BIGHORN RIVER ALLIANCE

Research Initiative

Aquatic Macroinvertebrate Monitoring Summary 2022 for the Bighorn River, Montana: Building a Long-term Data Set

Bighorn River restored Juniper Side Channel looking upstream, September 2022

David Stagliano, Aquatic Ecologist

Montana Biological Survey

Helena, Montana

December 31, 2022

BIGHORN RIVER ALLIANCE

Executive Summary

In 2022, the Bighorn River Alliance (BHRA) completed the 3rd year of the Bighorn River Benthic Macroinvertebrate (BMI) Monitoring program. Eight long-term monitoring sites plus two reconnected side channels (hereafter known as Juniper SC and Rattlesnake SC) were sampled in the spring and fall for BMI.

The goals of this study are: 1) to conduct standardized, replicated BMI surveys to serve as a baseline for future monitoring efforts within each Bighorn River section; 2) to evaluate both spatial and temporal BMI patterns to understand how the river's water quality, hydrology, flow management and temperature regimes affect the overall biological health of the river; and 3) to determine if significant biological changes have occurred since previous BMI samples were collected.

In both late-April and mid-September 2022, we collected 3 replicate Hess BMI samples at the 10 sites. Streamflow inputs from Soap Creek, Rotten Grass Creek, the Little Bighorn and other tributaries influenced by irrigation return flows can have significant effects on the water quantity, quality, turbidity and temperatures of the Bighorn River. During our Fall visit, Mallards Landing (Ml4) water clarity was so turbid that we could not see the stream bottom where we were sampling (river right) or the sediment grid. Likewise in the spring, Bighorn FAS, Two-Leggins, Arapooish and Custer were so turbid from Soap and Little Bighorn run-off that we could not use the sediment grid.

The abundance and diversity of a river's BMI communities, especially mayfly, stonefly and caddisfly species (EPT taxa), are important to assessing a river's biological health, salmonid growth and for fly-fishermen's matching the hatch. To evaluate these BMI communities, we use a variety of metrics known to be influenced by water quality and used by MDEQ in determining biological health or impairments for river assessments.

In 2022, 94 total BMI taxa were collected across the 10 Bighorn River sites, averaging 37 total taxa per site (range 25-47 species). Mayfly (E), caddisfly (T), plus one stonefly (P) (Total EPT Taxa) averaged 34% (5 to 78%) of the total community per site and 12 species per site (2-23 spp.); Arapooish and Manuel Lisa reported the highest total EPT richness in the Fall with 23 and 20 species, respectively.

Twenty-one species of mayflies (E) were recorded throughout the study section in 2022 (6 more than in 2021): the dominant four groups were Blue Winged Olives *(Baetis spp.*), Tiny BWOs (*Acentrella spp.*) and Tricos (*Tricorythodes explicatus*) followed by Pale Morning Duns (PMDs) (*Ephemerella excrucians, Serratella micheneri*). Abundant Trico nymph densities in the fall samples from Two-Leggins downstream, may indicate some large hatches to come in 2023.

Of the 15 total species of caddisflies (T) collected in 2022, the micro-caddis, *Hydroptila spp.*, net-spinning caddisflies, *Hydropsyche spp. and Cheumatopsyche*, and long-horned caddis, *Oecetis avara,* were collected across the most sites, while the western weedy sedge, *Amiocentrus asplius* can be quite abundant upstream of St Xavier.

Total taxa richness, EPT diversity, and % EPT significantly increased in the Fall compared to the Spring 2022 samples (T-test, p=0.001). The highest %EPT and EPT taxa per site ever reported occurred in the Fall sites from Two-Leggins downstream to Manuel Lisa. Species diversity and EPT taxa richness, increase with increasing distance from Yellowtail Dam until the Little Bighorn River enters, then nutrient, sediment and temperature tolerant BMI species increase.

Overall, BMI densities averaged 16,776 individuals per m^2 (± 2,873 SE) across all Bighorn River sites in Fall 2022; these densities were significantly lower (T-test, p=0.012) than values reported in Spring 2022 (31,399 ind. per m^2 ± 5,370 SE). BMI communities collected across the upper Bighorn, Split Island and Three Rivers sites, were building up significant densities in Fall of 2021 and Spring of 2022 until the flushing flows of June significantly removed large portions of interstitial fine sediments which housed the midges and aquatic worms. This led to not only decreased BMI densities in the Fall, but higher percentages of mayflies and caddisflies.

The restored side channels, Rattlesnake and Juniper, BMI densities averaged ~30,000 ind. per m^2 during both seasons and were very comparable to the adjacent Bighorn River mainstem; these BMI densities reached these levels since Fall 2021, just ~6 months colonization time.

The similarity of the side channel BMI community to the adjacent mainstem Bighorn River was fairly low, Fall 2022 Rattlesnake SC samples had a 46% Community Similarity (CS) and 65% Taxa Similarity with the Split Island site, and Juniper SC had a 42% Community Similarity (CS) and 51% Taxa Similarity with the 3-Rivers site. We expect side channel BMI communities to become fully colonized and more similar to the mainstem Bighorn in the coming year.

In 2022, we have now documented the invasive New Zealand mudsnails at 7 of the 10 sites including the two restored side channels: 2022 densities averaged ~120 NZMS per m^2, while occupied sites in 2021 averaged ~540 per m^2.

Hilsenhoff Biotic Index (HBI) Scores >5.0 reported at all Bighorn River sites from 2020 through 2022 indicate that the BMI communities are experiencing at least moderate nutrient and/or sediment enrichment. All 8 monitoring sites in the Fall 2021 and 60% of sites (6 of 10) in the Spring 2022 were exhibiting significant impairment with HBI scores >6.0, but these decreased to <6.0 in Fall 2022 (healthier) across all sites, except Mallards Landing and Custer FAS, indicating that the flushing flows of June greatly improved the BMI communities.

The implications of this research are that regulated river conditions compounded by multiple years of sustained flushing flows (2017-2019) followed by late-season, drought-like flows can significantly alter BMI communities within different sections of the Bighorn River. We observed that BMI densities are significantly reduced with continued flushing flows and then populations explode during periods of low discharge. The lack of flushing flows in 2021 have caused exponential increases in benthic BMI densities across BHRA sites.

We conclude this 2022 analysis by documenting that the BMI communities are consistently expressing the sediment impaired biological health of the Bighorn River, especially at Mallards Landing FAS and sites downstream of the Little Bighorn River. Only when a flushing flow discharge occurs (as in June 2022) does the BMI community reflect a less impaired salmonid-bearing stream. Without a significant flush every year, high nutrient levels can increase aquatic plant growth and sediment accumulations. During these ‘low-flow’ years, population increases of more silt-tolerant BMI taxa (midges, scuds and aquatic worms) tends to shift the BMI community away from mayfly and caddisflies.

Table of Contents

Tables

Figures

APPENDICES

Acknowledgements

We would like to thank Anne Marie Emery, James Chalmers and the BHRA Board for funding this project through Montana Biological Survey (MBS) for another year. Initial Report review and editing was provided by Phil Sawatzki. Field site visit coordination and logistics continue to be expedited by Dennis Fischer. James Paris and Dennis Fischer were instrumental in collecting samples at the upper Split Island site MI1 and at the Juniper Side Channel MI10 in the spring.

All photos in the report were taken by MBS, unless otherwise noted.

BHRA Aquatic Macroinvertebrate Studies

1.0 Introduction

The Bighorn River (HUC 10080015 Lower Bighorn) is the largest tributary to the Yellowstone River at approximately 481 miles (770 km) long with a watershed of ~22,000 square miles; it flows through the states of Wyoming and Montana and represents about 32 percent of the Yellowstone River basin (Petersen et al. 2001). The upper watershed lies within the Wyoming Basin ecoregion transitioning to the Northwestern Great Plains grasslands. Montana Department of Environmental Quality's (MDEQ) stream classification of the Bighorn River is as a prairie river with elevations of the entire study section <1400m in elevation (MDEQ 2012). The lower Bighorn River, a large prairie river, has been transformed into a salmonid bearing river by Yellowtail Dam's cold-water releases. Trout fishermen are most familiar with the upper 43-mile river section below Afterbay Dam downstream to Hardin, MT. This section is one of the most heavily fished trout fisheries in Montana and consistently ranks in the top three with the most angler days in the state (MFWP 2020). Despite this popularity, the Bighorn River has lacked recent comprehensive studies of benthic macroinvertebrate (BMI) populations and community structure that other Montana rivers have had.

In 2019, the Research Initiative of the Bighorn River Alliance (BHRA) identified multiple areas of scientific importance to the health of the river and BHRA funded this project to quantitatively sample the BMI communities of the main-stem Bighorn River from downstream of Afterbay Dam to its confluence with the Yellowstone River, a reach of ~84 miles.

1.1 Objectives. The objectives of the benthic macroinvertebrate (BMI) research are:

1) To develop a long-term data set on BMI populations and community assemblage structure along the study reach of the Bighorn River, including restored side channels (**Map 1**), by collecting repeatable, quantitative, baseline data using standardized methods (**Photo 1).**

2) To evaluate both temporal and spatial BMI patterns as they are affected by water quality, hydrology, flow management and temperature regimes. The result will be a better understanding of the overall biological health of the Bighorn River and its determinants.

3) To evaluate temporal and spatial BMI colonization patterns in the restored side channels.

2.0 Methods

During each sampling visit at a site, an Oakton 10 water quality multi-meter was used to collect *in situ* measurements of water temperature, specific conductance and pH. Ambient air temperature was recorded with a thermometer. These measurements and site photographs (**Appendix B**) were taken prior to the collection of BMI or other disturbances to the water column or substrate.

2.1 Benthic Macroinvertebrate Collections: Three replicate Hess (33 cm diameter, 500-micron mesh) samples were collected within a designated riffle at each site to quantitatively sample macroinvertebrates at measured distances from the bank (**Photo 1**). Three Hess samples typically capture 90% of the total taxa present in a riffle (Vinson and Hawkins 1996). Each Hess sample constitutes a benthic area of 0.1 m^2, so a multiplier of 10 is applied to the numbers of total invertebrates in each sample to achieve a per meter squared estimate. At each sampling point, the Hess sampler was pushed into the stream bottom to form an effective seal and all cobbles (>64 mm) within the sampler were scrubbed clean of organisms and removed; then the entire area within the sampler frame was raked for one minute until all organic matter and macroinvertebrates were washed into the collection net of the Hess sampler (**Photo 1**).

Photo 1. *Hess macroinvertebrate sample being taken at Split Island (left) and fine sediment grid count at the Bighorn FAS (right).*


Photos taken by Jim Chalmers

2.2 Physical Stream Habitat Measures:

Stream channel depths at each Hess sample point (n=3) were recorded at the time of sampling. Substrate size-classes (based on Wolman 1954), embeddedness of cobbles (>64mm) and % vegetation coverage within the Hess sampler frame was qualitatively estimated by relative percentage. A MDEQ fine sediment grid count was performed at each Hess site (n=3); this quantifies the number of fine sediment particles (<8mm) located at the 49 grid intersections (**Photo 1**). This number provides an estimation of % fine sediment in each riffle being sampled. Photo points were taken, and visual estimates of the aquatic vegetation were noted. Stream discharge (CFS) during our sampling was recorded from the USGS gauge at St. Xavier (***Figure 1***).

2.3 Sample Processing and Taxonomic Analysis

Samples were processed and analyzed at the Montana Biological Survey laboratory in Helena. BMI were picked from the samples on a random-selected grid pattern until 500-600 individuals were obtained, placed in vials and then identified to the lowest taxonomic level possible (genus/species) with a dissecting microscope (10-40x) following MDEQ (2012) protocols.

MDEQ's Low Valley (LVAL) and plains (PLN) ecoregional multi-metric macroinvertebrate indices (MMIs) and other metrics were calculated after data was entered into the Ecological Database Access System (EDAS) (Jessup 2006), including EPT taxa, % EPT, % Non-insect, % Chironomidae and the Hilsenhoff Biotic Index (HBI). The MMIs use different suites of these metrics: LVAL (5 metrics) and PLNS (7 metrics) to give a composite score by which impairment is judged. If the composite score is below the threshold scores of 48 for LVAL or 37 for PLNS then the community is considered impaired. MDEQ no longer uses the MMI to evaluate site impairment (MDEQ 2012), but it is still a useful tool to evaluate trends in communities.

The combined mayfly, caddisfly and stonefly species (EPT taxa) and the percentage of these in the sample (% EPT) are always informative metrics, as EPT taxa contain some of the more intolerant aquatic insects. Generally, 20 or more EPT taxa collected at a site in the mountain streams of Montana is considered an unimpaired and healthy community (Bukantis 1996). EPT richness metrics typically decrease with increasing sediment (Barbour et al. 1999); although, Tricos (*Tricorythodes and Caenis*) and burrowing mayflies are silt tolerant and can increase their numbers with increasing siltation.

One informative stand-alone metric is the Hilsenhoff Biotic Index (HBI) which measures the tolerance of a BMI community to organic enrichment (Hilsenhoff 1987); this has also been used as a surrogate for sediment tolerance (MDEQ 2012). Tolerance values are based on a 0-10 scale, where zero-ranked taxa are most sensitive and 10-ranked taxa are most tolerant to pollutants (*Low HBI scores are better*). Values of 0.0-3.0 indicate no apparent organic pollution (excellent), 3.0-4.0 possible slight organic pollution (very good), 4.0-5.0 moderate pollution (good), 5.0-6.0 fairly significant (fair), 6.0-7.0 significant pollution (fairly poor), 7.0-8.0 very significant organic pollution 8.0-10 severe organic pollution. HBI scores are evaluated using a threshold value of ≥4.0 as an indicator of organic or sediment impairment (MDEQ 2011).

Macroinvertebrate optimal and maximum thermal tolerances (Brandt 2001, Ott and Maret 2003), and categorical classifications (Apfelbeck 2007), were used to categorize 225 taxa in the Missouri River system (McGuire 2016). Community temperature metrics were calculated using pooled data (all replicates combined) where optimal and maximum temperature values were applied to the abundance of each taxon (where values are available) for each site.

BMI Community and Taxa Similarity Indices were used to compare taxa and community composition in side-channel and mainstem samples. We quantify community similarity from the overlap in taxa composition using all pooled Hess data (all replicates combined) (***Appendix C***)

2.4 Sampling Locations. Eight long-term, baseline monitoring sites were established for sampling water quality and benthic macroinvertebrates from ~2 miles below Yellowtail Dam to its confluence with the Yellowstone River. Four sites were chosen because they had been previously sampled in a MSU graduate study (Brammer, 1986-87) and by the Montana Department of Environmental Quality (MDEQ) (2001-2005). Two reconnected side channels (SC), Rattlesnake and Juniper, were added in 2022 between MI1 and MI2 (***Table 1, Map 1***).

***Table 1. BHRA Sampling locations. Agency that originally sampled site. WQS=water quality samples*.**

Station ID	Agency	Site Name	Latitude	Longitude	Parameter
BGHNR_RM82	MSU	MI 1: Bighorn River @ RM82 **Split Island** (Upper Brammer) (Appendix B, Photo 1,2)	45.32863	-107.8985	Macroinverts, WQS
BGHNR_RM79	BHRA	Rattlesnake Side Channel	45.35212	-107.86997	Macroinverts
BGHNR_RM76	BHRA	Juniper Side Channel	45.36941	-107.82127	Macroinverts
BGHNR _RM75	MSU	MI 2: Bighorn River @ RM75 **Three Rivers** (Lower Brammer) (Appendix B, Photo 3 & 4)	45.38232	-107.8125	Macroinverts, WQS
BGHNR _RM72	New BHRA	MI 3: Big Horn River @ **Bighorn** FAS (Appendix B, Photo 5,6,18)	45.41634	-107.7898	Macroinverts, WQS
Y11BGHNR01	MDEQ	MI 4: Big Horn River @ **Mallards Landing FAS** (Photos 11 & 12)	45.52166	-107.7258	Macroinverts, WQS
BGHNR _RM52	New BHRA	MI 5: Big Horn River @ **Two Leggins FAS** (Photo 6)	45.64449	-107.6599	Macroinverts, WQS
BGHNR _RM40	New BHRA	MI 6: Big Horn River @ **Arapooish** (Photo 13, 14, 15)	45.75664	-107.5653	Macroinverts, WQS
BGHNR _RM24	New BHRA	MI 7: Big Horn River @ **General Custer FAS** (Photo 16)	45.92737	-107.5744	Macroinverts, WQS
Y17BIGHNR01	MDEQ	MI 8: Bighorn River at **Manuel Lisa FAS** (river left) (Photo 17)	46.14486	-107.4644	Macroinverts, WQS

***Map 1**: Bighorn River 2022 BMI sampling sites (MI1-MI10) from Yellowtail Dam to the Yellowstone River confluence.*

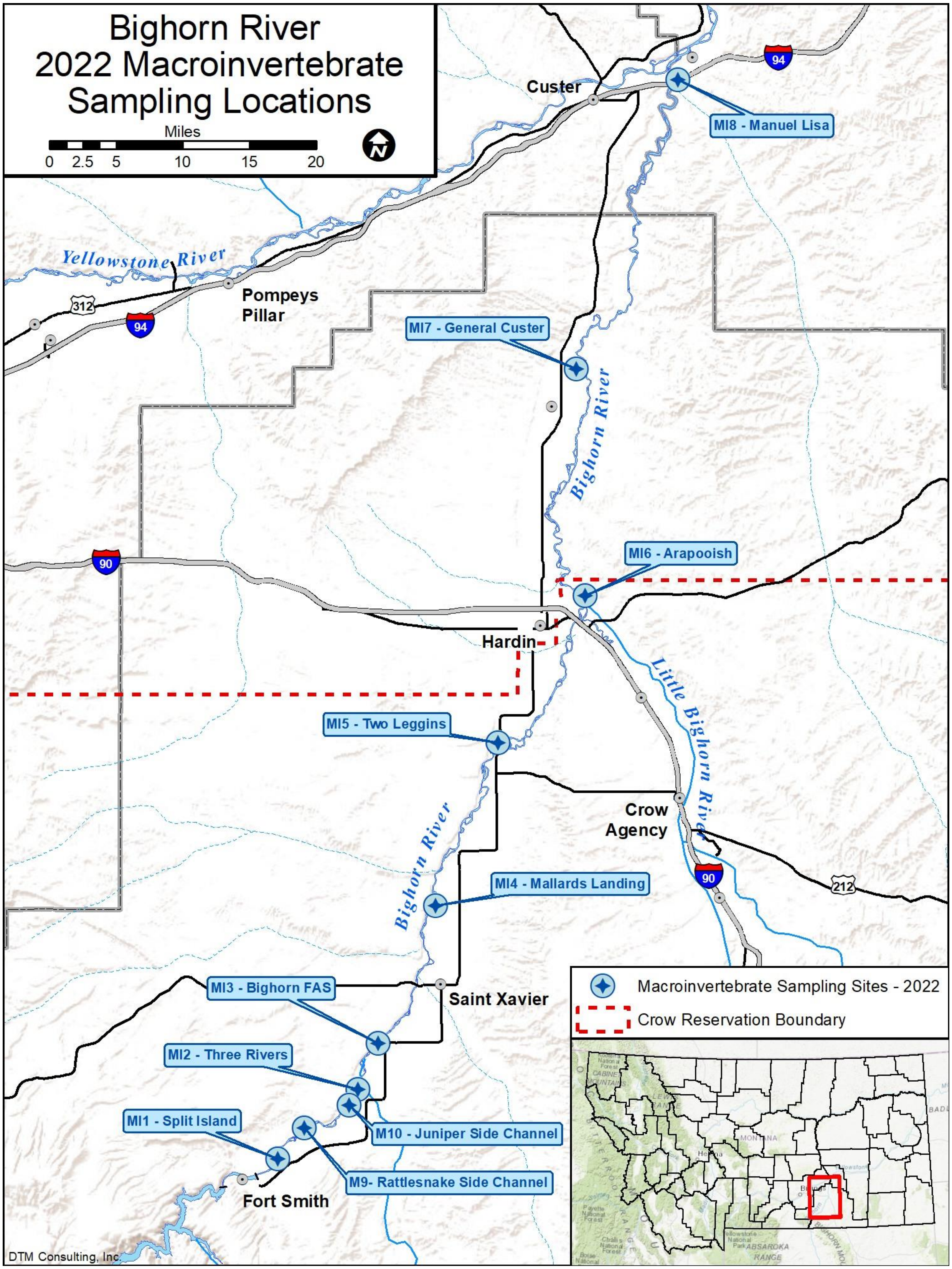
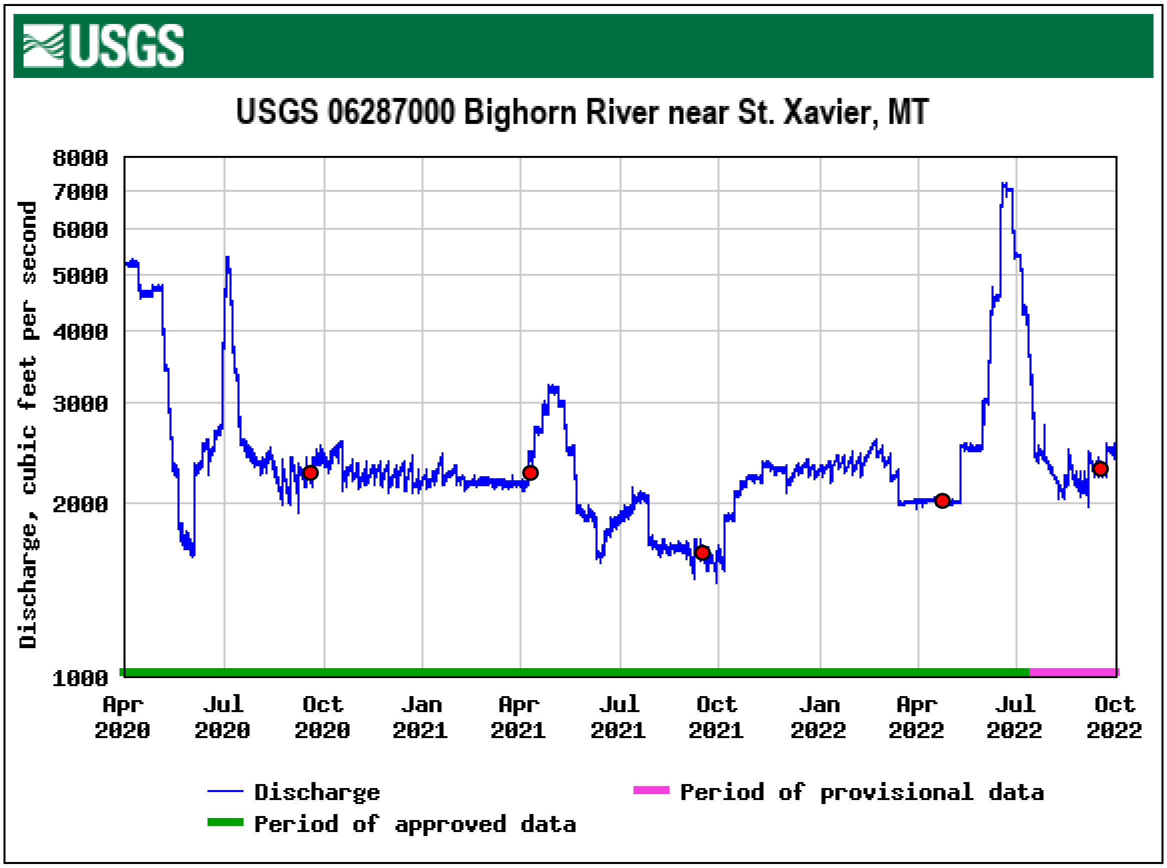


Figure 1. *Discharge reported during the 2020-2022 Seasonal BMI Sampling Visits (Red Dots).*



3.0 Results

3.1 General BMI Community. Overall, 94 total BMI taxa were collected across the 8 Bighorn River + 2 restored side channel (SC) sites in 2022 (***Appendix A***); average total number of taxa per site was 37 taxa (range 24-47 spp.); this is 3 more taxa per site than reported in 2021 (***Figure 2***). Restored side channels have been colonized by BMI quickly since Fall 2021 averaging 30 total BMI taxa in the Spring and 37 taxa in the Fall 2022. High points of overall taxa richness in 2022 were at Arapooish FAS and Two Leggins with 47 and 45 total taxa, respectively (***Figure 2*****).** Mayfly (E), caddisfly (T), plus one stonefly (P) (Total EPT Taxa) averaged 34% (5 to 78%) of the total BMI community per site and 12 species per site (2-23 spp.); Arapooish and Manuel Lisa reported the highest total EPT richness in the Fall with 23 and 20 species, respectively (***Figure 2***). EPT taxa richness in Fall 2022 (avg. 12.8 taxa) has significantly increased across all sites (T-test, p=0.029), since the Spring 2022 sampling which averaged 7.6 EPT species per site (***Figure 2***).

Figure 2*. Total BMI taxa, and EPT taxa (Mayflies + Stoneflies + Caddisflies) at the Bighorn River sites from 2020-2022. Sites are arranged from upstream (left) to downstream (right).*

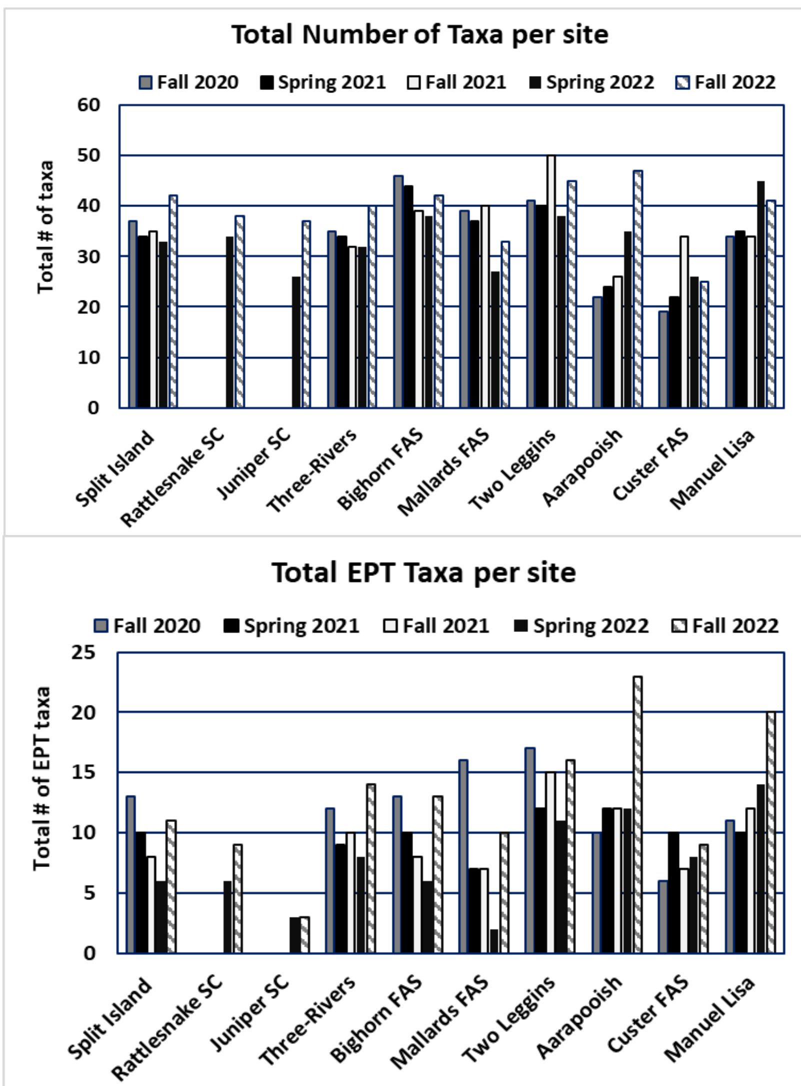
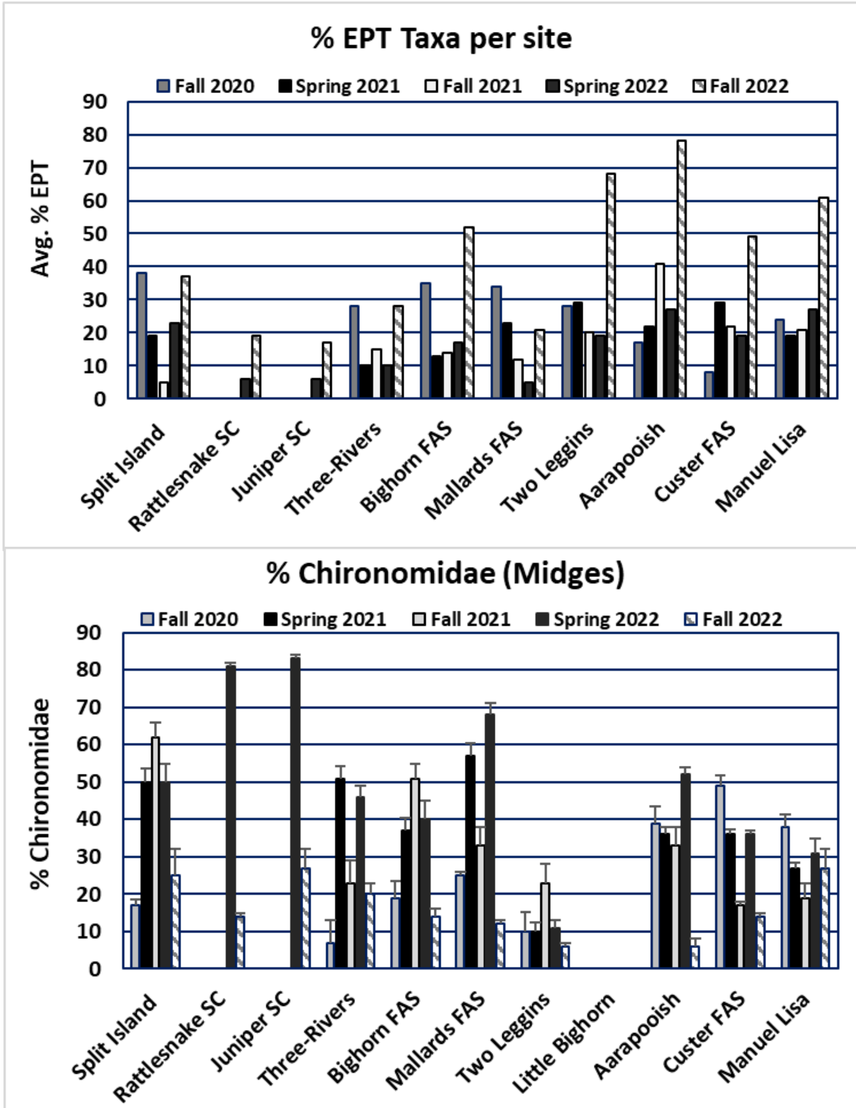


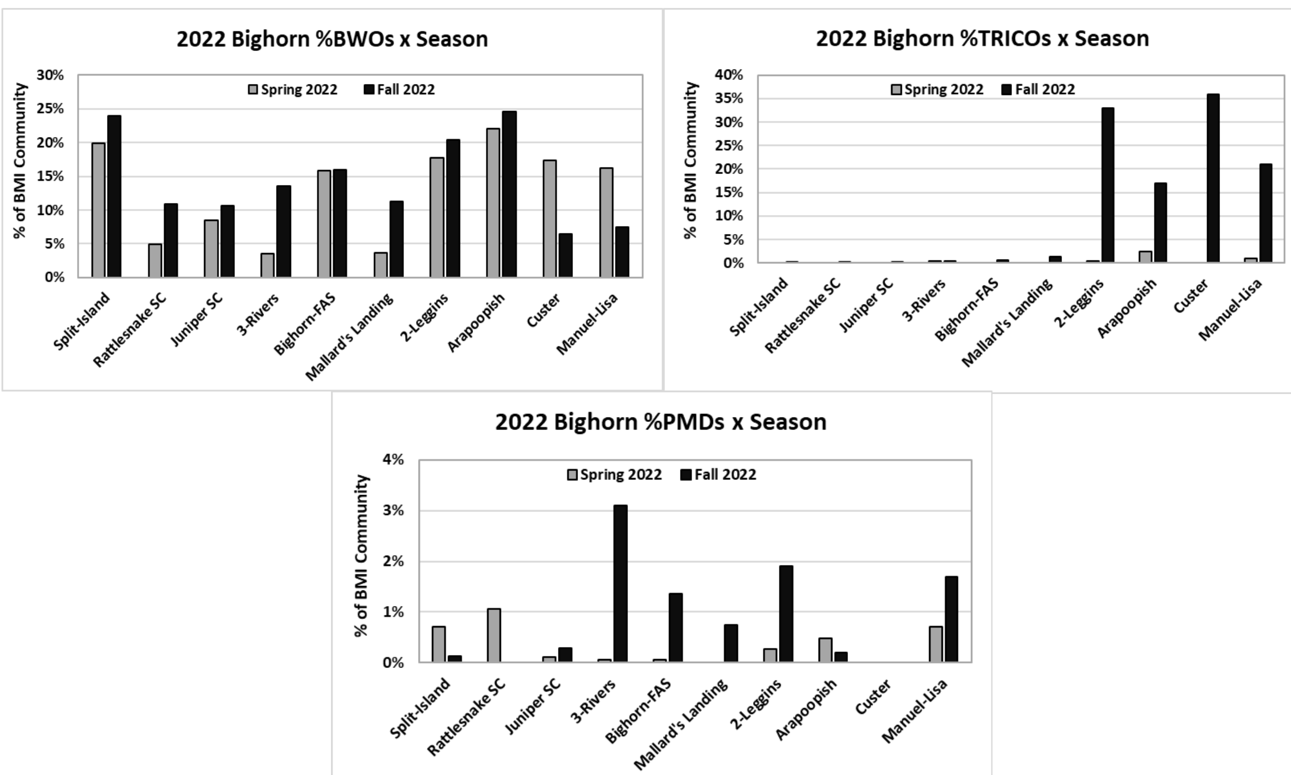
Figure 3*. Macroinvertebrate % EPT (Mayflies + Stoneflies + Caddisflies) (top) and % Chironomidae (bottom)*



Percent EPT taxa in the BMI community significantly increased across all sites in Fall 2022 compared to the Spring 2022 samples (T-test, p=0.0001); Arapooish FAS reported the highest %EPT (78%) in the Fall 2022 samples (***Figure 3***). Significant decreases in Chironomidae (Midges) abundance (T-test, p=0.0003) occurred concurrently with increases in Mayfly and Caddisfly numbers between Spring 2022 and Fall 2022; the restored side channels reported the highest % of Chironomidae in Spring 2022 (avg. 82%) along with Mallard’s Landing (68%), but in Fall 2022 reported some of the lowest midges (avg. 18%), but larval Blackflies (*Simulium sp.*) dominated these sites (***Figure 3***). Large increases in % EPT in the Fall 2022 at the Split Island and Three-Rivers sites now resemble the BMI community from Fall 2020, while Bighorn FAS and all downstream sites, except Mallard's Landing, have increased % EPT and EPT taxa richness to the highest values reported since monitoring began (***Figure 3***).

3.2) Mayflies. Twenty-one species of mayflies (E) were recorded throughout the study section in 2022: the dominant four groups were Blue Winged Olives *(Baetis tricaudatus, B. flavistriga*), Tiny BWOs (*Acentrella turbida, A. insignificans*) and Tricos (*Asioplax edmundsi*, *Tricorythodes explicatus*) and Pale Morning Duns (PMDs) (*Ephemerella excrucians* and *Serratella micheneri*) exhibited various spatial and temporal patterns in 2022 (***Figure 4***). BWO abundance has been most consistent between seasonal sampling in 2022, while Trico, and to a lesser extent PMD, nymph densities significantly increased between the Spring and Fall 2022 samples, indicating some large Trico hatches to come in 2023, especially downstream of Two-Leggins (***Figure 4***).

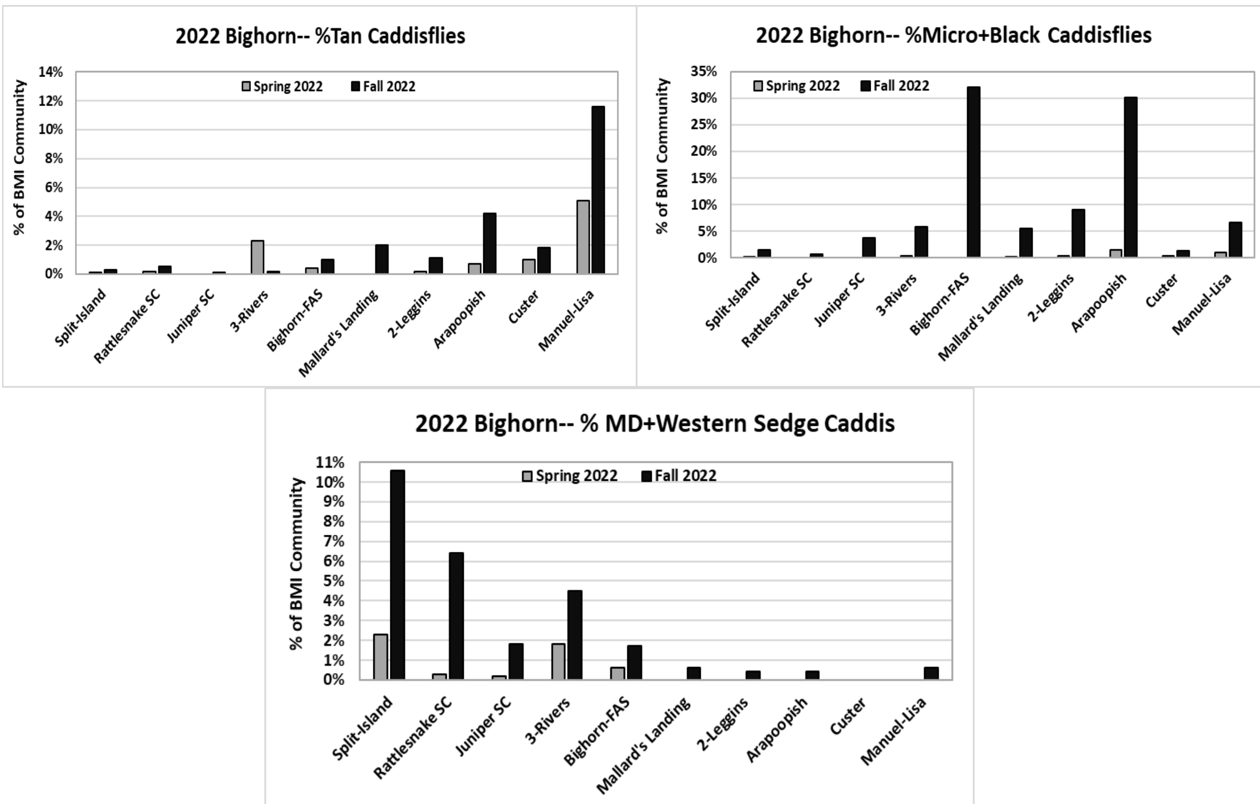
***Figure 4**. Percent of mayfly species contributing to the whole BMI community for Spring and Fall 2022. Note differences in y-axis values between taxa groups.*



3.3) Caddisflies. Of the 15 total species of caddisflies (T) collected in 2022, three dominant groups of caddisflies provide the most visible hatching adults in the summer: Tan Caddisflies *(*Hydropsychidae*: Cheumatopsyche spp. Hydropsyche occidentalis, Hydropsyche morosa gr., Hydropsyche bronta),* Black Caddis, Micro-caddis & Long-horned (*Oecetis avara, Ceraclea, Hydroptila spp.*) and the Mother's Day and Western Weedy Sedge Caddis (Brachycentridae:

Brachycentrus occidentalis and *Amiocentrus aspilus*). Similar to the Mayflies, most caddisflies groups were more abundant in the Fall 2022 after the high flows of June, and *Mother's Day Caddis and Western Sedges* should have a good hatch at the upper sites in the summer of 2023 (***Figure 5***).

Figure 5*. Percent of caddisfly groups contributing to the BMI community for Spring and Fall 2022. MD= Mother's Day Caddis. *Note differences in the y-axis values between taxa groups.*



3.4) Benthic Macroinvertebrate Densities. BMI densities averaged 16,776 individuals per m^2 (± 2,873 SE) across all Bighorn River sites in Fall 2022; these densities were significantly lower (T-test, p=0.012) than values reported in Spring 2022 (31,399 ind. per m^2 ± 5,370 SE) (***Figure 6, Table 2***). BMI densities reported at Three Rivers and Mallard's Landing in Spring 2022 averaged ~60,000 individuals per m^2; this is approaching the highest densities reported in September 1987 (75,670 ind. per m^2) after multiple years of no flushing flows (***Figure 6 & 7, Table 2***). BMI densities reported in the Bighorn River restored side channels (SC), Rattlesnake and Juniper, averaged ~30,000 individuals per m^2 during both seasons and were very comparable to densities in the adjacent Bighorn River mainstem; these SC benthic densities reached these levels since Fall 2021, just 6 months (***Figure 6, Table 2***). Large numbers of Chironomidae (Midges) were the initial colonizers of the Rattlesnake and Juniper Side channels (***Figure 3***), but in the Fall, blackfly larvae (*Simulium spp.*) were the dominant Diptera in the samples (***Appendix A***).

Figure 6*. Mean BMI densities at the Bighorn River sites across 2022 seasons. SC= restored side channel*

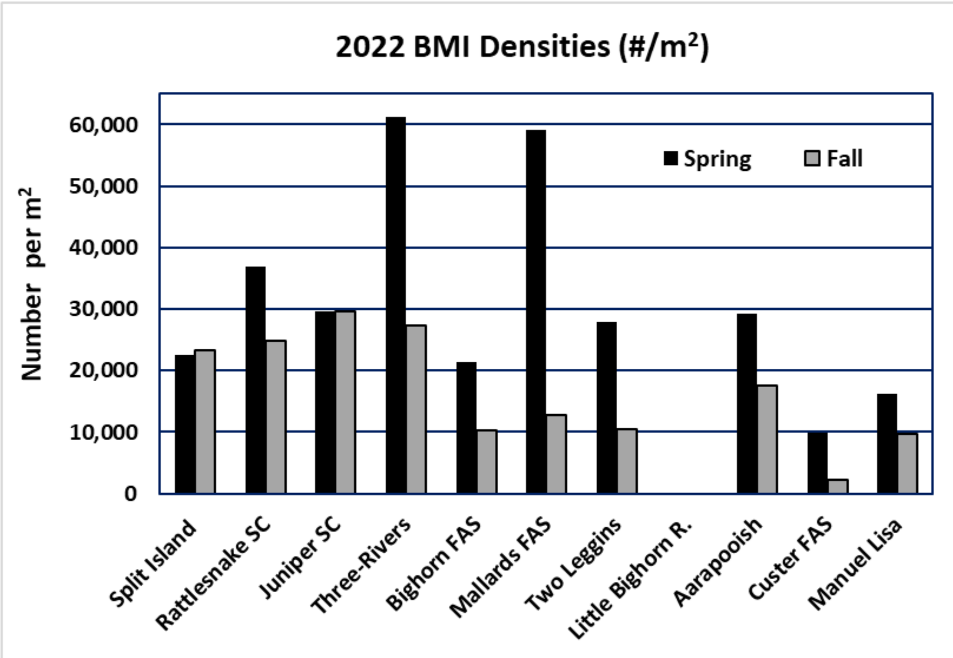
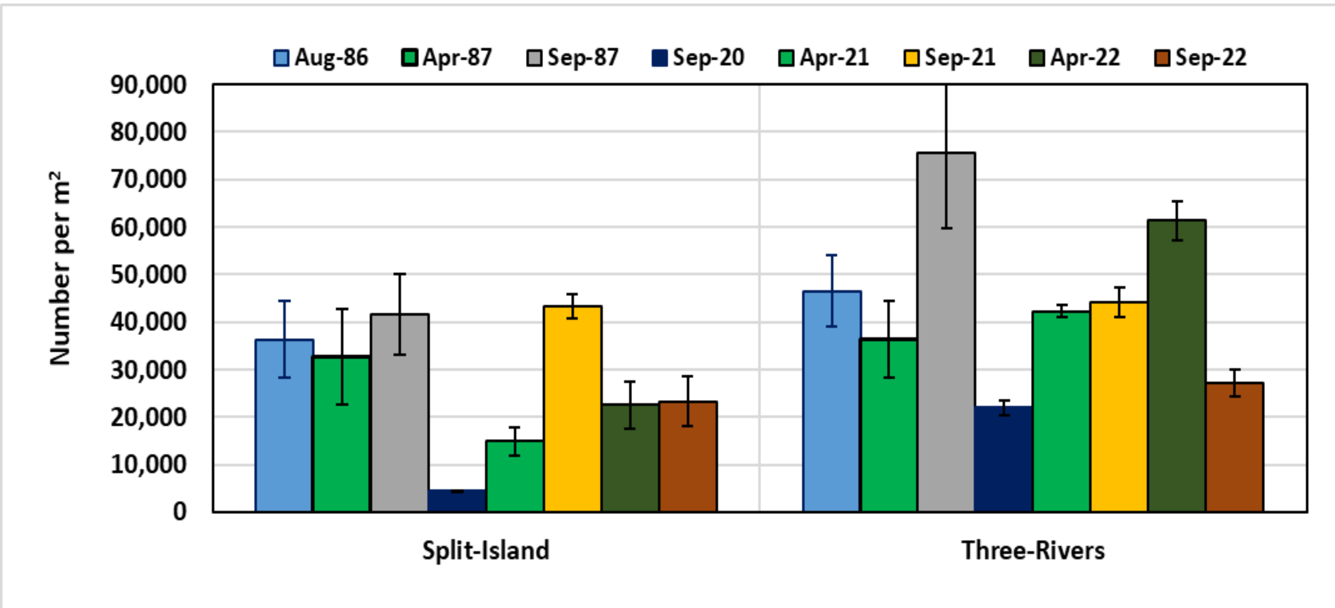


Table 2*. Macroinvertebrate Hess sample (n=3) numbers/densities at 10 Bighorn River sites from April & Sept. 2022. SC= restored side channel*

					Spring	Fall
	Hess Number				2022	2022
Site Name	**H1**	**H2**	**H3**	**Avg.**	**#/m2**	**#/m2**
BHR_Split Island	2,024	3,212	1,521	2,252	22,523	23,352
BHR_Rattlesnake SC	3,535	3,571	3,947	3,684	36,843	24,827
BHR_Juniper SC	3,200	2,188	3,488	2,959	29,587	29,544
BHR_Three Rivers	6,855	6,108	5,434	6,133	61,325	27,253
BHR_BighornFAS	2,324	2,872	2,436	2,147	21,470	10,270
BHR_MallardsFAS	5,800	6,330	5,636	5,922	59,220	12,667
BHR_TwoLegginsFAS	3,147	2,752	2,444	2,781	27,810	10,436
BHR_Arapooish	2,136	3,359	3,276	2,924	29,238	17,494
BHR_Custer	976	1,027	942	982	9,818	2,233
BHR_Manuel Lisa	624	2,512	1,709	1,615	16,151	9,680
				avg.	**31,399**	**16,776**
				SE	**5,370**	**2,873**

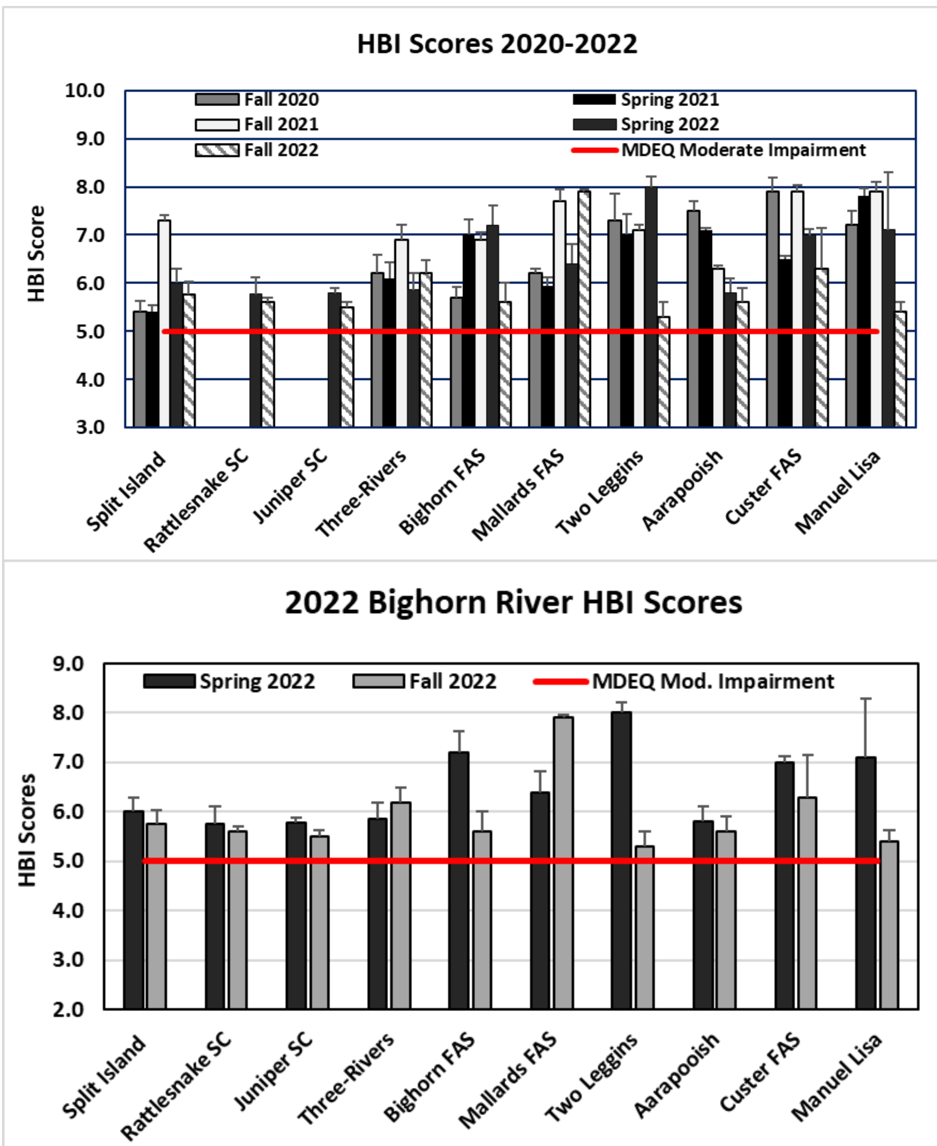
The Fall 2021 and Spring 2022 BMI densities across most of the upper Bighorn River sites have significantly increased during the 'less than normal' discharge year of 2021 compared to BMI densities reported from the Fall 2020 data (which had lower numbers attributed to large flushing flows of 2017-2019), (***Table 2, Figure 6***). We documented that the largest increases in BMI densities during the April 2021-April 2022 period are attributed to the silt-tolerant invertebrate taxa groups (Aquatic worms and midges) (***Figure 9***).

Figure 7*. Mean BMI densities at the 2 upper Bighorn River sites across 1986-2022 seasons. Error Bars = ± standard error.*



3.5) Tolerance Index Scores. HBI Scores >5.0 reported at all Bighorn River sites from 2020 to 2022 indicate that the BMI communities are experiencing moderate sediment/nutrient enrichment (***Figure 8***). Fall 2022 HBI scores across all sites, except for Mallard's Landing, averaged the lowest (5.9) (i.e., better health) of any other seasonal samples. In Fall 2021, HBI scores averaged 7.3 with all eight of the sites (100%) exhibiting significant enrichment scores >6.0. The biological integrity at the sites, as measured by the HBI, has significantly decreased (i.e., <u>gotten better</u>) from Fall 2021 to Fall 2022 (T-test, p=0.02); likely attributed to the flushing flows of June 2022.

Figure 8. *Macroinvertebrate HBI scores for the BHRA sites. Scores <u>above</u> the red line thresholds are moderately (>5.0) and significantly impaired (>6.0), respectively. Error bars are ±SE.*



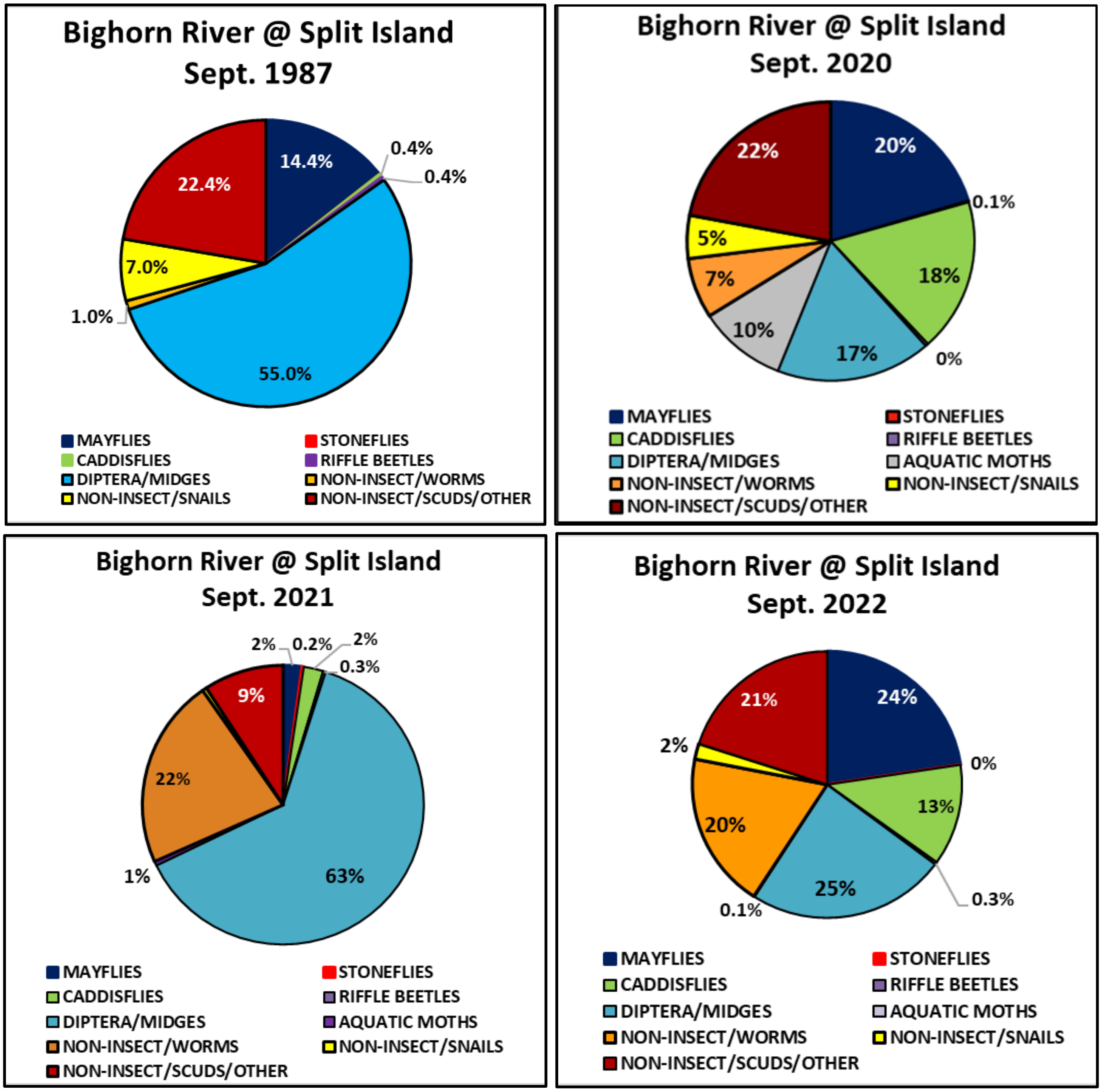
While most the Bighorn River sites exhibited HBI tolerance increases in 2021, Arapooish FAS has experienced a steady decline of the HBI (increasing BMI health, but still ranked moderately impaired) including its lowest scores ever during this Fall 2022 sampling (***Figure 8***).

3.6) NZMS. In 2022, non-native, New Zealand mudsnails (NZMS) were observed at seven of the 10 Bighorn River sites averaging ~120 individuals per m^2, including low densities in the newly restored side channels (***Appendix A*****).** NZMS in the newly constructed Rattlesnake and Juniper SC averaged 80 per m^2 and 13 per m^2 respectively, in the both the Spring and Fall 2022, indicating that these snails colonized the newly created habitats relatively quickly.

In 1987, the NZMS was not present at either of the upper sites, but during our first sampling in 2020, Three Rivers had NZMS densities of 880 ind. per m^2 and in 2021 we reported more than 2x those densities (1,730 per m^2), while in the Fall 2022 samples, NZMS densities averaged ~440 per m^2, a four-fold decrease (***Appendix A*****).** The presence of NZMS at these densities in the Bighorn River is not a surprise; they were first reported in the Afterbay river reach in 2002 and at the Three Mile and Bighorn FAS in 2005. NZMS typically occur at heavily fished access points (brought in on fisherman's boots and gear) and appear to have reached an equilibrium point (not too dominant) within the Bighorn River BMI community.

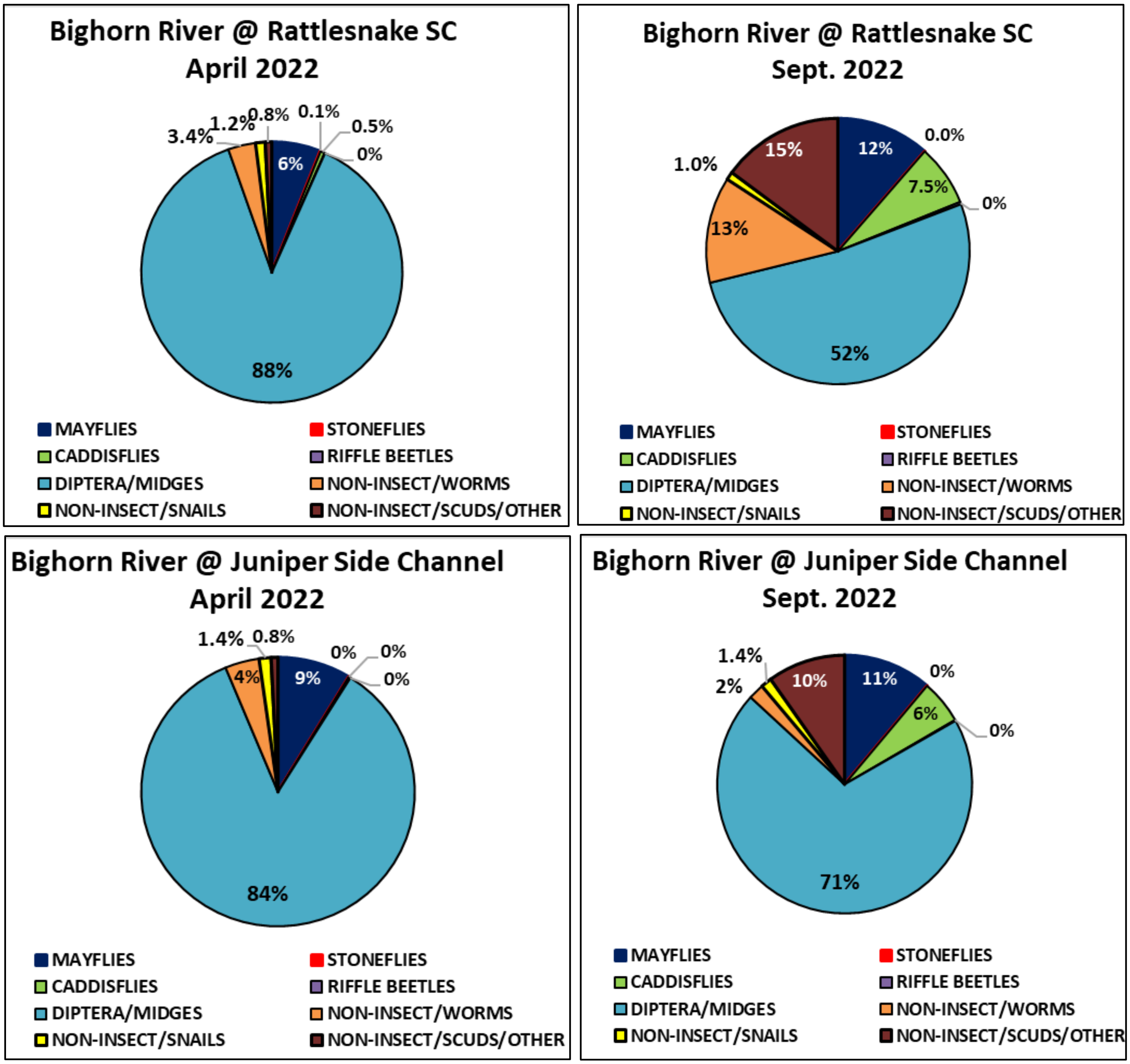
3.7) Split Island: The biggest differences observed in the composition of the BMI community at Split Island in 2020-2022 vs. 1987 were shifts from Mayflies and Caddisflies in 2020 to the increasing dominance of the midges and aquatic worms in 2021 (similar to 1987), and then a reversal back to more Mayflies and Caddisflies in Fall 2022. In Fall 2020, we reported an increase in abundance of the caddisflies, *Amiocentrus aspilus* and *Hydropsyche spp.* (which were both present in low numbers in 1987), but now contribute ~18% to the community. But, by Fall 2021, caddisflies and mayflies have declined to only 2% of the BMI community. Additionally, the aquatic moth *Petrophila* which was not reported in 1987, contributed ~10% of the BMI community in 2020, but is <1% in 2021 and 2022 (***Figure 9***). Aquatic worms have increased from 7% to 22% during the low-flow year between 2020 and 2021, and still are 20% in 2022; while caddisflies, mayflies and scuds are more evenly contributing to the BMI community in Fall 2022 (***Figure 9***).

Figure 9. *Macroinvertebrate Composition represented at Split Island Fall 1987 vs. 2020-2022.*

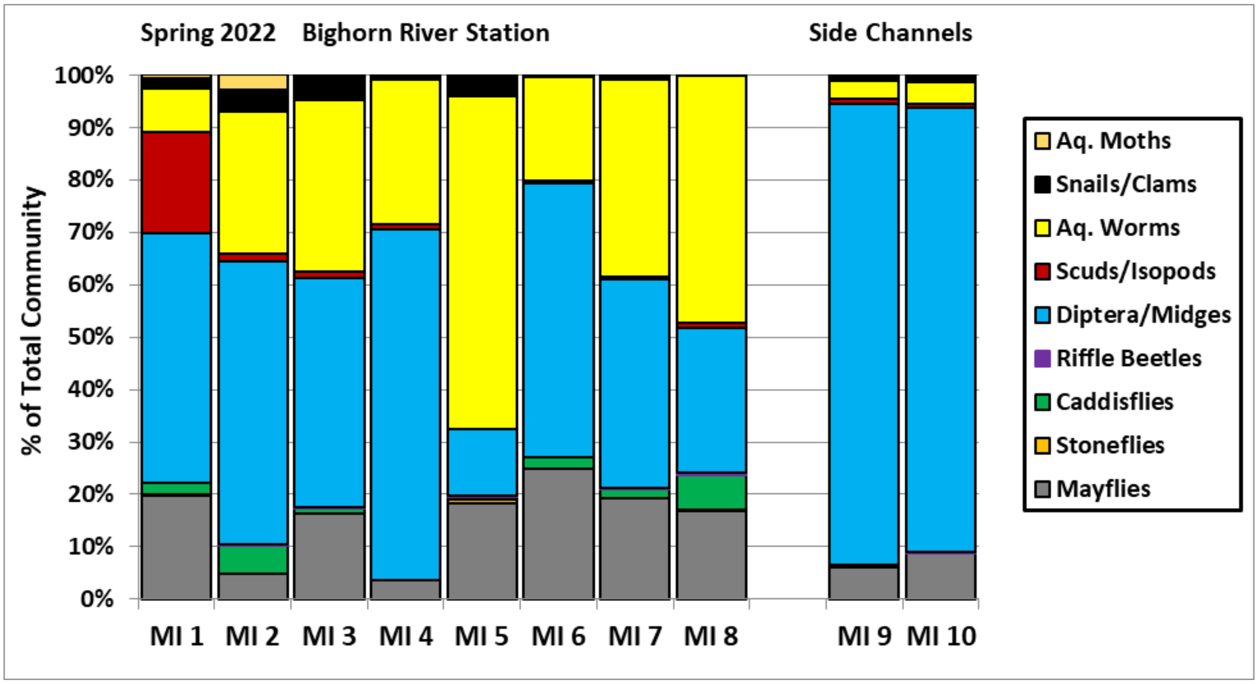


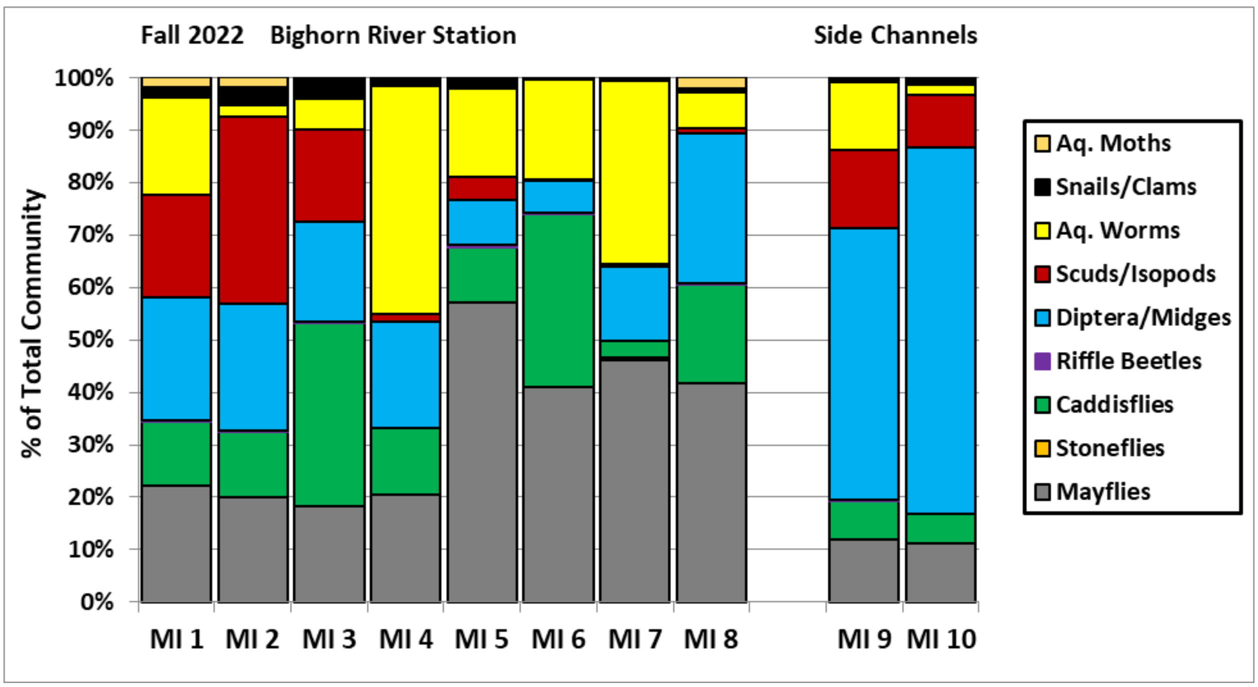
3.8) Rattlesnake and Juniper Side Channels (SC). The biggest differences observed in the composition of the BMI community at Rattlesnake and Juniper SC in Spring vs. Fall 2022 were increases in % Mayflies, Amphipods (Scuds) and Caddisflies in the Fall with the decreasing dominance of the midges (Diptera) which were mostly replaced by Blackfly larvae in the Fall (***Figure 10, Appendix A***).

Figure 10. *Seasonal Macroinvertebrate Composition documented at Rattlesnake (top) and Juniper (bottom) Side Channels (SC) in 2022.*



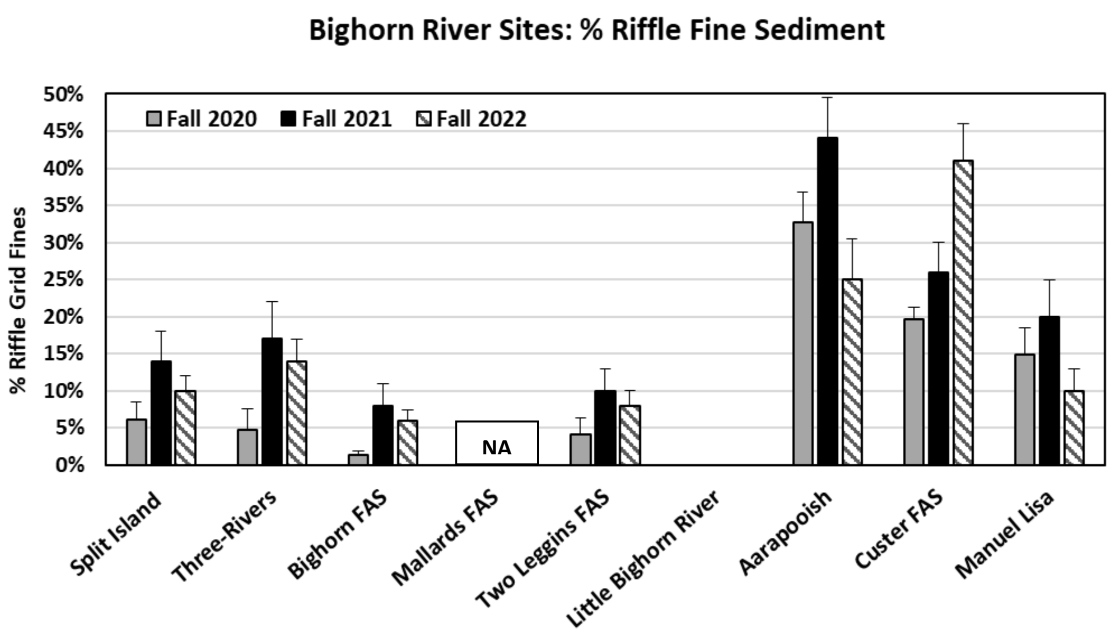
In terms of the similarity of the side channel BMI community to the adjacent mainstem Bighorn River, the Fall 2022 Rattlesnake SC samples had a 46% Community Similarity (CS) and 65% Taxa Similarity with the Split Island site, and Juniper SC had a 42% Community Similarity (CS) and 51% Taxa Similarity with the 3-Rivers site (**Appendix C**).


Spring 2022 Bighorn River Station
Side Channels
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% of Total Community
MI 1 MI 2 MI 3 MI 4 MI 5 MI 6 MI 7 MI 8 MI 9 MI 10
Aq. Moths
Snails/Clams
Aq. Worms
Scuds/Isopods
Diptera/Midges
Riffle Beetles
Caddisflies
Stoneflies
Mayflies


Fall 2022 Bighorn River Station
Side Channels
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% of Total Community
MI 1 MI 2 MI 3 MI 4 MI 5 MI 6 MI 7 MI 8 MI 9 MI 10
Aq. Moths
Snails/Clams
Aq. Worms
Scuds/Isopods
Diptera/Midges
Riffle Beetles
Caddisflies
Stoneflies
Mayflies

3.9) Stream Habitat Results. Benthic fine sediment in riffles increases substantially downstream of the Little Bighorn River (***Figure 11***). We documented a significant increase in riffle sediments at sites in Fall 2021 compared to Fall 2020 (T-test, p-value=0.0003). Fall 2022 riffle sediment has shown declines (improvements) across most sites, except for at Custer FAS, but this was not significantly less than Fall 2021 (T-test, p=0.39). We recorded the 'cleanest' riffle sediments in Fall 2020, especially at the Bighorn FAS (MI3) with <2% fines in the gravels, but this site has increased to >5% riffle sediments in 2021 and 2022 (***Figure 11***). Mallards Landing FAS was so turbid from the irrigation returns during all years' September visits that we could not see the grids for measurements (***Figure 11***).

Figure 11. *Percent riffle fine sediment calculated from the MDEQ grid tosses for the Fall 2020-2022 Bighorn River sites arranged u/s to d/s. NA* = too turbid to see the grid count.*



Three sites where aquatic vegetation (including filamentous algae *Cladophora*) may be adversely affecting benthic riffle habitats in the Fall 2022 were at the Bighorn FAS with an average of 55% vegetation coverage, Three Rivers with ~40% coverage and Split Island avg. 20% (***Appendix B, Photo 18***).

4.0 Conclusions

- Spring and Fall 2022 BMI sample data across the 10 Bighorn River sites revealed that the flushing flows of June have significantly reduced the densities of macroinvertebrates, especially Chironomidae (Midges) and Aquatic worms, and increased the richness and percent of EPT taxa (mayfly and caddisflies) across the sites.
- Bighorn River restored side channels (SC), Rattlesnake and Juniper, were colonized very quickly with BMI and averaged ~30,000 individuals per m^2 during both seasons which was comparable to densities in the adjacent Bighorn River mainstem; these SC benthic densities reached these levels in just ~6 months post restoration.
- Initial colonizers of the restored Rattlesnake and Juniper SC were large numbers of Chironomidae (Midges) in Spring 2022, while in the Fall, blackfly larvae (*Simulium spp*.) were the dominant Diptera in the benthic samples. These side channels averaged a fairly low 58% species similarity with the Bighorn River mainstem BMI community.
- Species diversity and EPT taxa richness increased with increasing distance from Yellowtail Dam; in the Fall 2022, Two-Leggins and Arapooish sites had the highest total taxa richness (avg. 45 taxa) and EPT richness was highest at Arapooish and Manuel Lisa FAS (avg. 21.5 species).
- By comparing historical data at Split Island and Three Rivers, we have documented large decreases in BMI densities following years with flushing flows 2017-2019 and 2022 with large population increases occurring during the non-flushing flow years (Fall 2020-Spring 2022) since the late 1980's, and shifts in the benthic community, including decreases of midges and mayflies, increases in caddisflies and the addition of New Zealand mudsnails, the aquatic moth, *Petrophila* and the isopod, *Caecidotea*.
- The non-native, New Zealand mudsnails were observed at seven of the 10 Bighorn River sites in 2022 (4 sites in 2021), including low densities in the newly restored side channels. NZMS densities averaged ~120 per m^2 in Fall 2022 samples; these are lower than reported in 2021 and they may have been distributed to new sites by the high flows of June.
- A dominant result of this research is that dynamic, regulated river conditions, especially with multiple years of sustained flushing flows (2017-2019) followed by drought-type

late-season flows can significantly alter macroinvertebrate communities within different sections of the river. Fall 2020 BMI numbers have been significantly reduced from high flushing flows within a couple miles of the dam (MI1, Split Island and MI2, Three Rivers) compared to previous years (1986-87), but these densities can increase quickly with the low river flows, as we documented in 2021 and then decrease with the next flushing flow, as documented between Spring and Fall 2022.

- We conclude this 3rd year of analysis by indicating that BMI communities are exhibiting improved biological health after the flushing flows throughout the Bighorn River in June 2022 but are still moderately impaired from sediments or nutrients based on the HBI. The use of the HBI is a good surrogate for determining nutrient and/or sediment enrichment compared to the MDEQ's Plains and Low Valley MMI's because the Bighorn River is such a unique ecosystem that does not fit perfectly into either classification.
- The main reasons for the observed seasonal ecological changes in the BMI communities between Fall 2020 and Fall 2022 can be causally linked to prior high discharge years (2017-2019) followed by lower discharge flows in 2021, which increased water temperatures, nutrient levels, sediment accumulations; then in June 2022 the subsequent flushing flows reversed these trends.

5.0 Literature Cited.

Apfelbeck, R. 2007. Water quality and biological characteristics of Montana streams in a statewide monitoring network, 1999-2005. Montana Department of Environmental Quality, Helena.

Barbour, M., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. United States Environmental Protection Agency; Office of Water: Washington, D.C.

Bollman, W. 2006. Summary of aquatic invertebrate data from the Montana statewide-monitoring network, 2001-2005. Rhithron Associates, Inc. Missoula, Montana

Brandt, D. 2001. Temperature preferences and tolerances for 137 common Idaho macroinvertebrate taxa. Idaho Department of Environmental Quality. Coeur d'Alene, Idaho.

Bukantis, R. 1996. Rapid bioassessment macroinvertebrate protocols: Sampling and sample analysis SOPs. MT Dept. of Environmental Quality, Planning, Prevention and Assistance Division, Helena, MT.

Jessup, B. 2006. Ecological Data Application System (EDAS) Version MT 3.3.2k A User's Guide. Tetra Tech, Inc.

MDEQ 2016. Montana Final 2016 Water Quality Integrated Report. Report to the U.S. Environmental Protection Agency. Doc# WQPBIMTSTR-011f. 72 pages

MDEQ 2012. Sample Collection, Sorting, and Taxonomic Identification of Benthic Macroinvertebrates Standard Operating Procedure. Helena, MT: Montana Department of Environmental Quality. WQPBWQM-012.
http://deq.mt.gov/wqinfo/qaprogram/PDF/SOPs/WQPBWQM-009rev2_final_web.pdf

MDEQ 2007. Water Quality and Biological Characteristics of Montana Streams in a Statewide Monitoring Network, 1999-2005. Water Quality Planning Bureau. Helena, MT.

MFWP 2020. Statewide Angling Pressure Report. Fisheries Division. Helena, MT.

Stagliano, D.M. 2016. Baseline Macroinvertebrate Monitoring Report 2016 for the Upper Missouri River, MT. 27 pages + appendices. Prepared for UMOWA, Wolf Creek, MT

Vinson, M.R. and C.P. Hawkins. 1996. Effects of sampling area and subsampling procedure on comparisons of taxa richness among streams. Journal of the North American Benthological Society 15: 393-400.

APPENDIX A. MACROINVERTEBRATE TAXA LIST AND ABUNDANCE AT ALL SITES

Appendix A. Macroinvertebrate taxa list and avg. number per meter squared (#/m2) for the Bighorn Sites Spring 2022

River Mile from Yellowstone R.	RM82	RM79	RM76	RM75	RM72	RM63	RM52	RM40	RM24	RM1.8	
	Split Island	**Rattlesnake SC**	**Juniper SC**	**Three-Rivers**	**Bighorn FAS**	**Mallards FAS**	**Two Leggins FAS**	**Aarapooish FAS**	**Custer FAS**	**Manuel Lisa FAS**	**# of Sites**
COLEOPTERA (Riffle Beetles)											
Optioservus quadrimaculatus	0	0	0	40	13	0	13	0	0	10	4
Zaitzevia parvula	0	0	0	0	0	0	0	0	0	24	1
Microcylloepus pusillus	0	0	0	0	0	0	0	0	0	10	1
Dubiraphia minima	0	0	0	0	20	37	0	20	22	10	5
Haliplus sp.	0	20	20	0	0	0	0	0	0	0	2
DIPTERA (midges/flies)	11302	33000	25207	27960	12560	39270	3468	15472	3850	5393	10
Chironomidae (midges)	11185	30320	24914	27920	11687	39197	2901	15453	3762	5349	10
Diamesa spp.	570	15700	8187	1680	2873	147	844	421	33	39	10
Cricotopus spp.	7706	6080	5860	8400	3813	2383	385	1690	176	503	10
Phaenopsectra sp	20	280	220	120	500	2713	242	204	715	1181	10
Cardiocladius spp.	148	120	2367	160	160	0	67	72	66	74	9
Parakiefferiella	973	80	1380	1280	0	25667	1161	7005	1870	936	9
Eukiefferiella spp.	827	3700	1333	560	267	183	52	0	0	0	7
Dicrotendipes sp.	60	20	0	640	640	3263	0	178	0	127	7
Monodiamesa sp.	11	0	0	0	20	37	91	20	0	13	6
Parametriocnemus sp.	44	4240	487	5520	13	0	0	171	0	0	6
Potthastia sp.	51	0	20	0	13	37	0	0	22	0	5
Orthocladius spp.	494	0	5060	7320	140	0	0	0	33	0	5
Tvetenia sp.	180	0	0	2160	0	1980	33	382	0	0	5
Cryptochironomus sp.	0	0	0	0	0	110	13	112	209	50	5
Microtendipes sp	0	0	0	0	0	183	13	4315	407	473	5
Endochironomus sp.	0	0	0	0	0	440	0	33	33	23	4
Rheotanytarsus sp.	0	0	0	0	100	0	0	440	198	1888	4
Micropsectra spp.	0	20	0	80	53	0	0	0	0	0	3
Pagastia sp	0	80	0	0	73	0	0	0	0	0	2
Polypedilum spp.	0	0	0	0	0	257	0	0	0	40	2
Tanytarsus sp.	100	0	0	0	0	220	0	0	0	0	2
Nanocladius sp.	0	0	0	0	0	0	0	410	0	0	1
Paracladius sp.	0	0	0	0	3020	0	0	0	0	0	1
Psectrocladius sp.	0	0	0	0	0	1577	0	0	0	0	1
Tipula sp. (Cranefly)	33	0	20	0	153	0	335	0	0	0	4
Ceratopogoninae (Biting Midges)	0	0	0	0	33	0	191	0	0	0	2
Limnophora	0	20	13	40	27	0	0	0	0	0	4
Simulium spp. (Blackflies)	84	2640	260	0	660	73	27	0	0	34	7
Hemerodromia sp. (Danceflies)	0	0	0	0	0	0	13	20	0	10	3
Tabanidae	0	20	0	0	0	0	0	0	0	0	1
EPHEMEROPTERA (Mayflies)	4486	2260	2807	2520	4373	2090	5141	7269	1859	1898	10
Acentrella insignificans (Tiny BWO)	0	20	0	0	13	0	13	0	0	0	3
Acentrella turbida (Tiny BWO)	20	0	0	0	0	0	0	0	11	0	2
Baetis flavistriga (BWO)	0	0	0	0	0	0	67	0	44	41	3
Baetis tricaudatus (BWO)	4282	1840	2767	2200	4333	2090	4852	6394	1804	1638	10
Ephemerella excrucians (PMDs)	184	400	40	40	27	0	67	132	0	69	8
Tricorythodes explicatus (Tricos)	0	0	0	280	0	0	143	742	0	150	4
LEPIDOPTERA (Aquatic Moths)											
Petrophila sp.	175	0	0	1440	73	0	0	0	0	14	4
ODONATA (Dragonflies)											
Ophiogomphus sp.	0	0	0	0	0	37	0	0	0	17	2
PLECOPTERA (Stoneflies)											
Isoperla quinquepunctata (Little Yellow Stones)	13	20	0	0	0	0	183	0	0	4	4

Appendix A. (cont.) Macroinvertebrate taxa list and avg. number per meter squared (#/m2) for the Bighorn Sites Spring 2022

River Mile from Yellowstone	RM82	RM79	RM76	RM75	RM72	RM63	RM52	RM40	RM24	RM1.8	
	Split Island	**Rattlesna ke SC**	**Juniper SC**	**Three-Rivers**	**Bighorn FAS**	**Mallards FAS**	**Two Leggins FAS**	**Aarapooish FAS**	**Custer FAS**	**Manuel Lisa FAS**	**# of Sites**
TRICHOPTERA (Caddisflies)	604	200	60	2760	280	36	159	593	154	984	10
Hydropsyche occidentalis	11	60	0	1280	53	0	0	99	44	71	7
Amiocentrus aspilus	573	120	60	880	180	0	46	0	11	0	7
Hydroptila spp.	20	0	0	0	0	37	0	277	11	67	5
Brachycentrus occidentalis	0	0	0	240	0	0	13	39	22	37	5
Cheumatopsyche spp.	0	0	0	0	47	0	40	0	66	14	4
Oecetis avara	0	0	0	240	0	0	0	20	0	37	3
Glossosoma sp.	0	0	0	0	0	0	13	39	0	4	3
Ceratopsyche spp.	0	20	0	0	0	0	0	66	0	0	2
Hydropsyche nr bronta	0	0	0	0	0	0	0	13	0	103	2
Nectopsyche sp.	0	0	0	0	0	0	0	20	0	18	2
Hydropsyche morosa gr.	0	0	0	0	0	0	0	0	0	633	1
Hydropsyche C. cockerelli	0	0	0	120	0	0	0	0	0	0	1
Leucotrichia pictipes	0	0	0	0	0	0	13	0	0	0	1
Lepidostoma sp.	0	0	0	0	0	0	0	20	0	0	1
Helicopsyche borealis	0	0	0	0	0	0	33	0	0	0	1
ANNELIDA (Worms/Leeches)	877	1260	1210	13760	8747	15990	17614	5798	4620	7544	10
Tubificidae (Aquatic Worm)	823	1180	1140	4960	8513	15547	17173	4352	3894	7385	10
Lumbricidae (Aquatic Worm)	53	20	67	8800	127	0	361	0	0	21	7
Naididae	0	0	0	0	0	0	0	1447	726	0	2
Erpobdellidae	0	60	0	0	0	0	53	0	0	124	3
Glossophonia complanata	0	0	0	0	107	0	13	0	0	10	3
Helobdella stagnalis	0	0	0	0	0	440	13	0	0	4	3
CRUSTACEA (Scuds/Isopods)	5175	100	233	10800	220	0	0	0	0	37	6
Caecidotea sp.	3788	20	153	5600	187	0	0	0	0	37	6
Gammarus spp.	1386	80	80	4960	33	0	0	0	0	0	5
Hyalella azteca	0	0	0	240	0	0	0	0	0	0	1
MOLLUSCA (Snails/Clams)	64	460	340	800	1100	73	921	46	143	104	10
Physella sp. (Pouch snails)	44	300	187	480	993	73	675	33	132	43	10
Ferrissia rivularis (Limpets)	0	0	0	0	0	0	0	13	0	51	2
Potamopyrgus antipodarum (NZMS)	20	100	13	320	107	0	53	0	0	0	6
Gyraulus sp.	0	40	140	0	0	0	73	0	11	0	4
Fossaria sp.	0	20	0	0	0	0	0	0	0	0	1
Pisidium sp. (Fingernail Clams)	0	0	0	0	0	0	120	0	0	10	2
OTHER Non-Insects	492	260	160	1000	1747	220	165	105	44	37	10
Turbellaria (Flatworms)	435	20	127	720	1747	183	165	33	44	37	10
Nematoda (Horsehair Worms)	11	100	0	120	0	0	0	39	0	0	4
Hydracarina (Water-Mites)	47	140	33	160	0	37	0	33	0	0	6
Total Taxa per site	**32**	**33**	**25**	**32**	**35**	**24**	**36**	**34**	**24**	**44**	**32.1**
EPT Taxa per site	**7**	**7**	**3**	**12**	**13**	**16**	**17**	**10**	**6**	**11**	**10.2**

Appendix A. Macroinvertebrate taxa list and avg. number per meter squared (#/m2) for the Bighorn Sites Fall 2022

River Mile from Yellowstone R.	RM82	RM79	RM76	RM75	RM72	RM63	RM52	RM40	RM24	RM1.8	
	Split Island	**Rattlesnake SC**	**Juniper SC**	**Three-Rivers**	**Bighorn FAS**	**Mallards FAS**	**Two Leggins FAS**	**Aarapooish FAS**	**Custer FAS**	**Manuel Lisa FAS**	**# of Sites**
COLEOPTERA (Riffle Beetles)											
Optioservus quadrimaculatus	27	30	0	26	0	0	12	40	0	0	5
Zaitzevia parvula	0	0	0	0	0	0	11	0	0	0	1
Microcylloepus pusillus	0	0	13	0	13	0	0	40	0	26	4
Stenelmis	0	0	0	0	0	0	8	0	0	13	2
Dubiraphia minima	0	0	0	0	0	0	8	0	0	0	1
Helichus lithophilus	0	0	0	0	0	0	8	0	0	0	1
Agabus sp.	10	27	20	0	13	0	0	0	0	0	4
Haliplus sp.	23	0	0	0	8	0	0	0	0	0	2
DIPTERA (midges/flies)	5927	13853	21193	5698	1936	1707	879	1127	303	2787	10
Chironomidae (midges)	5803	1653	8487	5441	1429	1507	645	1093	303	2720	10
Cricotopus spp.	2023	207	2140	513	381	107	269	153	60	1320	10
Polypedilum spp.	87	360	2173	2721	376	160	174	293	67	947	8
Dicrotendipes sp.	310	47	287	924	200	853	56	73	0	27	9
Eukiefferiella spp.	117	407	980	0	91	40	0	0	0	0	5
Cardiocladius spp.	990	53	0	154	0	0	0	73	0	0	4
Orthocladius spp.	743	67	453	0	0	0	0	0	0	0	3
Phaenopsectra sp	43	0	627	257	93	53	0	80	0	0	6
Thienemannimyia gr.	0	160	427	385	0	27	4	0	0	27	6
Diamesa spp.	930	20	0	0	0	0	0	0	0	0	2
Potthastia sp.	213	100	153	282	27	0	0	0	0	0	5
Parametriocnemus sp.	0	67	400	103	0	0	0	93	0	0	4
Rheotanytarsus sp.	0	0	0	103	40	53	0	60	117	280	6
Parakiefferiella	233	47	107	0	0	40	16	67	0	13	7
Tvetenia sp.	43	67	253	0	0	13	16	0	0	0	5
Rheocricotopus sp.	0	47	287	0	0	0	0	0	0	0	2
Micropsectra spp.	0	7	200	0	0	0	77	0	13	0	4
Microtendipes sp	0	0	0	0	53	0	11	133	33	53	5
Cryptochironomus sp.	13	0	0	0	128	0	21	13	13	13	6
Endochironomus sp.	0	0	0	0	0	80	0	53	0	27	3
Prodiamesa sp.	10	0	0	0	0	80	0	0	0	13	3
Tanytarsus sp.	47	0	0	0	0	0	0	0	0	0	1
Paracladius sp.	0	0	0	0	40	0	0	0	0	0	1
Ceratopogoninae (Biting Midges)	0	20	0	0	0	0	8	0	0	0	2
Tipulidae (Cranefly)											
Tipula sp.	43	47	13	0	27	0	84	0	0	0	5
Limnophora	37	307	220	0	83	13	0	0	0	0	5
Simulidae (Blackflies)											
Simulium spp.	23	11827	12453	231	397	187	142	33	0	53	9
Hemerodromia sp. (Danceflies)	0	0	0	0	0	0	0	0	0	13	1
EPHEMEROPTERA (Mayflies)	6227	2067	3500	4440	1840	1680	6042	7613	983	4040	10
Acentrella insignificans (Tiny BWOs)	0	33	0	26	40	0	0	1973	30	413	6
Acentrella turbida (Tiny BWOs)	13	0	0	26	0	227	142	1347	0	53	6
Baetis flavistriga (BWO)	0	47	0	0	0	0	19	113	0	0	3
Baetis intercalaris (BWO)	0	0	0	0	0	0	0	60	0	0	1
Baetis tricaudatus (BWO)	6153	1927	3300	3465	1619	1200	2157	493	103	27	10
Camelobaetidius sp.	0	0	0	0	0	0	0	200	0	0	1
Centroptilum bifurcatum	0	0	0	0	0	0	0	0	10	27	2
Fallceon quilleri	0	0	0	0	0	0	0	33	0	280	2
Attenella margarita (PMDs)	0	0	0	0	69	93	0	20	0	0	3
Ephemerella excrucians (PMDs)	0	0	33	26	45	0	0	0	0	160	4
Serratella micheneri (PMDs)	33	0	93	796	0	0	167	0	0	0	4
Ecdyonurus sp. (Flat-Headed Mayflies)	0	0	0	0	0	0	45	0	0	0	1
Leucrocuta sp. (Flat-Headed Mayflies)	0	0	0	0	0	0	0	53	0	27	2
Macaffertium terminatum (Flat-Headed M	0	0	0	0	0	0	24	0	20	733	3
Rhithrogena sp. (Flat-Headed Mayflies)	0	0	0	0	0	0	0	160	27	0	2
Choroterpes albiannulata	0	0	0	0	0	0	0	40	0	173	2
Traverella albertana	0	0	0	0	0	0	0	20	0	67	2
Paraleptophlebia bicornuta	0	0	0	0	0	0	0	0	0	13	1
Ephoron album	0	0	0	0	0	0	0	13	0	0	1
Asioplax edmundsi (Tricos)	0	0	0	0	0	0	0	33	0	40	2
Tricorythodes explicatus (Tricos)	27	60	73	103	67	160	3487	3053	793	2027	10
LEPIDOPTERA (Aquatic Moths)											
Petrophila sp.	40	0	0	50	0	0	0	37	0	21	4
ODONATA (Dragonflies)											
Ophiogomphus severus	0	0	0	0	0	0	50	20	0	16	3
PLECOPTERA (Stoneflies)											
Isoperla quinquepunctata (Little Yellow Stones)	0	0	0	0	0	0	0	0	13	0	1

Appendix A. (cont.) Macroinvertebrate taxa list and avg. number per meter squared (#/m2) for the Bighorn Sites Fall 2022

River Mile from Yellowstone	RM82	RM79	RM76	RM75	RM72	RM63	RM52	RM40	RM24	RM 1	
	Split Island	**Rattlesnake SC**	**Juniper SC**	**Three-Rivers**	**Bighorn FAS**	**Mallards FAS**	**Two Leggins FAS**	**Aarapooish FAS**	**Custer FAS**	**Manuel Lisa FAS**	**# of Sites**
TRICHOPTERA (Caddisflies)	2643	2187	1680	2823	3699	1027	1107	6280	70	1088	10
Hydroptila spp.	60	107	1113	1309	3421	693	936	5247	30	336	10
Brachycentrus occidentalis	1973	1593	260	1078	93	40	23	73	0	24	9
Amiocentrus aspilis	293	280	267	103	80	27	20	0	0	0	7
Hydropsyche slossonae	0	0	0	0	29	240	20	580	0	560	5
Hydropsyche occidentalis	10	0	0	26	37	13	46	73	40	40	8
Cheumatopsyche spp.	0	160	40	26	8	0	35	0	0	0	5
Hydropsyche morosa gr.	0	0	0	0	0	0	12	113	0	72	3
Oecetis avara	113	47	0	128	16	0	4	73	0	48	7
Lepidostoma sp.	120	0	0	77	0	0	0	0	0	0	2
Glossosoma sp.	0	0	0	77	0	0	0	100	0	0	2
Hydropsyche C. cockerelli	73	0	0	0	0	0	0	0	0	0	1
Polycentropus sp.	0	0	0	0	0	13	0	0	0	8	2
Nectopsyche sp.	0	0	0	0	0	0	0	20	0	0	1
Onocosmoecus unicolor	0	0	0	0	13	0	0	0	0	0	1
Ochrotrichia sp.	0	0	0	0	0	0	8	0	0	0	1
ANNELIDA (Worms/Leeches)	4503	3620	687	4107	552	7733	1739	2420	590	424	10
Lumbricidae (Aquatic Worm)	1257	600	147	411	168	0	25	53	40	0	8
Naididae	0	0	0	0	0	2307	54	0	0	0	2
Tubificidae	3247	3020	540	3696	357	5267	1545	2367	453	360	10
Erpobdellidae	0	0	0	0	13	0	82	0	37	64	4
Glossophonia complanata	0	0	0	0	13	13	33	0	0	0	3
Helobdella stagnalis	0	0	0	0	0	147	0	0	60	0	2
CRUSTACEA (Scuds/Isopods)	3670	2620	2073	7135	544	53	24	0	33	0	8
Hyalella azteca	0	0	0	51	0	40	0	0	0	0	2
Gammarus spp.	650	540	400	1617	171	0	0	0	0	0	5
Caecidotea sp.	3020	2080	1673	5467	373	13	24	0	33	0	8
MOLLUSCA (Snails/Clams)	237	167	173	796	443	400	161	107	50	32	10
Physella sp. (Pouch snails)	217	107	107	308	163	293	131	80	50	0	9
Ferrissia rivularis (Limpets)	0	0	0	26	0	13	0	13	0	32	4
Fossaria sp.	0	0	53	26	8	0	12	13	0	0	5
Stagnicola sp.	0	0	0	0	8	0	0	0	0	0	1
Potamopyrgus antipodarum (NZMS)	20	60	13	411	237	93	17	0	0	0	7
Pisidium sp. (Fingernail Clams)	0	0	0	26	27	0	0	0	0	0	2
OTHER Non-Insects	217	680	633	1155	1240	67	413	67	17	0	9
Turbellaria (Flatworms)	140	527	260	1052	1232	67	409	33	17	0	9
Nematoda (Horsehair Worms)	10	7	133	51	0	0	4	33	0	0	6
Hydracarina (Water-Mites)	67	147	240	51	8	0	0	0	0	0	5
Total Taxa per site	**41**	**38**	**36**	**39**	**42**	**33**	**45**	**45**	**22**	**37**	**37.8**
EPT Taxa per site	**11**	**9**	**8**	**14**	**13**	**10**	**16**	**23**	**9**	**20**	**12.8**

APPENDIX B. BIGHORN RIVER SITE PHOTOS

Photo 1. Bighorn River: Split Island Fall 2022 looking d/s.

Photo 2. Bighorn River: Split Island Fall 2021 looking d/s.

Photo 3. Bighorn River-Three Rivers Spring 2022 looking downstream.

Photo 4. Bighorn River-Three Rivers Fall 2022 downstream.

Photo 5. Bighorn River FAS Spring 2022 looking u/s.

Photo 6. Bighorn River FAS Fall 2022 looking upstream.

Photo 7. Bighorn River FAS Fall 2022 Hess cleaned vegetation area

Photo 8. Bighorn River Two Leggins FAS Fall 2022 looking downstream

Photo 9. Bighorn River Two Leggins FAS Fall 2022 looking upstream

Photo 10. Bighorn River Two Leggins FAS Fall 2021 looking upstream

Photo11. Bighorn River Mallard's Landing FAS Fall 2022 looking upstream.

Photo12. Bighorn River Mallards Landing FAS Fall 2021 looking upstream.

Photo 13. Bighorn River Arapooish FAS Spring 2022.

Photo 14. Bighorn River Arapooish FAS site Fall 2022 looking downstream.

Photo 15. Bighorn River Rattlesnake SC site looking upstream April 2022

Photo 16. Bighorn River Custer FAS site looking upstream Fall 2022

Photo 17. Bighorn River Rattlesnake SC site looking upstream Fall 2022

Photo 18. Bighorn River Rattlesnake SC site Hess sample April 2022.

Photo 19. Bighorn River Juniper SC site looking d/s Fall 2022

Photo 20. Bighorn River Juniper SC site looking u/s Fall 2022.

Photo 21. Bighorn River Manual Lisa FAS looking downstream Fall 2022

Photo 22. Bighorn River Manual Lisa FAS looking upstream Fall 2022

Photo 23. Bighorn River Manual Lisa FAS site Spring 2022 looking downstream.

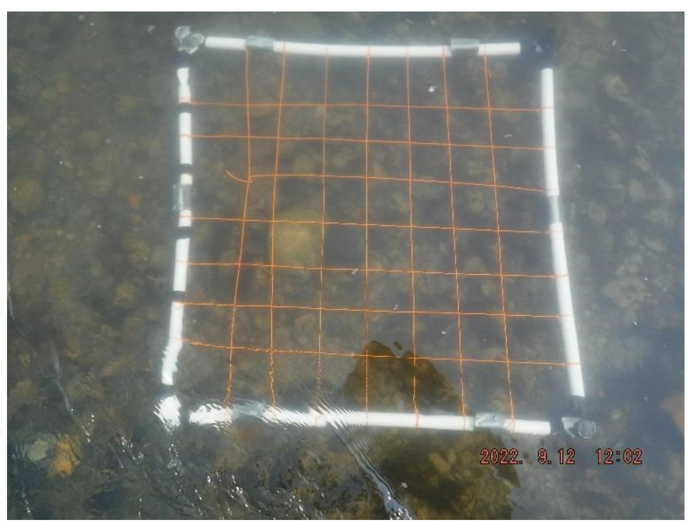


Photo 24. Bighorn River Manual Lisa FAS site grid count Fall 2022.

APPENDIX C. MACROINVERTEBRATE COMMUNITY AND TAXA SIMILARITY BETWEEN THE SIDE CHANNELS AND MAINSTEM RIVER

Appendix C. Bighorn River Similarity Indices based on total organisms from 3 Hess Samples per site

Total	7,057	7,562	42	37	31	48	108.6
Cuml Total		14,619					
Community Similarity	46						
TAXA SIMILARITY	65		Fall 2022 Split Island vs. Rattlesnake SC				
Taxon	**Spl Isl**	**Rattlesnake**	**TAXA 1**	**TAXA 2**	**COMMON**	**TAXA 1+2**	**,ai-bi,**
Optioservus quadrimaculatus	8	10	1	1	1	1	0.02
Agabus sp.	3	8	1	1	1	1	0.06
Haliplus sp.	7	0	1	0	0	1	0.10
Thienemannimyia gr.	0	48	0	1	0	1	0.63
Diamesa spp.	279	6	1	1	1	1	3.87
Potthastia sp.	64	30	1	1	1	1	0.51
Prodiamesa sp.	3	0	1	0	0	1	0.04
Cardiocladius spp.	297	16	1	1	1	1	4.00
Cricotopus spp.	607	62	1	1	1	1	7.78
Eukiefferiella spp.	35	122	1	1	1	1	1.12
Orthocladius spp.	223	20	1	1	1	1	2.90
Parakiefferiella	70	20	1	1	1	1	0.73
Parametriocnemus sp.	0	14	0	1	0	1	0.19
Tvetenia sp.	13	20	1	1	1	1	0.08
Cryptochironomus sp.	4	0	1	0	0	1	0.06
Dicrotendipes sp.	93	14	1	1	1	1	1.13
Phaenopsectra sp	13	0	1	0	0	1	0.18
Polypedilum spp.	26	108	1	1	1	1	1.06
Tanytarsus sp.	14	0	1	0	0	1	0.20
Micropsectra spp.	0	2	0	1	0	1	0.03
Tipula sp.	13	14	1	1	1	1	0.00
Ceratopogoninae	0	6	0	1	0	1	0.08
Limnophora	11	92	1	1	1	1	1.06
Simulium spp.	7	3,548	1	1	1	1	46.82
Stratiomyiidae	6	0	1	0	0	1	0.09
Acentrella turbida	4	10	1	1	1	1	0.08
Baetis flavistriga	0	14	0	1	0	1	0.19
Baetis tricaudatus Complex	1,846	578	1	1	1	1	18.51
Serratella micheneri	10	0	1	0	0	1	0.14
Tricorythodes explicatus	8	18	1	1	1	1	0.12
Petrophila sp.	12	0	1	0	0	1	0.17
Cheumatopsyche spp.	0	48	0	1	0	1	0.63
Hydropsyche occidentalis	3	0	1	0	0	1	0.04
Hydropsyche C. cockerelli	22	0	1	0	0	1	0.31
Hydroptila spp.	18	32	1	1	1	1	0.17
Lepidostoma sp.	36	0	1	0	0	1	0.51
Oecetis avara	34	14	1	1	1	1	0.30
Amiocentrus aspilis	88	84	1	1	1	1	0.14
Brachycentrus occidentalis	592	478	1	1	1	1	2.07
Lumbricidae	377	180	1	1	1	1	2.96
Tubificidae	974	906	1	1	1	1	1.82
Gammarus spp.	195	162	1	1	1	1	0.62
Caecidotea sp.	906	624	1	1	1	1	4.59
Physella sp.	65	32	1	1	1	1	0.50
Potamopyrgus antipodarum	6	18	1	1	1	1	0.15
Turbellaria	42	158	1	1	1	1	1.49
Nematoda	3	2	1	1	1	1	0.02
Hydracarina	20	44	1	1	1	1	0.30

Appendix C. Bighorn River Similarity Indices based on total organisms from 3 Hess Samples per site							
Total	7869	8992	40	37	26	51	115.9004569
Cuml Total		16861.4					
Community Similarity	42.0						
TAXA SIMILARITY	51.0			Fall 2022 3-River vs. Juniper SC			
Taxon	**3-Rivers**	**Juniper SC**	**TAXA 1**	**TAXA 2**	**COMMON**	**TAXA 1+2**	**,ai-bi,**
Optioservus quadrimaculatus	8	0	1	0	0	1	0.10
Microcylloepus pusillus	0	4	0	1	0	1	0.04
Agabus sp.	0	6	0	1	0	1	0.07
Thienemannimyia gp.	116	128	1	1	1	1	0.04
Potthastia sp.	85	46	1	1	1	1	0.56
Cardiocladius spp.	46	0	1	0	0	1	0.59
Cricotopus spp.	154	642	1	1	1	1	5.18
Eukiefferiella spp.	0	294	0	1	0	1	3.27
Orthocladius spp.	0	136	0	1	0	1	1.51
Parakiefferella sp.	31	120	1	1	1	1	0.94
Paraphaenocladius sp.	0	32	0	1	0	1	0.36
Parametriocnemus sp.	0	86	0	1	0	1	0.96
Tvetenia sp.	0	76	0	1	0	1	0.85
Dicrotendipes sp.	277	86	1	1	1	1	2.57
Phaenopsectra sp	77	188	1	1	1	1	1.11
Polypedilum spp.	816	652	1	1	1	1	3.12
Rheotanytarsus sp.	31	0	1	0	0	1	0.39
Micropsectra spp.	0	60	0	1	0	1	0.67
Tipula sp.	0	4	0	1	0	1	0.04
Limnophora	0	66	0	1	0	1	0.73
Simulium spp.	69	3736	1	1	1	1	40.67
Pericoma sp.	0	6	0	1	0	1	0.07
Stratiomyiidae	8	0	1	0	0	1	0.10
Acentrella insignificans	8	0	1	0	0	1	0.10
Acentrella turbida	8	0	1	0	0	1	0.10
Baetis tricaudatus	1040	990	1	1	1	1	2.20
Serratella micheneri	239	28	1	1	1	1	2.72
Ephemerella sp.	8	10	1	1	1	1	0.01
Tricorythodes sp	31	22	1	1	1	1	0.15
Petrophila sp.	15	0	1	0	0	1	0.20
Cheumatopsyche spp.	8	12	1	1	1	1	0.04
Hydropsyche occidentalis	8	0	1	0	0	1	0.10
Hydroptila spp.	393	334	1	1	1	1	1.28
Lepidostoma sp.	23	0	1	0	0	1	0.29
Oecetis avara	39	0	1	0	0	1	0.49
Amiocentrus aspilus	31	80	1	1	1	1	0.50
Brachycentrus occidentalis	323	78	1	1	1	1	3.24
Glossosoma sp.	23	0	1	0	0	1	0.29
Lumbricidae	123	44	1	1	1	1	1.08
Tubificidae	1109	162	1	1	1	1	12.29
Hyalella azteca	15	0	1	0	0	1	0.20
Gammarus spp.	485	120	1	1	1	1	4.83
Caecidotea sp.	1640	502	1	1	1	1	15.26
Physella sp.	92	32	1	1	1	1	0.82
Ferrissia sp.	8	0	1	0	0	1	0.10
Fossaria sp.	8	16	1	1	1	1	0.08
Potamopyrgus antipodarum (NZMS)	123	4	1	1	1	1	1.52
Pisidium sp.	8	0	1	0	0	1	0.10
Turbellaria	316	78	1	1	1	1	3.14
Nematoda	15	40	1	1	1	1	0.25
Hydracarina	15	72	1	1	1	1	0.61