BIGHORN RIVER ALLIANCE

Research Initiative

Bighorn River Aquatic Macroinvertebrate Monitoring Summary for 2023: High Flows and Side Channels

Bighorn River restored Juniper Side Channel looking upstream, September 2023

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Executive Summary

In 2023, the Bighorn River Alliance (BHRA) completed the 4th year of the Bighorn River Benthic Macroinvertebrate (BMI) Monitoring program. Eight long-term monitoring sites plus two reconnected side channels (hereafter Rattlesnake SC {MI9} and Juniper SC {MI10}) were sampled in the spring and fall for BMI communities.

The goals of this study are: 1) to conduct quantitative, replicated BMI surveys to serve as a baseline for future monitoring efforts within this Bighorn River section; 2) to evaluate spatial and temporal BMI patterns to understand how the river's water quality, hydrology, flow management and temperatures affect the overall biological health of the river; and 3) to determine if and when BMI communities within the reconnected (Fall 2021) side channels evolve to reflect the mainstem river.

In both mid-April and mid-September 2023, we collected 3 replicate Hess BMI samples in riffle areas at the 10 sites minus 1 site in the Fall. Early run-off streamflow from the Little Bighorn River affected our ability to Hess sample the Custer FAS site in April (we used multiple dipnets) and the record high discharge in July restructured this site so that we could not locate suitable substrate for the Fall sampling. Conditions in 2023 brought higher average flows, more aquatic vegetation, and turbidity than in previous years.

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 2023, 99 total BMI taxa were collected across the 10 Bighorn River sites, averaging 34 total taxa per site (range 25-49 species). Mayfly (E), caddisfly (T), plus one stonefly (P) (Total EPT Taxa) averaged 33% (2 to 87%) of the total community per site and 11 species per site (range 5-23 spp.). In Fall 2023, Two Leggings (MI5) tied Arapooish (MI6) (Fall 2022) for the highest ever reported total EPT taxa richness at a site with 23 species.

We must caveat that this monitoring study uses standardized, targeted-riffle methods and is not an exhaustive attempt to categorize every aquatic species in the river; we are fully aware that some species are not collected with these sampling protocols. Mayflies, *Pseudocloen/Isweon* (Pseudos or Anokas) and *Paraleptophlebia* (Mahoganys) are rarely observed in our riffle samples as these species prefer slower velocity, weedy, river edges and can be quite abundant in these habitats. Additional caddisfly species likely occur only in these marginal habitats or in greater numbers than what we find in the riffles, e.g. *Amiocentrus aspilus* (Western Weedy Sedge). Therefore, we cannot presume to know or predict how well every species' population is doing or will hatch out in the coming year.

Eighteen species of mayflies (E) were recorded throughout the study section in 2023 (3 less than in 2022): 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, and BWO's in the 2 side channels may indicate some good hatches to come in 2024.

Of the 17 total species of caddisflies (T) collected in 2023, the micro-caddis, *Hydroptila spp*., net-spinning (Tan) caddisflies, *Hydropsyche spp. and Cheumatopsyche*, and long-horned caddis, *Oecetis avara,* were collected across the most Bighorn sites, while the western weedy sedge, *Amiocentrus asplius* are usually more abundant upstream of St Xavier.

Tan caddisflies were very abundant at the Bighorn and Two-Leggins FAS sites in Fall 2023 after being virtually absent in the spring, this may portend a decent hatch in this area in summer of 2024.

Total taxa richness, EPT diversity, and % EPT significantly increased in the Fall compared to the Spring 2023 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 12,995 individuals per m^2 (± 2,461 SE) across all Bighorn River sites in Fall 2023; these densities were significantly lower (T-test, p=0.04) than values reported in the Spring (17,116 ind. per m^2 ± 1,229 SE). BMI communities collected across Split Island Three Rivers and Mallards Landing sites, were building up significant densities in Fall of 2021 and Spring of 2022 until the flushing flows of June 2022 and July 2023 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 BMI densities in the restored side channels, Rattlesnake and Juniper, averaged ~14,000 ind. per m^2 during both seasons in 2023 and were very comparable to the adjacent Bighorn River mainstem densities (~15,000 per m2); although, SC BMI densities were reduced significantly after the July-August high flows, whereas the mainstem sites increased in BMI densities.

The similarity of the side channel BMI communities to the adjacent mainstem Bighorn River has increased after 2 years since being reconnected but is still low; Fall 2023 Rattlesnake SC samples had a 72% Community Similarity (CS) and 48% Taxa Similarity with the Split Island site, and Juniper SC had a 84% Community Similarity (CS) and 45% Taxa Similarity with the 3-Rivers site in Spring. Side channel riffle BMI communities have become more fully colonized in 2023 but are not likely to continue increasing in taxa similarity with the mainstem beyond ~50% due to habitat differences and interspecific competition.

In 2023, only 4 of the 10 sites were occupied by invasive New Zealand mudsnails and they were not detected in the side channels in the

Fall sampling. NZMS averaged ~90 per m^2 across the 4 sites in 2023; this reduction in riffle occupancy from 7 sites is related to summer high flows. In 2022, we had documented NZMS at 7 of the 10 sites including the two restored side channels: 2022 densities averaged ~120 NZMS per m^2.

Fall 2023 was the 1st period of this study that Biotic Index (HBI) Scores dropped below 5.0 at 5 of the 9 sites sampled, including Juniper SC; this indicates a healthier BMI community ranking of moderately impaired. HBI scores of >5.0 have been reported at all Bighorn River sites from 2020 through 2022 and indicate that the BMI communities are experiencing significant 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 at Mallards Landing and Custer FAS, indicating that the flushing flows of June 2022 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 has resulted in exponential increases in BMI densities across BHRA sites.

We conclude this 2023 analysis by documenting that the BMI communities are consistently expressing the sediment impaired biological health of the Bighorn River, until a flushing flow discharge occurs (as in June 2022 and July 2023). Only then does the BMI community reflect a less impaired salmonid-bearing stream. Without a significant flush every year, high nutrient levels quickly increase aquatic plant growth and sediment accumulations.

During 'low-flow' years like 2021, population increases of more silt-tolerant BMI taxa (midges, scuds and aquatic worms) tend to shift the BMI community away from mayflies and caddisflies. Unfortunately, the same conditions that promote salmonid biomass and growth tend to cause fewer hatches and classify the benthic community as impaired. We'll soon determine if the prolonged flushing flows of summer 2023 translate into better hatches in 2024, but as of Fall, some sites contain very robust mayfly and caddisfly densities going into the winter.

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Acknowledgements

We would like to thank Anne Marie Emery, James Chalmers and the BHRA Board for continuing this project with 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. Dennis was instrumental in helping us to collect samples at the upper BHRA sites, Split Island and 3-Rivers sites MI1 and MI2, and at the Rattlesnake and Juniper Side Channels in the spring and fall.

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 due to Yellowtail Dam's hypolimnetic, cold-water releases. Trout fishermen are most familiar with the upper 43-mile river section below Afterbay Dam downstream to Hardin, MT. The top 10-mile 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 replicated, 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 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 randomized 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**). Dennis Fischer made a great video illustrating this process: Hess Sampling Bighorn River

***Photo 1**. Hess macroinvertebrate sample being taken at Split Island (left) Photo by Jim Chalmers and at Two-Leggins FAS (right).*

2.2 Sample Processing and Taxonomic Analysis

BMI 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.

Most BMI metrics were calculated after the data was entered into the Ecological Database Access System (EDAS) (Jessup 2006), including EPT taxa, % EPT, % Non-insect, % Chironomidae, Diversity Indices and the Hilsenhoff Biotic Index (HBI).

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 salmonid-bearing 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; Barbour et al 1999; MDEQ 2012); 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).

BMI Community and Taxa Similarity Indices (Lydy et al. 2000) were used to compare taxa and community composition in side-channel and mainstem samples. We quantified community similarity from the overlap in taxa composition using all pooled Hess data (all replicates combined) (following Heino et al. 2009; Lydy et al 2000). (***Appendix B***).

2.3 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. RM= River Mile. WQS = water quality samples.

Station ID	Agency	Site Name	Latitude	Longitude	Parameter
BGHNR_RM82 (MI1)	MSU	MI 1: Bighorn River @ RM82 **Split Island** (Upper Brammer)	45.32863	-107.8985	Macroinverts, WQS
BGHNR_RM79 (MI9)	BHRA	Rattlesnake Side Channel	45.35212	-107.86997	Macroinverts + Fish
BGHNR_RM76 (MI10)	BHRA	Juniper Side Channel	45.36941	-107.82127	Macroinverts + Fish
BGHNR _RM75 (MI2)	MSU	MI 2: Bighorn River @ RM75 **Three Rivers** (Lower Brammer)	45.38232	-107.8125	Macroinverts, WQS
BGHNR _RM72 (MI3)	New BHRA	MI 3: Big Horn River @ **Bighorn** FAS	45.41634	-107.7898	Macroinverts, WQS
Y11BGHNR01 (MI4)	MDEQ	MI 4: Big Horn @ **Mallards Landing FAS**	45.52166	-107.7258	Macroinverts, WQS
BGHNR _RM52 (MI5)	New BHRA	MI 5: Big Horn @ **Two Leggins FAS**	45.64449	-107.6599	Macroinverts, WQS
BGHNR _RM40 (MI6)	New BHRA	MI 6: Big Horn @ **Arapooish FAS**	45.75664	-107.5653	Macroinverts, WQS
BGHNR _RM24 (MI7)	New BHRA	MI 7: Big Horn River @ **General Custer FAS**	45.92737	-107.5744	Macroinverts, WQS
Y17BIGHNRM01 (MI8)	MDEQ	MI 8: Bighorn River at **Manuel Lisa FAS**	46.14486	-107.4644	Macroinverts, WQS

***Map 1**: Bighorn River 2023 BMI sampling sites (MI1-MI10) from Yellowtail Dam to the Yellowstone River confluence.*

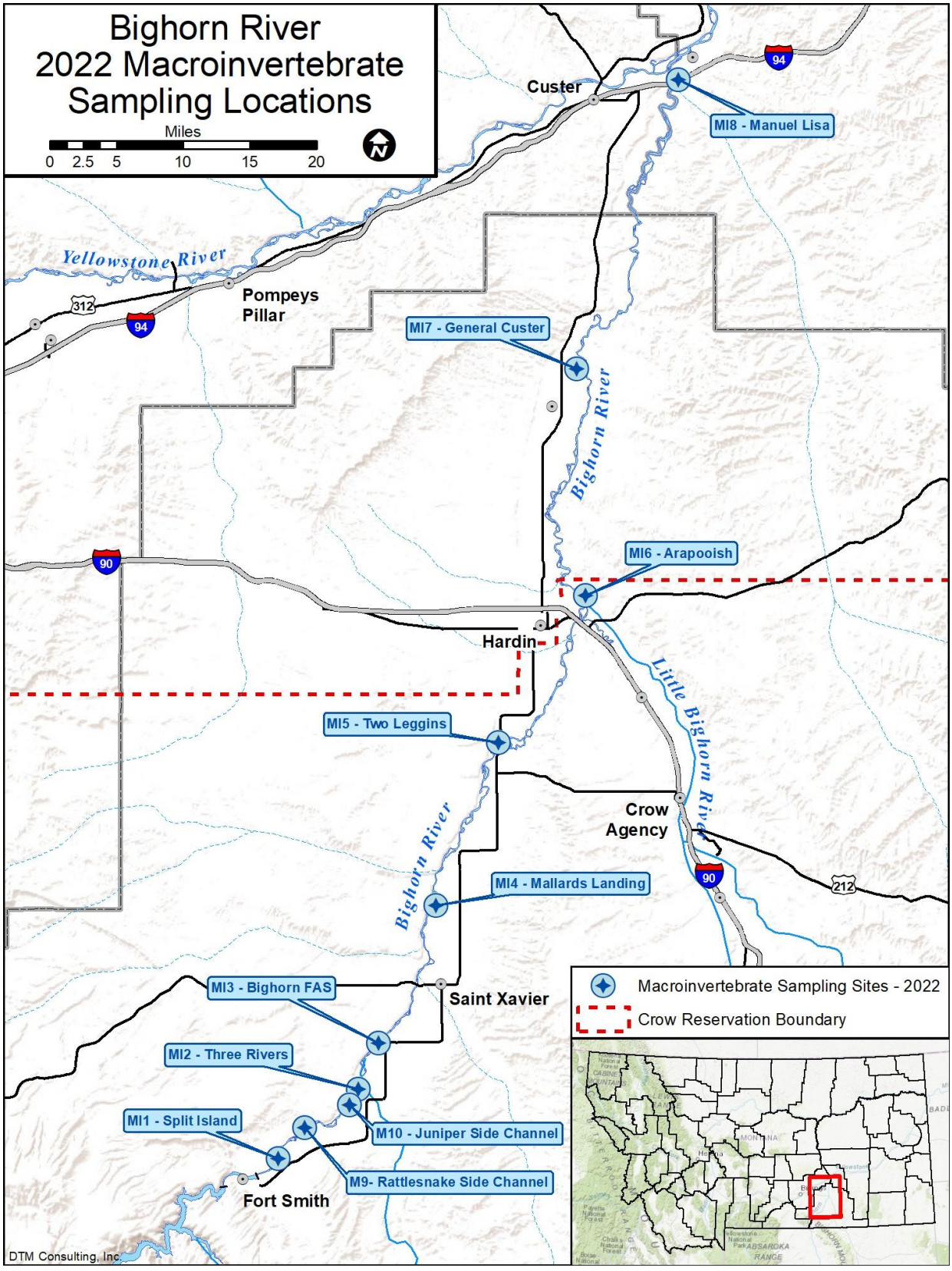
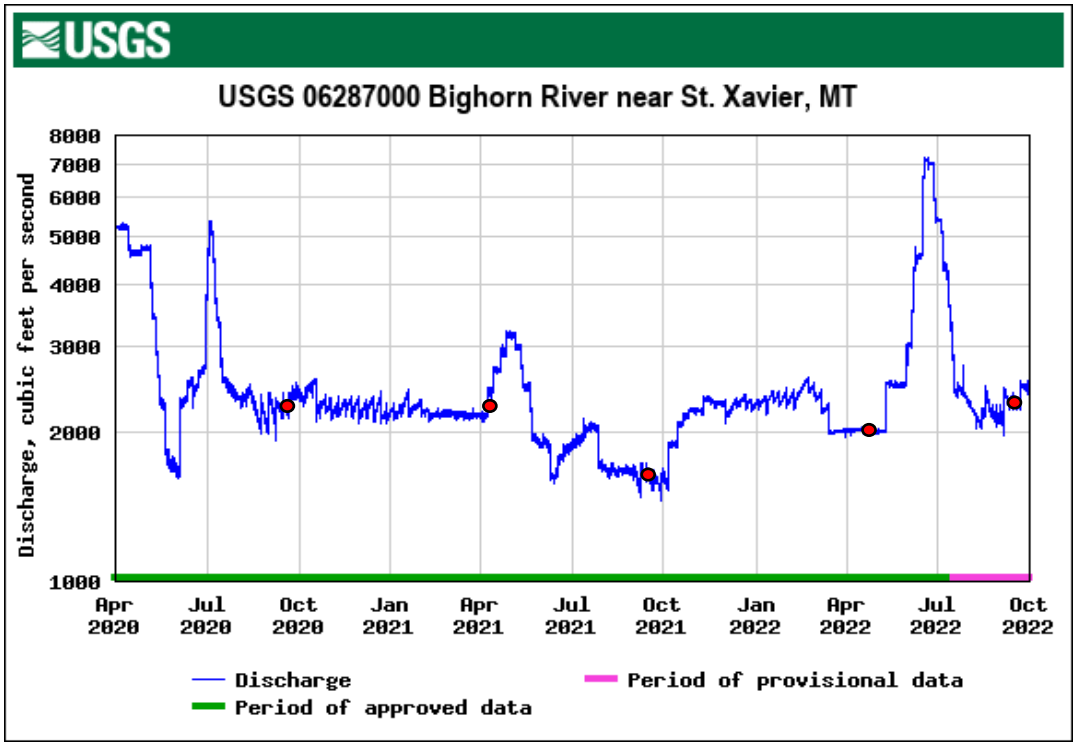
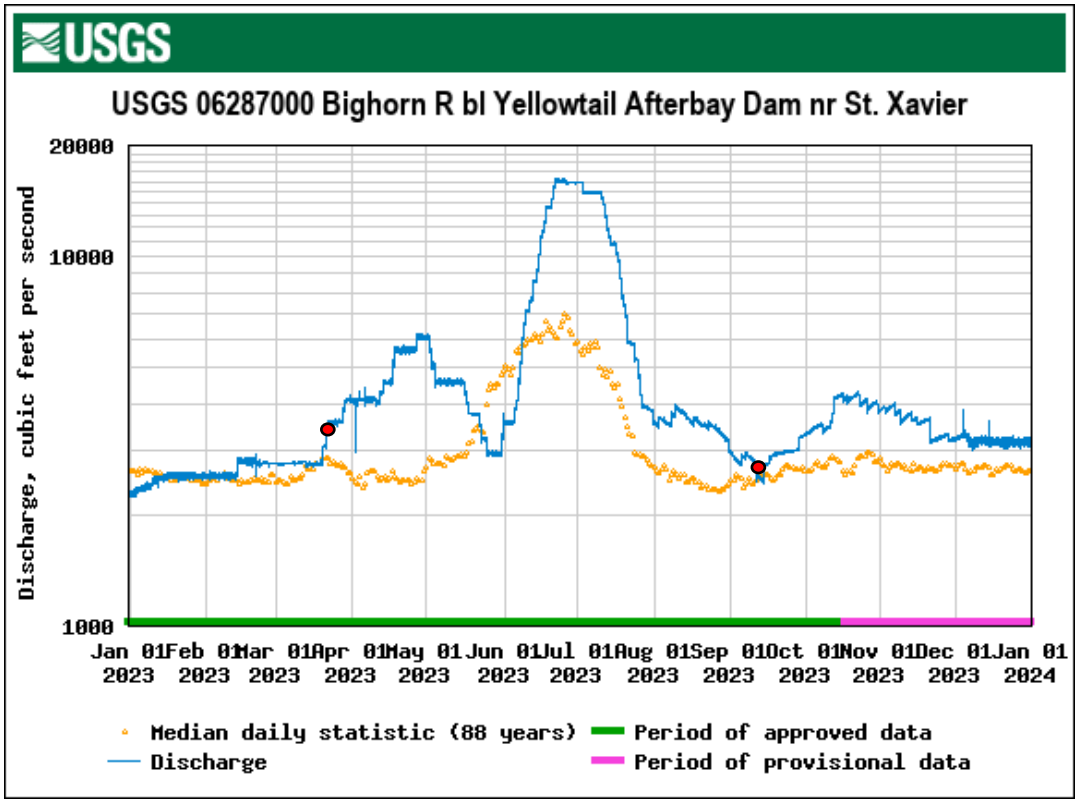


Figure 1. *Discharge reported during 2020-2022 (top) and 2023 (bottom). Seasonal BMI Sampling Visits are represented by Red Dots.*





3.0 Results

3.1 General BMI Community. Overall, 99 total BMI taxa were collected across the 8 Bighorn River mainstem + 2 restored side channel (SC) sites in 2023 **(*Appendix A***); average total number of taxa per site was 34 taxa (range 25-49 spp.); this is 3 fewer taxa per site than reported in 2022 (***Figure 2***). Restored SC have been colonized by BMI quickly since Fall 2021 averaging 30 total BMI taxa in the Spring and 37 taxa in the Fall 2022, with slightly lower total taxa (avg. 30) in 2023. High points of overall taxa richness in 2023 were at Bighorn FAS with 47 total taxa in the Fall (***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.); Two-Leggins FAS reported the highest total EPT richness in the Fall 2023 with 23 species (***Figure 2***). EPT taxa richness in Fall 2023 (avg. 13.3 taxa) has significantly increased across all sites (T-test, p=0.01) since the Spring 2023 sampling which averaged 8.0 EPT species per site (***Figure 2***).

Figure 2*. Total BMI taxa, and EPT taxa (Mayflies + Stoneflies + Caddisflies) at the Bighorn River sites from 2020-2023. Sites are arranged from upstream (left) to downstream (right).*

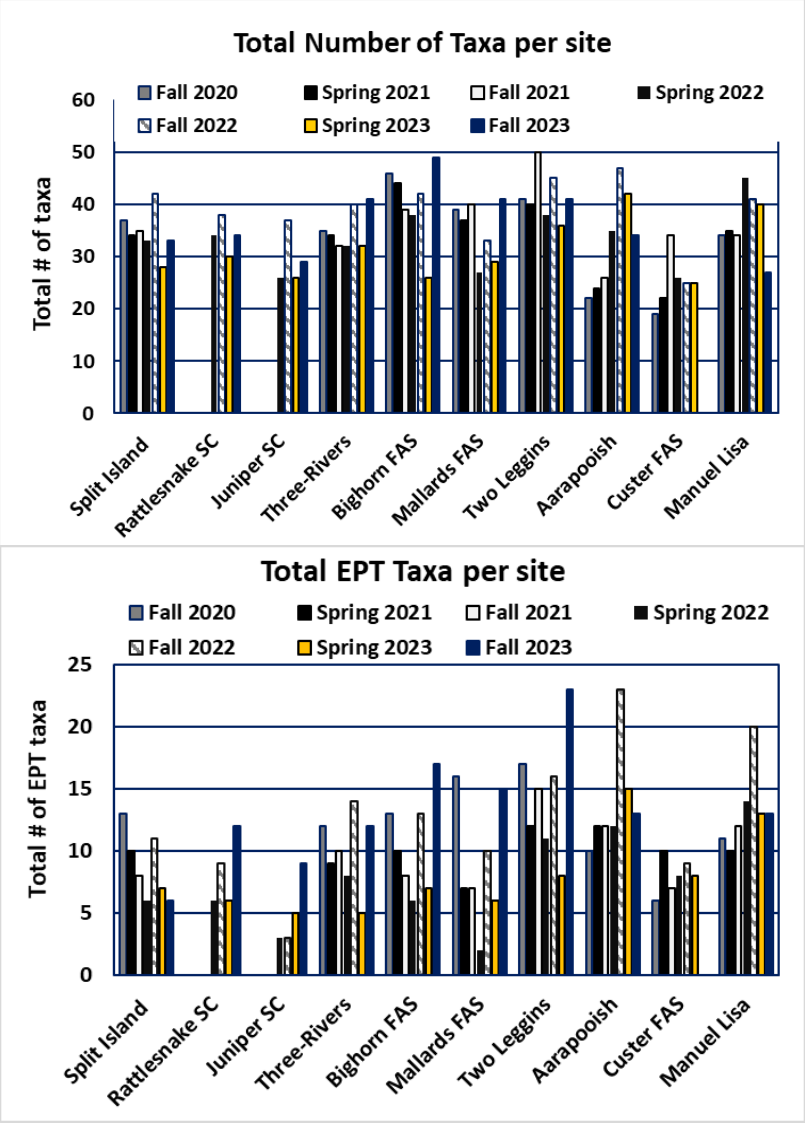
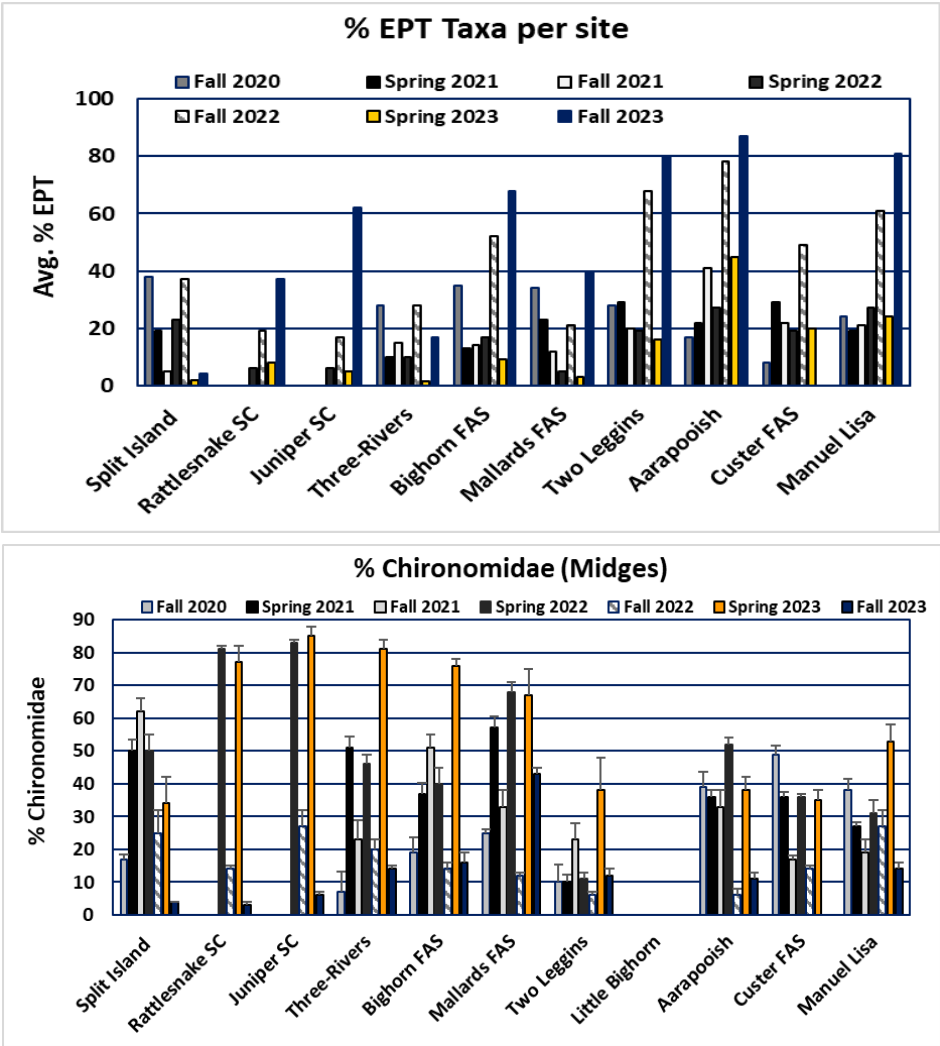


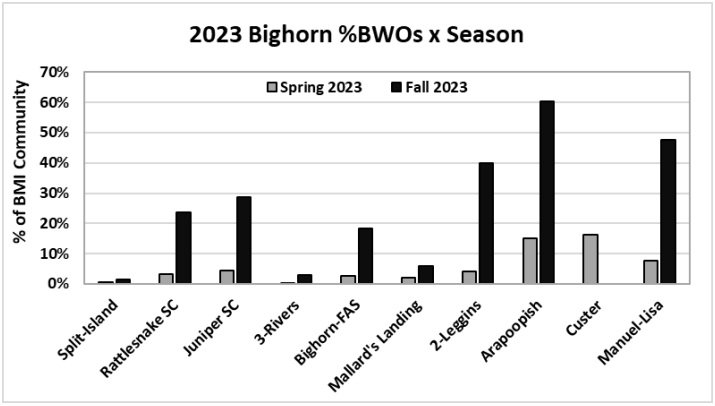
***Figure 3**. Macroinvertebrate % EPT (Mayflies + Stonefly + Caddisflies) (top) and % Chironomidae (bottom).*

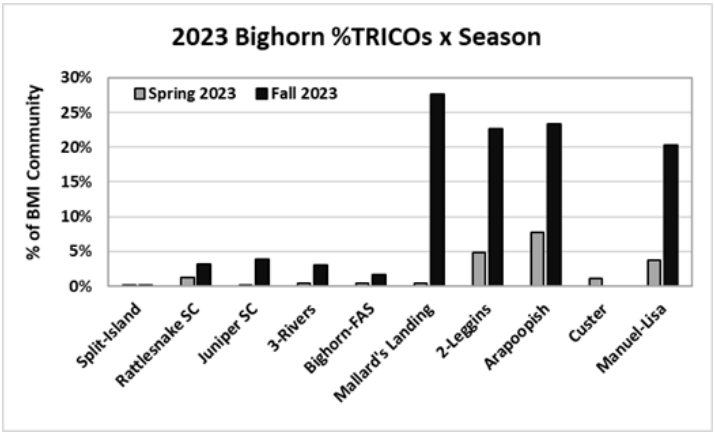


Percent EPT taxa in the BMI community significantly increased across 7 of the 9 sites in Fall 2023 compared to the Spring 2023 samples (T-test, p=0.0001); Two Leggins, Arapooish and Manual Lisa reported the highest ever %EPT (≥80%) in the Fall 2023 samples (***Figure 3***). Significant decreases in Chironomidae (Midges) abundance (T-test, p=0.00003) occurred concurrently with increases in Mayfly and Caddisfly numbers between Spring 2023 and Fall 2023; the restored side channels reported the highest % of Chironomidae in Spring 2023 (avg. 82%) along with Three-Rivers (81%), Bighorn FAS (76%), Mallard's Landing (68%), but in Fall 2023, these sites reported some of the lowest midge percentages (avg. 16%) (***Figure 3***). Large increases in the % EPT in the Fall 2023 at both Side Channels, the Bighorn FAS and all downstream sites have increased % EPT to the highest values reported since monitoring began, exceeding the Fall 2022 %EPT increases (***Figure 3***).

3.2) Mayflies. Eighteen mayfly (E) species of were recorded throughout the study section in 2023: the dominant four groups were Blue Winged Olives *(Baetis tricaudatus, B. flavistriga*), Tiny BWOs (*Acentrella turbida, A. insignificans*), Tricos (*Asioplax edmundsi*, *Tricorythodes explicatus*) and Pale Morning Duns (PMDs) (*Ephemerella excrucians* and *Serratella micheneri*) exhibited various spatial and temporal patterns in 2023 (***Figures 4a & b***). Mayfly abundance across the sites, in general, has significantly increased between Spring and Fall 2023, except PMD densities which were higher in the Spring (***Figure 4a & b***). We can expect some large BWO/Tiny BWO and Trico hatches around Mallard's Landing downstream to Arapooish in 2024 (***Figure 4b***).

Figure 4a*. Percent of mayfly species contributing to the whole BMI community for Spring and Fall 2023. Note differences in y-axis values between taxa groups.*





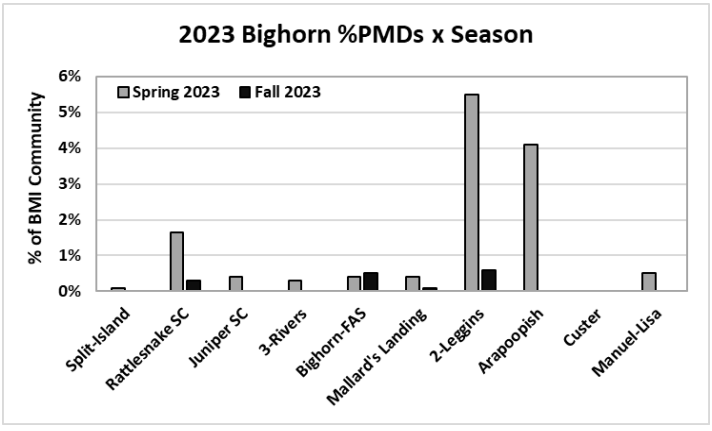
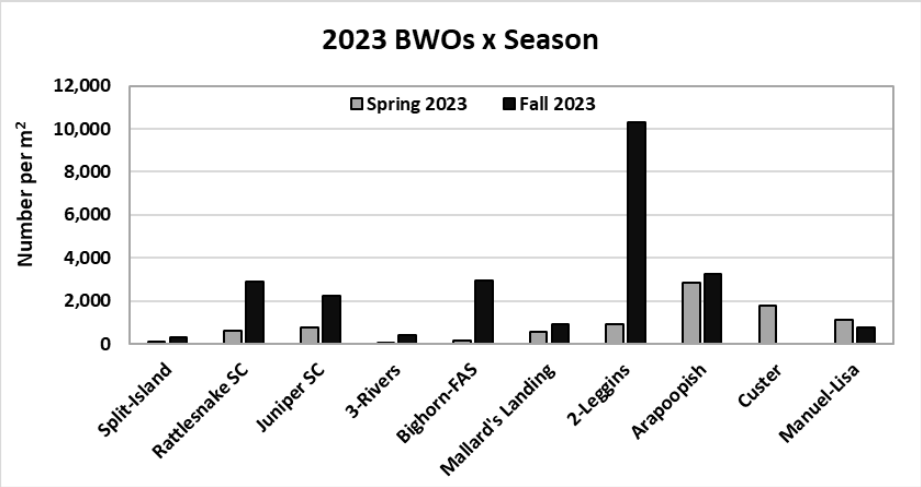
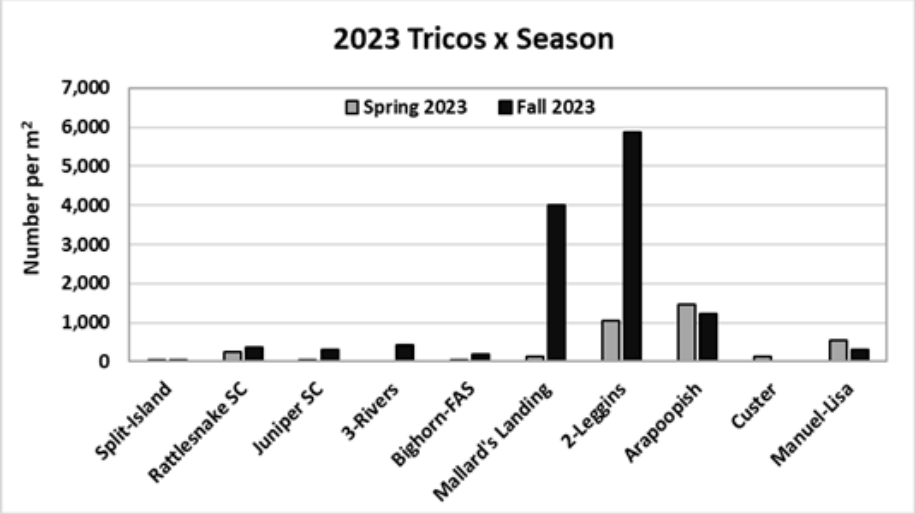
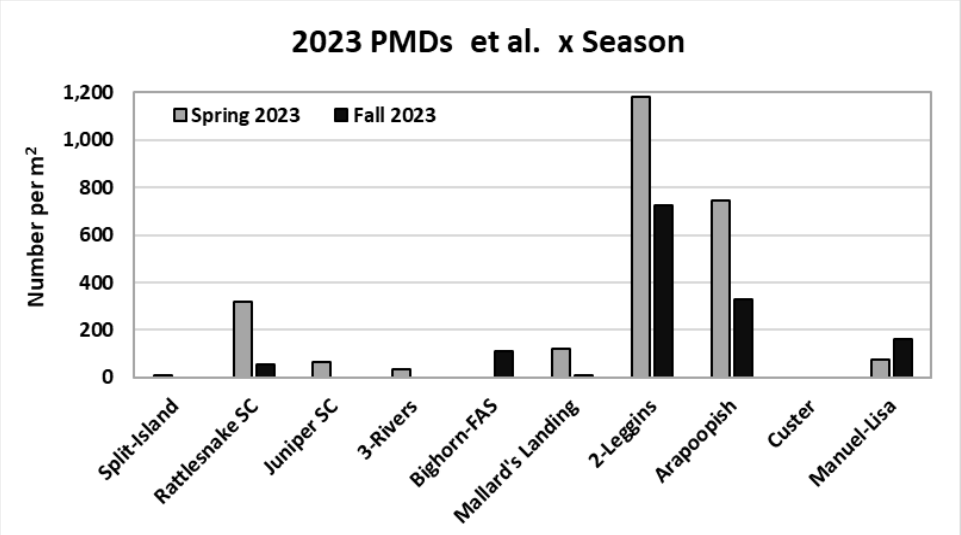


***Figure 4b**. Mayfly species group abundance (Numbers per m^2) for Spring and Fall 2023 and (right). Note differences in y-axis values between mayfly groups.*

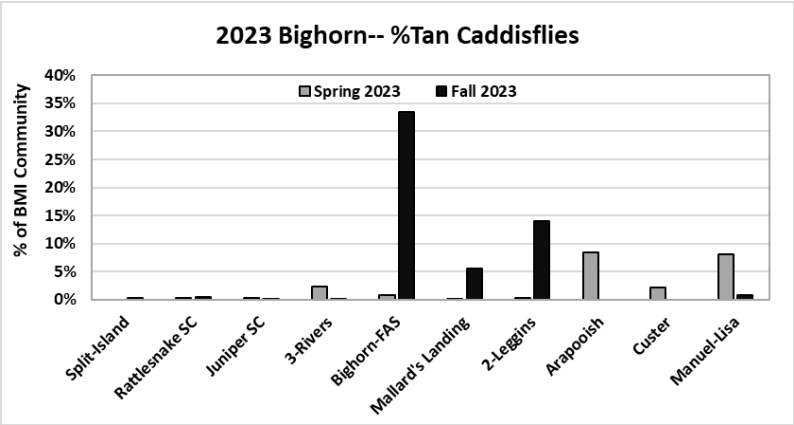


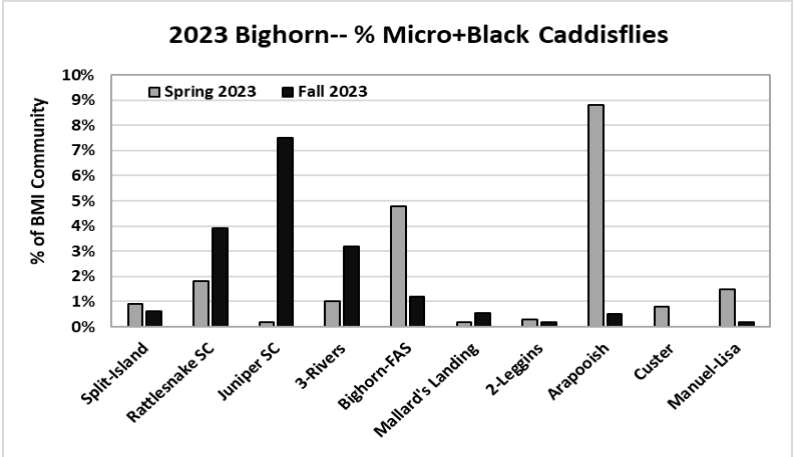


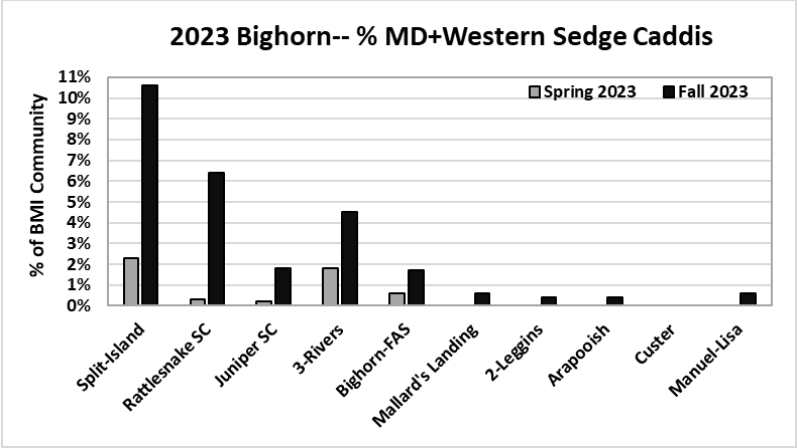


3.3) Caddisflies. Of the 17 total species of caddisflies (T) collected in 2023, three dominant groups of caddisflies provide the most visible hatching adults in the summer: Tan Caddisflies (Hydropsychidae*: Cheumatopsyche spp. Hydropsyche occidentalis, Hydropsyche morosa gr., H. bronta),* Black Caddis, Micro-caddis & Long-horned (*Oecetis avara, Ceraclea, Hydroptila spp.*) and the Mother's Day (MD) and Western Sedge Caddisfly (Brachycentridae: *Brachycentrus occidentalis* and *Amiocentrus aspilus*). Similar to the Mayflies, most caddisflies groups were more abundant in the Fall 2023 after the high flows of July, and *Mother's Day Caddis and Western Sedges* should have a good hatch at the upper sites in the summer of 2024 *(**Figure 5a & b***).

***Figure 5a**. Percent of caddisfly groups contributing to the BMI community for Spring and Fall 2023. MD= Mother's Day Caddis. *Note differences in the y-axis values between taxa groups.*

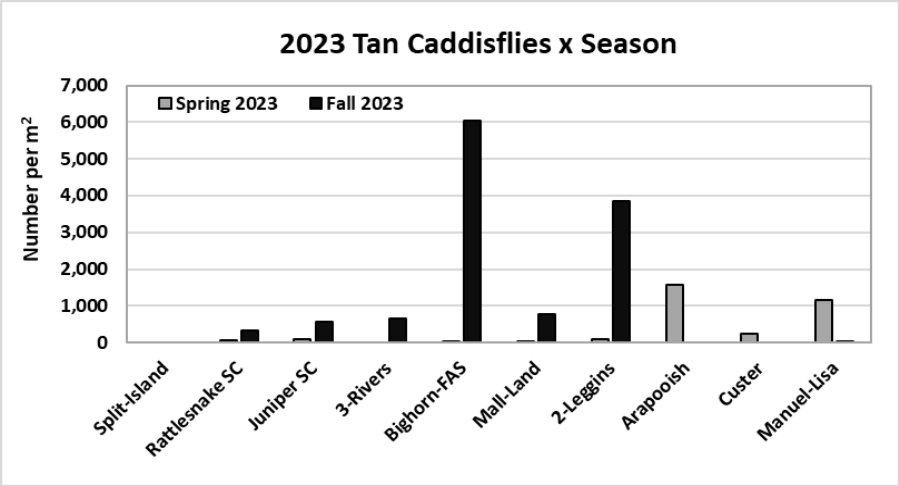


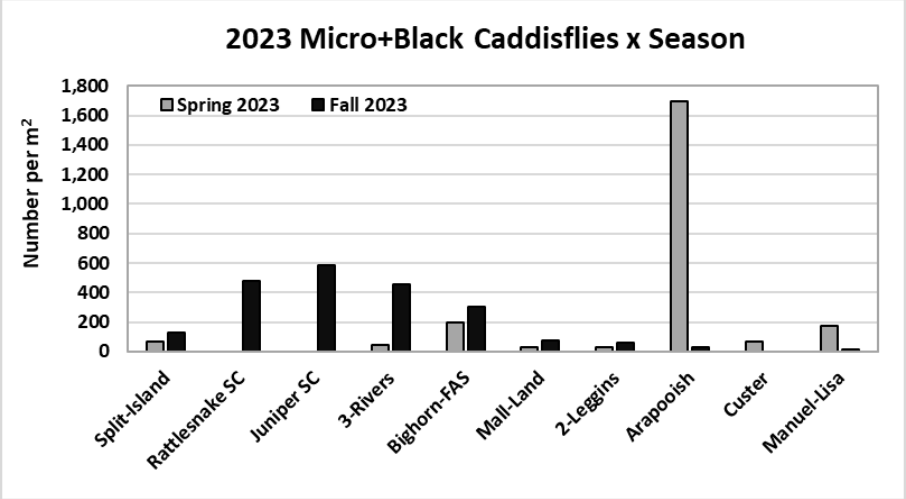


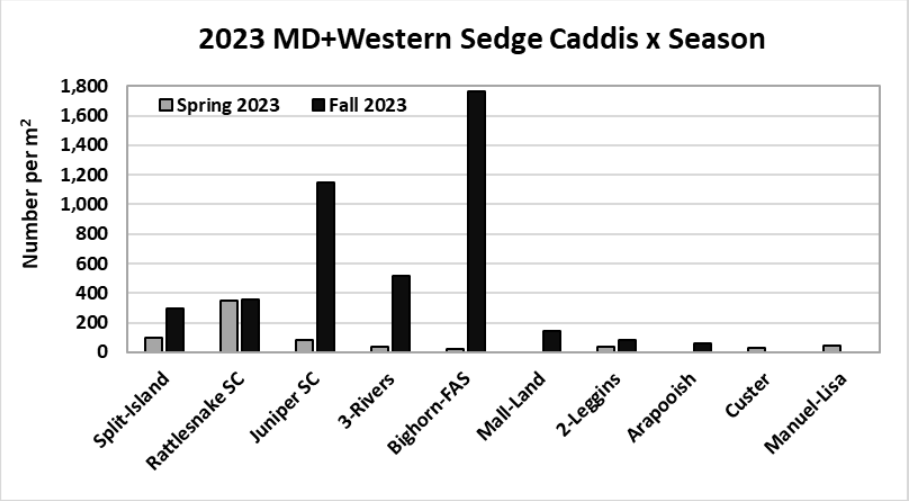


Tan caddisflies were very abundant at the Bighorn and Two-Leggins FAS sites in the Fall after being virtually absent in the spring (**Figure 5b**), so this may indicate a decent hatch in this area in summer of 2024.

***Figure 5b**. Caddisfly species group abundance (Numbers per m^2) for Spring and Fall 2023. MD= Mother's Day Caddis. *Note differences in the y-axis values between taxa groups.*





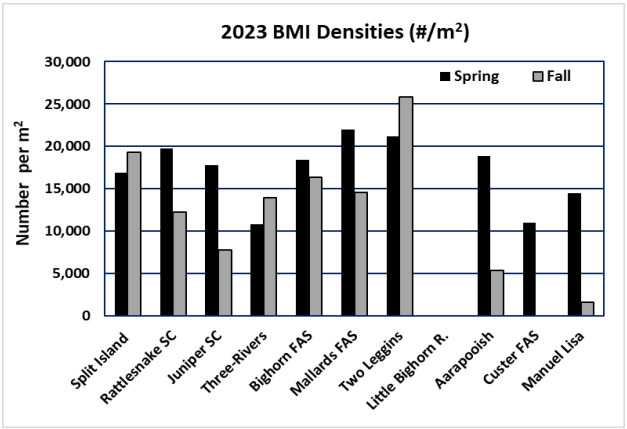


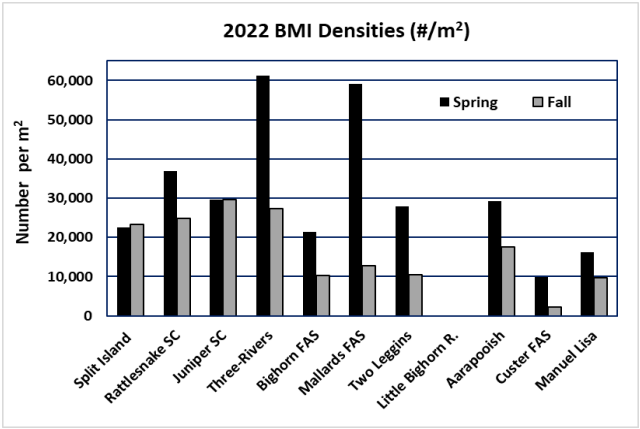
3.4) Benthic Macroinvertebrate Densities. BMI densities averaged 12,995 individuals per m^2 (± 2,461 SE) across all Bighorn River sites in Fall 2023; these densities were significantly lower (T-test, p=0.04) than values reported in Spring 2023 (17,116 ind. per m^2 ± 1,229 SE) (***Figure 6, Table 2***). 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) in 2022, averaged ~30,000 individuals per m^2 during both seasons and were very comparable to densities in the adjacent Bighorn River mainstem; these SC BMI densities reached these levels in just 6 months, since Fall 2021 (***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***).

Table 2 *. Macroinvertebrate Hess sample (n=3) densities at 10 Bighorn River sites from April & Sept. 2022-2023. SC= restored side channels*

	Spring 2022	Fall 2022	Spring 2023	Fall 2023	2-year Average
Site Name	**#/m2**	**#/m2**	**#/m2**	**#/m2**	
BHR_Split Island	22,523	23,352	16,855	19,275	20,501
BHR_Rattlesnake SC	36,843	24,827	19,773	12,240	23,421
BHR_Juniper SC	29,587	29,544	17,787	7,778	21,174
BHR_Three Rivers	61,325	27,253	10,795	13,920	28,323
BHR_BighornFAS	21,470	10,270	18,417	16,339	16,624
BHR_MallardsFAS	59,220	12,667	21,960	14,606	27,113
BHR_TwoLegginsFAS	27,810	10,436	21,152	25,864	21,316
BHR_Arapooish	29,238	17,494	18,892	5,354	17,744
BHR_Custer	9,818	2,233	11,039		7,697
BHR_Manuel Lisa	16,151	9,680	14,494	1,580	10,476
Avg.	**31,399**	**16,776**	**17,116**	**12,995**	**19,439**
SE	**5,370**	**2,873**	**1,229**	**2,461**	**2,083**

Figure 6*. Mean BMI densities at the Bighorn River sites across 2023 and 2022 seasons. SC= restored side channel*





Historical Perspective: In 2023, we documented the lowest BMI densities at the upper 2 sites (MI1 & MI2) since the beginning of the study (Fall 2020) (***Figure 7***). The Fall 2021 (both sites) and Spring 2022 (@3-Rivers) BMI densities resembled the high-density values reported from the late-1980's (37 years ago), presumably taken during drought conditions. BMI abundance across most of the upper Bighorn River sites has significantly increased during the 'below normal' discharge year of 2021 compared to BMI densities reported from the Fall 2020 data (which had lower benthic 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, scuds and midges) (Stagliano 2023)**.**

Figure 7*. Mean BMI densities at the 2 upper Bighorn River sites across 1986-2023 seasons. Error Bars = ± standard error.*

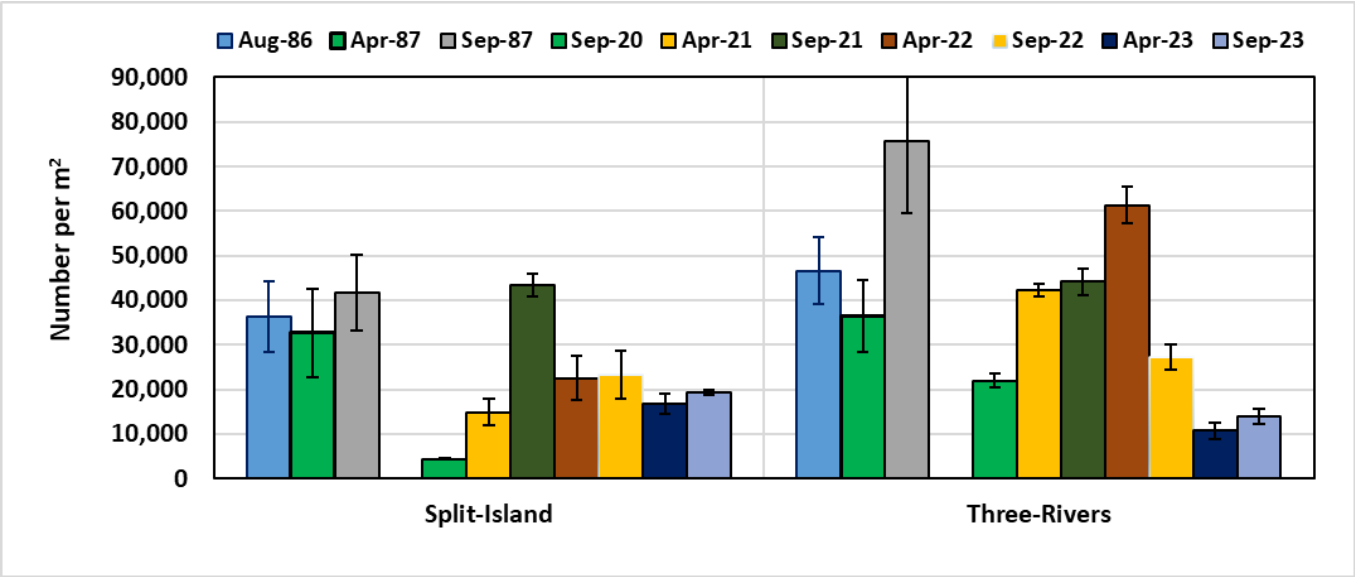
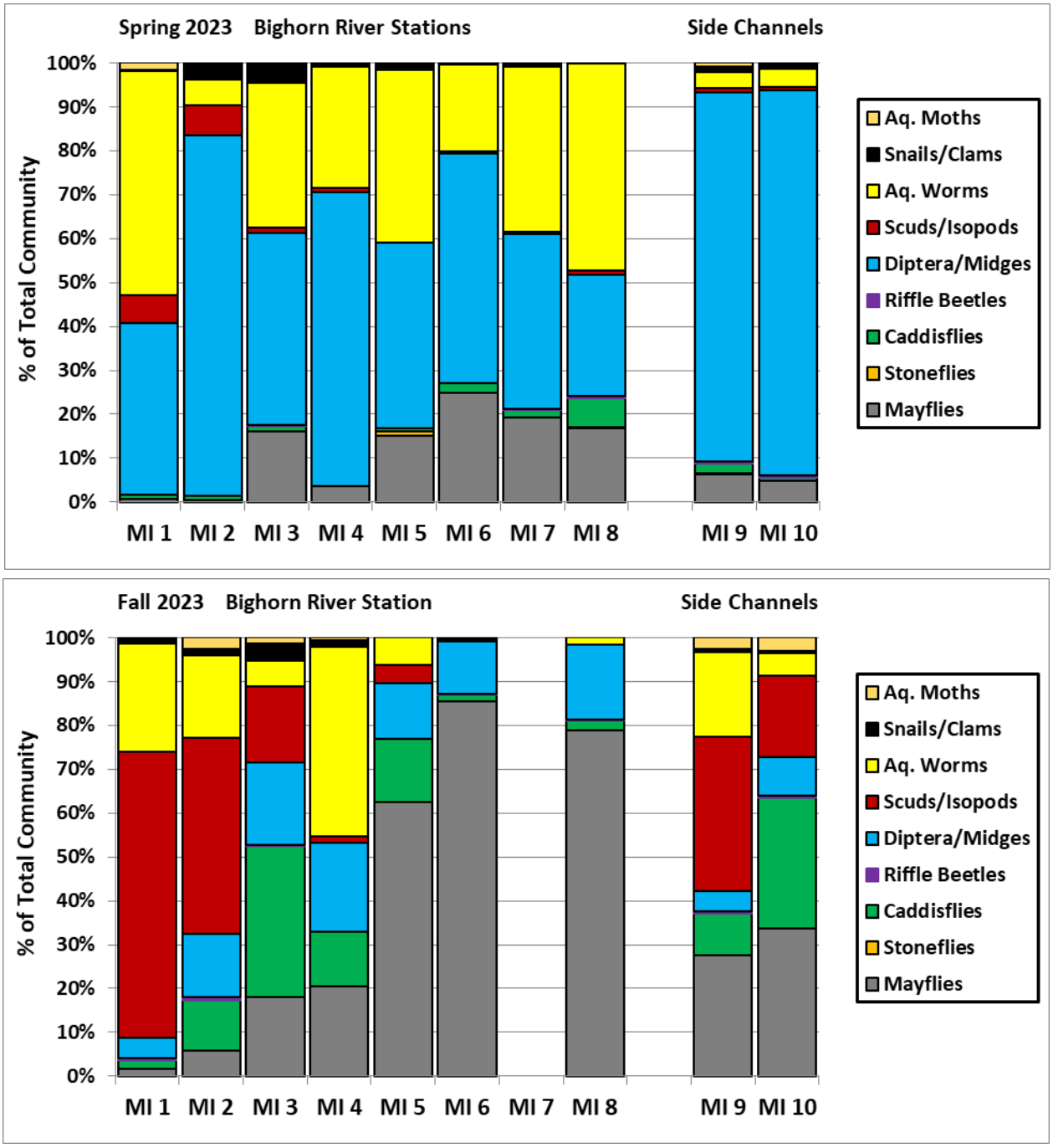
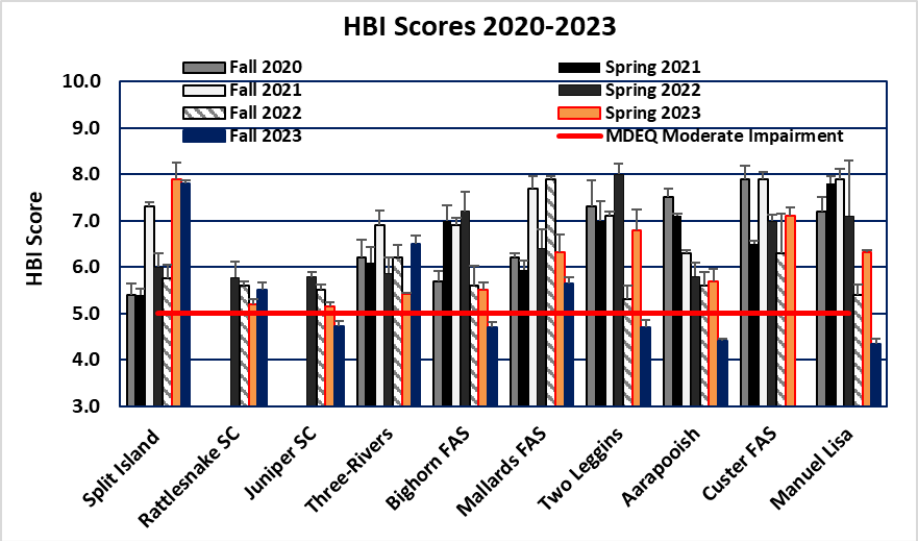


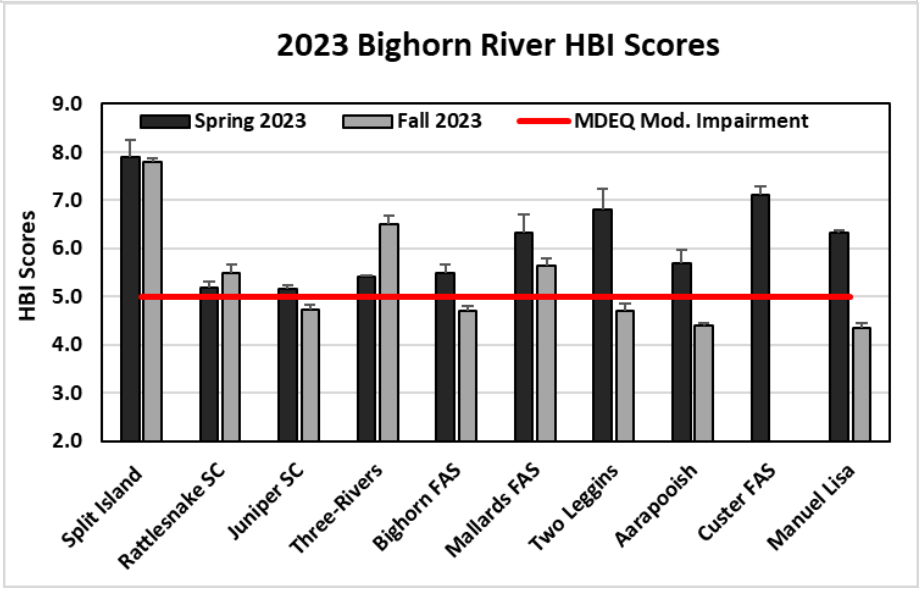
Figure 8. *BMI community composition at Bighorn River Sites (MI1-8) arranged upstream to downstream with Rattlesnake (MI9) and Juniper SC (MI10) in Spring (Top) vs. Fall (bottom) 2023*



3.5) Tolerance Index Scores. HBI Scores >5.0 were reported at all Bighorn River sites during both seasons from 2020 to 2022 indicating that the BMI communities are experiencing moderate to significant sediment/nutrient enrichment (***Figure 9***). In Fall of 2023, HBI scores were calculated at <5.0 at 5 of the 9 sites and this season collectively averaged the lowest HBI scores (5.4) (i.e., better health) of any other study season. Although, the BMI community at Split Island site (MI1) reported the most severely impacted HBI scores (>7.5) during both seasons (***Figure 9***) due to the dominance of the Isopod, *Caecidotea* and Aquatic Worms (***Figure 8***). In Fall 2021, HBI scores averaged 7.3 with all eight of the sites (100%) exhibiting significant enrichment scores >6.0.

Figure 9. *Macroinvertebrate HBI scores for the BHRA sites. Scores above the red line thresholds are moderately (>5.0) and significantly impaired (>6.0), respectively. Error bars are ±SE.*





The biological integrity at the sites, as measured by the HBI, has significantly decreased (i.e., improved) from Fall 2021 to Fall 2022 (T-test, p=0.02); likely attributed to the flushing flows of June 2022. While most of the Bighorn River sites exhibited HBI tolerance increases in 2021, Arapooish FAS has experienced a steady decline of the HBI (increasing BMI health) but was still ranking moderately impaired until the Fall 2023 when it reported the lowest HBI score ever at this site (4.4) (***Figure 9***): this was the 2nd lowest of the 2023 study with Manual Lisa reporting the lowest score (4.3) during this Fall 2023 sampling (***Figure 9***).

3.6) NZMS. In Fall 2023, non-native, New Zealand mudsnails (NZMS) were observed at four of the 10 Bighorn River sites averaging ~90 individuals per m^2 (***Appendix A*).** We did detect low spring densities of NZMS in Rattlesnake, but we did not detect these snails in either Side channel in the Fall 2023, indicating that the flushing flows of July-August likely sweep them out of the riffle habitats. In 2022, NZMS colonized the newly constructed Rattlesnake and Juniper SC relatively quickly and averaged 80 per m^2 and 13 per m^2 respectively.

In 1987, NZMS were 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) Rattlesnake and Juniper Side Channels (SC). The biggest differences observed in the composition of the BMI community at the Rattlesnake and Juniper SC in Spring vs. Fall 2023 were increases in % Mayflies, Amphipods and Isopods (Scuds) and Caddisflies in the Fall with the decreasing dominance of the midges (Diptera); this pattern was seen in 2022 as well (***Figure 8, Appendix A***). In terms of the similarity of the side channel BMI communities to the adjacent mainstem Bighorn River, the highest community similarity of the side channels to the mainstem occurred in the Fall 2023 at the Rattlesnake SC (72%) and Spring 2023 at the Juniper SC at 84%

(**Table 3, Appendix C**). Lowest BMI taxa and community similarity (~6 months post-connection) was observed in the Spring 2022 for both the Rattlesnake and Juniper SC samples. The largest increases in Community Similarity with the mainstem occurred at the Juniper SC site between Fall 2022 and 2023 (30%), and between Spring and Fall 2023 which reported 35% increased similarity with the BMI community at the 3-Rivers site (**Table 3**). Taxa similarity to the mainstem has never been more than 50% for either side channel which means there is still ~10-12 species that are not shared between sites (**Appendix C**). Taxa similarity increased between spring and fall indicating more colonization from the mainstem into the side channels post-runoff period. Both side channels reported higher densities of mayflies and caddisflies than the adjacent mainstem sites during the Fall 2023 (**Figure 8**).

Table 3*. BMI Community Similarity (CS) and Taxa Similarity (TS) for the side channel (SC) versus adjacent Bighorn River sites from April & Sept. 2022-2023. Green shading indicates increased similarity while red shading represents a decrease.*

Bighorn River Adjacent vs. SC Restoration Sites									
		Similarity 2022		Similarity 2023		Δ Similarity x Year		Δ Similarity x Season %TS/%CS	
Site Name	Season	% TS	%CS	% TS	%CS	% TS	%CS	2022	2023
Rattlesnake	Spring	34	60	31	45	-3.0	-15.0	+12	+17
	Fall	46	65	48	72	+2	+7	+5	+27
Juniper	Spring	43	54	45	84	+2	+30	+1	+1
	Fall	42	51	46	49	+4	-2	-3	+35

4.0 Conclusions

- Spring and Fall 2023 BMI data across the 10 Bighorn River sites revealed that the record high flushing flows of July-August has significantly reduced the densities of macroinvertebrates, especially Chironomidae (Midges) and Aquatic worms, while increasing the richness and percent of EPT taxa (mayfly and caddisflies) across most sites.
- BMI densities in the Bighorn River restored side channels (SC), Rattlesnake and Juniper, decreased to ~14,000 ind. per m^2 during both seasons in 2023 and were very comparable to the adjacent Bighorn River mainstem densities (~15,000 per m^2); although, SC BMI densities were reduced significantly after the July high flows, whereas the adjacent mainstem BMI densities increased in the Fall. This indicates that BMI communities in the side channels are likely more affected by scouring flows and higher velocities.
- Initial BMI colonizers of 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. Spring 2023 looked very similar to 2022 with a dominance of midges, but by Fall 2023, a more diverse BMI community has evolved.
- Even though these restored side channels averaged only about 50% species similarity with the Bighorn mainstem BMI community, 2 years of monitoring is sufficient to document the successful BMI colonization and productivity of these habitats.
- Species diversity and EPT taxa richness increased with increasing distance from Yellowtail Dam; in the Fall 2022 and 2023, Two-Leggins and Arapooish sites had the highest EPT species richness ever reported during this study (23 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 2022-2023 with large population increases occurring during the non-flushing flow years (Fall 2020-Spring 2022). Since the late 1980's, large shifts have occurred in the BMI community, including decreasing densities of midges and mayflies, increases in some caddisflies and the addition of NZ mudsnails, the aquatic moth, *Petrophila* and the isopod, *Caecidotea*.
- The non-native, New Zealand mudsnails were observed at fewer sites in 2023 compared to 2022 and even fewer between the Spring and Fall sampling of 2023, including not being

observed in the side channels; this leads us to conclude that flushing flows can certainly redistribute these snails out of the riffle areas where we focus our sampling.

- A large conclusion 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 multiple 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 historical years (1986-87), but these BMI densities can increase quickly with the low river flows, as we documented in Fall 2021 and then decrease with the next flushing flows, as we've documented between Spring and Fall 2022 and 2023.
- We conclude this 4th year of analysis by indicating that BMI communities are exhibiting improved biological health after the flushing flows throughout the Bighorn River in June 2022 and July 2023 and have reported the lowest HBI scores of all years (5.4, Fall 2023) which still indicates 'moderate' impairment from sediments or nutrients. The use of the HBI is a good surrogate compared to MDEQ's Plains and Low Valley MMI's because the Bighorn River is such a unique ecosystem that does not fit easily into either classification.
- The main reasons for the observed seasonal ecological changes in the BMI communities between Fall 2020 and Fall 2023 can be causally linked to annual discharge. In particular, previous high discharge years (2017-2019) were followed by decreased discharge in 2020 and 2021 which increased water temperatures, nutrient levels, aquatic vegetation and sediment accumulations; then in June 2022 and during July 2023, large flushing flows tended to reverse these low-flow BMI community trends.

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APPENDIX A. MACROINVERTEBRATE TAXA LIST AND ABUNDANCE AT ALL SITES SPRING AND FALL 2023

Appendix A. Macroinvertebrate taxa list and avg. number per meter squared (#/m2) for the Bighorn Sites Spring 2023

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	0	0	0	11	0	0	107	2
Zaitzevia parvula	0	0	0	0	0	0	0	53	0	75	2
Microcylloepus pusillus	0	0	0	0	0	0	0	27	27	10	3
Dubiraphia minima	0	0	0	0	0	0	40	0	0	0	1
Haliplus	0	80	160	14	0	0	0	0	0	0	3
DIPTERA (midges/flies)	6750	15546	15560	8248	4420	14200	10052	7440	3848	8168	10
Chironomidae (midges)	5990	15138	15093	8172	4129	14147	9110	7200	3848	7598	10
Diamesa spp.	2580	11287	13467	7528	3267	2440	3460	1573	60	123	10
Cricotopus spp.	2037	640	307	300	165	253	1091	1413	273	1280	8
Parakiefferiella	1083	2439	880	86	400	8973	3844	1533	1550	2073	10
Eukiefferiella spp.	103	171	93	33	0	13	22	93	13	0	8
Microtendipes sp	27	0	0	9	0	13	33	1173	533	157	7
Phaenopsectra sp	90	0	0	0	165	1413	183	413	660	2767	7
Prodiamesa sp.	7	20	27	0	0	53	53	320	0	0	6
Cardiocladius spp.	0	53	120	0	0	0	33	53	137	115	6
Dicrotendipes sp.	7	0	0	85	0	227	22	93	0	130	6
Cryptochironomus sp.	0	0	0	0	0	147	198	387	254	50	5
Thienemannimyia gr.	0	20	0	23	0	0	22	27	0	0	4
Orthocladius spp.	37	0	0	37	0	0	33	0	120	0	4
Potthastia sp.	0	0	160	9	0	0	0	107	0	0	3
Parametriocnemus sp.	0	112	0	0	0	0	97	13	0	0	3
Rheotanytarsus sp.	0	39	0	0	0	0	0	0	207	863	3
Micropsectra spp.	0	0	0	61	66	13	0	0	0	0	3
Tvetenia sp.	0	0	40	0	66	0	0	0	0	0	2
Endochironomus sp.	0	0	0	0	0	293	0	0	40	0	2
Polypedilum spp.	20	0	0	0	0	0	0	0	0	40	2
Cladotanytarsus sp.	0	0	0	0	0	27	18	0	0	0	2
Pagastia sp	0	358	0	0	0	0	0	0	0	0	1
Psectrocladius sp.	0	0	0	0	0	280	0	0	0	0	1
Tipulidae (Craneflies)											
Tipula sp.	7	20	0	0	66	0	84	53	0	0	5
Ceratopogoninae (Biting Midges)	723	0	0	5	0	13	815	0	0	0	4
Limnophora	30	105	133	0	0	0	0	0	0	0	3
Simulium spp. (Blackflies)	0	283	213	18	231	40	11	80	0	547	8
Hemerodromia sp. (Danceflies)	0	0	0	0	0	0	33	80	0	23	3
Stratiomyidae											
EPHEMEROPTERA (Mayflies)	150	1190	853	43	231	813	3159	5067	1903	1728	10
Acentrella turbida (Tiny BWOs)	60	0	0	0	0	0	0	0	0	0	1
Acerpenna pygmaea (Tiny BWOs)	0	0	0	0	0	13	0	0	0	0	1
Baetis flavistriga (BWO)	0	0	0	0	0	0	0	0	0	65	1
Baetis tricaudatus (BWO)	60	612	773	9	165	547	914	2827	1730	1047	10
Baetis intercalaris (BWO)	0	0	0	0	0	0	0	0	53	0	1
Tricorythodes explicatus (Tricos)	20	256	13	0	33	133	1062	1467	120	540	9
Ephemerella excrucians (PMDs)	10	322	67	34	33	120	1183	747	0	77	9
Macaffertium terminatum (Flat-Headed Ma	0	0	0	0	0	0	0	13	0	0	1
Paraleptophlebia (Mahoganys)	0	0	0	0	0	0	0	13	0	0	1
LEPIDOPTERA (Aquatic Moths)											
Petrophila sp.	230	178	53	5	0	0	0	27	0	41	6
ODONATA (Dragonflies)											
Ophiogomphus sp.	0	0	0	0	0	0	11	27	13	21	4
PLECOPTERA (Stoneflies)											
Isoperla quinquepunctata (Little Yellow Stones)	0	13	0	0	0	0	212	187	0	0	3

Appendix A. (cont.) Macroinvertebrate taxa list and avg. number per meter squared (#/m2) for Bighorn Spring 2023

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)	170	414	107	98	330	53	152	3265	329	1395	10
Cheumatopsyche (Tan Caddis)	0	0	0	0	0	27	68	0	105	73	4
Hydropsyche occidentalis (Tan Caddis)	0	66	80	0	66	0	11	787	130	397	7
Hydropsyche slossonae (Tan Caddis)	0	0	0	0	0	0	0	0	0	600	1
Hydropsyche C. cockerelli (Tan Caddis)	0	0	0	0	0	0	0	707	0	0	1
Hydropsyche morosa gr. (Tan Caddis)	0	0	0	0	0	0	0	80	0	103	2
Dicosmoecus (Sedges)	0	0	0	0	0	0	0	13	0	0	1
Onocosmoecus unicolor (Sedges)	0	0	0	0	0	0	0	27	0	0	1
Hydroptila spp. (Micro-Caddisflies)	60	0	0	4	132	27	33	1013	53	73	8
Ceraclea (Long-horned Caddis)	0	0	0	0	0	0	0	79	0	0	1
Ceraclea (Long-horned Caddis)	0	0	0	0	0	0	0	0	0	40	1
Oecetis avara (Black Caddis)	10	0	0	36	0	0	0	27	0	50	4
Amiocentrus aspilis (Western Sedge)	100	348	27	57	132	0	40	0	13	0	7
Brachycentrus occidentalis (MD Caddis)	0	0	0	0	0	0	0	0	27	45	2
Glossosoma (Black Caddis)	0	0	0	0	0	0	0	533	0	13	2
ANNELIDA (Worms/Leeches)	9070	723	253	192	231	6707	8168	2600	4720	2726	10
Lumbricidae (Aquatic Worm)	0	152	40	4	33	0	106	0	0	43	6
Naididae	0	0	0	0	0	0	0	387	727	90	3
Tubificidae	9057	551	213	178	198	6613	8063	2213	3993	2500	10
Erpobdellidae	0	20	0	9	0	93	0	0	0	65	4
Glossophonia complanata	13	0	0	0	0	0	0	0	0	23	2
Helobdella stagnalis	0	0	0	0	0	0	0	0	0	8	1
CRUSTACEA (Scuds/Isopods)	1143	1308	320	700	264	80	46	0	0	43	8
Hyalella azteca	0	0	0	0	0	27	0	0	0	0	1
Gammarus spp.	47	171	27	29	132	27	0	0	0	0	6
Caecidotea sp.	1097	1137	293	671	132	27	46	0	0	43	8
MOLLUSCA (Snails/Clams)	60	119	160	315	0	40	308	120	146	117	9
Physella sp. (Pouch snails)	53	86	160	166	66	40	236	13	146	33	10
Ferrissia rivularis (Limpets)	0	0	0	0	0	0	0	107	0	60	2
Fossaria (Pond Snails)	7	13	0	90	0	0	29	0	0	0	4
Stagnicola (Pond Snails)	0	0	0	13	0	0	0	0	0	0	1
Potamopyrgus antipodarum (NZMS)	0	20	0	40	198	0	44	0	0	0	4
Pisidium sp. (Fingernail Clams)	0	0	0	5	99	0	0	0	0	23	3
OTHER Non-Insects	87	204	320	374	33	66	88	80	50	60	10
Turbellaria (Flatworms)	87	204	53	374	33	53	88	0	53	63	9
Nematoda (Horsehair Worms)	0	0	0	0	0	0	0	53	0	0	1
Hydracarina (Water-Mites)	0	0	267	0	0	13	0	27	0	0	3
Total Taxa per site	**28**	**30**	**26**	**32**	**26**	**29**	**36**	**42**	**25**	**40**	**31.4**
EPT Taxa per site	**7**	**6**	**5**	**5**	**7**	**6**	**8**	**15**	**8**	**13**	**8.0**

Appendix A. Macroinvertebrate taxa list and avg. number per meter squared (#/m2) for the Bighorn Sites Fall 2023

River Mile from Yellowstone R.	RM82	RM79	RM76	RM75	RM72	RM63	RM52	RM40	RM1.8	
	Split Island	**Rattlesnake SC**	**Juniper SC**	**Three-Rivers**	**Bighorn FAS**	**Mallards FAS**	**Two Leggins FAS**	**Aarapooish FAS**	**Manuel Lisa FAS**	**# of Sites**
COLEOPTERA (Riffle Beetles)										
Optioservus quadrimaculatus	0	0	27	83	107	0	16	12	0	5
Zaitzevia parvula	11	13	0	0	5	0	0	0	0	3
Microcylloepus pusillus	0	0	0	7	29	0	0	0	0	2
Stenelmis	0	13	0	9	34	0	0	0	0	3
Dubiraphia minima	0	0	0	0	0	0	0	0	3	1
Agabus sp.	34	13	0	0	0	0	0	0	0	2
Haliplus sp.	79	0	0	0	0	0	0	0	0	1
DIPTERA (midges/flies)	963	533	587	2019	3255	6276	3475	644	287	9
Chironomidae (midges)	759	360	480	2009	2387	6201	3222	627	220	9
Cricotopus spp.	79	67	53	365	549	1242	999	20	3	9
Polypedilum spp.	0	213	253	1085	1446	2978	1415	210	83	8
Eukiefferiella spp.	68	13	0	39	172	311	50	0	0	6
Microtendipes sp	113	0	13	152	53	50	0	173	0	6
Parakiefferiella	68	0	0	46	5	825	0	21	0	5
Cryptochironomus sp.	0	13	0	0	15	117	0	88	30	5
Phaenopsectra sp	113	0	27	0	5	315	0	11	0	5
Dicrotendipes sp.	261	13	80	203	0	0	0	0	0	4
Rheotanytarsus sp.	0	0	0	10	0	132	656	20	0	4
Micropsectra spp.	0	0	0	25	0	72	0	12	3	4
Thienemannimyia gr.	0	0	13	35	0	0	0	0	10	3
Prodiamesa sp.	23	0	0	0	0	0	0	31	80	3
Cardiocladius spp.	0	0	0	13	57	0	18	0	0	3
Orthocladius spp.	34	40	40	0	0	0	0	0	0	3
Endochironomus sp.	0	0	0	0	0	109	0	16	7	3
Sublettea sp.	0	0	0	0	0	50	69	18	0	3
Diamesa spp.	0	0	0	0	0	0	0	3	0	1
Paracladius sp.	0	0	0	0	53	0	0	0	0	1
Parametriocnemus sp.	0	0	0	34	0	0	0	0	0	1
Rheocricotopus sp.	0	0	0	0	0	0	16	0	0	1
Thienemanniella sp.	0	0	0	0	0	0	0	4	0	1
Tvetenia sp.	0	0	0	0	32	0	0	0	0	
Parachironomus sp.	0	0	0	0	0	0	0	0	3	
Ceratopogoninae (Biting Midges)	0	0	0	0	5	0	0	0	0	1
Tipulidae (Cranefly)										
Hexatoma sp.	0	0	0	0	0	0	0	13	0	1
Tipula sp.	0	0	0	0	0	0	50	0	0	1
Limnophora	57	13	13	10	0	0	0	0	0	4
Simulidae (Blackflies)										
Simulium spp.	147	133	93	0	848	75	169	4	53	8
Empididae (Danceflies)										
Hemerodromia sp.	0	27	0	0	15	0	34	0	13	4
EPHEMEROPTERA (Mayflies)	329	3320	2533	800	3659	4937	16875	4596	1230	9
Acentrella insignificans (Tiny BWOs)	0	0	0	0	838	0	0	2922	710	3
Acentrella turbida (Tiny BWOs)	79	253	0	7	0	677	8741	0	0	5
Baetis flavistriga (BWO)	0	67	0	0	0	0	0	0	0	1
Baetis tricaudatus Complex (BWO)	238	2573	2227	380	2130	222	1537	0	7	8
Camelobaetidius sp.	0	0	0	0	0	10	16	329	10	4
Fallceon quilleri	0	0	0	0	0	0	128	4	23	3
Attenella margarita (PMDs)	0	0	0	0	94	0	0	0	0	1
Ephemerella excrucians (PMDs)	0	0	0	0	0	10	53	0	0	2
Serratella micheneri (PMDs)	0	40	0	0	0	0	98	0	0	2
Ecdyonurus sp. (Flat-Headed Mayflies)	0	0	0	0	0	0	132	0	0	1
Leucrocuta sp. (Flat-Headed Mayflies)	0	0	0	0	0	0	16	67	0	2
Macaffertium terminatum (Flat-Headed Mayflie	0	0	0	0	0	0	16	4	0	2
Rhithrogena sp. (Flat-Headed Mayflies)	0	0	0	0	0	0	85	33	3	3
Choroterpes albiannulata (Mahoganys)	0	13	0	0	15	0	144	0	7	4
Traverella albertana (Mahoganys)	0	0	0	0	0	0	36	0	0	1
Ephoron album (White Mayflies)	0	0	0	0	0	0	0	0	150	1
Asioplax edmundsi (Tricos)	0	0	0	0	5	27	0	12	23	4
Tricorythodes explicatus (Tricos)	11	373	307	413	197	3990	5873	1224	297	9
LEPIDOPTERA (Aquatic Moths)										
Petrophila sp.	79	307	227	347	204	60	0	9	0	7
ODONATA (Dragonflies)										
Ophiogomphus severus	0	0	0	0	0	25	66	13	0	3
PLECOPTERA (Stoneflies)										
Isoperla quinquepunctata (Little Yellow Stones)	0	0	0	0	0	0	16	0	0	1

Appendix A. (cont.) Macroinvertebrate taxa list and avg. number per meter squared (#/m2) for the Bighorn Sites Fall 2023										
River Mile from Yellowstone	RM82	RM79	RM76	RM75	RM72	RM63	RM52	RM40	RM 1	
	Split Island	**Rattlesnake SC**	**Juniper SC**	**Three-Rivers**	**Bighorn FAS**	**Mallards FAS**	**Two Leggins FAS**	**Aarapooish FAS**	**Manuel Lisa FAS**	**# of Sites**
TRICHOPTERA (Caddisflies)	419	1173	2293	1615	7950	1009	3994	88	30	9
Hydroptila spp.	113	480	587	131	221	67	32	12	0	8
Amiocentrus aspilis	295	360	1147	499	1394	115	34	32	0	8
Cheumatopsyche spp.	0	67	13	220	1296	297	1125	0	7	7
Hydropsyche occidentalis	0	173	360	383	3205	383	1820	0	7	7
Hydropsyche morosa gr.	0	80	160	43	1290	70	90	0	0	6
Oecetis sp.	11	0	0	205	23	0	24	11	3	6
Brachycentrus occidentalis	0	0	0	17	371	20	50	28	0	5
Hydropsyche nr bronta	0	13	27	0	37	0	40	0	0	4
Hydropsyche C. cockerelli	0	0	0	0	103	37	780	0	0	3
Ceraclea sp.	0	0	0	99	10	0	0	0	0	2
Onocosmoecus unicolor	0	0	0	0	0	10	0	0	0	1
Lepidostoma sp.	0	0	0	19	0	0	0	0	0	1
Nectopsyche sp.	0	0	0	0	0	0	0	4	0	1
Polycentropus sp.	0	0	0	0	0	0	0	0	13	1
Helicopsyche borealis	0	0	0	0	0	10	0	0	0	1
ANNELIDA (Worms/Leeches)	4182	347	173	961	605	1309	1530	29	23	9
Lumbricidae (Aquatic Worm)	23	267	120	90	446	0	64	0	0	6
Naididae	0	0	0	0	0	220	500	0	0	2
Tubificidae	4091	80	53	806	98	600	950	29	23	9
Erpobdellidae	11	0	0	17	31	52	0	0	0	4
Glossophonia complanata	45	0	0	42	31	40	0	0	0	4
Helobdella stagnalis	11	0	0	7	0	397	16	0	0	4
CRUSTACEA (Scuds/Isopods)	13645	4307	1360	6449	540	314	0	0	0	6
Hyalella azteca	0	0	0	0	0	120	0	0	0	1
Gammarus spp.	283	293	67	183	52	0	0	0	0	5
Caecidotea sp.	13362	4013	1293	6266	488	194	0	0	0	6
MOLLUSCA (Snails/Clams)	306	80	40	207	294	131	34	20	0	8
Physella sp. (Pouch snails)	193	67	27	158	79	27	0	12	0	7
Ferrissia rivularis (Limpets)	0	0	0	0	0	29	0	4	0	2
Gyraulus parvus	23	0	0	0	15	0	0	0	0	2
Fossaria sp. (Pond Snails)	0	13	13	0	12	0	0	3	0	4
Stagnicola sp. (Pond Snails)	0	0	0	0	12	0	0	0	0	1
Potamopyrgus antipodarum (NZMS)	91	0	0	34	163	75	0	0	0	4
Pisidium sp. (Fingernail Clams)	0	0	0	15	13	0	34	0	0	3
OTHER Non-Insects	1201	2133	453	1707	62	377	217	4	7	9
Turbellaria (Flatworms)	941	2027	427	1673	50	343	178	4	3	9
Nematoda (Horsehair Worms)	34	53	27	25	0	35	40	0	3	7
Hydracarina (Water-Mites)	227	53	0	9	12	0	0	0	0	4
Total Taxa per site	**33**	**34**	**29**	**41**	**49**	**41**	**41**	**34**	**27**	**36.5**
EPT Taxa per site	**6**	**12**	**9**	**12**	**17**	**15**	**23**	**13**	**13**	**13.3**

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

Appendix B. Bighorn River Similarity Indices based on total organisms from 3 Hess Samples per site							
Total	5,298	5,932	28	30	18	40	138.3
Cuml Total		11,230					
Community Similarity	31						
TAXA SIMILARITY	45				**Spring 2023**	**Split Island vs. Rattlesnake SC**	
Taxon	**Spl Isl**	**Rattlesnake**	**TAXA 1**	**TAXA 2**	**COMMON**	**TAXA 1+2**	**,ai-bi,**
Acentrella turbida	18	0	1	0	0	1	0.34
Amiocentrus aspilis	30	104	1	1	1	1	1.19
Baetis tricaudatus	18	184	1	1	1	1	2.76
Brillia sp.	0	0	0	0	0	0	0.00
Caecidotea sp.	329	341	1	1	1	1	0.46
Cardiocladius spp.	0	16	0	1	0	1	0.27
Ceratopogoninae	217	0	1	0	0	1	4.10
Cricotopus spp.	611	192	1	1	1	1	8.30
Diamesa spp.	774	3,386	1	1	1	1	42.47
Dicrotendipes sp.	2	0	1	0	0	1	0.04
Ephemerella sp.	3	97	1	1	1	1	1.57
Erpobdellidae	0	6	0	1	0	1	0.10
Eukiefferiella spp.	31	51	1	1	1	1	0.28
Fossaria sp.	2	4	1	1	1	1	0.03
Gammarus spp.	14	51	1	1	1	1	0.60
Glossophonia complanata	4	0	1	0	0	1	0.08
Haliplus sp.	0	24	0	1	0	1	0.40
Hydropsyche occidentalis	0	20	0	1	0	1	0.33
Hydroptila spp.	18	0	1	0	0	1	0.34
Isoperla fulva	0	4	0	1	0	1	0.07
Limnophora	9	32	1	1	1	1	0.36
Lumbricidae	0	46	0	1	0	1	0.77
Microtendipes sp	8	0	1	0	0	1	0.15
Oecetis sp.	3	0	1	0	0	1	0.06
Orthocladius spp.	11	0	1	0	0	1	0.21
Pagastia sp	0	107	0	1	0	1	1.81
Parakiefferiella	325	34	1	1	1	1	5.57
Parametriocnemus sp.	0	732	0	1	0	1	12.33
Petrophila sp.	69	53	1	1	1	1	0.40
Phaenopsectra sp	27	0	1	0	0	1	0.51
Physella sp.	16	26	1	1	1	1	0.13
Polypedilum spp.	6	0	1	0	0	1	0.11
Potamopyrgus antipodarum	0	6	0	1	0	1	0.10
Potthastia sp.	0	0	0	0	0	0	0.00
Prodiamesa sp.	2	6	1	1	1	1	0.06
Rheotanytarsus sp.	0	12	0	1	0	1	0.20
Simulium spp.	0	85	0	1	0	1	1.43
Thienemannimyia gr.	0	6	0	1	0	1	0.10
Tipula sp.	2	6	1	1	1	1	0.06
Tricorythodes sp	6	77	1	1	1	1	1.18
Tubificidae	2,717	165	1	1	1	1	48.50
Turbellaria	26	61	1	1	1	1	0.54

Appendix B. Bighorn River Similarity Indices based on total organisms from 3 Hess Samples per site							
Total	6,375	3,736	33	35	23	45	108.3
Cuml Total		10,111					
Community Similarity	46						
TAXA SIMILARITY	51				**Fall 2023 Split Island vs. Rattlesnake SC**		
Taxon	**Spl Isl**	**Rattlesnake**	**TAXA 1**	**TAXA 2**	**COMMON**	**TAXA 1+2**	**,ai-bi,**
Acentrella sp.	24	76	1	1	1	1	1.66
Agabus sp.	10	4	1	1	1	1	0.05
Amiocentrus aspilis	88	108	1	1	1	1	1.50
Baetis flavistriga	0	20	0	1	0	1	0.54
Baetis tricaudatus	71	772	1	1	1	1	19.54
Caecidotea sp.	4,009	1,204	1	1	1	1	30.65
Ceratopsyche spp.	0	24	0	1	0	1	0.64
Cheumatopsyche spp.	0	20	0	1	0	1	0.54
Choroterpes sp.	0	4	0	1	0	1	0.11
Cricotopus spp.	24	20	1	1	1	1	0.16
Cryptochironomus sp.	0	4	0	1	0	1	0.11
Dicrotendipes sp.	78	4	1	1	1	1	1.12
Erpobdellidae	3	0	1	0	0	1	0.05
Eukiefferiella spp.	20	4	1	1	1	1	0.21
Fossaria sp.	0	4	0	1	0	1	0.11
Gammarus spp.	85	88	1	1	1	1	1.02
Glossophonia complanata	14	0	1	0	0	1	0.21
Gyraulus sp.	7	0	1	0	0	1	0.11
Haliplus sp.	24	0	1	0	0	1	0.37
Helobdella stagnalis	3	0	1	0	0	1	0.05
Hemerodromia sp.	0	8	0	1	0	1	0.21
Hydracarina	68	16	1	1	1	1	0.64
Hydropsyche morosa gr.	0	4	0	1	0	1	0.11
Hydropsyche occidentalis	0	52	0	1	0	1	1.39
Hydroptila spp.	34	144	1	1	1	1	3.32
Limnophora	17	4	1	1	1	1	0.16
Lumbricidae	7	80	1	1	1	1	2.03
Microtendipes sp	34	0	1	0	0	1	0.53
Nematoda	10	16	1	1	1	1	0.27
Oecetis sp.	3	0	1	0	0	1	0.05
Orthocladius spp.	10	12	1	1	1	1	0.16
Parakiefferiella	20	0	1	0	0	1	0.32
Petrophila sp.	24	92	1	1	1	1	2.09
Phaenopsectra sp	34	0	1	0	0	1	0.53
Physella sp.	58	20	1	1	1	1	0.37
Polypedilum spp.	0	64	0	1	0	1	1.71
Potamopyrgus antipodarum	27	64	1	1	1	1	1.29
Prodiamesa sp.	7	0	1	0	0	1	0.11
Serratella micheneri	0	12	0	1	0	1	0.32
Simulium spp.	44	40	1	1	1	1	0.38
Stenelmis sp.	0	4	0	1	0	1	0.11
Tricorythodes sp	3	112	1	1	1	1	2.94
Tubificidae	1,227	24	1	1	1	1	18.61
Turbellaria	282	608	1	1	1	1	11.85
Zaitzevia sp.	3	4	1	1	1	1	0.05

Appendix B. Bighorn River Similarity Indices based on total organisms from 3 Hess Samples per site							
Total	2998	5336	32	26	18	40	32.6
Cuml Total		8334					
Community Similarity	83.7						
TAXA SIMILARITY	45.0			**Spring 2023 3-Rivers vs. Juniper SC**			
Taxon	**3-Rivers**	**Juniper SC**	**TAXA 1**	**TAXA 2**	**COMMON**	**TAXA 1+2**	**,ai-bi,**
Amiocentrus aspilus	17	8	1	1	1	1	0.42
Baetis tricaudatus	3	232	1	1	1	1	4.26
Caecidotea sp.	201	88	1	1	1	1	5.06
Cardiocladius spp.	0	36	0	1	0	1	0.67
Ceratopogoninae	2	0	1	0	0	1	0.05
Cricotopus spp.	90	92	1	1	1	1	1.27
Diamesa spp.	2259	4040	1	1	1	1	0.37
Dicrotendipes sp.	26	0	1	0	0	1	0.85
Enallagma sp.	1	0	1	0	0	1	0.04
Ephemerella sp.	10	20	1	1	1	1	0.03
Erpobdellidae	3	0	1	0	0	1	0.09
Eukiefferiella spp.	10	28	1	1	1	1	0.20
Fossaria sp.	27	0	1	0	0	1	0.90
Gammarus spp.	9	8	1	1	1	1	0.14
Haliplus sp.	4	48	1	1	1	1	0.76
Hydracarina	0	80	0	1	0	1	1.50
Hydropsyche occidentalis	0	24	0	1	0	1	0.45
Hydroptila spp.	1	0	1	0	0	1	0.04
Limnophora	0	40	0	1	0	1	0.75
Lumbricidae	1	12	1	1	1	1	0.18
Micropsectra spp.	18	0	1	0	0	1	0.61
Microtendipes sp	3	0	1	0	0	1	0.09
Monodiamesa sp.	0	8	0	1	0	1	0.15
Oecetis sp.	11	0	1	0	0	1	0.36
Orthocladius spp.	11	0	1	0	0	1	0.37
Parakiefferella sp.	26	264	1	1	1	1	4.08
Paraphaenocladius sp.	0	28	0	1	0	1	0.52
Petrophila sp.	2	16	1	1	1	1	0.25
Physella sp.	50	48	1	1	1	1	0.77
Pisidium sp.	2	0	1	0	0	1	0.05
Potamopyrgus antipodarum	12	0	1	0	0	1	0.40
Potthastia sp.	3	48	1	1	1	1	0.81
Simulium spp.	5	64	1	1	1	1	1.02
Stagnicola sp.	4	0	1	0	0	1	0.13
Stratiomyiidae	16	8	1	1	1	1	0.38
Thienemannimyia gp.	7	0	1	0	0	1	0.23
Tricorythodes sp	0	4	0	1	0	1	0.07
Tubificidae	54	64	1	1	1	1	0.59
Turbellaria	112	16	1	1	1	1	3.44
Tvetenia sp.	0	12	0	1	0	1	0.22

Appendix B. Bighorn River Similarity Indices based on total organisms from 3 Hess Samples per site							
Total	4261	2333	41	29	23	47	107.6
Cuml Total		6594					
Community Similarity	46.2						
TAXA SIMILARITY	48.9			**Fall 2023 3-Rivers vs. Juniper SC**			
Taxon	**3-Rivers**	**Juniper SC**	**TAXA 1**	**TAXA 2**	**COMMON**	**TAXA 1+2**	**,ai-bi,**
Acentrella turbida	2	1	1	1	1	1	0.00
Amiocentrus aspilus	150	344	1	1	1	1	11.23
Baetis tricaudatus	114	668	1	1	1	1	25.96
Brachycentrus occidentalis	5	0	1	0	0	1	0.12
Caecidotea sp.	1880	388	1	1	1	1	27.49
Cardiocladius spp.	4	0	1	0	0	1	0.09
Ceraclea sp.	30	0	1	0	0	1	0.70
Cheumatopsyche spp.	66	4	1	1	1	1	1.38
Cricotopus spp.	109	16	1	1	1	1	1.88
Dicrotendipes sp.	61	24	1	1	1	1	0.40
Erpobdellidae	5	0	1	0	0	1	0.12
Eukiefferiella spp.	12	0	1	0	0	1	0.28
Fossaria sp.	0	4	0	1	0	1	0.17
Gammarus spp.	55	20	1	1	1	1	0.43
Glossophonia complanata	13	0	1	0	0	1	0.30
Helobdella stagnalis	2	0	1	0	0	1	0.05
Hydracarina	3	0	1	0	0	1	0.06
Hydropsyche morosa gr.	13	48	1	1	1	1	1.76
Hydropsyche nr bronta	0	8	0	1	0	1	0.34
Hydropsyche occidentalis	115	108	1	1	1	1	1.93
Hydroptila spp.	39	176	1	1	1	1	6.62
Lepidostoma sp.	6	0	1	0	0	1	0.13
Limnophora	3	4	1	1	1	1	0.10
Lumbricidae	27	36	1	1	1	1	0.91
Microcylloepus sp.	2	0	1	0	0	1	0.05
Micropsectra spp.	8	0	1	0	0	1	0.18
Microtendipes sp	46	4	1	1	1	1	0.90
Narpus sp.	3	0	1	0	0	1	0.06
Nematoda	8	8	1	1	1	1	0.16
Oecetis sp.	61	0	1	0	0	1	1.44
Optioservus quadrimaculatus	25	8	1	1	1	1	0.24
Orthocladius spp.	0	12	0	1	0	1	0.51
Parakiefferella sp.	10	0	1	0	0	1	0.24
Paraphaenocladius sp.	14	0	1	0	0	1	0.32
Pericoma sp.	0	24	0	1	0	1	1.03
Petrophila sp.	104	68	1	1	1	1	0.47
Phaenopsectra sp	0	8	0	1	0	1	0.34
Physella sp.	47	8	1	1	1	1	0.77
Pisidium sp.	5	0	1	0	0	1	0.11
Polypedilum spp.	326	76	1	1	1	1	4.38
Potamopyrgus antipodarum	10	0	1	0	0	1	0.24
Rheotanytarsus sp.	3	0	1	0	0	1	0.07
Simulium spp.	0	28	0	1	0	1	1.20
Thienemannimyia gp.	11	4	1	1	1	1	0.08
Tricorythodes sp	124	92	1	1	1	1	1.03
Tubificidae	242	16	1	1	1	1	4.99
Turbellaria	502	128	1	1	1	1	6.30

Appendix B. Bighorn River Similarity Indices based on total organisms from 3 Hess Samples per site							
Total	2998	5932	32	30	20	42	55.33420308
Cuml Total		8929.6					
Community Similarity	72.3						
TAXA SIMILARITY	47.6			**Spring 2023 3-River vs. Rattlesnake SC**			
Taxon	**3-Rivers**	**Rattlesnake SC**	**TAXA 1**	**TAXA 2**	**COMMON**	**TAXA 1+2**	**,ai-bi,**
Amiocentrus aspilus	17	104	1	1	1	1	1.19
Baetis tricaudatus	3	184	1	1	1	1	3.01
Caecidotea sp.	201	341	1	1	1	1	0.96
Cardiocladius spp.	0	16	0	1	0	1	0.27
Ceratopogoninae	2	0	1	0	0	1	0.05
Cricotopus spp.	90	192	1	1	1	1	0.24
Diamesa spp.	2259	3386	1	1	1	1	18.26
Dicrotendipes sp.	26	0	1	0	0	1	0.85
Enallagma sp.	1	0	1	0	0	1	0.04
Ephemerella sp.	10	97	1	1	1	1	1.29
Erpobdellidae	3	6	1	1	1	1	0.01
Eukiefferiella spp.	10	51	1	1	1	1	0.54
Fossaria sp.	27	4	1	1	1	1	0.83
Gammarus spp.	9	51	1	1	1	1	0.57
Haliplus sp.	4	24	1	1	1	1	0.26
Hydropsyche occidentalis	0	20	0	1	0	1	0.33
Hydroptila spp.	1	0	1	0	0	1	0.04
Isoperla fulva	0	4	0	1	0	1	0.07
Limnophora	0	32	0	1	0	1	0.53
Lumbricidae	1	46	1	1	1	1	0.72
Micropsectra spp.	18	0	1	0	0	1	0.61
Microtendipes sp	3	0	1	0	0	1	0.09
Monodiamesa sp.	0	6	0	1	0	1	0.10
Oecetis sp.	11	0	1	0	0	1	0.36
Orthocladius spp.	11	0	1	0	0	1	0.37
Pagastia sp	0	107	0	1	0	1	1.81
Parakiefferella sp.	26	732	1	1	1	1	11.47
Paraphaenocladius sp.	0	34	0	1	0	1	0.56
Petrophila sp.	2	53	1	1	1	1	0.85
Physella sp.	50	26	1	1	1	1	1.23
Pisidium sp.	2	0	1	0	0	1	0.05
Potamopyrgus antipodarum	12	6	1	1	1	1	0.30
Potthastia sp.	3	0	1	0	0	1	0.09
Rheotanytarsus sp.	0	12	0	1	0	1	0.20
Simulium spp.	5	85	1	1	1	1	1.25
Stagnicola sp.	4	0	1	0	0	1	0.13
Stratiomyiidae	16	0	1	0	0	1	0.53
Thienemannimyia gp.	7	6	1	1	1	1	0.13
Tipula sp.	0	6	0	1	0	1	0.10
Tricorythodes sp	0	77	0	1	0	1	1.29
Tubificidae	54	165	1	1	1	1	1.00
Turbellaria	112	61	1	1	1	1	2.71