BIGHORN RIVER ALLIANCE

Research Initiative

Aquatic Macroinvertebrate Monitoring Summary 2021 for the Bighorn River, Montana: Building a Long-term Data Set

Bighorn River at Arapooish FAS looking upstream, September 2021

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BIGHORN RIVER ALLIANCE

Executive Summary

In 2021, the Bighorn River Alliance (BHRA) completed the 2nd year of the Bighorn River Baseline Benthic Macroinvertebrate (BMI) Monitoring program. Eight long-term, baseline monitoring sites were established for sampling water quality and benthic macroinvertebrates from ~2 miles below Afterbay Dam downstream to its confluence with the Yellowstone River. We chose four sites because they had been previously sampled by Brammer (1986-87) and Montana Department of Environmental Quality (MDEQ) (2001-2005). The goals of this study are: 1) to conduct standardized, replicated BMI surveys to serve as baseline standards for future monitoring efforts within this 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 have affected the overall biological health of the Bighorn River; and 3) to determine if significant aquatic biological changes have occurred over the last decade or longer since previous BMI samples were collected.

In both late-April and mid-September 2021, we collected 24 Hess BMI samples at eight sites. Streamflow inputs from Soap Creek, Rotten Grass Creek, the Little Bighorn and other tributaries influenced by irrigation return flows in the valley can have significant effects on the water quantity, quality and temperatures of the Bighorn River. During our Fall visits, the Mallards Landing (MI4) water clarity was so turbid from the 'African Queen' inflows that we could not see the stream bottom where we were sampling (river right) or the sediment grid in ~12 inches of water.

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

In 2021, 87 total BMI taxa were collected across the 8 Bighorn River sites, averaging 34 taxa per site**.** Mayfly (E), caddisfly (T), plus the occasional stonefly (P) (Total EPT Taxa) averaged 27% (5 to 41%) of the total community at a site and 12 species on average per site. Ten species of mayflies (E) were recorded throughout the study section: the dominant three were Blue Winged Olives *(Baetis tricaudatus, B. flavistriga*), Tiny BWOs (*Acentrella turbida*) and Tricos (*Tricorythodes explicatus*) followed by Pale Morning Duns (PMDs) (*Ephemerella excrucians*). Of the 13 total species of caddisflies (T) collected in 2021, the net-spinning caddisflies, *Hydropsyche occidentalis and Cheumatopsyche*, and long-horned caddis, *Oecetis avara,* were collected across the most sites, while the western weedy sedge, *Amiocentrus asplius* is quite abundant upstream of Hardin. Stoneflies (P) are minor components in the Bighorn River, only two species of Little Yellow Stones were reported in low numbers near the dam (MI1, Split Island) and at Two Leggins (MI5).

Total taxa richness, EPT diversity, % EPT and benthic densities of insects significantly decreased downstream of the Little Bighorn River confluence in 2020, but in 2021 there is an increase in BMI taxa richness and densities at sites from the Little Bighorn to the Yellowstone River confluence. EPT taxa and total species richness were both highest at Two Leggins FAS in the Fall of 2021 with 15 EPT and 50 total species richness. Species diversity and EPT taxa richness, in general, increased with increasing distance from Yellowtail Dam until the Little Bighorn River enters, then diversity declines and nutrient, sediment and temperature tolerant BMI species increase.

Overall, BMI communities collected across the upper Bighorn sites, Split Island and Three Rivers, in Fall 2021 were similar to those reported by Brammer (1987) with ~55% average taxa similarity and a similar benthic density at Split Island, (Three Rivers still has ~2x lower BMI densities than in 1987). Exceptionally low taxa similarity was reported at Mallards Landing (25%) and at Manuel Lisa (33%) between the 2020 and MDEQ's 2001 and 2005 samples. In 2001, the Manuel Lisa site contained a unique benthic community reporting six mayfly species that were collected nowhere else in the river, we only documented three of these persisting in 2021. Since 2020, we have now documented the invasive New Zealand mudsnail at 4 sites: 2021 densities are: Split Island (avg. 40 per m^2), Three Rivers (1,730 per m^2), Bighorn FAS (460 per m^2) and Two Leggins (20 per m^2); although the average of four sites is low, high densities in the Three Rivers section are beginning to look troubling.

Hilsenhoff Biotic Index (HBI) Scores >5.0 reported at all Bighorn River sites in 2020 and 2021 indicate that the BMI communities are experiencing at least moderate nutrient and/or sediment enrichment, and all (100%) monitoring sites in Fall 2021 are exhibiting significant organic impairment with HBI scores >6.0.

The implications of this research are that dynamic, 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 numbers were significantly depressed within three miles of the Afterbay Dam (MI1, Split Island and MI2, Three Rivers) in 2020 compared to previous years (1986-87) and at sites further downstream (MI4, Mallards Landing). But the lack of flushing flows in 2021 have caused exponential increases in benthic BMI densities at these sites.

We conclude this 2020-2021 analysis by documenting that the BMI communities are expressing the impaired biological health of the Bighorn River, especially at Mallards Landing FAS and downstream of the Little Bighorn River. Reasons for these observed ecological changes can be linked to increasing average water temperatures, high nutrient levels, aquatic plant growth and sediment accumulations exacerbated by depressed 2021 stream flows. Population increases of more silt-tolerant BMI taxa (midges and aquatic worms) appears to be shifting the community away from mayfly and caddisflies.

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APPENDICES

Appendix A Macroinvertebate Taxa List and Densities for All Sites.

Appendix B Site Photos

Acknowledgements

We would like to thank Anne Marie Emery, James Chalmers and the BHRA Board for funding this project with Montana Biological Survey (MBS). Initial Report review and editing was provided by Phil Sawatzki. Initial field site coordination and logistics were set up and expedited by Dennis Fischer and Emery Three Irons.

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 should be a large prairie river but has been transformed into a salmonid bearing river by Yellowtail Dam's cold-water releases. Trout fishermen are most familiar with the 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 is consistently ranked in the top three with the most angler days in the state (MFWP 2016). Despite this popularity, the Bighorn River has lacked recent comprehensive studies of benthic invertebrate (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 (**Map 1**) by collecting repeatable, quantitative, baseline data using standardized methods (**Photo 1).**

- What historical BMI data has been collected and how can this be used in comparison to current communities? How can we relate these biological indicators to spatial and temporal trends in aquatic habitat health as influenced by macrophyte beds, algae, aquatic invasive species, sedimentation, water quality, dissolved gases and regulated flows?

2) To evaluate both temporal and spatial BMI patterns to help BHRA understand how they have been 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.

2.0 Methods

During each sampling event at a site, an Oakton 10 water quality multi-meter was used to collect 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 macroinvertebrates 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 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 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 noted 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. Macroinvertebrates 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 more silt tolerant and can increase in numbers with increasing siltation.

One informative stand-alone metric is the Hilsenhoff Biotic Index (HBI) which measures the tolerance of a macroinvertebrate 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 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 a core 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.

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) (***Table 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 _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 2021 BMI sampling sites (MI1-MI8) from Yellowtail Dam to the Yellowstone River confluence*.

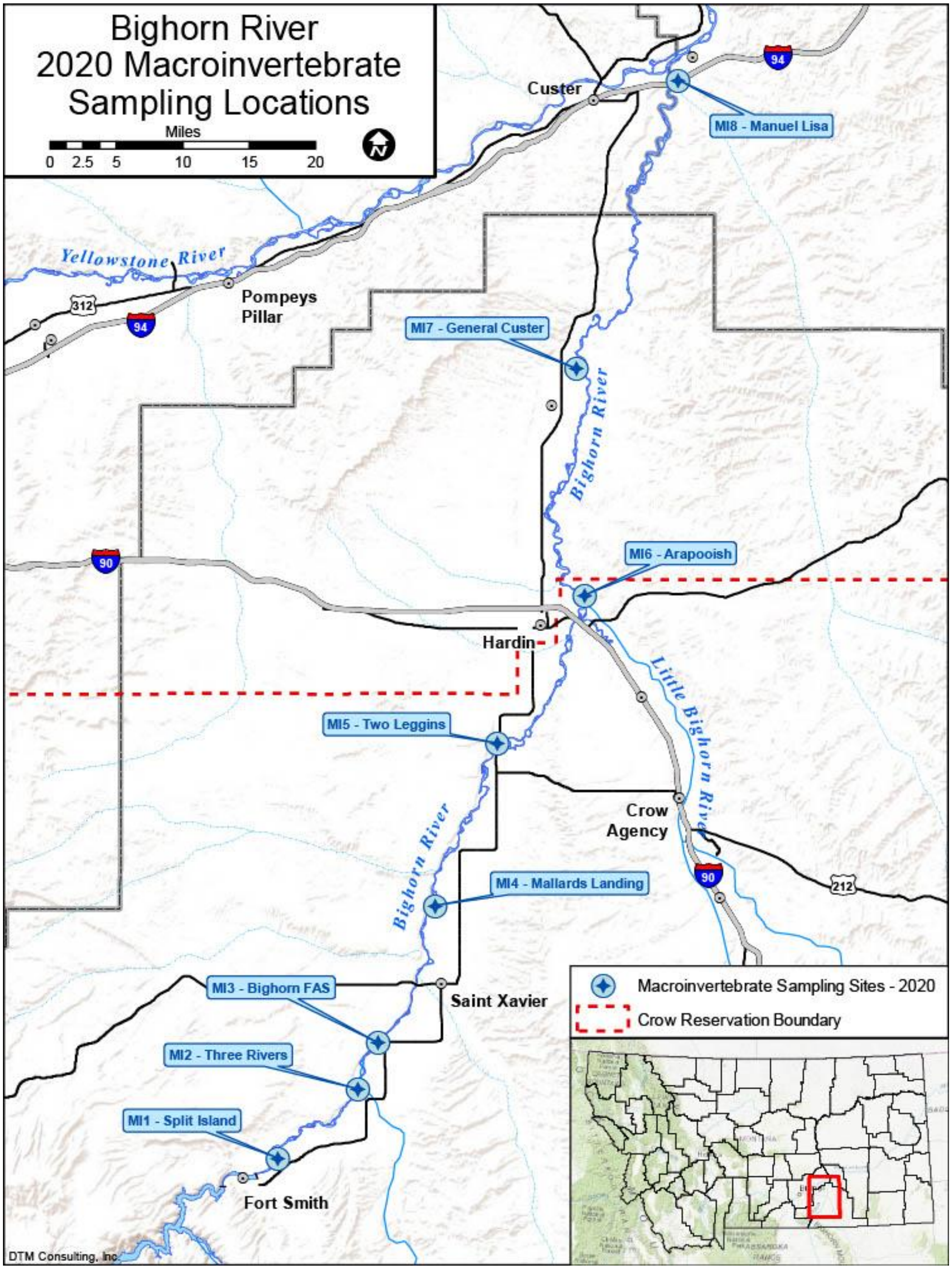
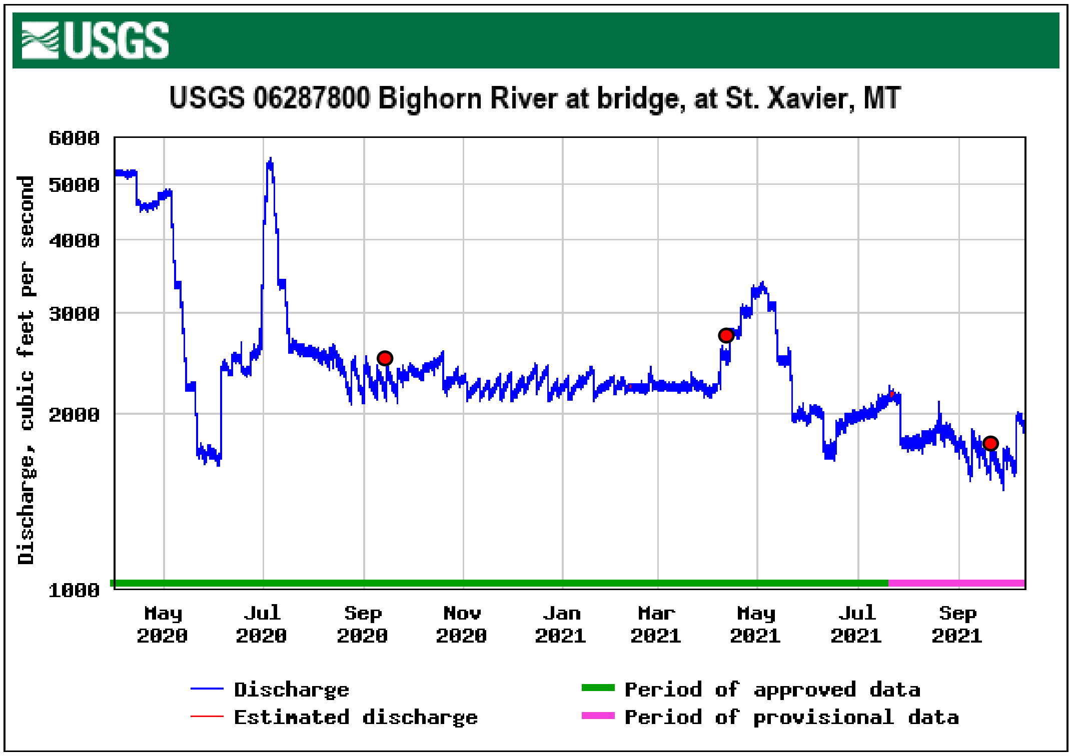


Figure 1. *Discharge reported during the 2020-2021 Seasonal BMI Sampling Visits (Red Dots).*



3.0 Results

1) Overall, 87 total macroinvertebrate taxa were collected across the 8 Bighorn River sites in 2021 (***Appendix A***); average total number of taxa per site was 35 (***Figure 2***). Mayfly (E), caddisfly (T), plus the occasional stonefly (P) (Total EPT Taxa) were in the range of about 5 to 41 percent of the total number of taxa at a site and averaged 12 species per site (***Figure 2***). High points of overall and EPT richness in 2021 were at Two Leggins FAS with 50 total taxa and 15 EPT taxa (***Figure 2*****).** In the Fall 2020, total taxa, EPT taxa and % EPT taxa tended to decline after the Little Bighorn River entered near Hardin (upstream of Arapooish FAS), but in 2021 declining EPT species richness and %EPT at the upper sites, meant that downstream sites, especially Arapooish, with 12 EPT species and 41% EPT, have increased since 2020 (***Figure 2, Map 1, Table 3***).

Figure 2*. Total BMI taxa, and EPT taxa (Mayflies + Stoneflies + Caddisflies) at the Bighorn River sites from 2020 & 2021. Sites are arranged from upstream (left) to downstream (right).*

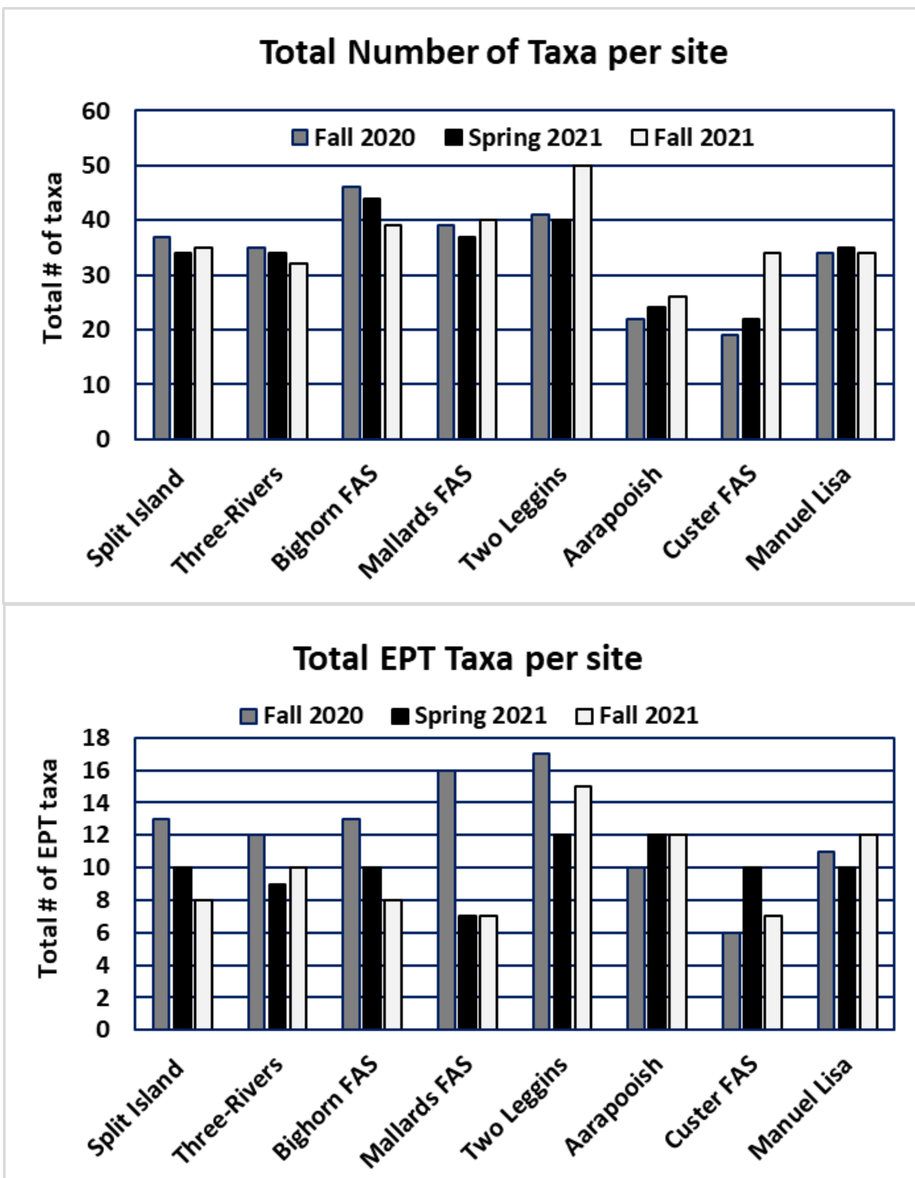
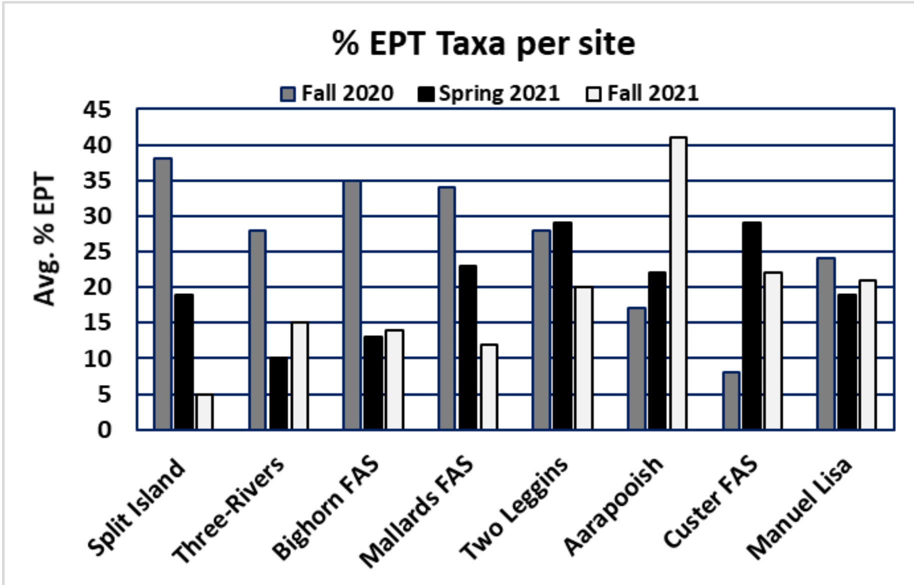
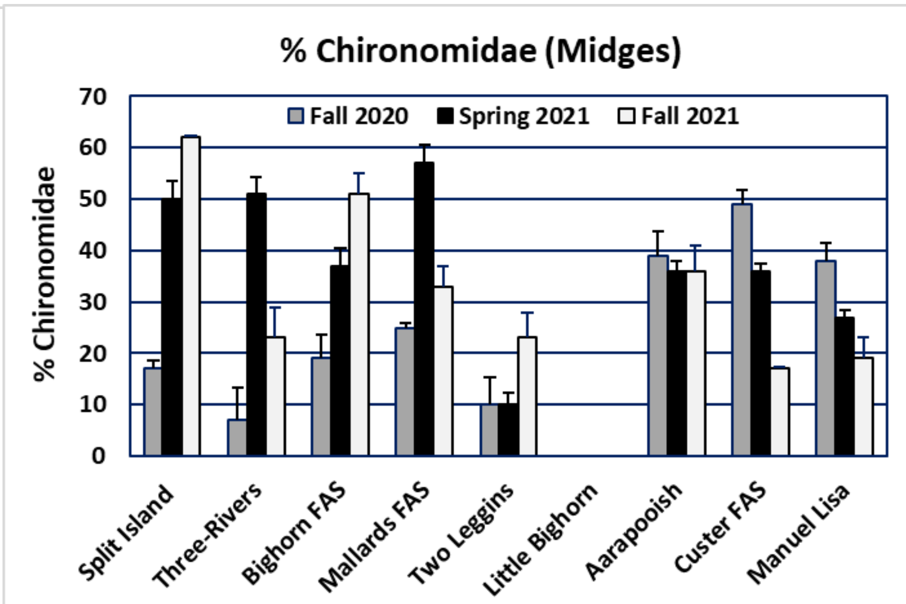


***Figure 2**. (Cont.) Macroinvertebrate % EPT (Mayflies + Stoneflies + Caddisflies) (top) and % Chironomidae (bottom)*

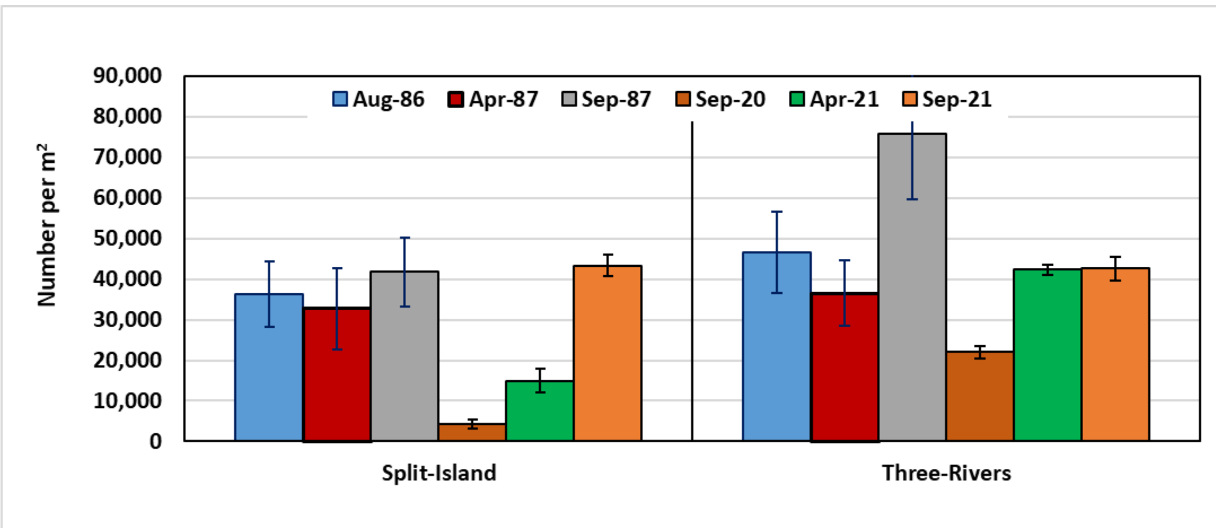




Mayflies and caddisflies (EPT taxa) dominated the upper sites in the Fall 2020, but not in the Spring or Fall 2021; Arapooish FAS reported the highest %EPT (41%) in the Fall 2021 samples. Significant increases in Chironomidae (Midges) abundance with subsequent decreases in Mayfly and Caddisfly numbers has occurred between Fall 2020 and both Spring and Fall 2021; Split Island reported the highest % of Chironomidae in Fall 2021, but in Fall 2020 reported the 2nd lowest (***Figure 2***). Large increases in midges and decreases in caddisflies between the seasonal sampling periods are most notable. Midges contribute a larger % of the BMI community during both 2021 seasons upstream of the Little Bighorn River.

2) **Benthic Macroinvertebrate Densities.** BMI densities reported at Split Island on September 2021 averaged 43,292 individuals per m^2 (± 2,560 SE); this is very similar to values reported in Sept. 1987 (41,761 ind. per m^2) and 10 times higher than reported in Fall 2020 (***Figure 3, Table 2***). BMI densities at Three Rivers in September 2021 averaged 42,570 individuals per m^2 (± 2,970 SE) which is very similar to the Spring 2021 Density value; but is still significantly (~2 times) lower than densities reported in Sept. 1987 (75,670 ind. per m^2) (***Figure 3, Table 2***). BMI densities at the Bighorn FAS site in Fall 2021 are ~4 times higher (32,000 more inverts per m^2) than reported during the Spring 2021 (***Table 2***).

Figure 3*. Mean BMI densities at the 2 upper Bighorn River sites across years and seasons. Error bars are ±1 Standard Error.*



The macroinvertebrate densities reported at Mallards Landing in 2021 were higher than in 2020 or 2005 (***Table 2***). We could not compare our Manuel Lisa FAS benthic densities with DEQ samples collected there in 2001-2005 because we could not find data on the Hess sample processing.

The 2021 BMI densities across most of the upper Bighorn River sites have significantly increased during this low flow year compared to BMI densities from the Fall 2020 data (attributed to large flushing flows of 2017-2019), (***Table 2, Figure 3***). We documented that the largest increases are attributed to the silt-tolerant invertebrate taxa groups (Aquatic worms and midges) (***Table 3***).

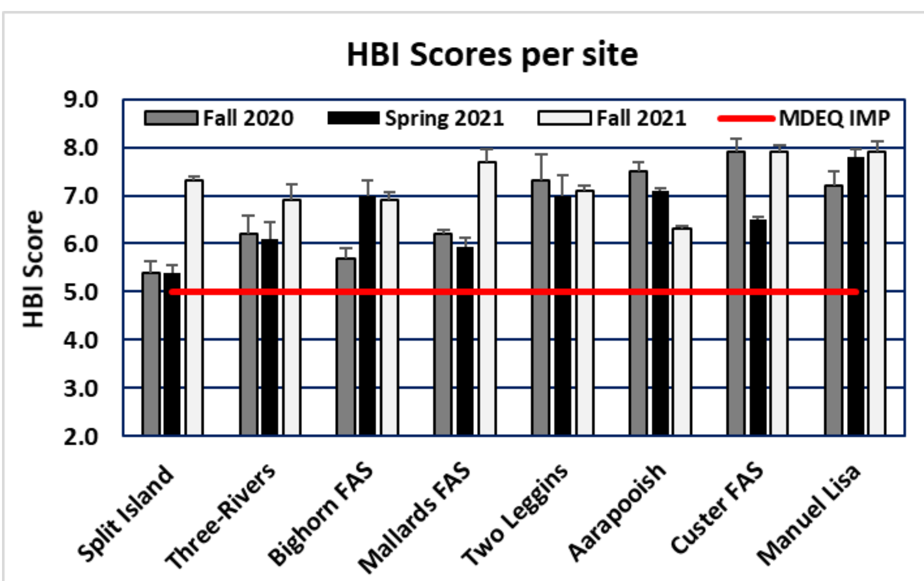
***Table 2**. Macroinvertebrate Hess sample (n=3) numbers/densities at 8 Bighorn River sites from Sept. 2021, April 2021, Sept. 2020 vs. 1987 or 2003-2005.*

	Fall 2021			Fall	Fall	Spring	Fall	Fall
	Hess Number			2021	2021	2021	2020	1987
Site Name	**H1**	**H2**	**H3**	**Avg.**	**#/m2**	**#/m2**	**#/m2**	**#/m2**
BHR_Split Island	4775	3,888	4,325	4,329	43,290	14,890	4,434	41,761
BHR_Three Rivers	4,807	4,145	3,819	4,257	42,570	42,250	21,970	75,670
BHR_BighornFAS	3,913	4,163	4,625	4,233	42,330	10,720	14,180	na
BHR_MallardsFAS	1,934	1,665	4,034	2,544	25,440	34,520	18,185	12,180*
BHR_TwoLegginsFAS	2,006	1,941	2,194	2,047	20,470	14,210	15,783	na
BHR_Arapooish	912	982	750	881	8,810	8,480	3,800	na
BHR_Custer	2,318	1871	2,053	2,080	20,800	10,320	3,950	na
BHR_Manuel Lisa	1,765	1,224	900	1,296	12,960	15,220	8,127	na

** DEQ samples from 2003-2005*

3) Tolerance Index Scores. HBI Scores >5.0 calculated at all Bighorn River sites in 2020 and 2021 indicate that the macroinvertebrate communities are experiencing moderate sediment/nutrient enrichment, and all eight (100%) of the sites exhibited significant enrichment scores >6.0 in the Fall 2021 (***Figure 4***). The biological integrity at the upper sites, as measured by the HBI increases, has significantly decreased (i.e., gotten worse) from Fall 2020 to Fall 2021 (T-test, p=0.02).

***Figure 4.** 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.*



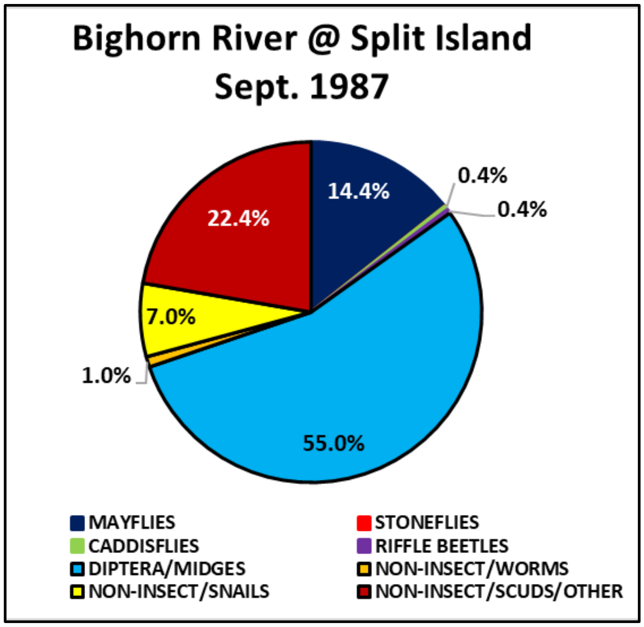
While most the Bighorn sites exhibited HBI tolerance increases in 2021, Arapooish FAS has experienced a steady decline of the HBI (increasing BMI health, but still ranked significantly impaired) over the last year of seasonal sampling (***Figure 4***).

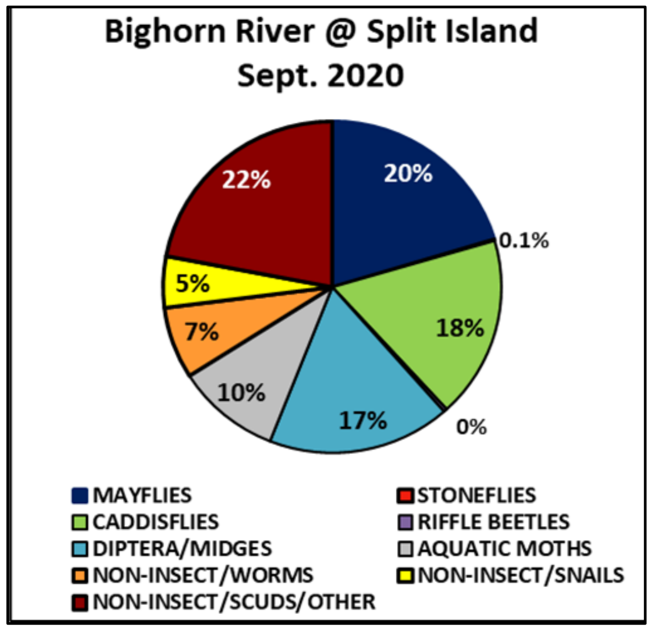
4) Species Losses/Additions. Multiple species reported from the Bighorn River samples in 2020 & 2021 were not observed during the 1986-1987 sampling. No individuals of the aquatic moth, *Petrophila,* were reported during any sample period in 1986-1987, but in 2021, we documented this species at densities of 208 individuals per m^2 (0.5% of the community) at Split Island and 3,458 per m^2 (7.8%) at Three Rivers (**Table 2**). Similarly, the Isopod, *Caecidotea* was not reported during 1986-1987, but we documented this taxon at moderately abundant numbers (3,333 ind. per m^2) at Split Island (7.7% of the community) and 8,617 ind. per m^2 at Three Rivers (19%). Likewise, the non-native, New Zealand mudsnail (NZMS) was not present at either upper site in 1987, but in 2020, Three Rivers had NZMS densities of 880 ind. per m^2 and in 2021 approximately 2x those densities (1,730 per m^2) (**Table 2)**. 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. So, it is surprising that we did not observe them at the Split Island sampling reach in 2020, but at low densities in 2021. They occur at similar densities up and down the Madison, Yellowstone and Missouri Rivers at heavily fished access points and appear to have reached an equilibrium point (not too dominant) within the benthic community. In 2001, the Manuel Lisa site contained a unique benthic community reporting six mayfly species that were collected nowhere else in the river, we only documented three of those taxa persisting in 2021.

5) Split Island: The biggest differences observed in the composition of the BMI community at Split Island in 2020/2021 vs. 1987 were the decreases in Mayflies and the increasing dominance of the midges and aquatic worms. 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) which now contribute ~18% of the community. But, by Fall 2021, caddisflies have declined to only 2% of the BMI community. Additionally, the aquatic moth *Petrophila* was not reported in 1987, contributed ~10% to the community in 2020, in 2021 is <1% (**Table 3, Figure**

5). Aquatic worms have continued to increase from 7% to 22% during the low-flow year between 2020 and 2021, while caddisflies, mayflies and even scuds are contributing less to the BMI community (**Figure 5**).

Figure 5. *Macroinvertebrate Composition represented at Split Island Fall 1987 vs. 2020/2021.*





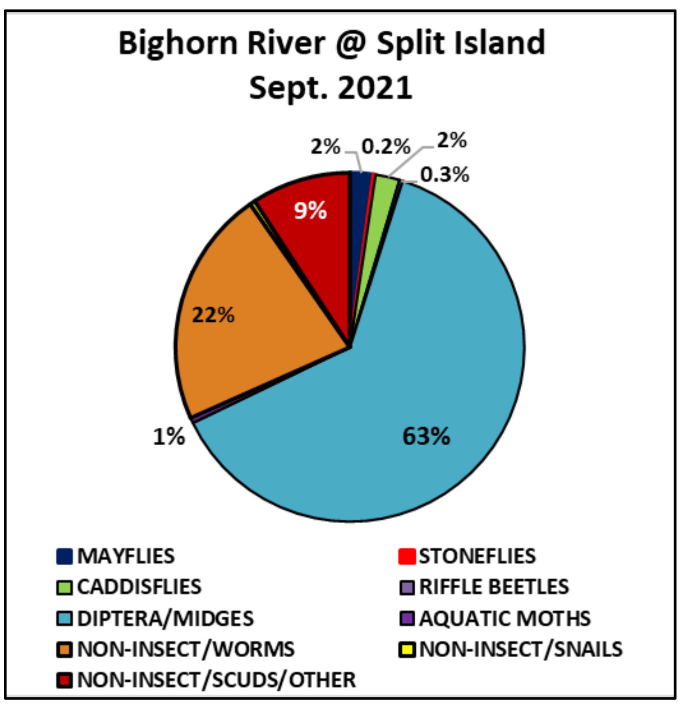


Table 3 *. Macroinvertebrate Ordinal Percentage (%) at Bighorn Sites for Sept. 2020, April 2021 and Sept. 2021. Large increases in midges and aquatic worms and decreases in caddisflies between seasonal sampling periods are most notable. Green shading are significant increases between seasons, while red shading are decreases.*

Site Name	Split Island			Three Rivers			Bighorn FAS			Mallards Landing FAS		
Invertebrate Orders	FALL 2020	SPRING 2021	FALL 2021	FALL 2020	SPRING 2021	FALL 2021	FALL 2020	SPRING 2021	FALL 2021	FALL 2020	SPRING 2021	FALL 2021
MAYFLIES	20%	13%	2%	19%	9%	4%	10%	9%	6%	24%	22%	9%
STONEFLIES	0.1%	0%	0.2%	0%	0%	0%	0%	0.1%	0%	0%	0%	0%
CADDISFLIES	18%	5%	2%	9%	2%	11%	25%	5%	8%	10%	1%	3%
RIFFLE BEETLES	0.3%	0.6%	0.3%	1.0%	0.1%	0.0%	3%	0.7%	0.8%	0.1%	0.0%	0.2%
DIPTERA/MIDGES	17%	50%	63%	7%	51%	25%	18%	39%	53%	24%	58%	39%
AQUATIC MOTHS	10%	8%	0.5%	2%	2%	8%	2.1%	2.5%	2.0%	0.1%	0.9%	0.3%
NON-INSECT/WORMS	7%	4%	22%	7%	19%	22%	5%	30%	18%	30%	16%	39%
NON-INSECT/SNAILS	5%	2%	0.5%	9%	4%	6%	19%	9%	6%	2%	1%	4.0%
NON-INSECT/SCUDS/OTH	22%	16%	9%	46%	15%	23%	19%	5%	5%	6%	1%	0.7%

Site Name	Two Leggins FAS			Arapooish FAS			Custer FAS			Manuel Lisa		
Invertebrate Orders	FALL 2020	SPRING 2021	FALL 2021	FALL 2020	SPRING 2021	FALL 2021	FALL 2020	SPRING 2021	FALL 2021	FALL 2020	SPRING 2021	FALL 2021
MAYFLIES	14%	24%	5%	9%	20%	25%	6%	28%	14%	8%	18%	10%
STONEFLIES	0.0%	0.6%	0.2%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
CADDISFLIES	14%	4%	14%	8%	2%	15%	2.0%	1.3%	8.4%	15%	1.1%	11.0%
RIFFLE BEETLES	0.5%	0.1%	1.0%	0.1%	0.0%	1.0%	0.0%	0.2%	0.0%	0.2%	0.1%	2%
DIPTERA/MIDGES	11%	14%	24%	38%	37%	37%	48%	36%	18%	28%	27%	20%
AQUATIC MOTHS	1.4%	3.3%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
NON-INSECT/WORMS	47%	46%	40%	43%	40.5%	21%	42%	34%	49%	47%	53%	55%
NON-INSECT/SNAILS	7%	2%	5%	0.7%	0.3%	0.4%	0.7%	0.7%	2%	1.0%	0.2%	2.0%
NON-INSECT/SCUDS/OTH	7.2%	4.5%	8%	2.0%	0.4%	0.9%	0.9%	0.4%	2%	0.5%	1.1%	0.4%

6) Mallards Landing FAS: The largest long-term differences observed in the 2020 composition of the BMI community at Mallards Landing FAS compared to 2003-2005 are the significant increase in the numbers of mayflies (E) and caddisflies (T), and the decrease in abundance of the non-insect taxa, especially aquatic worms during Fall of 2020 (**Figure 6**). This increase in EPT taxa has caused a decrease of the HBI tolerance scores, indicating an increase in the biological health of this river section in 2020 (**Figure 7**). But low river flows of 2021 have allowed the increase of silt-tolerant, non-insect taxa (Aquatic worms) and midges coupled with reductions of mayfly and caddisfly abundance by the Fall; this has resulted in a subsequent increase in the HBI scores back to significantly impaired rankings (**Figure 7**).

Figure 6. *Macroinvertebrate Composition represented at Mallards Landing 2004 vs. 2020/2021.*

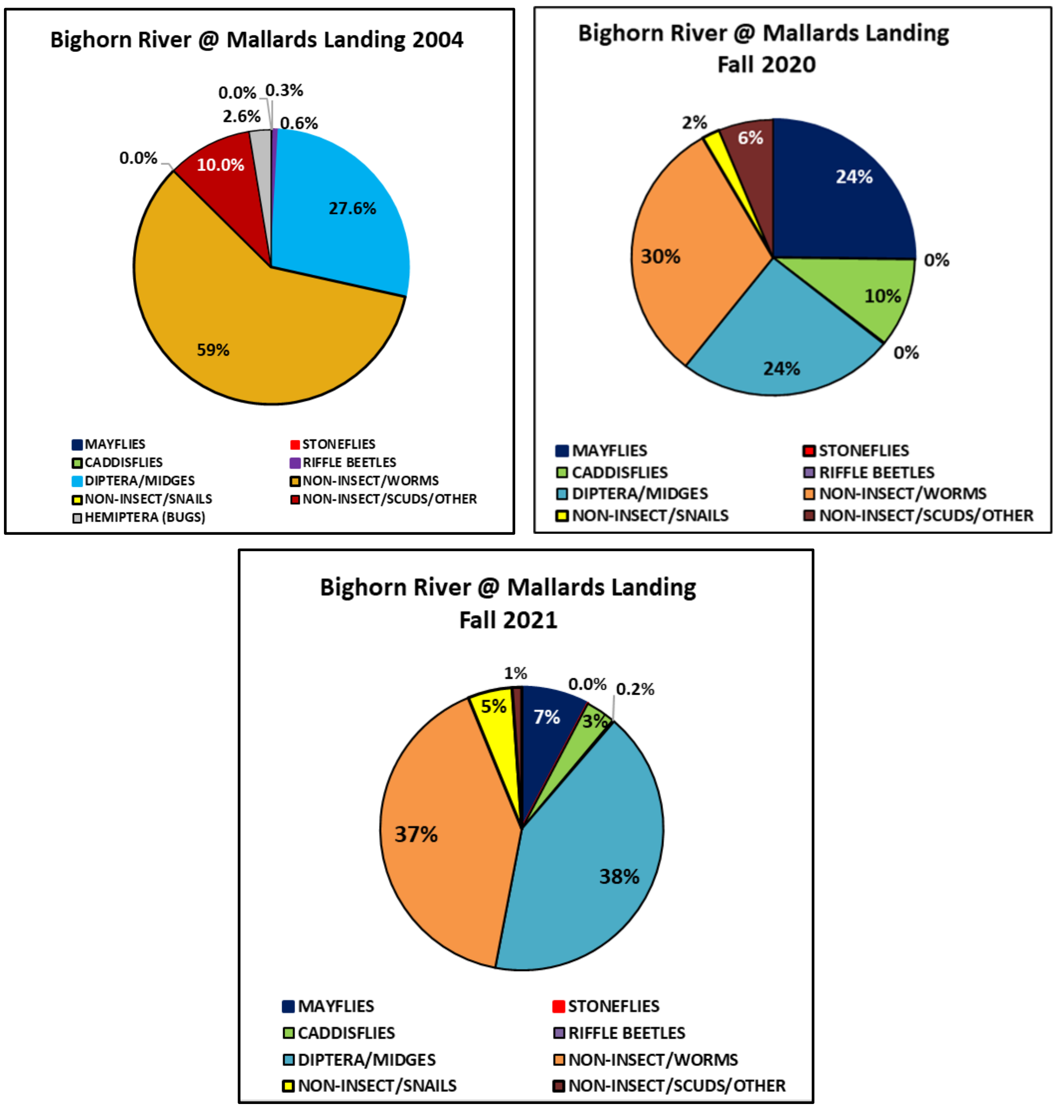
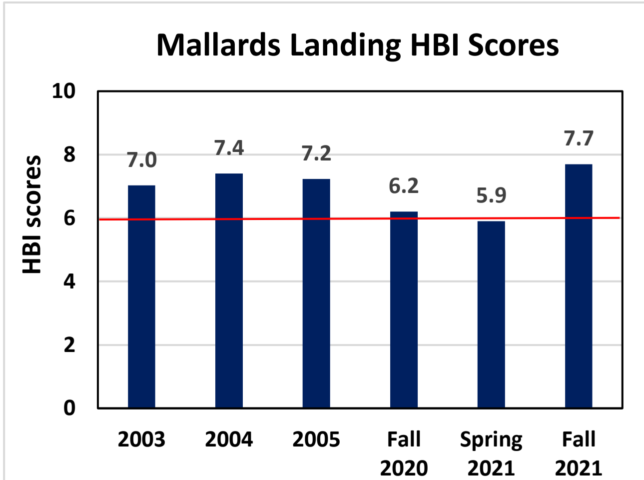
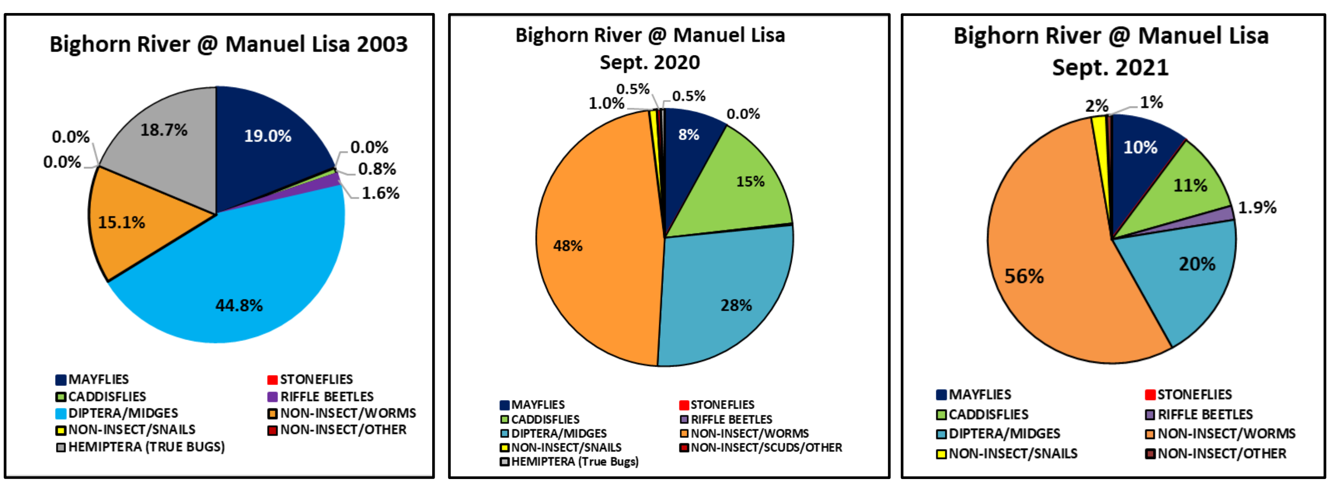


Figure 7. *Macroinvertebrate HBI scores calculated at Mallards Landing 2003-2005 vs. 2020/2021. Redline represents significant nutrient impairment with HBI scores >6.0.*



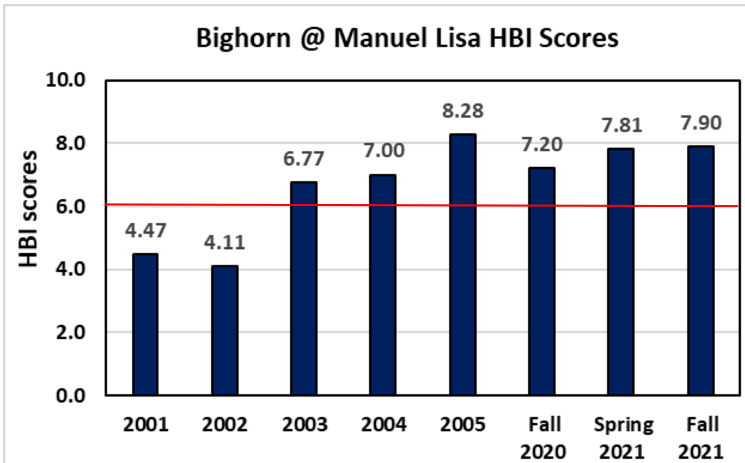
8) Manuel Lisa FAS: The biggest differences observed in the composition of the BMI community at Manuel Lisa FAS in 2020 compared to 2003 are the decreased dominance of mayflies, true bugs and midges, and the increase in abundance of non-insect taxa, especially aquatic worms (**Figure 8**). Net-spinning caddisflies, *Cheumatopsyche / Hydropsyche spp.* (which were both present in low numbers in 2001-2005), have increased in abundance in 2020 to contribute ~15% of the BMI community; these contributions were slightly lower (11%) in 2021. Mayflies had similar contributions to the community in 2020 (~8%) and 2021 (10%), as did the midges (**Figure 8**). Despite maintaining decent mayfly and caddisfly abundance in 2020 and 2021, the increase in Aquatic worms has caused the HBI scores to increase to their highest levels documented **(Figure 9**).

Figure 8. *Macroinvertebrate Composition represented at Manuel Lisa 2003 vs. 2020/2021*



HBI scores of >6.0 reported during all years since 2003 and >7.0 in the last 3 seasons are indicative of a significantly impaired BMI community and are ranking the biological integrity of this river section as unhealthy due to sediment and/or nutrient enrichment (**Figure 9**)

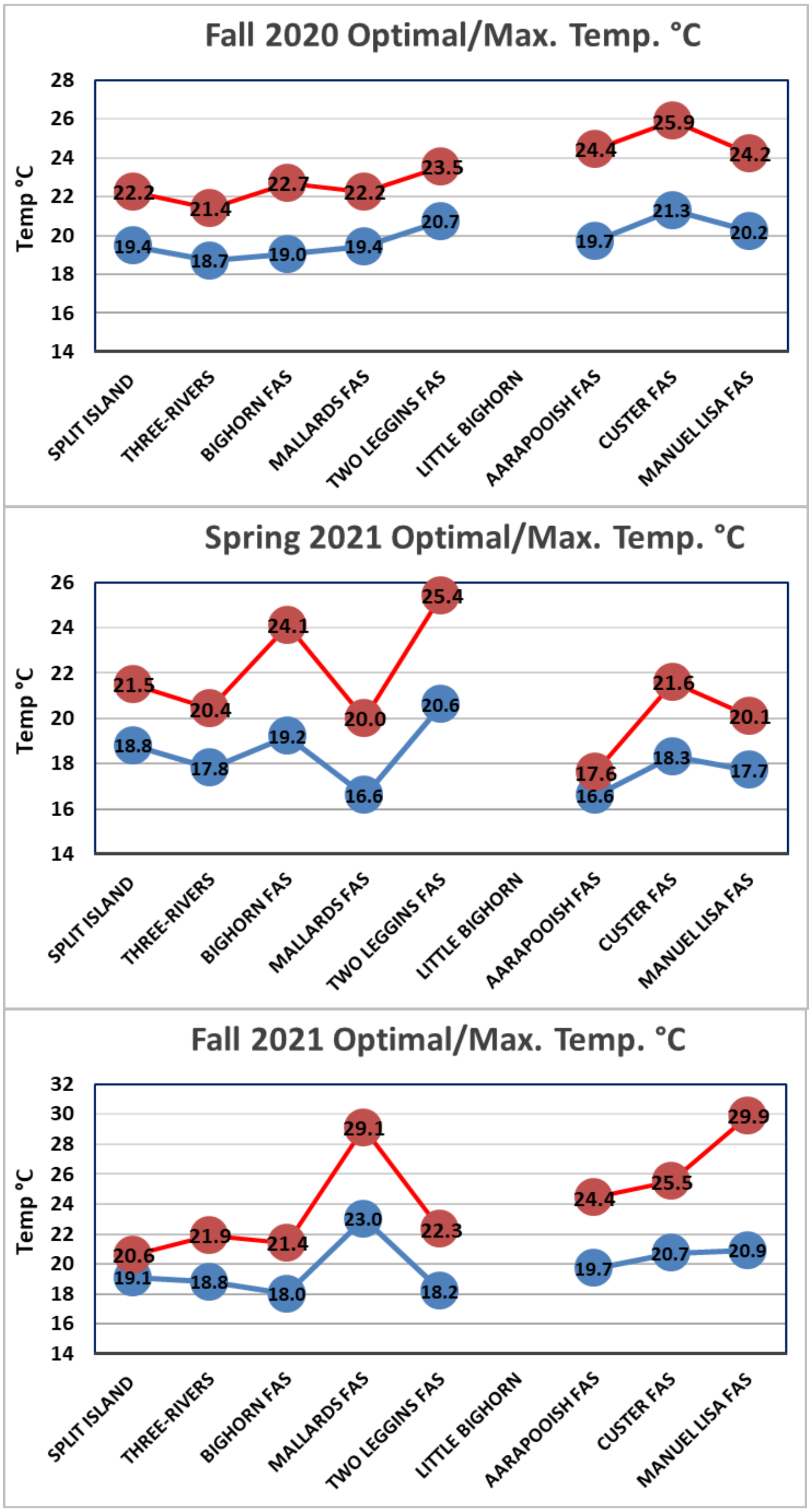
Figure 9. *Macroinvertebrate HBI scores calculated at Manuel Lisa 2001-2005 vs. 2020. Redline is >6.0 indicate significant nutrient impairment.*



9) Macroinvertebrate Community Temperatures

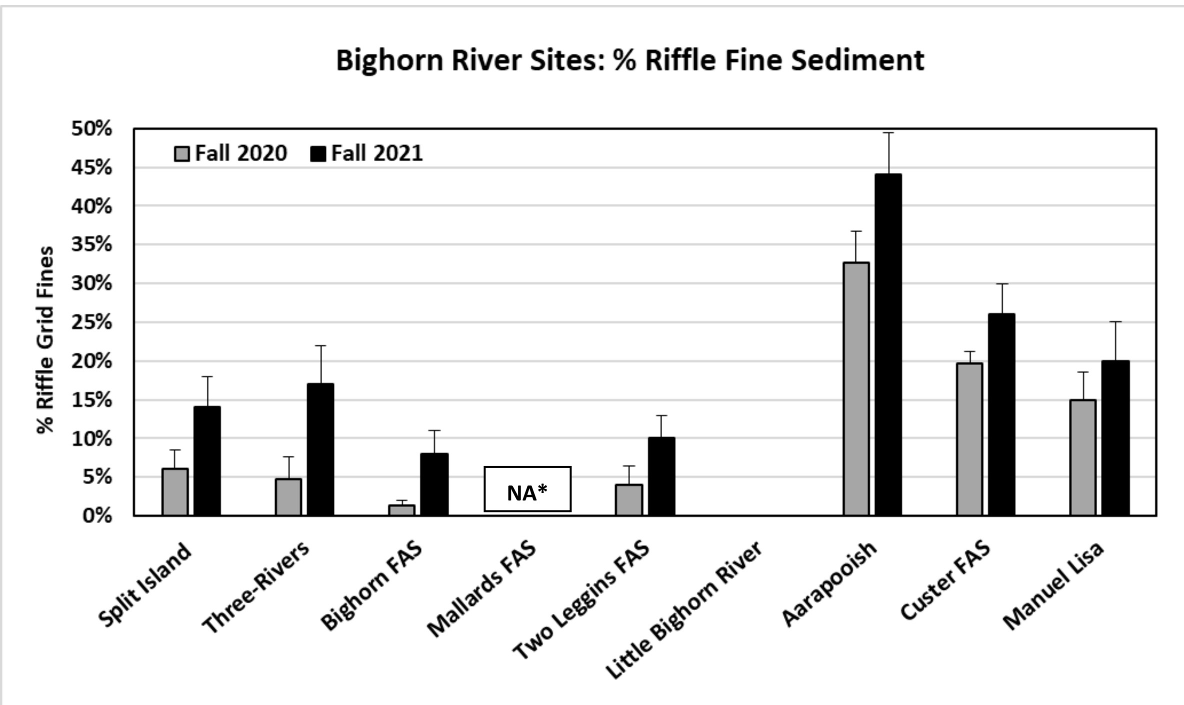
We evaluated the Bighorn River macroinvertebrate community tolerance to increasing temperatures and lower flows between the Fall 2020 & Fall 2021 samples. Increasing maximum temperature tolerances up to ~26°C (78.6°F) at the Custer FAS Bighorn River site begins downstream of Two Leggins FAS with a slight decrease at Manuel Lisa (24.2°C, 75.6°F), from the effects of Tullock Creek entering upstream (***Figure 10***). We observed the largest temperature shift from cool-water taxa to more temperature-tolerant, warm-water species during Fall 2021 samples at Mallards Landing (likely caused by warm irrigation returns), and all the way downstream at Manuel Lisa FAS with maximum temperatures >29°C (***Figure 10*****).**

Figure 10. *Macroinvertebrate Community Optimal (blue) and Maximum (red) Temperatures for Bighorn River sites during 2020 and 2021.*



10) Stream Habitat Results. The MDEQ fine sediment grid counts revealed that benthic fine sediment in riffles increases substantially downstream of Hardin after the Little Bighorn River enters (***Figure 11***). We also documented a significant increase in riffle sediments in the upper 5 sites in 2021 compared to 2020 (t-test, p-value=0.01). We recorded the 'cleanest' riffle sediments at the Bighorn FAS (MI 3) with less than 2% fines in the gravels in 2020, but this site has increased to ~10% riffle sediments in 2021 (***Figure 11***). Mallards Landing FAS was so turbid from the irrigation returns during both years' September visits that we could not see the grids to measure (***Figure 11***).

Figure 11. *Percent riffle fine sediment calculated from the MDEQ grid tosses for the fall 2020 & 2021 Bighorn River sites. NA* = too turbid to see the grid count.*



Three sites where aquatic vegetation may be adversely affecting benthic riffle habitats in the Fall 2021 were at the Bighorn FAS with 45% average coverage, Three Rivers with ~25% coverage and Split Island avg. 7% (**Appendix B, Photo 18).**

4.0 Conclusions

- Spring and Fall 2021 macroinvertebrate sample data at these 8 Bighorn River sites revealed that five sites upstream of Hardin contained more abundant and diverse macroinvertebrates and contain higher percentages of EPT taxa; although low flows in 2021 are reversing this trend with Arapooish reporting the highest %EPT in the Fall 2021.
- Species diversity and EPT taxa richness, in general, increased with increasing distance from Yellowtail Dam until the Little Bighorn River enters, then diversity declines and nutrient, sediment and temperature tolerant macroinvertebrates increase.
- By comparing historical data at Split Island and Three Rivers, we have documented large decreases in BMI densities during flushing flows (2020) 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*.
- Flow conditions in 2021 are more like those of 1987 and subsequently the BMI densities and dominant taxa are increasing to levels seen during that sampling year.
- Even though we have documented non-native, NZ mudsnails at four of the eight Bighorn River sites, the observed densities (avg. 580 NZMS per m^2 in Fall 2021) are low enough to not cause considerable worry or to compete with native benthic invertebrates for food or benthic cobble habitat.
- 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. 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) and sites further downstream (MI4, Mallards Landing), but these densities are building back up with the low river flows in 2021.
- We conclude this 2nd year of analysis by indicating that BMI communities are exhibiting impaired biological health throughout the Bighorn River in 2021, especially at Mallards

Landing FAS and downstream of the Little Bighorn River. 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.

- Reasons for these observed ecological changes between Fall 2020 and 2021 can linked to lower late-summer flows, increasing average water temperatures, high nutrient levels, sediment accumulations and higher aquatic plant coverage across the riffle habitats.

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.

Ott, D. S., & Maret, T. R. 2003. Aquatic assemblages and their relation to temperature variables of least-disturbed streams in the Salmon River basin, central Idaho, 2001. Boise, Idaho, U.S. Dept. of the Interior, U.S. Geological Survey. http://purl.access.gpo.gov/GPO/LPS98632.

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 2021									
River Mile u/s of Yellowstone R.	RM82	RM75	RM72	RM63	RM52	RM40	RM24	RM1.8	
	Split Island	**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	83	0	220	27	0	10	4
Zaitzevia parvula	0	0	125	0	0	0	0	0	1
Microcylloepus pusillus	0	0	42	0	0	20	0	27	3
Dubiraphia minima	0	0	0	0	0	0	0	13	1
Stenelmis sp.	0	0	83	37	20	0	0	243	4
Helichus lithophilus	0	0	0	0	20	0	0	0	1
Agabus (Diving Beetles)	125	0	0	37	0	0	0	0	2
DIPTERA (midges/flies)	26,120	10,880	22,250	10,600	5,040	3,290	3,860	2,890	8
Chironomidae (midges)	25,680	9,958	21,667	8,890	4,800	3,180	3,580	2,788	8
	11 spp.	8 spp.	12 spp.	7 spp.	12 spp.	12 spp.	15 spp.	8 spp.	0
Tipula sp. (Cranefly)	417	0	0	27	20	10	40	0	5
Ceratopogoninae (Biting Midges)	0	0	0	0	40	0	0	0	1
Limnophora	43	27	13	0	60	0	0	0	4
Simulium spp. (Blackflies)	50	93	40	27	0	88	100	0	6
Hemerodromia sp. (Danceflies)	4	0	0	133	120	10	80	97	6
EPHEMEROPTERA (Mayflies)	920	1,541	1,400	4,347	1,116	2,190	2,980	1,510	8
Acentrella turbida (Tiny BWO)	0	0	0	0	180	205	500	0	3
Baetis flavistriga (BWO)	0	0	27	373	373	92	80	0	5
Baetis tricaudatus (BWO)	880	500	1,040	960	787	492	1,000	105	8
Tricorythodes explicatus (Tricos)	40	958	240	2,560	760	1,397	1,400	1,121	8
Serratella tibialis (PMDs)	0	0	93	107	13	0	0	0	3
Ephemerella excrucians (PMDs)	0	83	0	0	13	0	0	0	2
Leucrocuta sp. (Flat-Headed Mayflies)	0	0	0	0	0	0	0	92	1
Ecdyonurus sp. (Flat-Headed Mayflies)	0	0	0	293	13	0	0	60	3
Macaffertium terminatum (Flat-Headed Ma	0	0	0	0	0	0	0	120	1
Paraleptophlebia (Mahoganys)	0	0	0	0	0	0	0	16	1
LEPIDOPTERA (Aquatic Moths)									
Petrophila sp.	200	3,458	293	27	20	0	0	0	5
HEMIPTERA (Corixidae/Water Boatman)									
Sigara sp.	0	0	0	0	0	50	1,060	0	
ODONATA (Dragonflies)									
Ophiogomphus severus	0	0	53	53	360	10	0	0	4
PLECOPTERA (Stoneflies)	7	0	0	0	0	0	0	0	1
Isoperla quinquepunctata (Little Yellow Stones)	3	0	0	0	0	0	0	0	1
TRICHOPTERA (Caddisflies)	1,000	5,000	3,417	817	2,900	1,393	1,800	887	8
Hydroptila spp.	125	250	250	640	1,780	1,043	1,680	424	8
Brachycentrus occidentalis	125	875	1,167	60	560	37	20	18	8
Hydropsyche occidentalis	0	125	1,292	80	60	38	100	0	6
Oecetis sp.	42	458	83	0	160	8	0	58	6
Cheumatopsyche spp.	0	83	458	0	80	60	0	0	4
Nectopsyche sp.	0	0	0	0	20	17	0	22	3
Amiocentrus aspilis	625	2000	167	0	0	0	0	0	3
Hydropsyche slossonae	0	0	0	37	0	0	0	118	2
Ceratopsyche spp.	0	0	0	0	80	117	0	0	2
Hydropsyche C. cockerelli	83	1208	0	0	0	0	0	0	2
Hydropsyche morosa gr.	0	0	0	0	0	73	0	247	2
Onocosmoecus unicolor	0	0	0	0	80	0	0	0	1
Helicopsyche borealis	0	0	0	0	60	0	0	0	1
Glossosoma sp.	0	0	0	0	20	0	0	0	

Appendix A. (cont.) Macroinvertebrate taxa list and avg. number per meter squared (#/m2) for the Bighorn Sites 2021									
River Mile u/s of Yellowstone	RM82	RM75	RM72	RM63	RM52	RM40	RM24	RM 1	
	Split Island	**Three-Rivers**	**Bighorn FAS**	**Mallards FAS**	**Two Leggins FAS**	**Aarapooish FAS**	**Custer FAS**	**Manuel Lisa FAS**	**# of Sites**
ANNELIDA (Worms/Leeches)	9540	9670	7750	9510	8340	1780	10400	4991	8
Lumbricidae (Aquatic Worm)	1542	1250	542	0	540	0	0	0	4
Tubificidae	7958	8333	7167	9253	7700	1773	9980	4920	8
Erpobdellidae	42	0	0	153	100	0	260	71	5
Glossophonia complanata	0	83	42	0	0	0	0	0	2
Helobdella stagnalis	0	0	0	100	0	10	160	0	3
CRUSTACEA (Scuds/Isopods)	3875	9625	0	0	20	0	180	36	5
Gammarus spp.	542	1208	0	0	0	0	0	0	2
Caecidotea sp.	3333	8417	0	0	20	0	180	36	5
MOLLUSCA (Snails/Clams)	208	2792	2583	1177	1060	38	520	154	8
Physella sp. (Pouch snails)	167	917	2125	1177	1020	28	360	60	8
Ferrissia rivularis (Limpets)	0	0	0	0	0	10	40	94	3
Gyraulus sp.	0	0	0	0	20	0	20	0	2
Fossaria sp.	0	0	0	0	0	0	100	0	1
Potamopyrgus antipodarum (NZMS)	42	1792	458	0	20	0	0	0	4
Pisidium sp. (Fingernail Clams)	0	83	0	0	0	0	0	0	1
OTHER Non-Insects	275	2287	2413	1347	1137	27	33	6	8
Turbellaria (Flatworms)	275	2280	2373	1093	1107	0	33	0	6
Nematoda (Horsehair Worms)	0	0	13	13	30	27	0	2	5
Hydracarina (Water-Mites)	0	7	27	240	0	0	0	4	4
Total Taxa per site	**32**	**29**	**35**	**27**	**45**	**33**	**33**	**32**	**33.1**
EPT Taxa per site	**8**	**10**	**10**	**9**	**17**	**12**	**7**	**12**	**10.6**

APPENDIX B. BIGHORN RIVER SITE PHOTOS

Photo 1. Bighorn River: Split Island April 2021 looking d/s.

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

Photo 3. Bighorn River-Three Rivers Fall 2021 looking d/s.

Photo 4. Bighorn River-Three Rivers Fall 2020 downstream.

Photo 5. Bighorn River FAS Spring 2021 looking u/s.

Photo 6. Bighorn River FAS Fall 20201 looking upstream.

Photo 7. Bighorn River: Two Leggins FAS Spring 20201 looking upstream

Photo 8. Bighorn River Two Leggins FAS Spring 20201 looking downstream

Photo 9. Bighorn River Two Leggins FAS Fall 2021 looking upstream

Photo 10. Bighorn River Two Leggins FAS Fall 20201 Hess sampler.

Photo11. Bighorn River Mallard's Landing FAS Fall 2021 looking downstream.

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

Photo 13. Bighorn River Arapooish Hess sample with Longnose Dace (released) and dragonfly nymphs 2021.

Photo 14. Bighorn River Arapooish FAS site Fall 2021 looking downstream.

Photo 15. Bighorn River Arapooish FAS site looking upstream.

Photo 16. Bighorn River Custer FAS Spring 2021 looking downstream.

Photo 17. Bighorn River Manual Lisa FAS site Spring 2021 looking downstream.

Photo 18. Bighorn River FAS a clean Hess sample circle surrounded by vegetation.