions of the river in an unusual manner. As a result, survey locations were chosen based on accessibility and time of day.

All survey sites occurred on sandbars off the mainstem of the river, though macroinvertebrate surveys were conducted along a combination of mainstem and back channel waters. The fact that all surveys were conducted on sandbars is significant and important to address. Classical macroinvertebrate stream survey methods recommend avoiding sandbars when attempting to assess overall stream abundance and diversity as sand bars typically have low insect species richness (Hickey & Quinn, 1990). Surveying sandbars may completely underestimate the macroinvertebrate species richness of the stream or river and bias survey results. Thus, data collected from surveying solely along sandbars can only be used to understand and define the insect communities on sandbars, not within the stream or river system as a whole.

Macroinvertebrate surveys were conducted at four sites along the Yampa River and at one site along the Green River. These five survey sites are indicated on the map in Figure 1. Survey sites 1-5 are labeled as Reach 1-5 with reference to the nearest landmark within Dinosaur National Monument (campsite, cave, etc.).

Reach 1 survey was conducted river left of mainstem Yampa River, upstream of Harding Hole Campsite 1. Reach 2 survey was conducted channel left of a backwater channel that flowed behind a sandbar river right of the mainstem Yampa River, several meters upstream of the trailhead leading to Signature Cave. Reach 3 survey was conducted river right of the mainstem Yampa River at Box Elder Campsite 3. Reach 4 survey was conducted along the shoreline of a sandbar river right of the mainstem Yampa. This sandbar was located at the confluence of the Yampa and Green Rivers. Reach 5 survey was conducted river right of the mainstem Green River at Jones Hole Campsite 4.

Figure 1. *Map with locations of the macroinvertebrate and water quality surveys conducted along the Yampa and Green River within Dinosaur National Monument.*



Study Period

Macroinvertebrate surveys at reach sites 1-5 occurred between June 20th-24th of 2019. This late spring to early summer study period occurred when peak flows in the unregulated Yampa River were very high. In the week of our sampling period a flash flood event within the tributaries occurred, which may have impacted the findings of our study. Major floods have been documented to cause substantial reductions in taxonomic richness and total invertebrate biomasses and densities in New Zealand rivers (Hickey and Quinn, 1990). High flows impact macroinvertebrate sampling by scouring and submerging important substrate and by temporarily improving water quality that favors intolerant species (Bureau of Fisheries Management, 2000). Thus, macroinvertebrate samples taken during late spring to early summer during seasonal high flows may not provide a representative sample of general abundance and diversity within the environment year round. These factors must be acknowledged as potential sources of bias in the methods of data collection and results for this assessment.

Sampling Equipment
To sample macroinvertebrates, a kick net of opening dimensions 0.26m * 0.50m was used. Three sampling trays were utilized as the receptacle for emptying the collection content from the kick net. An integrated spout wash bottle was used to wash any remaining contents from the kick net into these sampling trays. Forceps were used to pick up bugs in the trays and sort them into the two sets of plastic ice cube trays based on family and order.

Sampling Procedure
A. Technique
Three sampling techniques were used to assess the macroinvertebrate abundance and diversity along the sandbars of the mainstem and backwater channels of the Yampa and Green Rivers. Kick net sampling was used for transect surveys at reaches 1, 2 and 4, drift net sampling was used for transect surveys at reach 3, and vegetation sweep sampling was used for transect surveys at reach 5. Brief descriptions on the sampling protocol for each survey technique is summarized below:

- Kick net: procedure requires the dip net opening be positioned upstream. For thirty seconds, the surveyor then disturbs and kicks the substrate directly upstream of the net opening in an area of a square with a width equivalent to the length of the net. Then, for another thirty seconds, the surveyor uses their hands to comb through the substrate in the same area. Net is then lifted out of the water with care to avoid losing any collection material.

- Drift net: procedure requires the dip net be positioned with the opening of the net facing upstream. The surveyor then holds the net suspended in the water column for one minute. Net is then lifted out of the water with care to avoid losing any collection material.

- Vegetation sweep: Same procedure as the drift net, but in a vegetated zone of the river instead of over a sand or gravel bar.

B. Interval Samples and BMI identification

For a majority of the kick net samples, three sub-samples were collected at each reach. The first sub-sample was collected at the downstream-most interval of the reach. The second sub-sample was taken at an interval roughly a meter or two upstream of the first, and the third, at the most upstream interval on the reach. The sub-samples occurred in increasingly upstream intervals in order to avoid disturbing or influencing subsequent sub-samples. These three sub-samples were then composited into one sample per study reach.

The drift net and vegetation sweep samples each had one interval along the reach. The decision for this was for time constraint during survey hour.

Once samples were composited, BMI were manually sorted in the field into partitioned trays until no further individuals could be found. Individuals were identified to order and counted.

Photos: After each sampling effort, net contents were parsed into catch trays, and surveyors used forceps to catch BMI and place them into an ice tray well for identification and count.

C. Sampling Errors

Several errors occurred during the sampling of the macroinvertebrates that must be acknowledged before the findings of our research are presented. Twice, survey members threw out sub-samples before they were processed (first at Reach 1 and again at Reach 4). This accidental discard of data likely affected the results and calculations, biasing the representation of BMI at these two reaches. Additionally, inconsistency and bias of survey members varied by team. Three teams of surveyors rotated sampling responsibility, and there may be a wide range in ability to pick and sort through sub-samples and count BMI in the allotted time intervals. Smaller, faster BMI were more difficult to catch and count, and their representation in the samples may appear less abundant in our data than they were in reality. Therefore, some variation in our results by reach location may be attributed to variation in bias by respective sampling teams and sampling errors.

Statistical Data Calculations

Data collected from the field surveys were compiled and statistically analyzed through the following methods (see Table 1 and Table 3):

1. Total # Individuals: Represents the sum of all individual macroinvertebrates collected for a given reach site.
2. Total Abundance: Many reach sites had multiple sampling intervals (approximately three sub-samples per survey reach). The total abundance represents the sum for each order across the different intervals.
3. Average abundance: Total abundance divided by the number of sampling intervals in reach. For instance, if 46 Ephemeroptera were found in reach 1, and three intervals were taken in reach 1, the average abundance would be 46/3 = 15.3.
4. Alpha-diversity- The number of different orders in a sample.
5. Orders per Area: Alpha Diversity divided by area of the net. Value represents the order density per 1 square meter of river bed.
6. Total # of EPT: Total number of EPT represents the total sum of E, P, and T orders in a given reach.
7. Percent EPT: Total # of EPT divided by the total number of individuals.
8. Bootstrapping: Bootstrapping resamples our data with replacement, adding variation to each bootstrap sample and mimicking resampling from the population. Bootstrap distributions are data-driven, and do not assume a particular null hypothesis to be true.

Results

Diversity by Survey Site

Results of the analyses of macroinvertebrate density and abundance trends conducted across survey sites along the Yampa and Green Rivers are presented below (Table 1, Figure 2). As Ephemeroptera were the only order of the EPT index that were detected in this study, EPT abundance provided below is synonymous with Ephemeroptera abundance.

Figure 2: Summary of BMI abundance by order for Yampa & Green River survey sites

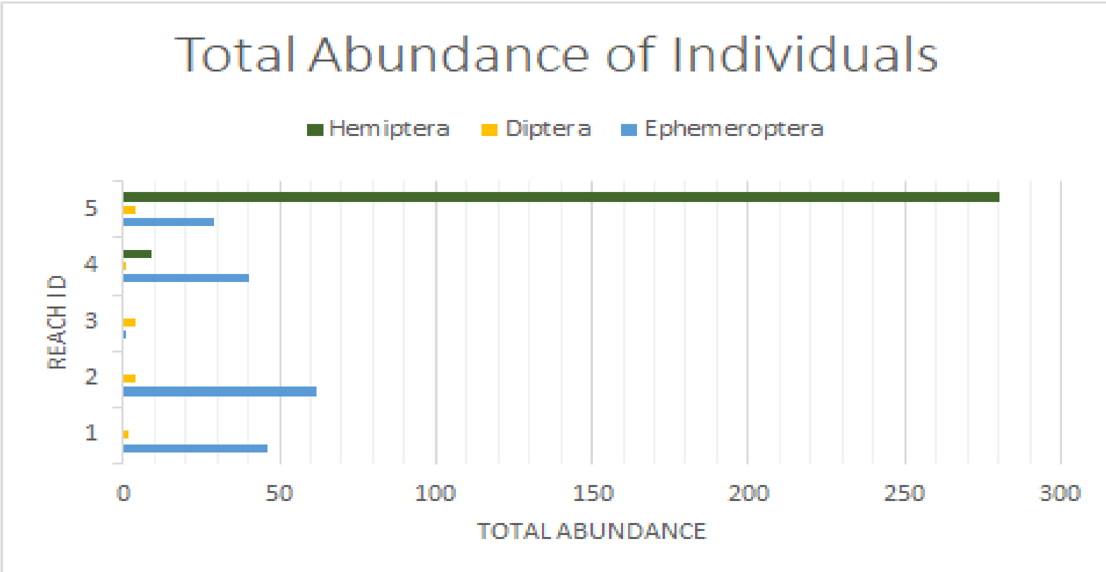


Table 1: Macroinvertebrate metrics for Yampa & Green River survey sites

Metric	Reach 1	Reach 2	Reach 3	Reach 4	Reach 5
Total # Individuals	48	66	5	50	313
Ephemeroptera Total Abundance	46	62	1	40	29
Ephemeroptera Average Abundance	15.3	20.7	1	13.3	14.5
Diptera Total Abundance	2	4	4	1	4
Diptera Average Abundance	0.7	1.3	4	0.3	2
Hemiptera Total Abundance	0	0	0	9	280
Hemiptera Average Abundance	0	0	0	3	140
Alpha Diversity	2	2	2	3	3
Species per Area	8	8	8	12	12
Total # EPT	46	62	4	40	29
Percent EPT	95.8%	93.9%	20%	80%	9.3%

Total numbers of Ephemeroptera and Diptera appear to be fairly similar across most reaches, however there is a large increase in Hemiptera at Reach 5, and very few Ephimeroptera at Reach 3. Hemiptera are not found in any sample until the confluence of the Yampa and Green Rivers at Reach 4, and their abundance increased considerably at the Green River site, Reach 5. Hemiptera were most abundant at Reach 5; Diptera at Reaches 2, 3, and 5; Ephemeroptera at Reach 2. The EPT index was highest at Reach 1 (95.83%) and lowest at Reach 5 (9.27%).

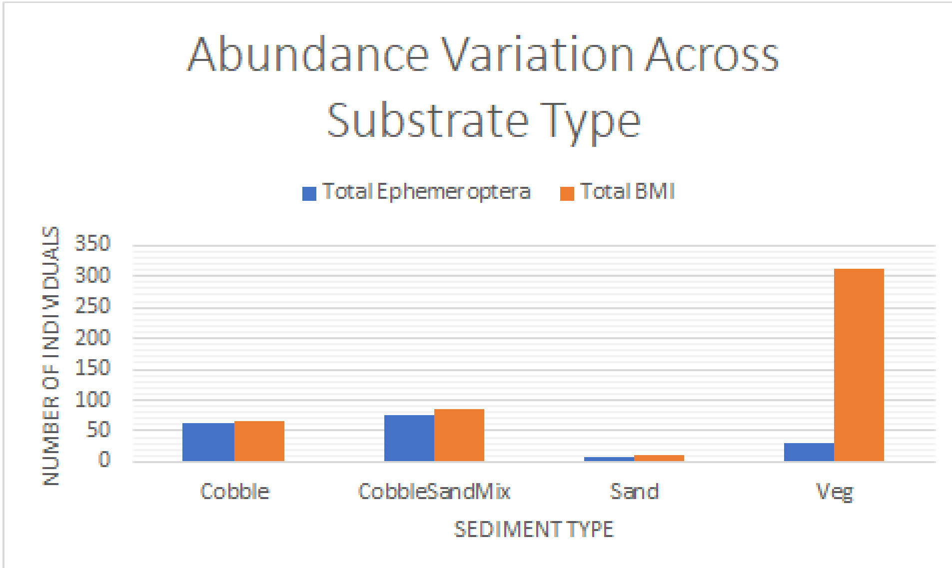
Diversity by Sediment Type

In addition to analyzing trends longitudinally between survey locations, analyses of macroinvertebrate density and abundance between substrate types were conducted. Results from this analysis are presented below. Raw data, averages, and density calculation are listed in Table 2, and summary results are shown in figure 4.

Table 2: Macroinvertebrate metrics across sediment type in the Yampa and Green Rivers.

Metric	Cobble Dominant	Sand Dominant	Cobble Sand Mix	Vegetation
n	2	2	5	2
Total # Taxa	67	10	86	313
Ephemeroptera Total Abundance	64	9	75	29
Ephemeroptera Average Abundance	32	4.5	15	14.5
Diptera Total Abundance	3	1	2	4
Diptera Average Abundance	1.5	1	1	2
Hemiptera Total Abundance	0	0	9	280
Hemiptera Average Abundance	0	0	3	140
Alpha Diversity	2	2	3	3
Orders per Area	8	8	12	12
Total # EPT Taxa	64	9	75	29
Percent EPT Taxa	95.5	90	87.2	9.2

Figure 3: Comparison of substrate types for raw data on total Ephemeroptera and BMI



The "n" row of Table 2 indicates the number of sampling intervals for each substrate type. Each substrate type had two sampling intervals, except for the cobble-sand mixed substrate which had 5 sampling intervals. The raw data shows highest overall BMI abundance occurred in vegetated substrates, while the highest Ephemeroptera abundance was in the cobble-sand mixture. The small number of repetitions, and the varying amount of sampling intervals across samples raises issues for statistical inferences to be made.

To resolve these issues, bootstrapping was performed to enhance our ability to analyze this data. Table 3 contains 90% bootstrap BCA confidence intervals for comparing the number of Ephemeroptera and the total number of BMI collected between each substrate type. To achieve this comparison, the bootstrap was run B = 4,000 times. Our parameter of interest, theta hat, is the difference in averages between groups (substrate types).

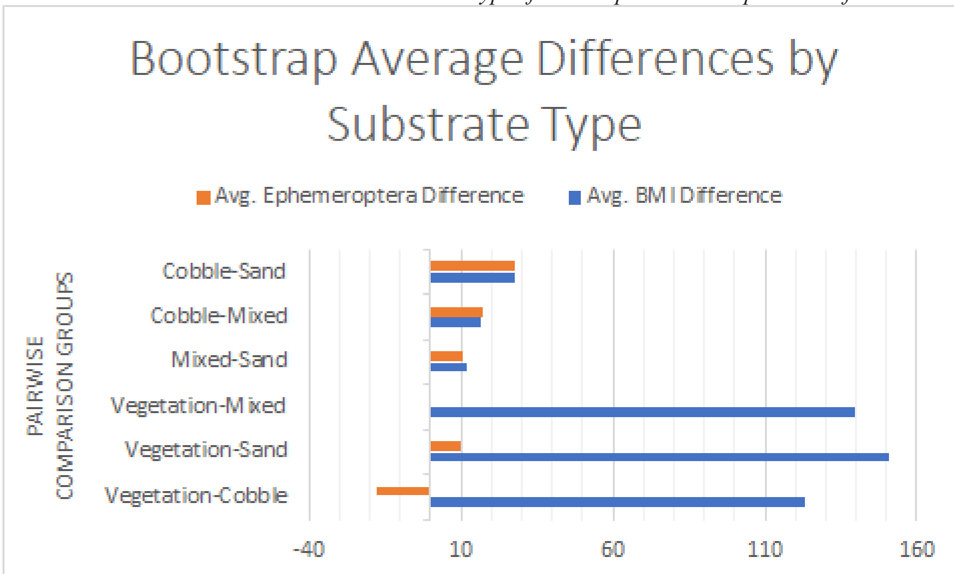
Table 3: Results of 90% BCA CI comparing group (substrate) averages for Ephemeroptera and overall BMI, based on bootstrapping (B=4,000).

Comparison	**Group 1**	**Group 2**	**90% BCA CI**	**Theta hat (group 1 mean - group 2 mean)**
Ephemeroptera	Cobble	Sand	(26.5, 29)	27.5
Ephemeroptera	Cobble	Mixed	(12.8, 20.8)	17
Ephemeroptera	Sand	Mixed	(-15, -7)	-10.5

Ephemeroptera	Vegetation	Mixed	(-11, 10.2)	-.05
Ephemeroptera	Vegetation	Sand	(1.5, 19)	10
Ephemeroptera	Vegetation	Cobble	(-26, -8)	-17.5
Total BMI	Cobble	Sand	(26.5, 30)	28
Total BMI	Cobble	Mixed	(11.8, 21.4)	16.3
Total BMI	Sand	Mixed	(-15.9, -7.1)	-11.7
Total BMI	Vegetation	Mixed	(37.8, 241.4)	139.3
Total BMI	Vegetation	Sand	(51.5, 251)	151
Total BMI	Vegetation	Cobble	(23.5, 224)	123
Summarized Ephemeroptera abundance comparison results:	Higher in cobbles than sand (estimated difference in means is 27.5 individuals); Higher in cobbles than mixed substrate (estimated difference in means is 17 individuals); Higher in mixed than sand substrate (estimated difference in mean is 10.5 individuals); Vegetation and mixed substrate not significantly different; Higher in vegetation than sand (estimated difference in mean is 10 individuals); Higher in cobble than vegetation (estimated difference in mean is 17.5 individuals).			
Summarized total BMI abundance comparison results:	Higher total BMI individuals in cobble than sand (estimated difference in mean is 28 individuals); Higher total BMI individuals in cobble than mixed (estimated difference in mean is 16.3 individuals); Higher total BMI individuals in mixed than sand (estimated difference in mean is 11.7 individuals); Higher total BMI individuals in vegetation than mixed (estimated difference in mean is 139.3 individuals); Higher total BMI individuals in vegetation than sand (estimated difference in mean is 151 individuals); More total BMI individuals in vegetation than cobble (estimated difference in mean is 123 individuals).			

The 90% bootstrap BCA confidence intervals show that average Ephemeroptera counts were largest in cobble substrata, whereas average total BMI was largest in vegetated substrata. For both Ephemeroptera and overall BMI counts, sand had the lowest average abundances. The table above summarizes each pairwise comparison made between substrate types (cobble dominated, mixed cobble/sand, sand dominated, and vegetated) for just Ephemeroptera as well as for overall BMI. Figure 4 visually demonstrates the differences seen in group (substrate) means. Negative differences indicate group 2 had higher average abundance.

Figure 4. *Differences in the total abundance between substrate types from the pairwise comparisons of the bootstrap averages.*

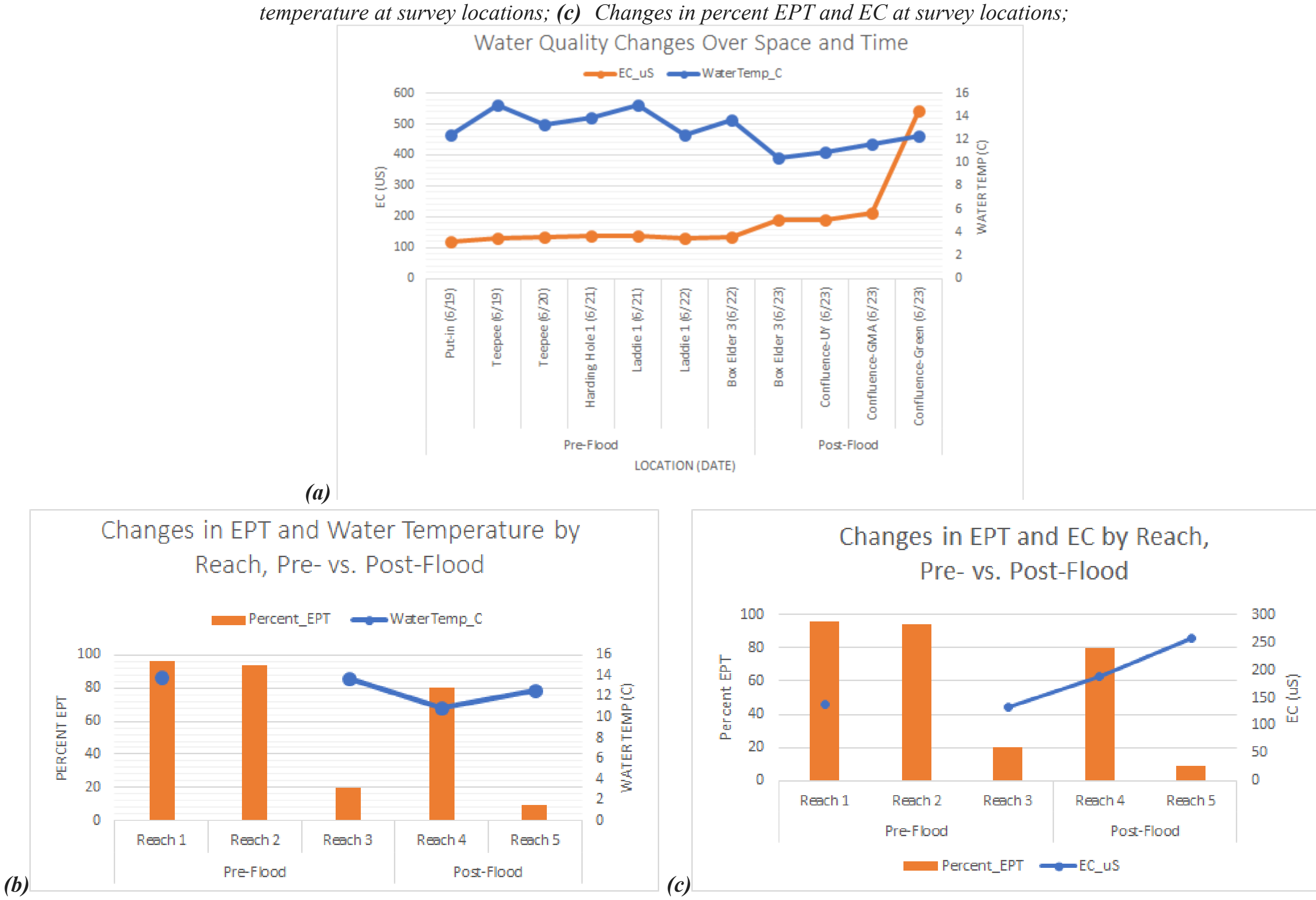


Diversity by Water Quality

Locations were surveyed for BMI sequentially by day: Reach 1 (Harding Hole 1) was sampled on 06/20/2019, Reach 2 (Signature Cave) on 06/21/2019, Reach 3 (Box Elder 3) on 06/22/2019, Reach 4 (Confluence) on 06/23/2019, and finally Reach 5 (Jones Hole 4) on 06/24/2019. Please note that water quality data for Reach 1 was sampled on 06/21/2019, a day after the bug collection, and Reach 2 was not sampled for water quality. A flash flood event started the evening of 06/22/2019, after sampling efforts had concluded that day. Post-flood, we observed a decrease in water temperature from 13.7℃ on 06/22/2019 to 10.9℃ on 06/23/2019 (Figure 5). The temperature increased the next day at the Green River, where the temperature were 12.6℃. Percent EPT appears inversely related to temperature for Reaches 3, 4, and 5, but higher EPT values were observed at reach 1 where water tempearature was similar to reach 3. Although interesting to note, the study data are insufficient to make statistical comparisons between water temperature and EPT.

The flood event was also associated with an increase in EC from 134 uS at Reach 3 to 189 uS at the confluence. Although we observed an increasing trend in EC from Reach 3 through Reach 5, we did not see a corresponding trend in the EPT data. However, the study data are insufficient to make statistical comparisons between EC and EPT.

Figure 5. ***(a)*** *Changes in water quality (temperature and EC) over space and time;* ***(b)*** *Changes in percent EPT and water temperature at survey locations;* ***(c)*** *Changes in percent EPT and EC at survey locations;*



Discussion

Spatial Trends

Relating macroinvertebrate data to longitudinal downstream trends was informative in analyzing the health of the Yampa-Green system. Patterns were observed within the water temperature, EC, sediment type, and BMI downstream.   In particular, we noted the exclusive presence of *Hemiptera* in the Green River. Hemiptera were not present in the first three survey reaches on the Yampa River, and only were present in the survey sites below the confluence, appearing at Reach 4 and increasing in abundance at Reach 5. Though technically the Yampa River was surveyed at the confluence, it is likely that mixing of Green River water introduced this macroinvertebrate order into the Yampa River at this location. In general, the Hemiptera order is a tolerant one, and their presence indicates lower stream health (Berg, 2008). However, no definitive literature exists on whether the family Corixidae in the Hemiptera order is a tolerant species and/or representative of specific water quality conditions. Hence, no definitive conclusions can be made about the health of the stream from the presence of Corixidae

Hemiptera, only that their presence is likely exclusively characteristic of the Green River, rather than the Yampa River. Similarly, the continuous downstream presence of *Diptera* is of questionable significance. Diptera are known for being a tolerant order of macroinvertebrate (University of Minnesota, 2009). However, mutual presence of both Ephemeroptera, a sensitive species, and Diptera, a tolerant species, rule out the quick conclusion that the water quality in either river is poor.

In general, EPT % decreased downstream, with the exception of Reach 3. However, Reach 3 was the location of a drift sample, the only unique sample method compared to samples collected at the other four study sites. Thus, the EPT% may be an outlier on the decreasing EPT trend due to sampling bias. A decrease in the sensitive EPT species typically indicates that the water quality of the river is decreasing (NRCS National Water and Climate Center, n.d.). As water quality decreases in a freshwater body, the EPT percentage decreases because intolerant species can no longer survive in the poor conditions. Hence, the data may suggest a decrease in water quality from Deerlodge Park to Split Mountain. However, other factors such as substrate type, localized flow conditions, or other factors may have contributed to the observed trend.

Further, the downstream trend in EPT% may also be confounded by the high flows of the 2019 spring runoff. Studies have shown that a prolonged spring flow recession with sustained high flows late into the season disturbs BMI habitat and can be linked with declines in invertebrate density. Populations can have difficulty establishing after winter flows if long spring flows persist (Steel et al., 2017). Because high spring peak flows lasted beyond the typical late May-early June time frame and continued into late June when this study was conducted, it is possible that BMI populations were hindered. Future studies with consistent sampling techniques, repeated samples, and additional sample sites would provide additional insight.

BMI Distribution by Water Quality

Electrical Conductivity (EC) remained constant throughout the course of the survey trip, until the flash flood event on 06/22/2019. EC was significantly higher following the event, continued to increase as we entered the Green River, and reached the maximum reading on the last day. Water temperature fluctuated day to day, but had the most rapid change after the flash flood event.

Water temperature appeared to have an inverse relationship with the percentage of EPT in our samples in the more downstream sites, however, the data were too limited to make any statistical conclusions. EC did not appear to have an association with the proportion of EPT in our samples. This lack of relationship was likely due to multiple factors, including the limited choice in survey sites for this assessment. Further, because the BMI results observed here were likely not representative of the true BMI abundance in the Yampa and Green Rivers, any perceived relationships between BMI or EPT abundances and water quality measures were inconclusive.

BMI Distribution by Sediment Type

The results from bootstrap pairwise comparisons indicated total abundance of BMI was greatest in vegetation and cobble-dominated substrates and lowest in sand-dominated substrates. Ephemeroptera abundance was greatest in cobble-dominated substrates. These results support general notions that cobble and vegetation substrates provide beneficial habitat for food, oxygen, and refuge for BMI.

The substrate data also indicate why abundance of total BMI and Ephemeroptera were relatively low throughout the assessment. Due to high flows, all surveys took place on sandbars at the outer channel edges. Though some transects on the sandbars were classified as having vegetated or cobble-dominated substrate, the majority (7 of 11) of transects were sand-dominated or sand-mixed. More than 65 percent of the total number of individual BMIs collected in this assessment were collected at one transect with vegetated substrate. Furthermore, seven of the eleven transects surveyed in this assessment were completely or partially sand-dominated and only contributed 16 percent of the total number of individual BMI collected. Since the dominant substrate type surveyed was partially or completely dominated by sand-sized grains, BMI abundances were low and data was limited.

Conclusion

Studying and analyzing benthic macroinvertebrates can give insight into the health and conditions of a river. During this study, longitudinal trends in BMI, water quality, and sediment were evaluated using data collected on the Yampa and Green rivers. In summary, not many spatial (upstream vs. downstream) and (Yampa vs. Green) trends were observed. The varied sampling methods and efforts, as well as sustained high spring flows, significantly limited our ability to both collect abundant data and confidently determine significant trends. Our hypothesis that sandy substrates would support the lowest abundances of BMI was supported by our raw data and bootstrap results comparing BMI abundance and substrate type. As predicted, cobble-dominated and vegetated substrates supported more BMI than sand-dominated and sand-cobble mixed substrates. Ephemeroptera appeared to prefer cobble dominated substrates. Overall, while this study could not make many definitive findings, it did nevertheless highlight the complexity of aquatic habitat in the Yampa-Green system and how BMI abundance and richness may be affected by water quality, substrate type, and flow conditions.

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