Ghost Watershed

Water Monitoring Program

CABiN/STREAM Project

2021

BIOTA CONSULTANTS

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fRI Research shared the results of their sampling in 2020, which allowed us to compare their two sites with ours.

We are very grateful to those who reviewed the draft report and provided helpful comments and suggestions, particularly Cal Hill and Sharlene Fritz. Editing services were provided by Anne Holcroft Weerstra.

Cover photo: Anne Holcroft Weerstra – Kicknetting across upper Ghost River

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Executive Summary

The Ghost Watershed Alliance Society (GWAS) began a water monitoring program in 2020 to aid in determining aquatic ecosystem health. This followed a recommendation in the *Ghost River State of the Watershed Report 2018* to sample aquatic invertebrates using the Canadian Aquatic Biomonitoring Network (CABiN) protocols, and using Ephemeroptera, Plecoptera and Trichoptera (EPT) ratios as a proxy for water quality.

In 2019, GWAS began participation in the STREAM (Sequencing the Rivers for Environmental Assessment and Monitoring) three-year pilot project, which uses CABiN methods to collect water samples for environmental DNA (eDNA) testing to determine presence of benthic macro-invertebrates. Physical and chemical parameters of the samples also are measured. GWAS then developed a multi-year water monitoring plan that incorporated the STREAM pilot project.

The water monitoring program began in the fall of 2020 when Biota Consultants was contracted to oversee the sampling of ten sites, eight along Waiparous Creek (WAP02 to WAP09) and two on the Ghost River (GHO01 and GHO02). In the second year (2021) of the program, the focus was the Ghost River, with a slight modification due to the Devil's Head/ Black Rock wildfire (CWF-156-2020), which occurred in the fall of 2020. Two sites had been established by fRI Research, Alberta Agriculture and Forestry and the City of Calgary in the fall of 2020. One was located above the western extent of the wildfire and the other was immediately above the old TransAlta diversion structure, within the burnt zone. It was decided to resample these sites. Three additional sites were sampled downstream.

The southwest fork of the headwaters of Johnson Creek in the Waiparous Creek sub-basin also had been burnt in the wildfire. It was therefore decided to sample Johnson Creek just above its confluence with Waiparous Creek. In addition, sites WAP02 and WAP03, that were sampled in 2020 below and above the confluence with Johnson Creek, were resampled.

Field sampling occurred between August 26th and September 10th when there was low stream flow and stable weather conditions. Triplicate kicknet samples were taken at each site for use in the eDNA analysis. A fourth kicknet sample was collected for morphological analysis to determine benthic macroinvertebrate species abundance. This information was required to determine the EPT ratio, among other metrics. In addition, detailed descriptions were made of the site, stream characteristics and benthic macroinvertebrate habitat. Water chemistry was measured on-site or through subsequent lab analyses.

At the time of sampling, water quality was within the parameters acceptable for benthic macroinvertebrates and fish. The chemical and physical attributes were well below exceedance levels. Total suspended solids and turbidity were very low.

As measurements of diversity, Simpson's Index of Diversity and the Shannon-Weiner Index indicate that the sites were diverse in their community composition. The Hilsenhoff Biotic Index suggests that there was possible slight organic pollution at most of the Ghost River sites and the Johnson Creek site, rating water quality as very good. The remainder of the sites fell into the excellent category, with organic pollution unlikely.

The EPT ratio indicates high water quality at most of the sites, with EPT species at much higher abundance than the pollution-tolerant chironomid family. The exceptions were GHO06 and JOH01 where the ratio was 0.48 and 0.59, respectively, potentially raising concerns.

The percent of the more tolerant Hydropsychidae within the Trichoptera and Baetidae within the Ephemeroptera was highly variable. This raised potential concerns at the most downstream site on the Ghost River (GHO07) and the two sites on Waiparous Creek (WAP02 and WAP03).

The differences in the chemical and physical attributes of water samples at WAP02 and WAP03 in 2020 and 2021 were subtle, with values well below exceedance levels. The EPT ratio was similar between years. The Hilsenhoff Biotic Index was lower in 2021, but in both years was within the excellent water quality category.

The differences in the chemical and physical attributes of water samples at the upper Ghost River sites in 2020 and 2021 also were subtle, with values well below exceedance levels. However, the proportion of the key EPT taxa differed, particularly at the uppermost site (GR-20-01/GHO06). The high percentage of chironomids within the Diptera at GHO06 resulted in only a moderate EPT ratio compared to a high ratio at GR-20-01 the year before. This coupled with the higher Hilsenhoff Biotic Index at GHO06 suggests a potential concern, which is surprising considering the lack of obvious anthropogenic disturbances.

The results of the 2021 field sampling provide a baseline for comparison in future years. With more data, trends may become apparent. If issues with water quality are suggested, sampling effort may become more focussed.

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1.0 Introduction

1.1 Background

The mission of the Ghost Watershed Alliance Society (GWAS) is to protect the integrity of the Ghost Watershed. One means of accomplishing this is to monitor water quality to determine aquatic ecosystem health. This was a recommendation in the *Ghost River State of the Watershed Report 2018* (ALCES and GWAS 2018), specifically sampling aquatic invertebrates as per the Canadian Aquatic Biomonitoring Network (CABiN) protocols, and using Ephemeroptera, Plecoptera and Trichoptera (EPT) ratios as a proxy for water quality.

In 2019, GWAS began participating in a three-year environmental DNA (eDNA) project called STREAM (Sequencing the Rivers for Environmental Assessment and Monitoring), which is a collaboration between World Wildlife Fund (WWF) Canada, Living Lakes Canada (LLC) and Environment and Climate Change Canada (ECCC), led by the Hajibabaei Lab at Centre for Biodiversity Genomics (University of Guelph). STREAM employs the existing nationally standardized protocols of CABiN for freshwater monitoring. CABiN methods include assessing physical and chemical parameters, and collecting benthic macroinvertebrates for morphological analysis to determine species abundance. Through STREAM, rather than quantifying abundance, water samples are submitted for eDNA testing to determine presence or absence of benthic macroinvertebrates.

Four individuals from GWAS were trained in the summer of 2019 in CABiN wadeable stream protocol and STREAM protocol. One site on Waiparous Creek was sampled on July 18 (WAP01) as part of this field course. During the spring and summer of 2020, the GWAS CABiN team developed a strategic multi-year plan (*GWAS Water Monitoring Program Plan 2020*) to obtain information on the health of water courses within the Ghost River watershed. The intent was to augment existing information and to assist public land managers and other organizations tasked with water management responsibilities. This plan is a living document and continues to be updated. It adopts water quality indicators as per the CABiN protocol, using the *CABiN Field Manual – Wadeable Streams* (Environment Canada 2012), as well as committing to the STREAM three-year pilot project.

The water monitoring program began in the fall of 2020, when ten sites were sampled, eight along Waiparous Creek (WAP02 to WAP09) and two on the Ghost River (GHO01 and GHO02). In this first year of the plan, the focus was mainly on sites above and below creek tributaries and other possible point source sites which might affect water quality as a result of land use activities (see Biota Consultants 2022).

1.2 Field Plan

In the second year (2021) of the water monitoring program, the focus was the Ghost River, with a slight modification due to the Devil's Head/Black Rock wildfire (CWF-156-2020)[1], which occurred in the fall of 2020. Just after the wildfire, on October 29th, fRI Research and Alberta Agriculture and Forestry (AAF), in conjunction with the City of Calgary, sampled the Ghost River using the CABiN protocol immediately above the wildfire (GR-20-001) and at the lower end of the fire zone (GR-20-002). The downstream site was located above the old TransAlta water diversion structure in an area where the north side of the river had been burned by the fire. The purpose of this sampling was to evaluate the effect of the fire on the quality of source water for the City of Calgary. The group has since collected regular water samples at these sites, timed to coincide with sampling at an established station near Benchlands.

GWAS decided to resample these sites in 2021 using the CABiN and STREAM protocols. The downstream site was named GHO05 and the upstream site was named GHO06, which is located within the Ghost River Wilderness Area where motorized vehicle access is restricted. To access this area, special permission was granted by AAF, and transportation was provided by Michael Wagner of AAF. Within the GWAS water monitoring program, GHO05 represents the river at the lower end of the region where the fire burned the valley and mountain slopes. GHO06 represents the river upstream of the western extent of the fire, approximately 9 km from GHO05. Both sites occur in a broad fluvial flood plain where stream channelling and repositioning is common during spring runoff and flood events.

Site GHO06 was considered to be a potential reference site since it was located in a region considered to be minimally affected by anthropogenic factors. Environment and Climate Change Canada have the authority to decide if it can be considered a reference site.

Since the fire also encroached on the southwest fork of the headwaters of Johnson Creek in the Waiparous Creek sub-basin, it was decided to sample this creek above its confluence with Waiparous Creek. In addition, the paired sites on Waiparous Creek that were sampled in 2020 below and above the confluence of Johnson Creek, WAP02 and WAP03, were resampled.

A further three sites were sampled on the Ghost River. Site GHO03 was upstream of Richards Road bridge and represented the river upstream of its confluence with Waiparous Creek. Site

[1] Code assigned by Alberta Agriculture and Forestry.

GHO04 was upstream of the TransAlta berm at the hamlet of Benchlands, representing the river below the confluence with Waiparous Creek (8.3 km from GHO03). Both sites were chosen since they were near public roads, providing relatively easy access using motor vehicles. In addition, GHO03 was in the vicinity of a site sampled in 2010 by Environment Canada using the CABiN protocol. Environment Canada also has a stream gauge station upstream of the bridge for recording water attributes. The third sampling site (GHO07) was upstream of the Ghost Reservoir, close to where the river ends when the reservoir is at full volume capacity.

2.0 Methods

2.1 Field Sampling

The field sampling followed the same CABiN and STREAM protocols as in 2020, described in *Ghost Watershed Water Monitoring Program CABiN/STREAM Project 2020* (Biota Consultants 2022). In 2021, field sampling occurred between August 26th and September 10th mainly during sunny stable weather conditions. Site name codes, date of sampling and geographical locations are presented in Table 1, and locations are mapped in Figure 1. Air and water temperatures at the time of sampling are provided.

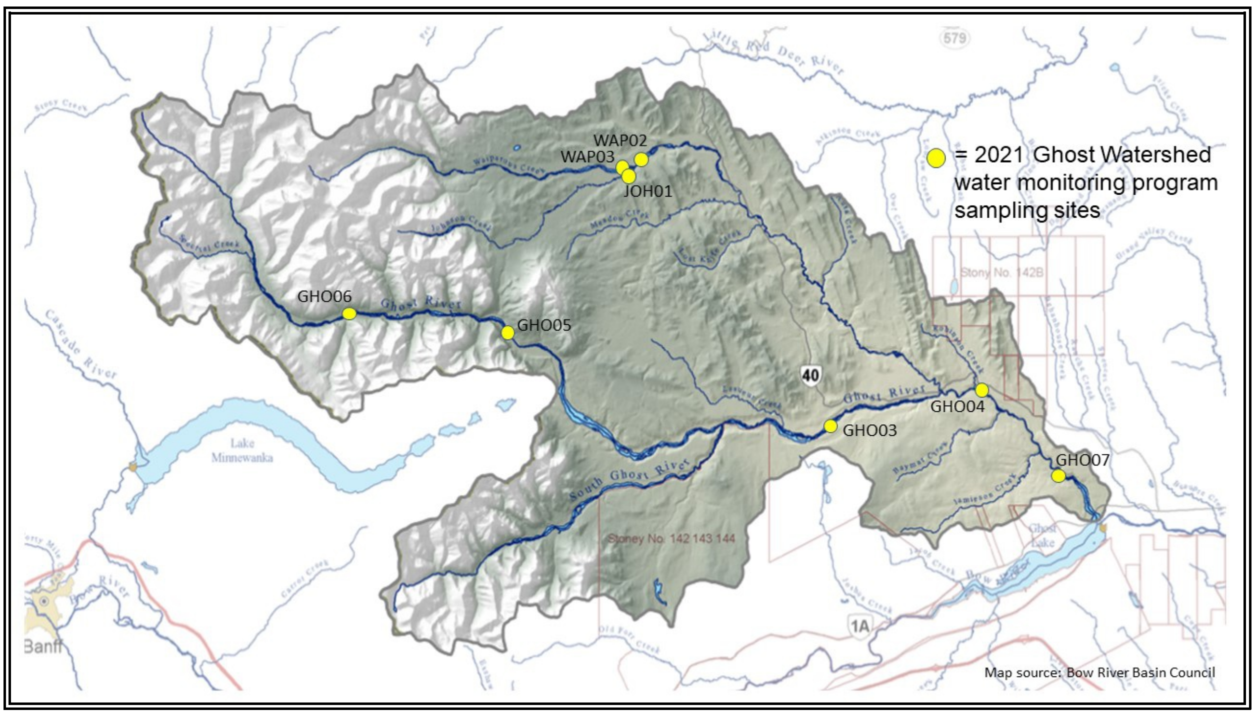

Figure 1. Sampling locations in 2021 within the Ghost River watershed.

Following the sampling of site GHO04, it was noted that Robinson Creek enters the Ghost River immediately above the sampling location. An abundance of periphyton occurred in the shallow water along the river's edge downstream of the creek. To determine the possible reason and the potential impact of the creek, water samples were collected in Robinson Creek above its confluence with the Ghost River on September 10th (ROB-WS). This creek drains a sub-basin where there are private properties containing homes, agricultural operations (ranching) and logging. A second water sample also was collected on the Ghost River above the confluence with Robinson Creek (GHO04A).

Table 1. Location of 2021 Ghost River (GHO), Waiparous Creek (WAP), Johnson Creek (JOH) and Robinson Creek (ROB) sites, plus sampling date, time of day, and conditions.

Code/ Date	Latitude	Longitude	Elevation (m)	Comments
GHO03 Aug. 26	51.2690°	114.9260°	1314	Ghost River upstream from Richards Road bridge Morning Sun and cloud, air temperature 17.0°C, water temperature 9.6°C
GHO04 Aug. 26	51.2836°	114.8094°	1240	Ghost River upstream of TransAlta berm at hamlet of Benchlands Afternoon Sunny, air temperature 22.0°C, water temperature 12.5°C
GHO05 Aug. 30	51.3099°	115.1928°	1610	Ghost River upstream of old TransAlta water diversion structure (destroyed in 2013 flood); area burned in 2020 wildfire (CWF-156) Morning Sunny, air temperature 18.0°C, water temperature 9.8°C
GHO06 Aug. 30	51.3202°	115.3197°	1732	Ghost River upstream of 2020 wildfire (CWF-156) Afternoon Sunny, air temperature 20.0°C, water temperature 8.2°C
WAP02 Sept. 2	51.3944°	115.0860°	1559	Waiparous Creek below confluence with Johnson Creek Morning Sun and cloud, air temperature 10.5°C, water temperature 7.5°C
WAP03 Sept. 2	51.3925°	115.0892°	1565	Waiparous Creek above confluence with Johnson Creek Afternoon Sunny, air temperature 19.0°C, water temperature 10.2°C
JOH01 Sept. 7	51.3916°	115.0895°	1569	Johnson Creek above confluence with Waiparous Creek Morning/Afternoon Sunny, air temperature 16.0°C, water temperature 6.2°C
GHO07 Sept. 10	51.2417°	114.7415°	1209	Ghost River above the Ghost Reservoir Morning/afternoon Sunny, air temperature 16.0°C, water temperature 10.9°C
GHO04A Sept. 10	51.2417°	114.7415°	1240	Second water sample for comparision to Aug. 26 sample Ghost River upstream of TransAlta berm at hamlet of Benchlands Afternoon Sunny, air temperature N/A, water temperature 13.7°C
ROB-WS Sept. 10	51.2836°	114.8094°	1240	Robinson Creek above confluence with Ghost River Afternoon Sunny, air temperature N/A, water temperature 10.8°C

When sampling the paired site on Waiparous Creek, the downstream site (WAP02) was sampled prior to the upstream site (WAP03) to ensure that the downstream site was not disturbed by upstream activities.

Biological sampling followed the CABiN/STREAM protocols used in 2020 (Biota Consultants 2022), with two minor modifications. Under a directive from the Hajibabaei Lab at Centre for Biodiversity Genomics (University of Guelph) in conjunction with Living Lakes Canada, Absolute Zero RV waterline antifreeze was approved as an alternate to 95% ethanol solution (Histoprep) for preserving the eDNA samples (R. Mallinson, pers. comm.). This non-hazardous biodegradeable solution does not require the strict "Transportation of Dangerous Goods" (TDS) labelling and handling. In addition, a different method of sealing the sample jars was recommended. Rather than using a square of parafilm just beneath the lid with a strip of duct tape around the outside of the lid, a strip of parafilm was wound tightly around the outside of the jar and lid.

The description of physical attributes of each site and the collection of water chemistry data followed the same protocols described by Biota Consultants (2022).

2.2 Data Entry

All of the data, except the benthic macroinvertebrate community structure information, were entered into the CABiN database by the Project Manager. To reduce potential errors, the morphologic consultant (Cordillera Consulting Inc.) entered the benthic macroinvertebrate community data. The data were also submitted to the head taxonomist at Environment and Climate Change Canada, CABiN taxonomic laboratory, located in British Columbia.

3.0 Results and Discussion

3.1 Physical Characteristics

The physical characteristics of the eight sample sites are presented in Table 2. This information was collected in the fall, under conditions of low stream flow and stable weather.

Table 2. Physical characteristics of sample sites.

Attributes	Site and Date of Sampling							
	GHO03	GHO04	GHO05	GHO06	WAP02	WAP03	JOH01	GHO07
	Aug. 26	Aug. 26	Aug. 30	Aug. 30	Sept. 2	Sept. 2	Sept. 7	Sept. 10
Elevation (m)	1314	1240	1610	1732	1554	1560	1569	1209
Bankfull width (m)	13.32	23.75	14.92	16.39	12.02	21.90	6.66	33.55
Wetted width (m)	12.52	22.55	13.20	10.45	10.35	8.90	5.64	30.60
Bankfull wetted depth (cm)	19.7	15.0	47.0	23.5	17.0	57.0	23.0	21.0
Maximum channel depth (cm)	45.5	34.5	31.0	29.7	21.2	24.2	23.5	34.5
Avg channel depth (cm)	36.6	30.4	24.6	19.1	17.2	15.6	18.5	28.9
Maximum velocity (m^3/s)	1.3435	1.3362	1.2450	0.9078	1.3065	1.0850	0.8287	1.4956
Avg velocity (m^3/s)	1.2165	1.0061	1.1288	0.7227	0.8630	0.7305	0.5658	1.0727
Slope (m/m)	0.0096	0.0023	0.0050	0.0050	0.0110	0.0087	0.0085	0.0073
Substrate embeddedness (%)	25	25	50	25	25	25	25	25
Dominant substrate (cm)	3.2-6.4	3.2-6.4	3.2-6.4	3.2-6.4	3.2-6.4	3.2-6.4	6.4-12.8	6.4-12.8
2nd dominant substrate (cm)	6.4-12.8	6.4-12.8	1.6-3.2	6.4-12.8	6.4-12.8	6.4-12.8	12.8-25.6	3.2-6.4
Surrounding material (cm)	0.1-0.2	0.1-0.2	0.2-1.6	0.2-1.6	0.1-0.2	0.2-1.6	0.2-1.6	0.2-1.6
Geometric median particle size (cm)	5.95	4.60	4.20	5.40	6.90	5.80	9.60	6.90
% Sand	0	0	0	0	0	0	0	0
% Gravel	0	3	2	3	0	1	0	1
% Pebble	60	73	79	56	49	57	29	35
% Cobble	39	24	19	41	51	37	67	64
% Boulder	1	0	0	0	0	2	4	0
% Bedrock	0	0	0	0	0	3	0	0

Note: Sand = fine sand, silt or clay (<0.1 cm), coarse sand (0.1 - 0.2 cm); Gravel = 0.2 - 1.6 cm; Pebble = small (1.6 - 3.2 cm), large (3.2 - 6.4 cm); Cobble = small (6.4 - 12.8 cm), large (12.8 - 25.6 cm); Boulder = >25.6 cm.

Substrate embeddedness refers to how deeply the dominant substrate is buried in the surrounding finer particles. Five categories of substrate embeddedness[2] were used. In areas modified by stream side activities (anthropogenic land uses), increased erosion can result in

[2] Embedded Categories:
1) Completely embedded: 100% embedded
2) 75% embedded
3) 50% embedded
4) 25% embedded
5) 0% embedded

the accumulation of fine material in the interstitial spaces. The more embedded the substrate, the fewer interstitial spaces for macroinvertebrates to occupy, which can reduce productivity (Environment Canada 2012).

3.1.1 Ghost River

The Ghost River at site GHO03 had a narrower bankfull and wetted width (13.32 m and 12.52 m, respectively) than site GHO04 (23.75 m and 22.55 m widths, respectively). The various depth measurements were higher at GHO03, however, the maximum velocity was similar at both sites (Table 2). Average velocity was greater at GHO03, which is likely a result of the narrower, deeper channel and greater slope compared to GHO04. The larger geometric median particle size at the GHO03 site is due to a higher percentage of cobble-sized substrate and lower percentage of pebble-sized substrate, possibly resulting from the higher velocity.

The approximate elevation of site GHO07, in the lowest section of the river, was recorded as 1209 m compared to 1732 m at the uppermost site (GHO06). The bankfull and wetted widths were greater than all other sites (33.55 m and 30.60 m, respectively). Also of note was a higher percentage of cobble-sized substrate and a lower percentage of pebble-sized substrate than all other sites, resulting in a higher geometric median particle size.

The lowest average channel depth and velocity was recorded at GHO06. This did not result in significantly higher gravel- and pebble-sized substrate than other sites, however.

3.1.2 Waiparous Creek

Sites WAP02 and WAP03 were resampled in 2021, however the exact location of the tape when it was stretched across the creek to determine the physical attributes was likely not identical to the previous year. Permanent marker stakes were not installed in 2020 for accurate relocation in subsequent years. This coupled with fluvial action altering the stream channel may have contributed to the variation in the data between years (Table 3). At the paired sites established in 2020 along Waiparous Creek, an attempt was made to select reach locations with similar stream channel characteristics above and below confluences. However, due to the heterogeneous nature of the sites, stream characteristics varied in both 2020 and 2021 (Table 3).

Table 3. Comparison of physical attributes at Waiparous Creek sites WAP02 and WAP03 in 2020 and 2021.

Attributes	Site and Date of Sampling			
	WAP02		WAP03	
	Sept. 1 2020	Sept. 2 2021	Sept. 3 2020	Sept. 2 2021
Elevation (m)	1554	1554	1560	1560
Bankfull width (m)	17.00	12.02	15.00	21.90
Wetted width (m)	9.60	10.35	6.90	8.90
Bankfull wetted depth (cm)	26.5	17.0	56.0	57.0
Maximum channel depth (cm)	27.0	21.2	22.0	24.2
Avg channel depth (cm)	17.40	17.24	16.40	15.20
Maximum velocity (m^3/s)	1.2530	1.3065	1.1290	1.0850
Avg velocity (m^3/s)	0.8760	0.8630	0.8650	0.7305
Slope (m/m)	0.0140	0.0110	0.0150	0.0087
Substrate embeddedness (%)	25	25	25	25
Dominant substrate (cm)	6.4-12.8	3.2-6.4	3.2-6.4	3.2-6.4
2nd dominant substrate (cm)	12.8-25.6	6.4-12.8	6.4-12.8	6.4-12.8
Surrounding material (cm)	0.2-1.6	0.1-0.2	0.2-1.6	0.2-1.6
Geometric median particle size (cm)	10.3	6.9	5.9	5.8
% Sand	0	0	0	0
% Gravel	1	0	1	1
% Pebble	23	49	56	57
% Cobble	68	51	41	37
% Boulder	8	0	2	2
% Bedrock	0	0	0	3

The bankfull width at high water was less at WAP02 in 2021 (Table 3). This was likely a result of measuring tape location. At WAP03, it was wider in 2021. Here, the stream width was affected by a new secondary channel flowing parallel to and above the main channel on the south side, with water flowing laterally into the main channel. Depth measurements also varied between years, particularly at WAP02 where the bankfull wetted depth and maximum channel depth were less in 2021 (Table 3).

The geometric median particle size of the substrate was once again higher at WAP02 but not to as great a degree as in 2020 (Table 3). Pebble-sized substrate was slightly higher upstream of Johnson Creek and cobble-sized substrate was less, suggesting again that below the tributary, the finer substrates were transported downstream. Accordingly, slightly higher maximum and average stream flows were recorded below Johnson Creek (Table 3).

3.1.3 Johnson Creek

The Johnson Creek sampling location was approximately 220 m upstream from its confluence with Waiparous Creek. This tributary creek was the narrowest of those previously sampled (Table 2). It had the largest geometric mean particle size of 9.6 cm. The dominant and second dominant substrate were among the coarsest sampled (small cobble and large cobble, respectively). Only the lowest site on the Ghost River (GHO07) had the same sized dominant substrate. At the time of sampling, it had the lowest flow velocity (Table 2).

3.2 Water Attributes and Chemical Analysis

The chemical attributes (i.e., pH, dissolved oxygen, anions, nutrients) along with the physical attributes (i.e., total suspended solids, turbidity, specific conductance, temperature) are presented for each site in Table 4.

Table 4. Chemical and physical attributes of water samples at each site.

Tests	Site and Date of Sampling									
	GHO03	GHO04	GHO05	GHO06	WAP02	WAP03	JOH01	GHO07	ROB-WS	GHO4A
	Aug. 26	Aug. 26	Aug. 30	Aug. 30	Sept. 2	Sept. 2	Sept. 7	Sept. 10	Sept. 10	Sept. 10
Total Suspended Solids (mg/L)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	2.5	<1.0
Turbidity (lab) (NTU)	0.14	0.23	0.13	0.10	0.22	0.10	0.11	0.16	3.20	0.19
Specific Conductance (µS/cm)	340.3	346.6	351.9	384.0	336.8	336.7	335.8	348.2	460.4	345.9
Air Temperature (°C)	17.0	22.0	18.0	20.0	10.5	19.0	16.0	16.0	-	-
Water Temperature (°C)	9.6	12.5	9.8	8.2	7.4	10.2	6.2	10.9	10.8	13.7
Dissolved Oxygen (mg/L)	9.53	9.14	9.18	9.36	9.92	9.28	10.24	9.89	9.49	8.97
pH	8.24	8.44	8.32	8.20	8.29	8.38	8.21	8.35	8.25	8.25
Anions										
Alkalinity (Total as $CaCO_3$) (mg/L)	140	150	120	130	140	130	150	150	220	150
Alkalinity (PP as $CaCO_3$) (mg/L)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	1.9	1.9	1.1
Bicarbonate (HCO_3) (mg/L)	180	190	150	160	170	160	190	180	270	170
Carbonate (CO_3) (mg/L)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	2.3	2.3	1.3
Hydroxide (OH) (mg/L)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Nutrients										
Total Phosphorus (P) (mg/L)	0.0040	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030
Dissolved Nitrogen (N) (mg/L)	0.25	0.17	0.21	0.17	0.13	0.10	0.16	0.15	0.23	0.17
Dissolved Nitrite (N) (mg/L)	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Dissolved Nitrate plus Nitrite (N)	0.21	0.16	0.21	0.23	0.13	0.13	0.17	0.17	0.15	0.16

The chemical analysis suggests that the water quality at the time of sampling was within the parameters acceptable for benthic macroinvertebrates and fish (Government of Alberta 2018). The water quality exceedance criteria, including a brief narrative, are presented in Table 5.

Table 5. Water quality exceedance criteria for water quality parameters.

Water Quality Variable (Substance or Condition)	**Short-term (Acute)**	**Long-term (Chronic)**	**Notes and Direction**
Alkalinity (as $CaCO_3$) (mg/L)	-	20	A minimum value, unless natural conditions are less, in which case the guideline cannot be lower than 25% of the natural level.
Bicarbonate (HCO_3)	-	-	
Carbonate (CO_3)	-	-	
Hydroxide (OH)	-	-	
Nitrate – N (mg/L)	>124	>3.0	As N. For protection from toxicity. Does not consider eutrophication effects .
Nitrite – N (mg/L)	Varies	Varies	As N. Varies with chloride.
Nitrogen – total (inorganic + organic)	-	Narrative	Nitrogen (total) and phosphorus concentrations should be maintained to prevent detrimental changes to algal and aquatic plant communities, aquatic biodiversity, oxygen levels and recreational quality. Where priorities warrant, develop site-specific nutrient objectives and management plans.
Dissolved Oxygen (mg/L) (Minimum values)	5	6.5	See Alberta Environmental Protection (1997) for guidance when natural conditions do not meet guidelines. Long-term is 7 day mean, short-term is instantaneous value.
	-	<8.3	For mid-May to end of June, to protect mayfly emergence.
	-	9.5	For areas and times where and when larval fish develop within gravel beds.
Total Phosphorous (mg/L)	-	-	For major rivers and for surface waters not covered by specific guidelines, nitrogen (total) and phosphorus concentrations should be maintained to prevent detrimental changes to algal and aquatic plant communities, aquatic biodiversity, oxygen levels, and recreational quality. Where priorities warrant, develop site-specific nutrient objectives and management plans.

Table 5. Continued

Water Quality Variable (Substance or Condition)	**Short-term (Acute)**	**Long-term (Chronic)**	**Notes and Direction**
pH	<6.5 or >9.0	+- 0.5 from baseline	Not to be altered by more than 0.5 units from background.
Total Suspended Solids (TSS) (mg/L)	Narrative	Narrative	During clear flows or for clear waters: Maximum increase of 25 mg/L from background for any short-term exposure (e.g., 24 hr period). Maximum average increase of 5 mg/L from background levels for longer term exposures (greater than 24 hr). During high flow or for turbid waters: Maximum increase of 25 mg/L from background levels at any time when background levels are between 25 and 250 mg/L. Should not increase more than 10% of background levels when background is ≥250 mg/L.
Specific Conductance	-	-	
Turbidity (NTU)	Narrative	Narrative	For clear waters: Maximum increase of 8 NTU from background for any short-term exposure (e.g., 24 hr period). Maximum average increase of 2 NTU from background levels for longer term exposures (greater than 24 hr). For high flow or turbid waters: Maximum increase of 8 NTU from background levels at any time when background levels are between 8 and 80 NTU. Should not increase more than 10% of background levels when background is > 80 NTU.

Source: Government of Alberta (2018)

The water quality exceedance criteria for Alberta surface waters (Government of Alberta 2018) does not provide values for specific conductivity or three main anions: bicarbonate (HCO_3), carbonate (CO_3) and hydroxide (HO). Further discussion is provided below on specific conductivity and on the relationship of the three anions to alkalinity and inorganic carbon.

3.2.1 Alkalinity, Inorganic Carbon, Hardness and pH

A full description of alkalinity, inorganic carbon, hardness and pH is given in the report on the 2020 monitoring program (Biota Consultants 2022). Alkalinity, as expressed by the total $CaCO_{3,}$ was lowest at the upstream sites on the Ghost River, GHO05 and GHO06 (120 mg/L and 130 mg/L , respectively). Similarly, the uppermost site sampled on Waiparous Creek in 2020 had the lowest alkalinity (Biota Consultants 2022). These values are well above the minimum 20 mg/L level indicated in Table 5. The pH of the samples varied from 8.20 to 8.44, which is in the safe range for acute toxicity according to Government of Alberta (2018) criteria (Table 5).

The hardness of a water body is regulated largely by the levels of calcium and magnesium salts. Hard water contains cations with a charge of 2+, especially Ca^{2+} and Mg^{2+} (Casiday and Frey 1998). The water at the majority of the sites sampled would be classified as hard according to the USGS (2021) classification:

Soft = 0 to 60 mg/L $CaCO_3$
Moderately hard = >60 to 120 mg/L $CaCO_3$
Hard = >120 to 180 mg/L $CaCO_3$
Very hard = >180 mg/L $CaCO_3$

3.2.2 Specific Conductance (Conductivity)

Specific conductance (conductivity) is a numerical expression of water's ability to conduct an electrical current, usually expressed in microsiemens per centimetre (µS/cm). Specific conductance is measured at, or corrected to, 25°C (Miller *et al.* 1988). Since conductivity increases with temperature, reporting conductivity at the reference temperature of 25°C allows data to be easily compared (FEI 2014a).

There is no set standard for the conductivity of water (Table 5) because conductivity can differ regionally and between neighbouring streams if there is enough difference in the surrounding geology, or if one source has a separate inflow (FEI 2014a). Freshwater that runs through granite bedrock will have a very low conductivity value. Clay- and limestone-derived soils can contribute to higher conductivity values in freshwater systems (LCRA 2014). Despite the lack of standards and the fact that the surrounding environment can affect conductivity, there are approximate values that can be expected based on the source of the water (American Public Health Assoc. *et al.* 1999, as cited in FEI 2014a; Clean Water Team 2004).

A full discussion on specific conductance is provided in Biota Consultants (2022). Specific conductance is one of the most useful and commonly measured water quality parameters (Miller *et al.* 1988). It is the basis of most salinity and total dissolved solids calculations, and is an early indicator of change in a water body. Most water bodies maintain a fairly constant conductivity that can be used as a baseline for future comparisons (EPA 2012, as cited in FEI 2014a). Therefore, conductivity is a useful tracer of point source discharges (Environment Canada 2012). A significant increase in conductivity, due to natural flooding, evaporation or man-made pollution, can be detrimental to water quality, hence to aquatic insects (FEI 2014a). The 2020 and 2021 data provide baseline measurements for comparison in the future.

3.2.3 Total Suspended Solids, Turbidity and Dissolved Oxygen

3.2.3.1 Total Suspended Solids

Total suspended solids (TSS) were <1 mg/L at all sites except the Robinson Creek site, where they were 2.5 mg/L (Table 4), but still well below the exceedence criteria. The higher level is probably related to the high amount of periphyton noted. The input of TSS into the Ghost River from Robinson Creek did not affect the TSS downstream, as the level was <1 mg/L at GHO04 and at GHO04A upstream.

Particles in the water column that are larger than 2 microns comprise TSS. Anything smaller (average filter size) is considered to be a dissolved solid. Most suspended solids are made up of inorganic materials such as sand and silt. However, bacteria, algae, plankton, and organic particles from decaying plants and animals can also contribute to the TSS concentration, i.e., anything drifting or floating in the water (Kentucky Water Watch n.d.; Murphy 2007; EPA 2012, as cited in FEI 2014b). Water clarity is significantly affected, declining as the amount of solids increases (FEI 2014b).

Suspended solids can adversely affect aquatic organisms in several ways:

- Clog the filtering systems of fish and some immature stages of insects (e.g., caddisfly larvae);
- Cause physical injury to delicate eye and gill membranes by abrasion;
- Restrict food availability to fish, affecting growth rates;
- Restrict normal movements and migrations of fish; and
- Inhibit egg development (Alabaster and Lloyd 1984, as cited in CCME 1999).

For further information on suspended and settleable solids, please see Biota Consultants (2022).

3.2.3.2 Turbidity

Turbidity is often reported as nephelometric turbidity units (NTU) and is a measure of relative water clarity. The turbidity of most samples ranged from 0.10 NTU to 0.23 NTU (Table 4), which is considered very low (Table 5). The exception was again the Robinson Creek site at 3.2 NTU. This may explain the slight increase in turbidity from 0.19 NTU at GHO04A to 0.23 NTU at GHO04 downstream of Robinson Creek.

Turbidity in water results from the presence of suspended matter such as clay, silt, finely divided inorganic and decaying organic material, soluble coloured organic compounds, and living organisms that are held in suspension by turbulent flow (McNeely *et al.* 1979, as cited in CCME 2008). Turbidity can also include coloured dissolved organic matter, also known as humic stain, which refers to the tea colour produced from decaying vegetation underwater due to the release of tannins and other molecules. This material causes water to appear red or brown, depending on the type of flora present. Discolouration is often found in water bodies, such as bogs and wetlands. These dissolved substances may be too small to be counted as suspended solids, but they still affect the turbidity measurement since they affect water clarity (FEI 2014b).

Turbid water can appear cloudy, murky, hazy, muddy, coloured or opaque. Turbidity and TSS are related, as both reduce water clarity. However, turbidity is not a direct measurement of the total suspended materials in water. It is often used to indicate changes in the TSS concentration without providing an exact measurement of solids (EPA 2012, as cited in FEI 2014b). Since the correlation between turbidity and the weight of suspended (or total suspended) and settleable solids is often tenuous, both should be assessed.

3.2.3.3 Dissolved Oxygen and Temperature

Dissolved oxygen (DO) is the concentration of free oxygen (O_2) present in water or other liquids and is usually measured in mg/L. An O_2 level that is too low or too high can affect water quality, harming aquatic life (Alberta Environmental Protection 1997). The amount of O_2 dissolved in water primarily depends on temperature, atmospheric (barometric) pressure and turbulence (e.g., rapids, waterfalls, waves), although salinity also has an effect (FEI 2013). Temperature is the main factor, as cold water can hold more oxygen (Environment Canada 2012). Therefore, water temperature and the amount of DO are important in assessing water quality due to their influence on organisms within a body of water. Please see Biota Consultants (2022) for a further discussion on factors influencing DO and the effects of DO on aquatic fauna.

The DO values in our samples were within acceptable limits, ranging from 9.14 to 10.24 mg/L (Table 4). Daily variation was noted. Site GHO03 was sampled in the morning and site GHO04 was sampled the same day in the afternoon when the water temperature of the Ghost River was higher. Accordingly, the DO in the water decreased slightly.

3.2.4 Comparison of Ghost River Sites Related to Devil's Head/Black Rock Wildfire

As with sites GHO05 and GHO06, the water quality at the sites sampled by staff from fRI Research, Alberta Agriculture and Forestry and the City of Calgary was within the parameters acceptable for benthic macroinvertebrates and fish (Government of Alberta 2018) (Table 5). The water sampling protocols and analytical laboratory at the City of Calgary were used to obtain the results (Table 6).

Table 6. Comparison of chemical and physical attributes of water samples at GR-20-02/ GHO05 and GR-20-01/GHO06.

Attributes	**Site and Date of Sampling**			
	GR-20-02*	**GHO05**	**GR-20-01***	**GHO06**
	Oct. 29 2020	**Aug. 30 2021**	**Oct. 29 2020**	**Aug. 30 2021**
pH	8.3	8.32	8.3	8.20
Total Suspended Solids (mg/L)	1	<1.0	1	<1.0
Turbidity (lab) (NTU)	0.08	0.13	0.06	0.10
Specific Conductance (µS/cm)	338.1	351.9	355.3	384.0
Dissolved Oxygen (mg/L)	11.95	9.18	12.24	9.36
Water Temperature (°C)	4.8	9.8	4.4	8.2
Air Temperature (°C)	2.5	18.0	3.0	20.0
Anions				
Alkalinity (Total as $CaCO_3$) (mg/L)	122	120	129	130
Alkalinity (PP as $CaCO_3$) (mg/L)	-	<1.0	-	<1.0
Bicarbonate (HCO_3) (mg/L)	-	150	-	160
Carbonate (CO_3) (mg/L)	-	<1.0	-	<1.0
Hydroxide (OH) (mg/L)	-	<1.0	-	<1.0
Nutrients				
Total Phosphorus (P) (mg/L)	0.0030	<0.0030	0.0025	<0.0030
Dissolved Nitrogen (N) (mg/L)	-	0.21	-	0.17
Dissolved Nitrite (N) (mg/L)	0.0025	<0.010	0.0025	<0.010
Dissolved Nitrate plus Nitrite (N)	0.18	0.21	0.124	0.23

* Source: fRI Research

3.2.5 Comparison Between Years of Waiparous Creek Sites

A comparison between years, before and after the Devil's Head/Black Rock wildfire, of the chemical and physical attributes of the water samples at sites WAP02 and WAP03 is presented in Table 7. The results for the nearby Johnson Creek site, approximately 220 m upstream of Waiparous Creek, also are included.

Table 7. Comparison between years of chemical and physical attributes of water samples at Waiparous Creek sites WAP02 and WAP03, and attributes at Johnson Creek in 2021.

Tests	Site and Date of Sampling				
	WAP02		WAP03		JOH01
	Sept. 1 2020	Sept. 2 2021	Sept. 3 2020	Sept. 2 2021	Sept. 7 2021
pH	8.18	8.29	8.38	8.38	8.21
Total Suspended Solids (mg/L)	1.2	<1.0	2.0	<1.0	<1.0
Turbidity (NTU)	<0.10	0.22	<0.10	0.11	0.11
Specific Conductance (µS/cm)	316.6	336.8	320.2	336.7	335.8
Dissolved Oxygen (mg/L)	8.97	9.92	8.77	9.28	10.24
Water Temperature (°C)	15.0	7.4	12.8	10.2	6.2
Air Temperature (°C)	22.5	10.5	17.5	19.0	16.0
Anions					
Alkalinity (Total as $CaCO_3$) (mg/L)	150	140	140	130	150
Alkalinity (PP as $CaCO_3$) (mg/L)	<1.0	<1.0	1.4	<1.0	<1.0
Bicarbonate (HCO_3) (mg/L)	180	170	160	160	190
Carbonate (CO_3) (mg/L)	<1.0	<1.0	1.7	<1.0	<1.0
Hydroxide (OH) (mg/L)	<1.0	<1.0	<1.0	<1.0	<1.0
Nutrients					
Total Phosphorus (P) (mg/L)	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030
Dissolved Nitrogen (N) (mg/L)	0.26	0.13	0.25	0.10	0.16
Dissolved Nitrite (N) (mg/L)	<0.010	<0.010	<0.010	<0.010	<0.010
Dissolved Nitrate plus Nitrite (N)	0.14	0.13	0.19	0.13	0.17

The alkalinity (total as $CaCO_3$) at both sites was 10 mg/L lower than in 2020, but in both years the site downstream of Johnson Creek was slightly higher in alkalinity than upstream. In 2021 at least, this was likely a result of Johnson Creek having higher alkalinity and bicarbonate values, and lower pH, than the Waiparous Creek sites.

The TSS readings in Waiparous Creek were lower in the 2021 (<0.10 mg/L) but the readings in both years are considered to be very low. In contrast, the turbidity readings were higher in

2021 (Table 7). The elevated turbidity at WAP02 in 2021 was likely a result of the extra volume of water and a higher velocity flow below the confluence with Johnson Creek.

The DO at both Waiparous Creek sites was higher in 2021, which may be explained by the lower water temperatures (Table 7).

3.3 Benthic Macroinvertebrate Morphological Analysis

In addition to measuring chemical and physical parameters, CABiN uses benthic macroinvertebrates as indicators of aquatic ecosystem health (Environment Canada 2012). Organisms in natural aquatic systems are continuously exposed to fluctuations in their environment. Some species adapt to these changes, whereas other species cannot (CCME 2008).

The orders Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) (EPT) are taxa sensitive to pollution or degraded aquatic environments. The EPT index is the proportion of these taxa in the benthic invertebrate community. In contrast, the family Chironomidae (non-biting midges) are tolerant of degraded waterbodies. Therefore, determining the ratio of chironomids to EPT species can be a good indicator of water quality. Monitoring the ratio over time can be used to determine whether the community is changing, either because of anthropogenic (using test sites) or naturally-caused (using reference condition sites) influences. Metric indices using the data collected in GWAS's water monitoring program can provide information on the abundance, richness, diversity and evenness of the community.

The community/population data and analyses are presented in appendices. Appendix B contains the common names of the orders and families of the benthic macroinvertebrates that were identified in this study. Appendix C contains the entire raw data set of the benthic macroinvertebrates identified based on morphological characteristics. Appendix D contains this taxonomic data at the family level. Appendix E contains the raw data set of the benthic macroinvertebrates at the upper Ghost River sites sampled by fRI Research in 2020 and GWAS in 2021. Appendix F contains the metric indices for the entire 2021 taxonomic data to the genus/species level based on morphological identification.

Within CABiN, the metrics are classified into four main groups: measurements of richness, measurements of abundance or composition, functional measurements, and biotic indices. A description of these taxonomic data analyses is provided in the report on the 2020 monitoring program (Biota Consultants 2022). All of the metric results are presented in Appendix F, and key results are summarized below.

3.3.1 Richness Measurements

The number of different species present is a measure of richness, or the number of species within a functional feeding group (i.e., predators, shredder-herbivores, collector-gatherers, scrapers, collector-filterers, omnivores, parasites, piercer-herbivores, gatherers or unclassified types). Species richness does not take into account the number of individuals of each species present, giving as much weight to those species represented by very few individuals as to those represented by many individuals.

Diversity/evenness measurements take into account the abundance and distribution among the taxa present (i.e., Simpson's Diversity/Evenness Index and Shannon-Weiner Diversity Index). Diverse communities are indicators of "good" water quality.

The results of the Simpson's Index of Diversity indicate the community composition of the sites sampled is diverse (Figure 2). On the Ghost River, values ranged from a low of 0.74 at site GHO06 to a high of 0.90 at site GHO07. Similar results are indicated by the Shannon-Weiner Index. On Waiparous Creek, the diversity at WAP02 was less than it was in 2020.

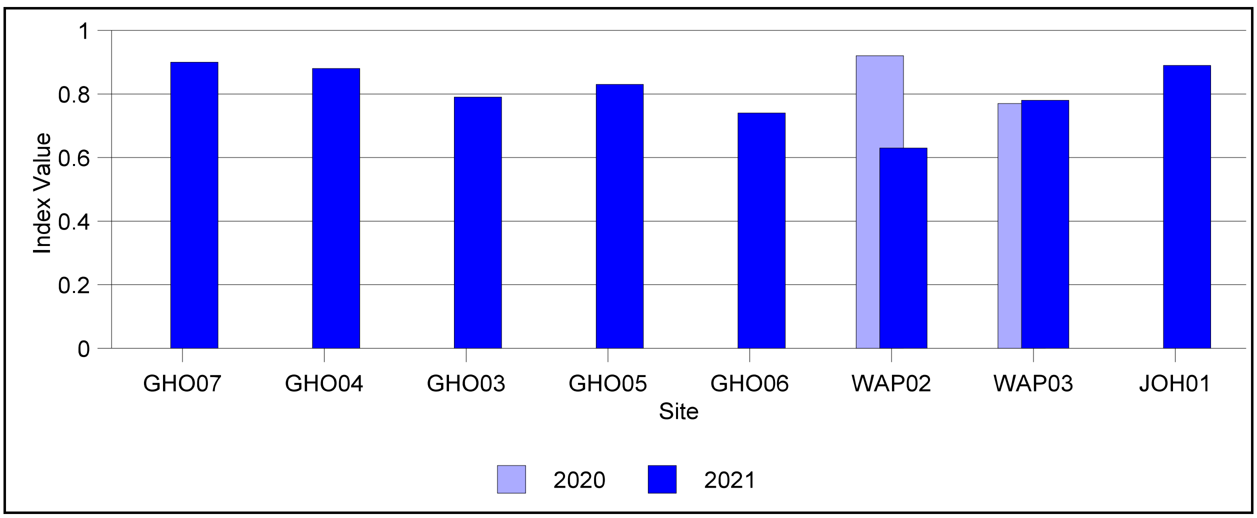

Figure 2. Simpson's Index of Diversity (1-D) for each site, ordered from downstream to upstream for each water course.

3.3.2 Abundance and Compositional Measures

Abundance can be expressed as the sum of all organisms present at a selected taxonomic level or within a specified group. Composition of taxa within the population can be expressed numerically or as a percentage within the population. Shifts within the population can alter the structure at various trophic levels, as certain species increase or decrease due to changes in the aquatic environment. The abundance and compositional measures presented include:

- Ratio: EPT/(chironomids+ EPT): the abundance of EPT individuals divided by the abundance of chironomids plus the EPT individuals (expressed as a value from 1 to 0).

- % Diptera that are Chironomidae: Chironomidae tend to be more tolerant than other families of Diptera.

- % Trichoptera that are Hydropsychidae: Hydropsychidae tend to be more tolerant than other families of Trichoptera.

- % Ephemeroptera that are Baetidae: Baetidae tend to be more tolerant than other families of Ephemeroptera.

The following graphs illustrate the relationship between the Ephemeroptera, Plecoptera, Trichoptera and Diptera at each site. Of the EPT species, the Ephemeroptera dominated all sites except the Johnson Creek site, where it was only 0.9% higher than the Plecoptera. Trichoptera were not detected in the sample from GHO06, the uppermost site on the Ghost River (Figure 3). The EPT species were far more abundant than the Diptera species at all sites except GHO06 and the Johnson Creek site (Figure 4), and the chironomid family comprised the majority of the taxa within the Diptera, and was the only family represented at site WAP02 (Figure 5).

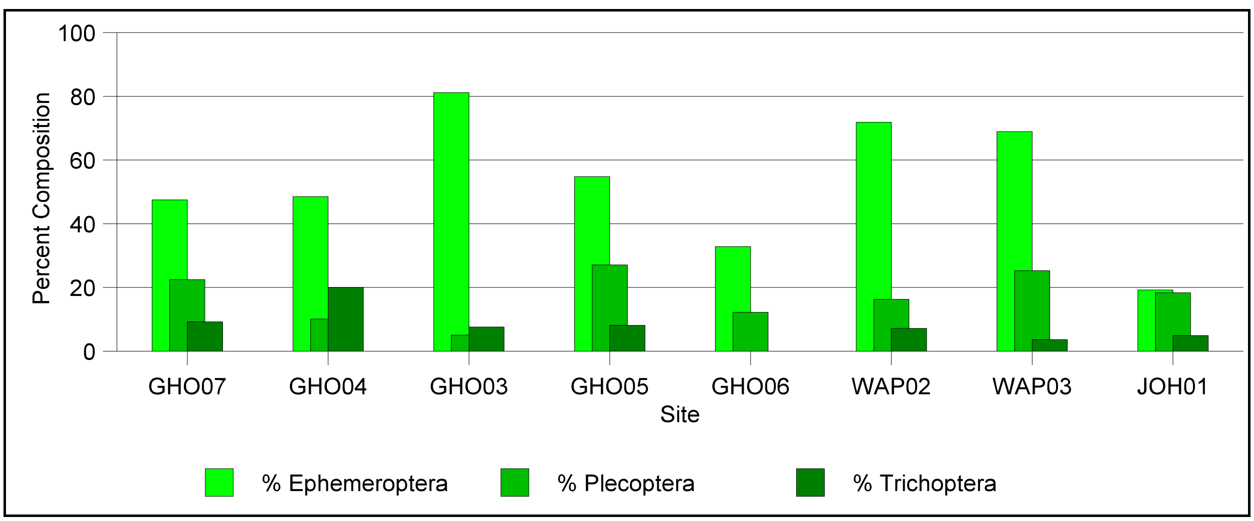

Figure 3. Percent composition of EPT orders at each site, ordered from downstream to upstream for each water course.

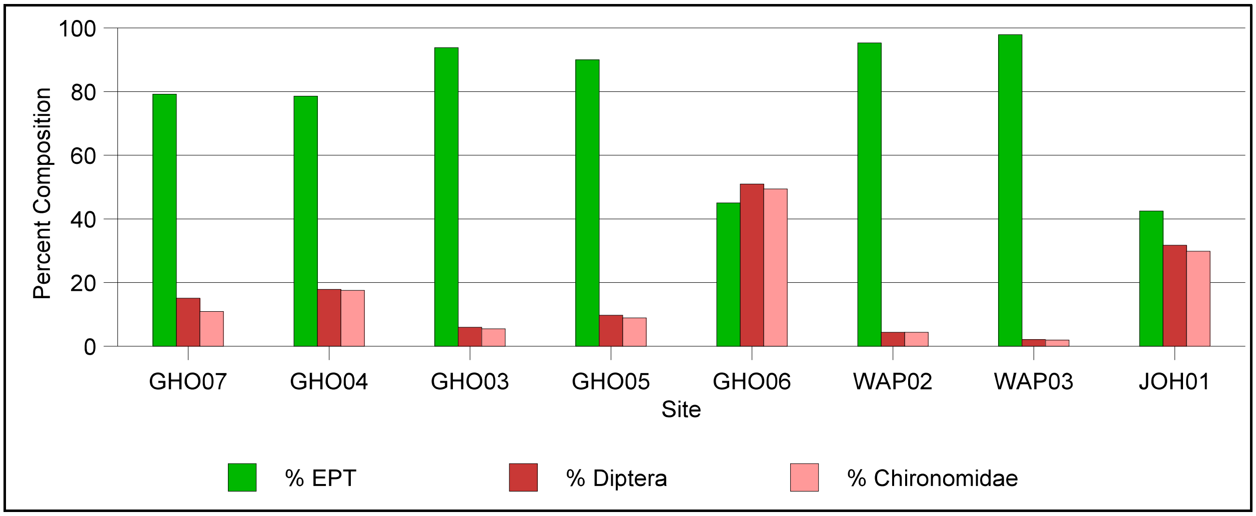

Figure 4. Percent composition of EPT orders, Diptera order and chironomid family at each site, ordered from downstream to upstream for each water course.

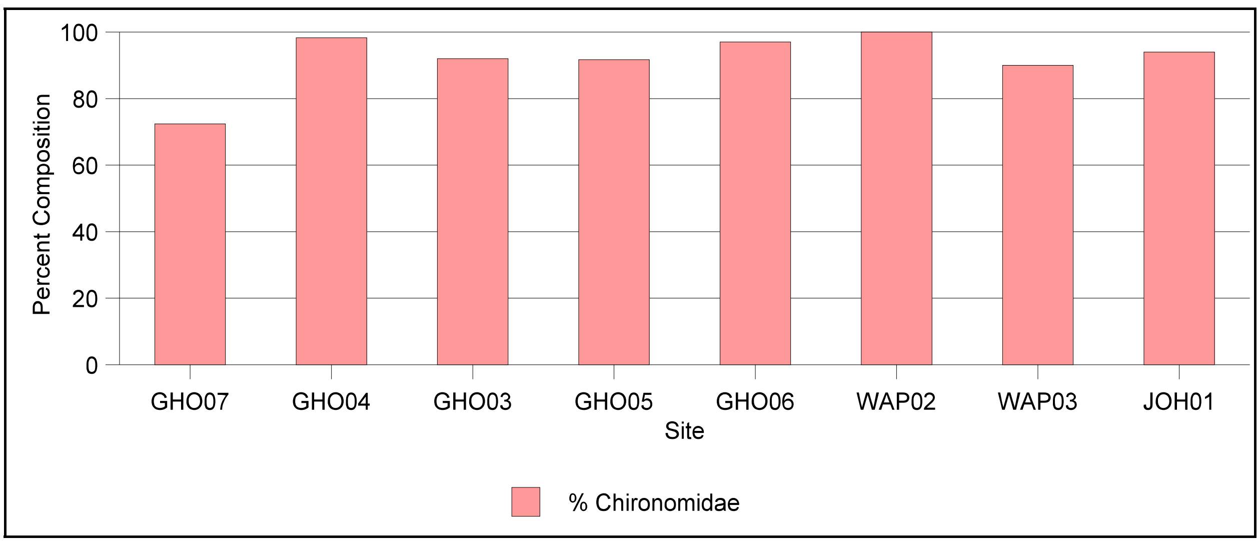

Figure 5. Percent of Diptera that were chironomid flies at each site, ordered from downstream to upstream for each water course.

The EPT ratio was very high at all sites except GHO06 at 0.48 and the Johnson Creek site (JOH01) at 0.59, potentially raising concerns (Figure 6). The low EPT ratio at GHO06 is surprising due to the lack of obvious anthropogenic disturbances. The high values at WAP02 and WAP03 in 2020 and 2021 suggest good water quality.

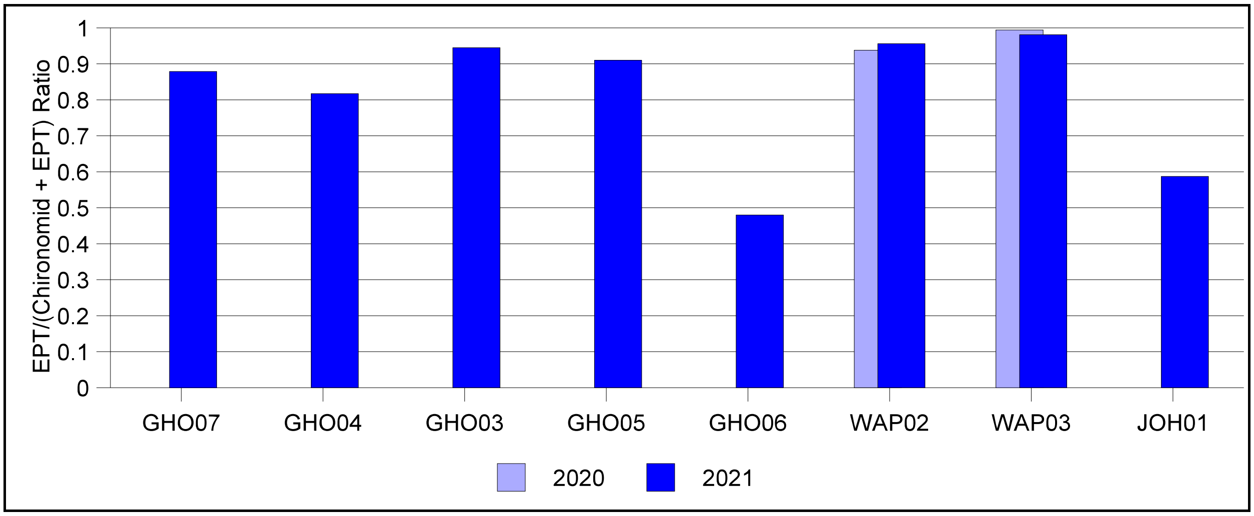

Figure 6. EPT/(chironomid + EPT) ratio using percent community composition for each site, ordered from downstream to upstream.

The abundance of Hydropsychidae within the Trichoptera was highly variable among sites on the Ghost River. The most downstream site (GHO07) had a much higher percentage than the other sites (Figure 7), suggesting a potential concern. At the paired sites on Waiparous Creek, Hydropsychidae comprised a higher proportion of the Trichoptera at WAP02 than in 2020 (Figure 7). No Trichoptera were detected by morphological identification at WAP03 in 2020, but in 2021, 59.5% of the Trichoptera identified were Hydropsychidae.

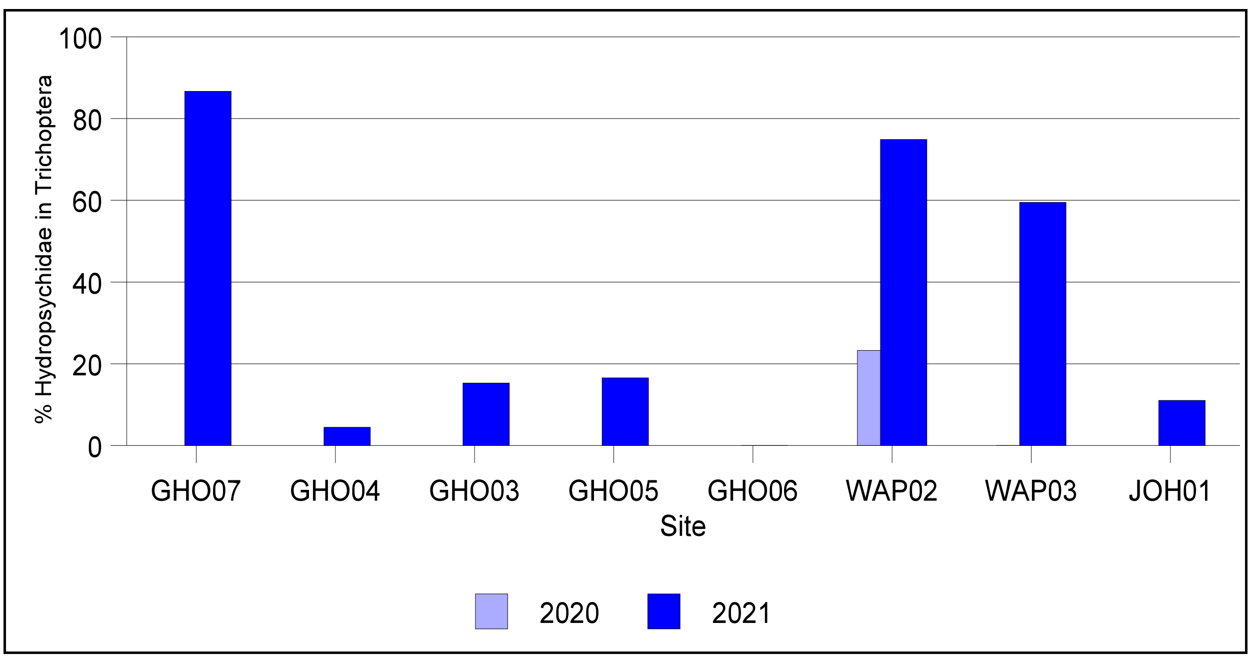

Figure 7. Percent of Trichoptera that were Hydropsychidae at each site, ordered from downstream to upstream for each water course.

The percent of Baetidae within the Ephemeroptera also was variable among the Ghost River sites. There were no or very few Baetidae at the two upstream sites (Figure 8). On Waiparous Creek, there was a lower proportion of Baetidae compared to 2020 (Figure 8).

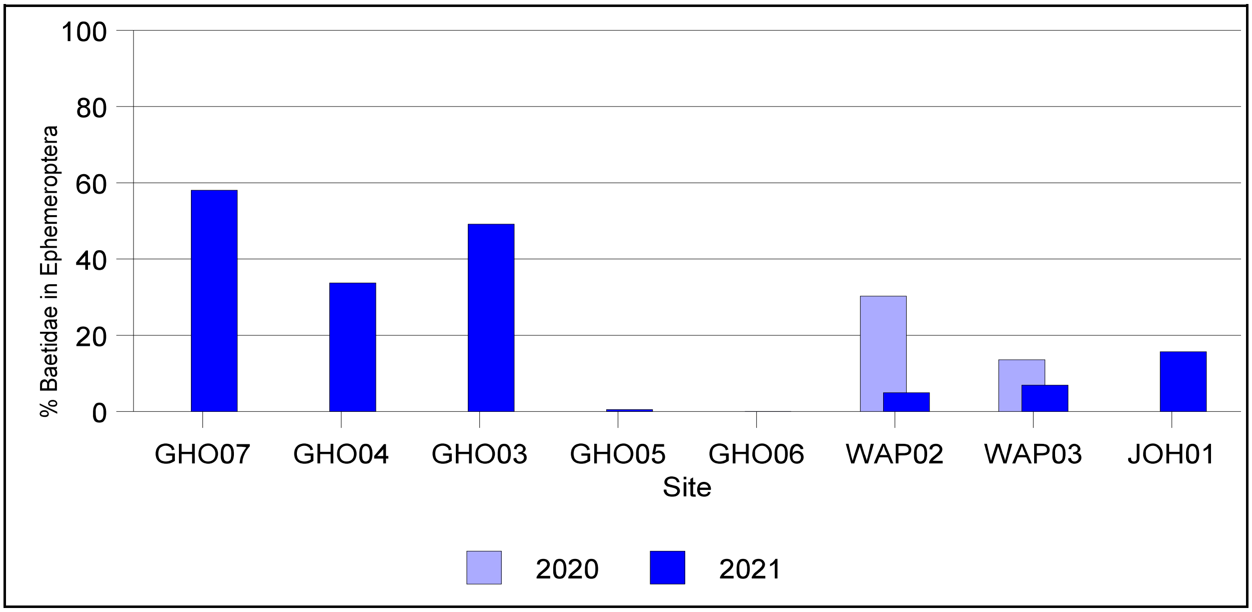

Figure 8. Percent of Ephemeroptera that were Baetidae at each site, ordered from down-stream to upstream for each water course.

3.3.3 Hilsenhoff Biotic Indices

The Hilsenhoff Family Biotic Index (FBI) estimates overall tolerance to organic pollution for each family within the community based on the proportion (abundance) of each, whereas the Hilsenhoff Biotic Index (HBI) estimates a score using taxa at the genus/species level (Appendix F). Biotic tolerance values are assigned to each taxa based on their response to organic pollution (Table 8). Index scores range from 0 to 10. Sensitive taxa have low scores and tolerant taxa have high scores, therefore an increase in the index suggests decreased water quality due to organic pollution.

Within CABiN, generalizing the level of detail to the family level may be adequate depending on the objectives of the study. According to Hilsenhoff (1988), the use of the FBI is advantageous for evaluating the general status of organic pollution in streams to help decide which streams should be studied further. Some accuracy is lost using the FBI, as it usually indicates greater pollution than the generic- and species-level biotic index (BI) in unpolluted or slightly polluted streams, and less pollution in organically polluted streams (Hilsenhoff 1988). Therefore, Hilsenoff recommends "for greatest sensitivity, everything should be identified to species" (Hilsenoff 1987).

Table 8. Hilsenhoff Biotic Index (HBI) categories.

Biotic Index	Water Quality	Degree of Organic Pollution
0.00–3.50	Excellent	Organic pollution unlikely
3.51–4.50	Very Good	Possible slight organic pollution
4.51–5.50	Good	Some organic pollution probable
5.51–6.50	Fair	Fairly substantial pollution likely
6.51–7.50	Fairly Poor	Substantial pollution likely
7.51–8.50	Poor	Very substantial pollution likely
8.51–10.00	Very Poor	Severe organic pollution likely

The water quality at two of the sites on the Ghost River was rated as excellent, and one came close (GHO07) at 3.60. It along with GHO03 and GHO06 fell into the "very good" category, where there was possible slight organic pollution (Figure 9). The water quality at the two sites sampled on Waiparous Creek remained in the "excellent" category, whereas the Johnson Creek site fell just outside the excellent range, at 3.57.

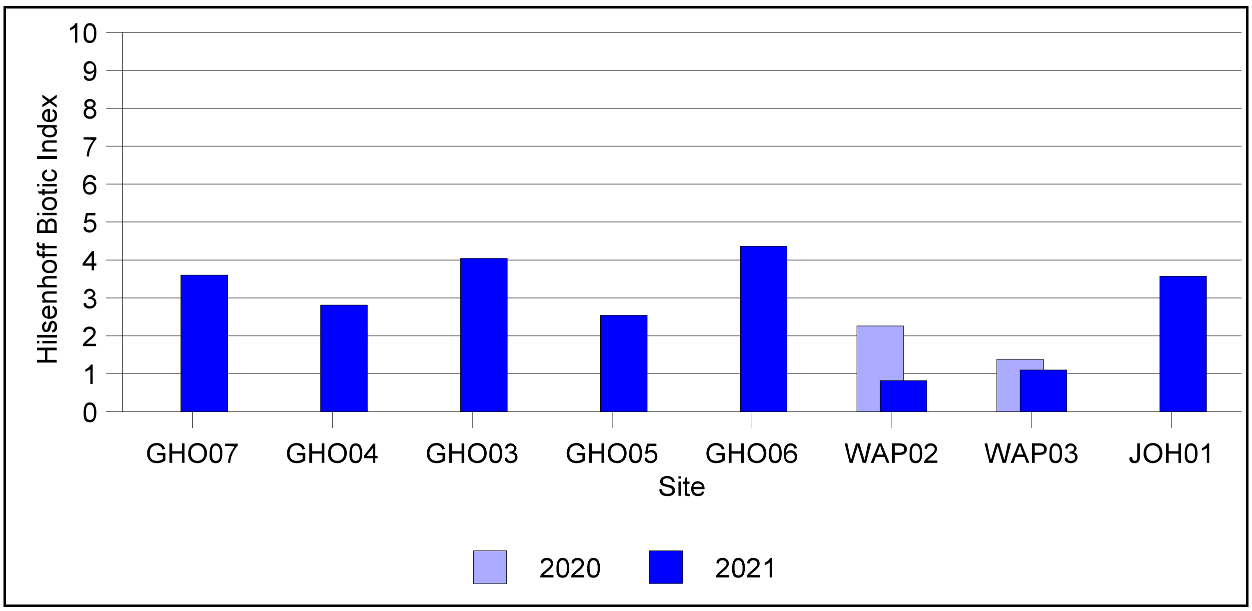

Figure 9. Hilsenhoff Biotic Index for each site, ordered from downstream to upstream for each water course.

3.4 Comparison of Ghost River Sites Related to Devil's Head/Black Rock Wildfire

The most striking difference between each of the upper Ghost River sites in 2020 versus 2021 was the percent of key taxa (Table 9). Percent of Ephemeroptera and Trichoptera was much higher at GHO05 in 2021 than GR-20-02 in 2020, and percent Plecoptera was lower; however, percent EPT was similar, as was the EPT ratio. The percent of Baetidae within the Ephemeroptera was higher in 2020. Balanced with the other metrics, there appear to be no reasons for concern though.

The species composition suggests water quality at the uppermost site on the Ghost River declined in 2021, which is hard to explain considering the lack of obvious anthropogenic effects. The percent of Plecoptera was lower in 2021 and the percent of chironomids within the Diptera was higher, resulting in a moderate EPT ratio. No Baetidae were recorded compared to over 29% in 2020, which is a positive sign (Table 9). Although no Trichoptera were recorded at GHO06 in the morphological analysis, the DNA of two species was detected in the eDNA analysis (Appendix G). The Hilsenhoff Biotic Index was higher in 2021, but as mentioned above, the index still fell into the "very good" category.

Table 9. Comparison of metrics for water samples at GR-20-02/GHO05 and GR-20-01/GHO06.

Metric	Site and Date of Sampling			
	GR-20-02*	GHO05	GR-20-01*	GHO06
	Oct. 29 2020	Aug. 30 2021	Oct. 29 2020	Aug. 30 2021
Simpson's Index of Diversity (1 - D)	0.80	0.83	0.91	0.74
% Ephemeroptera	27.71	54.79	30.92	32.84
% Plecoptera	57.32	27.10	65.54	12.19
% Trichoptera	2.23	8.12	0.54	0
% EPT	87.26	90.00	97.00	45.03
% Diptera	9.24	9.73	1.77	50.98
% Chironomidae in Diptera	82.76	91.72	57.58	97.00
EPT/(chironomids+ EPT) ratio	0.92	0.91	0.99	0.48
% Baetidae in Ephemeroptera	10.34	0.49	29.12	0
% Hydropsychidae in Trichoptera	14.29	16.54	100	N/A
Hilsenhoff Biotic Index	2.46	2.54	2.18	4.36

* Source: fRI Research; morphologic identification by Cordillera Consulting

3.5 STREAM eDNA Results

3.5.1 eDNA and Morphological Identification

The eDNA results complement the results of the morphological identification. An additional 136 species were identified, 24 of which were terrestrial species. The remainder were within 73 different genera. It was expected that more taxa would be identified by eDNA, partly because three kicknet samples were collected versus one, and partly because the method does not require a recognizable specimen. DNA trapped in the sediment, from gut contents and from animal waste is also detected (M. Wright, pers. comm.). The morphological identification produced 20 genera that were not detected by eDNA along with one phylum (Nemata).

There are a number of possible explanations for taxa to be identified in the morphological samples but not in the eDNA samples (M. Wright, pers. comm.). If the taxa are not in the eDNA reference database, they will not be detected. (This was the case for several taxa in 2020.) Other possible reasons include:

- The sequences in the reference database are from different species within the genus than those present in their sample, and are genetically distinct enough from each other that the species in their sample is not identified;
- The DNA primers that are used, which target the specific DNA region to be sequenced and compared, were not compatible with the species in their sample (three different primers are used in the workflow to overcome this known issue, but there are still sometimes taxa that are not compatible);
- The taxa may be too rare within the sample to be identified by DNA metabarcoding;
- The taxa may not be in the sample (since the samples collected for morphology and eDNA are different subsamples of the watercourse, and distribution of the taxa may be patchy).

The majority of the eDNA detections were to the species level, with only three at just the genus level. Morphological identifications were rarely to the species level, usually to the genus level, often to the family level and, in rare cases, only to the order, class or phylum level. Most direct comparisons, therefore, could only be made at higher taxonomic levels (Table 10). The more detailed combined presence/absence results of each method are presented in Appendix G. Only those taxa that spend at least part of their life cycle in aquatic habitats are included. It is likely when morphological identification indicates specimens at levels above genus and species, they are the same genus/species detected by eDNA, but this may not always be the case.

Table 10. Comparison of results of eDNA and morphological identification for benthic macroinvertebrates that were detected by both methods. (Note: results are given for the lowest taxonomic level of morphological identification, sometimes only at the order level. [Suffix "idae" = family level, "inae" = subfamily level, "ini" = tribe level] A blank line indicates that all specimens were identified at a lower level. Taxa were often detected by eDNA, and occasionally by morphological identification, at lower levels than is indicated.)

Taxa	Site							
	GHO03	GHO04	GHO05	GHO06	GHO07	WAP02	WAP03	JOH01
Class: Insecta								
Order: Diptera								
Chironomidae	Both	Both	Both	Both	Both	Both	Both	Both
Chironominae								
Tanytarsini	Morph	Both	Morph		Both	Both	Both	Morph
Diamesinae		Both	Morph	eDNA	Both			Both
Orthocladiinae	Both	Both	Both	Both	Both	Morph	Morph	Both
Empididae	eDNA	Both	Morph	Morph	Both			Morph
Simuliidae	Both	eDNA	Morph	eDNA	eDNA	eDNA		eDNA
Tipulidae	Both	eDNA	Both	eDNA	Morph		Morph	Morph
Order: Ephemeroptera								
Ameletidae								
Ameletus	eDNA	Both	Both	Both		eDNA	Both	Both
Baetidae	Both	Both	Both	eDNA	Both	Both	Both	Both
Acentrella		Both			Both	eDNA	eDNA	
Baetis	Both	Both	Both	eDNA	Both	Both	Both	Both
Ephemerellidae	Both	Both	Both	Both	Both	Both	Both	Both
Drunella	Both	Both	Both	eDNA	Both	Both	Both	Both
D. coloradensis	eDNA	eDNA	Both	eDNA		eDNA	eDNA	eDNA
D. doddsii	Both	Both	Both	eDNA	Both	Both	Both	eDNA
Ephemerella	Both	Both	eDNA	Morph	eDNA	eDNA		eDNA
Heptageniidae	Both	Both	Both	Both	Both	Both	Both	Both
Cinygmula	eDNA	Both	eDNA	Both	eDNA	eDNA	Both	eDNA
Leptophlebiidae	eDNA	Both	Morph		eDNA	eDNA	Morph	Both
Order: Plecoptera	Both	Both	Both	Both	Both	Both	Both	Both
Capniidae	eDNA	eDNA	Morph	Both	Both	Both	Both	Both
Chloroperlidae	Both	Both	Both	eDNA	Both	Both	Both	Both
Leuctridae	eDNA	eDNA			eDNA	eDNA	Morph	eDNA
Nemouridae	Both	Both	Both	Both	Both	Both	Both	Both
Zapada	Both	Both	Both	Both	Both	Both	Both	Both
Z. cinctipes	Both	eDNA	eDNA	eDNA	Both	Both	Both	Both
Z. columbiana	eDNA		Both	eDNA		Both	Both	eDNA

Taxa	Site							
	GHO03	GHO04	GHO05	GHO06	GHO07	WAP02	WAP03	JOH01
Perlidae	Both	Both	eDNA		Both	Both	Both	Both
Doroneuria	eDNA	eDNA	eDNA		Both	Both		eDNA
Hesperoperla	Both	Both			Both	eDNA	Both	Both
Perlodidae	eDNA	eDNA	eDNA	eDNA	eDNA	Both	Both	
Isogenoides		eDNA			eDNA	Both	Both	
Pteronarcyidae								
Pteronarcella		eDNA			eDNA	eDNA	Morph	
Taeniopterygidae	Both	Both	Both	eDNA	Both	Both	Both	eDNA
Order: Trichoptera	Both	Both	Both	eDNA	Both	Both	Both	Both
Brachycentridae		eDNA			Morph		Morph	Both
Brachycentrus americanus		eDNA			Morph		Morph	Both
Glossosomatidae	Both	Both	Both		Morph	Morph		
Glossosoma	eDNA	Both	Both		Morph			
Hydropsychidae	Both	Both	Morph	eDNA	Both	Both	Both	Both
Arctopsyche	eDNA	Both			Both	Both	Both	Both
Hydropsyche					Morph			eDNA
Parapsyche	Both		eDNA	eDNA		eDNA		
Lepidostomatidae								
Lepidostoma	eDNA	Both				eDNA		
Limnephilidae			Both				Morph	eDNA
Rhyacophilidae								
Rhyacophila	Morph	Both				eDNA	Morph	Both
R. brunnea/vemna group								Both
Class: Arachnida								
Order: Trombidiformes					Morph			Both
Torrenticolidae					Morph			Both
Testudacarus					Morph			Both
Class: Ostracoda	Morph	Morph			eDNA	Morph		Both
Class: Oligachaeta								
Order: Haplotaxida								
Naididae	Morph	eDNA	Morph		Morph			
Nais	Morph	eDNA						

Species richness is the only metric that can be used with presence/absence data. Figure 10 presents the results from each method. These are not expected to be the same due to the different techniques used. The combined results suggest a trend of higher species richness moving downstream on the Ghost River.

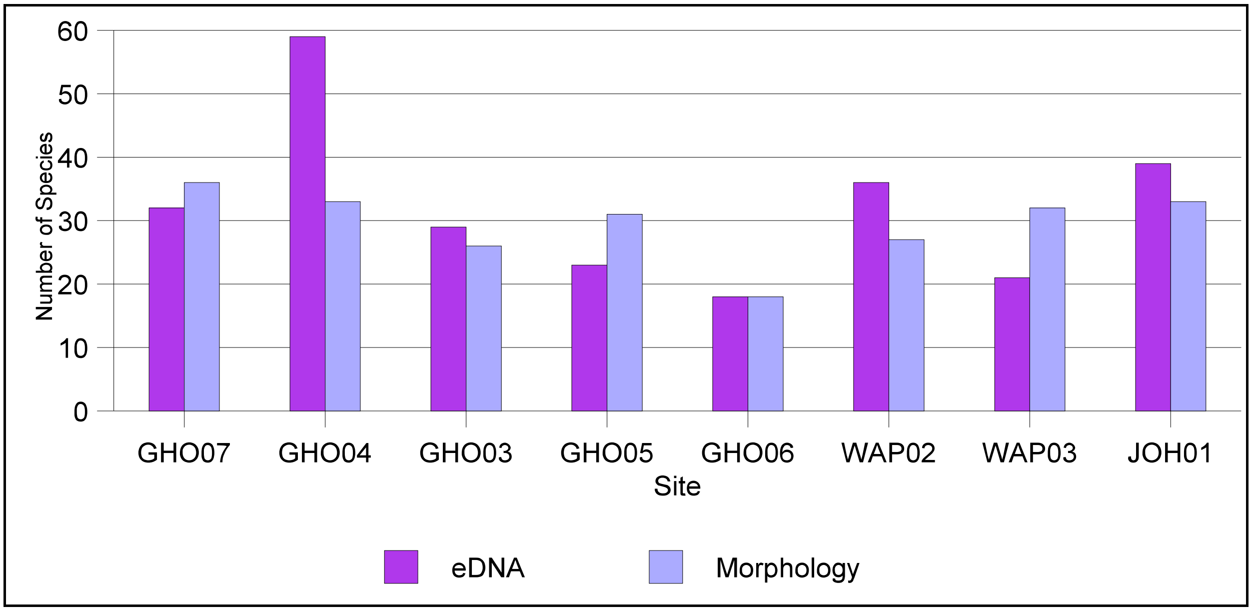

Figure 10. Species richness based on species taxonomically assigned by eDNA with high confidence based on normalized sequence data, and taxa identified morphologically.

3.5.2 Whirling Disease

Although whirling disease has been detected in the Ghost Watershed (Government of Alberta 2020), DNA of *Tubifex tubifex* (sludge worm), the intermediate host of the microscopic parasite that causes the disease, was not found at any of the eight sites. This suggests that the sampling locations may be outside of the confirmed zone for whirling disease (Hajibabaei Lab 2022).

4.0 Conclusions and Recommendations

4.1 Comparison of All Sites

The analyses of chemical and physical attributes of water samples at the eight sites indicate high water quality. The results were well below exceedance levels. TSS and turbidity were extremely low. Water quality parameters were all within acceptable limits for benthic macroinvertebrates and fish.

Despite the input of periphyton from Robinson Creek into the Ghost River at GHO04, there was little evidence that Robinson Creek was affecting the chemical and physical attributes of the river water. It is possible that the higher turbidity in Robinson Creek was responsible for the slightly higher turbidity in the river downstream of the confluence versus upstream, but the level was still very low.

There was evidence that Johnson Creek might have influenced the attributes of Waiparous Creek below the confluence. The higher alkalinity and bicarbonate values, and lower pH of JOH01 may explain the slightly higher alkalinity of WAP02 versus WAP03. Similarly, the higher turbidity at WAP02 was likely a result of the extra volume of water and a higher velocity flow below the confluence with Johnson Creek.

The Simpson's Index of Diversity and the Shannon-Weiner Index indicate that the sites were diverse in their benthic macroinvertebrate community composition. The Hilsenhoff Biotic Index suggests that there was possible slight organic pollution at most of the Ghost River sites, rating water quality as very good. Only water quality at GHO04 and GHO05 was rated as excellent, with organic pollution unlikely. The two sites sampled on Waiparous Creek also fell into the excellent category, whereas the Johnson Creek site was very good.

The EPT ratio suggests high water quality at most of the sites, with EPT species at much higher abundance than the pollution-tolerant chironomid family. The exceptions were GHO06 and JOH01 where the ratio was 0.48 and 0.59, respectively, potentially raising concerns.

The abundance of Hydropsychidae within the Trichoptera was highly variable. The most downstream site on the Ghost River (GHO07) and the two sites on Waiparous Creek (WAP02 and WAP03) had much higher percentages than the other sites, suggesting a potential concern. The percent of Baetidae within the Ephemeroptera also was variable, with the highest proportion at GHO07, GHO03 and GHO04. However, no other metrics suggested any concerns at the latter two sites.

The results of the 2021 field sampling provide a baseline for comparison in future years. With more data, trends may become apparent. If issues with water quality are suggested, sampling effort may become more focussed.

4.2 Comparison Between Years of Waiparous Creek Sites

The geometric median particle size of the substrate was once again higher at WAP02 but not to as great a degree as in 2020. This suggests that below the tributary, the finer substrates are transported downstream.

The differences in the chemical and physical attributes of water samples at WAP02 and WAP03 in 2020 and 2021 were subtle, with values well below exceedance levels. Although the abundance of Hydropsychidae within the Trichoptera was higher than in 2020, the percent of Baetidae within the Ephemeroptera was lower, and the EPT ratio was similar, suggesting no major concerns. Similarly, the Hilsenhoff Biotic Index was lower in 2021, but in both years was within the excellent water quality category.

4.3 Comparison of Ghost River Sites Related to Devil's Head/Black Rock Wildfire

The differences in the chemical and physical attributes of water samples at the upper Ghost River sites in 2020 and 2021 were subtle, with values well below exceedance levels. There were, however, differences in the proportion of the key EPT taxa. On balance at GHO05/GR-20-02, there was no evidence to suggest a concern. The percent EPT and the EPT ratio were similar. This was not the case with GHO06 and GR-20-01. The high percentage of chironomids within the Diptera at GHO06 resulted in only a moderate EPT ratio compared to a high ratio at GR-20-01 the year before. The reason is unclear and further monitoring is advisable. The Hilsenhoff Biotic Index suggested possible slight organic pollution at GHO06 in 2021, but organic pollution was unlikely at GR-20-01 the year before. Further sampling at this location will determine if these differences persist and become a concern.

4.4 General Recommendations

- Adequate annual funding for this program should be maintained.
- The *GWAS Water Monitoring Program Plan* should continue to be followed, allowing flexibility if circumstances materialize that suggest a deviation.
- The 2020 and 2021 sites should be monitored as frequently as possible as funds will allow, and as personnel are available, giving priority to those sites where water quality may be more comprised, e.g., GHO06, GHO07.

- Prior to conducting the field sampling, the survey team should read and fully understand the methodology presented in the *CABiN Field Manual – Wadeable Streams* and *Procedure for Collecting Benthic Macroinvertebrate DNA Samples in Wadeable Streams*.

- A practice run through all of the methods should be conducted prior to data collection.

- Certain tasks, such as kicknetting, should only be conducted by qualified personnel, whereas other tasks may be done by volunteers who have been trained by the CABiN-certified personnel or previously trained volunteers. Because not all of the trained volunteers may be present on each field day, they should be encouraged to try different tasks to become familiar with them in case they are required to perform them at some time.

- During the sampling, the field team must adhere to the order of events required to maintain quality assurance/quality control (QA/QC) of each sample.

- Absolute Zero RV antifreeze (propylene glycol) should be used for preservation of the STREAM eDNA samples versus 95% ethanol solution. Absolute Zero is less expensive, is not considered to be a dangerous good, and has been approved by STREAM.

- In order to maintain QA/QC of each sample, the same laboratories that were originally selected and used in 2020 and 2021 (water chemical and benthic macroinvertebrate analysis) should continue to be used.

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6.0 Personal Communications

Mallinson, Raegan	Program Manager, Living Lakes Canada, Nelson, BC. (Oct. 1, 2020)
Wright, Michael	Laboratory Manager, Hajibabaei Lab, Centre for Biodiversity Genomics, Biodiversity Institute of Ontario, University of Guelph. (Aug. 10, 2022)

Appendix A
CABiN Field Sheets

Field Crew: ______________________________ Site Code: ___________

Sampling Date: (DD/MM/YYYY) ____________________

☐ **Occupational Health & Safety: Site Inspection Sheet completed**

PRIMARY SITE DATA

CABIN Study Name: ____________________ Local Basin Name: ____________________

River/Stream Name: ____________________ Stream Order: (map scale 1:50,000) ___________

Select one: ☐ Test Site ☐ Potential Reference Site

Geographical Description/Notes:

Surrounding Land Use: (check those present) Information Source: ____________

☐ Forest ☐ Field/Pasture ☐ Agriculture ☐ Residential/Urban

☐ Logging ☐ Mining ☐ Commercial/Industrial ☐ Other ___________

Dominant Surrounding Land Use: (check one) Information Source: ____________

☐ Forest ☐ Field/Pasture ☐ Agriculture ☐ Residential/Urban

☐ Logging ☐ Mining ☐ Commercial/Industrial ☐ Other ___________

Location Data

Latitude: __________N Longitude: - __________W (DMS or DD)

Elevation: ________(fasl *or* masl) GPS Datum: ☐ GRS80 (NAD83/WGS84) ☐ Other: _________

Site Location Map Drawing

Note: Indicate north

Field Crew: ______________________________________ Site Code: ______________

Sampling Date: (DD/MM/YYYY) __________________________

Photos

☐ Field Sheet ☐ Upstream ☐ Downstream ☐ Across Site ☐ Aerial View

☐ Substrate (exposed) ☐ Substrate (aquatic) ☐ Other _____________________

REACH DATA *(represents 6 times bankfull width)*

1. Habitat Types: *(check those present)*
   ☐ Riffle ☐ Rapids ☐ Straight run ☐ Pool/Back Eddy

2. Canopy Coverage: *(stand in middle of stream and look up, check one)*
   ☐ 0 % ☐ 1-25 % ☐ 26-50 % ☐ 51-75 % ☐ 76-100 %

3. Macrophyte Coverage: *(not algae or moss, check one)*
   ☐ 0 % ☐ 1-25 % ☐ 26-50 % ☐ 51-75 % ☐ 76-100 %

4. Streamside Vegetation: (*check those present)*
   ☐ ferns/grasses ☐ shrubs ☐ deciduous trees ☐ coniferous trees

5. Dominant Streamside Vegetation: *(check one)*
   ☐ ferns/grasses ☐ shrubs ☐ deciduous trees ☐ coniferous trees

6. Periphyton Coverage on Substrate: *(benthic algae, not moss, check one*)

   ☐ **1** - Rocks are not slippery, no obvious colour (thin layer < 0.5 mm thick)
   ☐ **2** - Rocks are slightly slippery, yellow-brown to light green colour (0.5-1 mm thick)
   ☐ **3** - Rocks have a noticeable slippery feel (footing is slippery), with patches of thicker green to brown algae (1-5 mm thick)
   ☐ **4** - Rocks are very slippery (algae can be removed with thumbnail), numerous large clumps of green to dark brown algae (5 mm -20 mm thick)
   ☐ **5** - Rocks are mostly obscured by algal mat, extensive green, brown to black algal mass may have long strands (> 20 mm thick)

   Note: 1 through 5 represent categories entered into the CABIN database.

BENTHIC MACROINVERTEBRATE DATA

Habitat sampled: (*check one*) ☐ riffle ☐ rapids ☐ straight run

400 μm mesh Kick Net	
Person sampling	
Sampling time (i.e. 3 min.)	
No. of sample jars	
Typical depth in kick area (cm)	

Preservative used: ________________

Sampled sieved on site using “Bucket Swirling Method”:
☐ YES ☐ NO

If YES, debris collected for QAQC ☐

Note: Indicate if a sampling method other than the recommended 400 μm mesh kick net is used.

Field Crew: ______________________________________ Site Code: ______________

Sampling Date: (DD/MM/YYYY) __________________________

WATER CHEMISTRY DATA **Time:**_________ (24 hr clock) **Time zone**: ______________

Air Temp: _______________(ºC) Water Temp: __________(ºC) pH: ______________

Specific Conductance: _______(µs/cm) DO: __________(mg/L) Turbidity: _________(NTU)

Check if water samples were collected for the following analyses:

☐ TSS (Total Suspended Solids)

☐ Nitrogen (i.e. Total, Nitrate, Nitrite, Dissolved, and/or Ammonia)

☐ Phosphorus (Total, Ortho, and/or Dissolved)

☐ Major Ions (i.e. Alkalinity, Hardness, Chloride, and/or Sulphate) ☐ Other________________

Note: Determining alkalinity is recommended, as are other analyses, but not required for CABIN assessments.

CHANNEL DATA

Slope - Indicate how slope was measured: (check one)

☐ **Calculated from map**

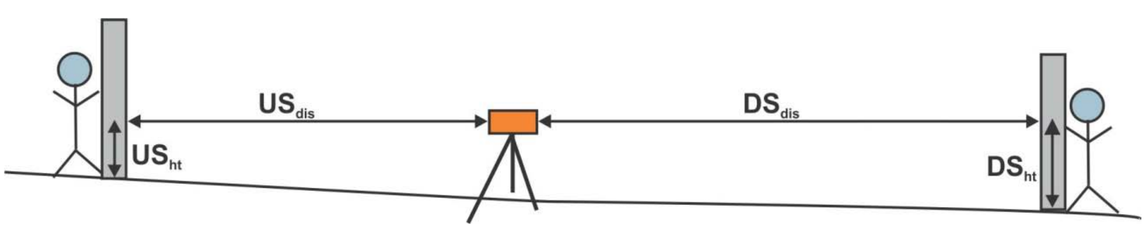
Scale: _______________ (Note: small scale map recommended if field measurement is not possible - i.e. 1:20,000).
contour interval (vertical distance) ___________ (m),
distance between contour intervals (horizontal distance) ___________ (m)
slope = vertical distance/horizontal distance =_________________

OR

☐ **Measured in field**

Circle device used and fill out table according to device:
a. Survey Equipment b. Hand Level & Measuring Tape

Measurements	Upstream (U/S)	Downstream(D/S)	Calculation
[a]Top Hairline (T)			
[a]Mid Hairline (ht) OR [b]Height of rod			
[a]Bottom Hairline (B)			
[b]Distance (dis) OR [a]T-B x 100	[a]US_{dis}=T-B	[a]DS_{dis}=T-B	$US_{dis}+DS_{dis}=$
Change in height (Δht)			$DS_{ht}-US_{ht}=$
Slope (Δht/total dis)			



Field Crew: ______________________________________ Site Code: ______________

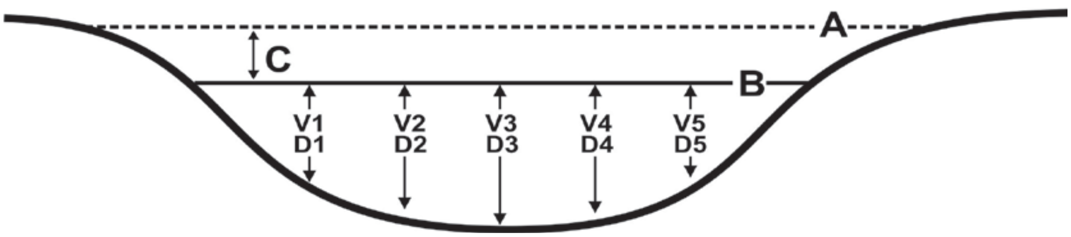
Sampling Date: (DD/MM/YYYY) __________________________

Widths and Depth

Location at site: _________________________________(Indicate where in sample reach, ex. d/s of kick area)

A - Bankfull Width: ___________(m) B - Wetted Stream Width: ____________(m)

C - Bankfull–Wetted Depth (height from water surface to Bankfull): __________________________(cm)



Note:
Wetted widths > 5 m, measure a minimum of 5-6 equidistant locations;
Wetted widths < 5 m, measure 3-4 equidistant locations.

Velocity and Depth

Check appropriate velocity measuring device and fill out the appropriate section in chart below. Distance from shore and depth are required regardless of method:

☐ **Velocity Head Rod (or ruler)**: Velocity Equation (m/s) = $\sqrt{[\,2(\Delta D/100) * 9.81]}$

☐ **Rotary meters**: Gurley/Price/Mini-Price/Propeller (Refer to specific meter conversion chart for calculation)

☐ **Direct velocity measurements**: ☐ Marsh-McBirney ☐ Sontek or ☐ Other_________________

	1	2	3	4	5	6	AVG
Distance from Shore (m)							
Depth (**D**) (cm)							
Velocity Head Rod (ruler)							
Flowing water Depth (**D_1**) (cm)							
Depth of Stagnation (**D_2**) (cm)							
Change in depth (**$\Delta D = D_2 - D_1$**) (cm)							
Rotary meter							
Revolutions							
Time (minimum 40 seconds)							
Direct Measurement or calculation							
Velocity (**V**) (m/s)							

Field Crew: ____ Site Code: ______________

Sampling Date: (DD/MM/YYYY) ___________________________

SUBSTRATE DATA

Surrounding/Interstitial Material

Circle the substrate size category for the surrounding material.

Substrate Size Class	Category
Organic Cover	0
< 0.1 cm (fine sand, silt or clay)	1
0.1-0.2 cm (coarse sand)	2
0.2-1.6 cm (gravel)	3
1.6-3.2 cm (small pebble)	4
3.2-6.4 cm (large pebble)	5
6.4-12.8 cm (small cobble)	6
12.8-25.6 cm (cobble)	7
> 25.6 cm (boulder)	8
Bedrock	9

100 Pebble Count & Substrate Embeddedness

- Measure the intermediate axis (100 rocks) and embeddedness (10 rocks) of substrate in the stream bed.
- Indicate B for bedrock, S for sand/silt/clay (particles < 0.2 cm) and O for organic material.
- Embededness categories (E): Completely embedded = 1, 3/4 embedded, 1/2 embedded, 1/4 embedded, unembedded = 0

	Diameter (cm)	E		Diameter (cm)	E		Diameter (cm)	E		Diameter (cm)	E
1			26			51			76		
2			27			52			77		
3			28			53			78		
4			29			54			79		
5			30			55			80		
6			31			56			81		
7			32			57			82		
8			33			58			83		
9			34			59			84		
10			35			60			85		
11			36			61			86		
12			37			62			87		
13			38			63			88		
14			39			64			89		
15			40			65			90		
16			41			66			91		
17			42			67			92		
18			43			68			93		
19			44			69			94		
20			45			70			95		
21			46			71			96		
22			47			72			97		
23			48			73			98		
24			49			74			99		
25			50			75			100		

Note: The Wolman D50 (i.e. median diameter), Wolman Dg (i.e. geometric mean diameter) and the % composition of the substrate classes will be calculated automatically in the CABIN database using the 100 pebble data. All 100 pebbles must be measured in order for the CABIN database tool to perform substrate calculations.

Field Crew: ____ Site Code: ______________

Sampling Date: (DD/MM/YYYY) ___________________________

SITE INSPECTION

Site Inspected by: ___________________________________

Communication Information

☐ Itinerary left with contact person (include contact numbers)

Contact Person: ______________________________ Time checked-in: _________

Form of communication: ☐ radio ☐ cell ☐ satellite ☐ hotel/pay phone ☐ SPOT

Phone number: (   ) ______________

Vehicle Safety

☐ Safety equipment (first aid, fire extinguisher, blanket, emergency kit in vehicle)

☐ Equipment and chemicals safely secured for transport

☐ Vehicle parked in safe location; pylons, hazard light, reflective vests if necessary

Notes:

Shore & Wading Safety

☐ Wading Task Hazard Analysis read by all field staff

☐ Wading Safe Work Procedures read by all field staff

☐ Instream hazards identified (i.e. log jams, deep pools, slippery rocks)

☐ PFD worn

☐ Appropriate footwear, waders, wading belt

☐ Belay used

Notes:

Appendix B
Benthic Macroinvertebrate Common Names

Order	Family	Common Name
Coleoptera		Beetles
	Dytiscidae	Predaceous diving beetles
	Elmidae	Riffle beetles
Diptera		Flies
	Athericidae	Water snipe flies
	Ceratopogonidae	Biting midges
	Chironomidae	Non-biting midges
	Empididae	Dance flies
	Simuliidae	Black flies
	Tipulidae	Craneflies
Ephemeroptera		Mayflies
	Ameletidae	Combmouthed minnow mayflies
	Baetidae	Small minnow mayflies
	Caenidae	Small squaregill mayflies
	Ephemerellidae	Spiny crawler mayflies
	Heptageniidae	Flat-headed mayflies
	Leptophlebiidae	Prong-gilled mayflies
	Siphlonuridae	Primitive minnow mayflies
Hemiptera		True bugs
	Corixidae	Water boatmen
Odonata		Dragonflies, damselflies
	Coenagrionidae	Damselflies
	Gomphidae	Club-tailed dragonflies
Plecoptera		Stoneflies
	Capniidae	Small winter stoneflies
	Chloroperlidae	Green stoneflies
	Leuctridae	Rolled-winged stoneflies
	Nemouridae	Spring stoneflies
	Perlidae	Common stoneflies
	Perlodidae	Springflies
	Pteronarcyidae	Giant stoneflies
	Taeniopterygidae	Winter stoneflies
Trichoptera		Caddisflies
	Brachycentridae	Humpless casemaker caddisflies
	Glossosomatidae	Saddle casemaker caddisflies
	Hydropsychidae	Net-spinning caddisflies

Order	Family	Common Name
	Lepidostomatidae	Bizarre caddisflies
	Limnephilidae	Tube-case caddisflies
	Rhyacophilidae	Free-living caddisflies
Oribatida		Oribatid mites
	Steganacaridae	Oribatid mites
Trombidiformes		Mites
	Lebertiidae	Water mites
	Sperchontidae	Water mites
	Torrenticolidae	Torrent mites
Anthoathecata		Athecate hydroids
	Hydridae	Hydra
Amphipoda		Amphipods
	Hyalellidae	Amphipods
Podocopida		Ostracods
	Candonidae	Freshwater ostracods
	Cyprididae	Freshwater ostracods
Veneroida		Bivalve molluscs
	Montacutidae	Clams
Lumbriculida		Microdrile oligochaetes (worms)
	Lumbriculidae	Aquatic worms
Haplotaxida		Haplotaxid worms
	Naididae	Clitellate oligochaete worms

Appendix C

Benthic Macroinvertebrates Identified Using Morphological Characteristics

Taxa	Site							
	GHO03	GHO04	GHO05	GHO06	WAP02	WAP03	JOH01	GHO07
Phylum: Arthropoda								
Subphylum: Hexapoda								
| Class: Insecta								
| Order: Ephemeroptera								
| Family: Ameletidae								
Ameletus	0	33	164	61	0	21	118	0
| Family: Baetidae	162	83	0	0	0	21	0	25
Acentrella	0	133	0	0	0	0	0	825
Baetis	615	683	9	0	44	79	91	212
Baetis fuscatus gr.	0	0	0	0	0	7	0	0
Baetis rhodani group	562	17	0	0	89	50	9	12
| Family: Ephemerellidae	8	17	0	22	89	100	100	38
Drunella	8	0	0	0	0	0	0	0
Drunella coloradensis	0	0	18	0	0	0	0	0
Drunella doddsii	115	33	391	0	78	164	0	25
Drunella spinifera	0	17	0	0	0	0	9	0
Ephemerella excrucians complex	8	17	0	6	0	0	0	0
| Family: Heptageniidae	1185	1600	1118	489	2056	1521	155	638
Cinygmula	0	17	0	17	0	21	0	0
Epeorus	0	0	0	6	22	50	0	0
Rhithrogena	62	50	127	0	311	236	0	75
| Family: Leptophlebiidae	0	17	9	0	0	7	155	0
| Order: Plecoptera	0	0	0	11	0	29	9	38
| Family: Capniidae	0	0	200	206	44	7	27	12
| Family: Chloroperlidae	77	133	0	0	89	36	45	388

Taxa	Site							
	GHO03	GHO04	GHO05	GHO06	WAP02	WAP03	JOH01	GHO07
Haploperla	0	0	9	0	0	0	0	0
Neaviperla	8	0	9	0	0	0	0	0
Suwallia	0	0	18	0	0	0	0	0
Sweltsa	15	117	18	0	89	79	18	125
| Family: Leuctridae	0	0	0	0	0	14	0	0
| Family: Nemouridae	15	33	0	0	0	0	0	0
Zapada	0	33	9	6	56	50	227	25
Zapada cinctipes	8	0	0	0	44	14	64	75
Zapada columbiana	0	0	27	0	33	14	0	0
| Family: Perlidae	23	17	0	0	44	0	200	38
Doroneuria	0	0	0	0	22	0	0	12
Hesperoperla	15	50	0	0	0	7	18	150
| Family: Perlodidae	0	0	0	0	11	0	0	0
Isogenoides	0	0	0	0	11	57	0	0
| Family: Pteronarcyidae								
Pteronarcella	0	0	0	0	0	7	0	0
| Family: Taeniopterygidae	8	183	618	0	167	521	0	12
| Order: Trichoptera	0	0	0	0	67	0	109	0
| Family: Brachycentridae	0	0	0	0	0	14	0	12
Brachycentrus americanus	0	0	0	0	0	7	9	12
Micrasema	0	0	0	0	0	0	9	0
| Family: Glossosomatidae	208	467	73	0	1	0	0	12
Glossosoma	0	517	145	0	0	0	0	12
| Family: Hydropsychidae	31	33	45	0	33	29	0	262
Arctopsyche	0	17	0	0	167	43	18	12
Hydropsyche	0	0	0	0	0	0	0	38
Parapsyche	8	0	0	0	0	0	0	0
| Family: Lepidostomatidae								
Lepidostoma	0	67	0	0	0	0	0	0

Taxa	Site							
	GHO03	GHO04	GHO05	GHO06	WAP02	WAP03	JOH01	GHO07
| Family: Limnephilidae	0	0	9	0	0	7	0	0
| Family: Rhyacophilidae								
Rhyacophila	0	17	0	0	0	14	0	0
Rhyacophila betteni group	0	0	0	0	0	7	0	0
Rhyacophila brunnea/vemna group	0	0	0	0	0	0	18	0
Rhyacophila narvae	8	0	0	0	0	0	0	0
| Order: Coleoptera								
| Family: Elmidae	0	0	0	0	0	0	264	12
Heterlimnius	0	67	0	0	11	0	518	0
| Order: Diptera								
| Family: Athericidae								
Atherix	0	0	0	0	0	0	0	25
| Family: Ceratopogonidae								
Culicoides	0	0	0	11	0	0	0	0
| Family: Chironomidae	46	117	73	222	33	7	45	100
| Subfamily: Chironominae								
| Tribe: Tanytarsini	0	0	18	0	0	0	0	0
Constempellina sp. C	0	0	0	0	0	0	36	0
Micropsectra	38	333	0	0	44	21	27	38
Stempellinella	46	100	0	0	0	0	0	0
| Subfamily: Diamesinae								
| Tribe: Diamesini								
Pagastia	0	33	9	0	0	0	100	38
Potthastia gaedii group	0	0	0	0	0	0	0	38
Pseudodiamesa	0	0	9	0	0	0	0	0
| Subfamily: Orthocladiinae								
Corynoneura	0	0	9	22	0	7	0	0
Eukiefferiella	15	50	18	0	0	21	109	50

Taxa	Site							
	GHO03	GHO04	GHO05	GHO06	WAP02	WAP03	JOH01	GHO07
Heleniella	0	0	0	0	0	0	0	12
Orthocladius complex	8	333	18	22	11	0	627	112
Parametriocnemus	0	0	0	0	22	0	0	0
Parorthocladius	0	0	45	611	11	0	0	0
Rheocricotopus	0	0	73	22	0	0	9	0
Tvetenia	31	17	27	6	44	7	36	38
| Family: Empididae	0	0	9	6	0	0	27	12
Neoplasta	0	17	0	11	0	0	27	75
| Family: Simuliidae	0	0	9	0	0	0	0	0
Simulium	8	0	0	0	0	0	0	0
| Family: Tipulidae								
Dicranota	8	0	0	0	0	0	0	0
Hexatoma	0	0	9	0	0	7	0	50
Rhabdomastix	0	0	0	0	0	0	9	0
Subphylum: Chelicerata								
| Class: Arachnida								
| Order: Trombidiformes								
| Family: Lebertiidae								
Lebertia	0	50	0	28	0	0	18	62
| Family: Sperchontidae								
Sperchon	0	50	0	0	0	0	0	125
| Family: Torrenticolidae								
Testudacarus	0	0	0	0	0	0	18	12
Torrenticola	0	0	0	0	0	0	45	0
Phylum: Annelida								
Subphylum: Clitellata								
| Class: Oligochaeta								
| Order: Lumbriculida								

Taxa	Site							
	GHO03	GHO04	GHO05	GHO06	WAP02	WAP03	JOH01	GHO07
| Family: Lumbriculidae	0	0	0	6	0	0	0	0
Rhynchelmis	0	0	0	39	0	0	0	0
| Order: Tubificida								
| Family: Naididae	0	33	9	0	0	0	0	12
Nais	8	0	0	0	0	0	0	0
Totals:	3357	5601	3351	1830	3743	3303	3323	3896
Taxa present but not included:								
Phylum: Arthropoda								
Subphylum: Crustacea								
| Class: Ostracoda	8	17	0	0	11	0	9	0
Phylum: Nemata	8	0	0	0	0	0	0	0
Totals:	16	17	0	0	11	0	9	0

Appendix D

Benthic Macroinvertebrates Identified at the Family Level Using Morphological Characteristics

Taxa	Site							
	GHO03	GHO04	GHO05	GHO06	WAP02	WAP03	JOH01	GHO07
Phylum: Arthropoda								
Subphylum: Hexapoda								
| Class: Insecta								
| Order: Ephemeroptera								
| Family: Ameletidae	0	33	164	61	0	21	118	0
| Family: Baetidae	1339	916	9	0	133	157	100	1074
| Family: Ephemerellidae	139	84	409	28	167	264	109	63
| Family: Heptageniidae	1247	1667	1245	512	2389	1828	155	713
| Family: Leptophlebiidae	0	17	9	0	0	7	155	0
| Order: Plecoptera	0	0	0	11	0	29	9	38
| Family: Capniidae	0	0	200	206	44	7	27	12
| Family: Chloroperlidae	100	250	54	0	178	115	63	513
| Family: Leuctridae	0	0	0	0	0	14	0	0
| Family: Nemouridae	23	66	36	6	133	78	291	100
| Family: Perlidae	38	67	0	0	66	7	218	200
| Family: Perlodidae	0	0	0	0	22	57	0	0
| Family: Pteronarcyidae	0	0	0	0	0	7	0	0
| Family: Taeniopterygidae	8	183	618	0	167	521	0	12
| Order: Trichoptera	0	0	0	0	67	0	109	0
| Family: Brachycentridae	0	0	0	0	0	21	18	24
| Family: Glossosomatidae	208	984	218	0	1	0	0	24
| Family: Hydropsychidae	39	50	45	0	200	72	18	312
| Family: Lepidostomatidae	0	67	0	0	0	0	0	0
| Family: Limnephilidae	0	0	9	0	0	7	0	0
| Family: Rhyacophilidae	8	17	0	0	0	21	18	0

Taxa	Site							
	GHO03	GHO04	GHO05	GHO06	WAP02	WAP03	JOH01	GHO07
| Order: Coleoptera								
| Family: Elmidae	0	67	0	0	11	0	782	12
| Order: Diptera								
| Family: Athericidae	0	0	0	0	0	0	0	25
| Family: Ceratopogonidae	0	0	0	11	0	0	0	0
| Family: Chironomidae	184	983	299	905	165	63	989	426
| Family: Empididae	0	17	9	17	0	0	54	87
| Family: Simuliidae	8	0	9	0	0	0	0	0
| Family: Tipulidae	8	0	9	0	0	7	9	50
Subphylum: Chelicerata								
| Class: Arachnida								
| Order: Trombidiformes								
| Family: Lebertiidae	0	50	0	28	0	0	18	62
| Family: Sperchontidae	0	50	0	0	0	0	0	125
| Family: Torrenticolidae	0	0	0	0	0	0	63	12
Phylum: Annelida								
Subphylum: Clitellata								
| Class: Oligochaeta								
| Order: Lumbriculida								
| Family: Lumbriculidae	0	0	0	45	0	0	0	0
| Order: Tubificida								
| Family: Naididae	8	33	9	0	0	0	0	12
Totals:	3357	5601	3351	1830	3743	3303	3323	3896

Taxa	Site							
	GHO03	GHO04	GHO05	GHO06	WAP02	WAP03	JOH01	GHO07
Taxa present but not included:								
Phylum: Arthropoda								
Subphylum: Crustacea								
| Class: Ostracoda	8	17	0	0	11	0	9	0
Phylum: Nemata	8	0	0	0	0	0	0	0
Totals:	16	17	0	0	11	0	9	0

Appendix E
Comparison of Benthic Macroinvertebrates Identified at Upper Ghost River Sites Sampled by fRI Research in 2020 and GWAS in 2021

Taxa	GR-20-002*	GHO05	GR-20-001*	GHO06
	Oct. 2020	Sept. 2021	Oct. 2020	Sept. 2021
Phylum: Arthropoda				
Subphylum: Hexapoda				
| Class: Insecta				
| Order: Ephemeroptera				
| Family: Ameletidae				
Ameletus	28	164	14	61
| Family: Baetidae				
Baetis	36	9	168	0
| Family: Ephemerellidae	24	0	59	22
Drunella coloradensis	0	18	0	0
Drunella doddsii	24	391	0	0
Ephemerella excrucians complex	0	0	0	6
| Family: Heptageniidae	140	1118	232	489
Cinygmula	0	0	36	17
Epeorus	0	0	59	6
Rhithrogena	96	127	9	0
| Family: Leptophlebiidae	0	9	0	0
| Order: Plecoptera	0	0	5	11
| Family: Capniidae	0	200	77	206
Utacapnia	8	0	0	0
| Family: Chloroperlidae	12	0	0	0
Haploperla	20	9	0	0
Neaviperla	0	9	0	0
Suwallia	0	18	0	0

Taxa	GR-20-002*	GHO05	GR-20-001*	GHO06
	Oct. 2020	Sept. 2021	Oct. 2020	Sept. 2021
Sweltsa	0	18	0	0
| Family: Leuctridae	0	0	5	0
Paraleuctra	0	0	9	0
| Family: Nemouridae	16	0	127	0
Zapada	24	9	145	6
Zapada columbiana	0	27	200	0
Zapada oregonensis group	4	0	159	0
| Family: Perlodidae				
Megarcys	0	0	5	0
| Family: Taeniopterygidae	116	618	241	0
Taenionema	520	0	250	0
| Order: Trichoptera				
| Family: Glossosomatidae	0	73	0	0
Glossosoma	24	145	0	0
| Family: Hydropsychidae	0	45	0	0
Cheumatopsyche	0	0	5	0
Parapsyche	4	0	0	0
Parapsyche elsis	0	0	5	0
| Family: Limnephilidae	0	9	0	0
| Order: Diptera				
| Family: Ceratopogonidae				
Culicoides	0	0	0	11
| Family: Chironomidae	0	73	0	222
| Subfamily: Chironominae				
| Tribe: Tanytarsini	0	18	0	0
| Subfamily: Diamesinae				
| Tribe: Diamesini				
Diamesa	8	0	14	0

Taxa	GR-20-002*	GHO05	GR-20-001*	GHO06
	Oct. 2020	Sept. 2021	Oct. 2020	Sept. 2021
Pagastia	0	9	0	0
Pseudodiamesa	0	9	0	0
| Subfamily: Orthocladiinae	4	0	0	0
Corynoneura	0	9	0	22
Eukiefferiella	0	18	0	0
Hydrobaenus	4	0	0	0
Orthocladius complex	8	18	0	22
Parorthocladius	44	45	5	611
Rheocricotopus	0	73	0	22
Thienemanniella	8	0	0	0
Tvetenia	20	27	0	6
| Family: Empididae	0	9	0	6
| Subfamily: Clinocerinae Unknown Genus A	0	0	5	0
Neoplasta	0	0	0	11
| Family: Simuliidae	0	9	0	0
| Family: Muscidae				
Limnophora	0	0	9	0
| Family: Oreoleptidae				
Oreoleptis	16	0	0	0
| Family: Tipulidae				
Hexatoma	4	9	0	0
Subphylum: Chelicerata				
| Class: Arachnida				
| Order: Trombidiformes	0	0	5	0
| Family: Lebertiidae				
Lebertia	4	0	18	28
| Order: Sarcoptiformes				
| Order: Oribatida	4	0	0	0

Taxa	GR-20-002*	GHO05	GR-20-001*	GHO06
	Oct. 2020	Sept. 2021	Oct. 2020	Sept. 2021
Phylum: Annelida				
Subphylum: Clitellata				
| Class: Oligochaeta				
| Order: Lumbriculida				
| Family: Lumbriculidae	0	0	0	6
Rhynchelmis	36	0	0	39
| Order: Tubificida				
| Family: Naididae	0	9	0	0
Totals:	1256	3351	1866	1830
<u>Taxa present but not included</u>:				
Phylum: Arthropoda				
Subphylum: Crustacea				
| Class: Ostracoda	4	0	0	0
Phylum: Platyhelminthes				
| Class: Turbellaria	4	0	0	0
Totals:	8	0	0	0

* Source: fRI Research; morphologic identification by Cordillera Consulting

Appendix E
Metric Indices of the Benthic Macroinvertebrates
(Genus/Species Level)

Metric	Site							
	GHO03	GHO04	GHO05	GHO06	WAP02	WAP03	JOH01	GHO07
Richness Measures								
Species Richness	26	33	31	18	27	32	33	36
EPT Richness	18	22	18	7	21	27	19	20
Ephemeroptera Richness	7	10	7	5	6	9	7	7
Plecoptera Richness	7	7	8	2	11	11	7	8
Trichoptera Richness	4	5	3		4	7	5	5
Chironomidae Richness	5	6	9	5	5	4	7	7
Oligochaeta Richness	1	1	1	2				1
Non-Chiro. Non-Olig. Richness								
Abundance Measures								
Corrected Abundance	3357	5601	3351	1830	3743	3303	3314	3896
EPT Abundance	3149	4401	3016	824	3567	3233	1408	3085
Dominance Measures								
1st Dominant Taxon	Heptageniidae	*Rhithrogena*	Heptageniidae	*Parorthocladius*	*Rhithrogena*	*Rhithrogena*	*Heterlimnius*	*Acentrella*
1st Dominant Abundance	1185	1244	1118	810	2232	1405	773	845
2nd Dominant Taxon	*Baetis*	*Glossosoma*	Taeniopterygidae	*Cinygmula*	Taeniopterygidae	Taeniopterygidae	*Orthocladius* complex	Heptageniidae
2nd Dominant Abundance	700	984	618	378	167	540	657	638
3rd Dominant Taxon	*Baetis rhodani* group	*Baetis*	*Drunella doddsii*	Capniidae	*Arctopsyche*	*Epeorus*	*Zapada*	Chloroperlidae
3rd Dominant Abundance	639	751	391	217	167	298	230	406
% 1 Dominant Taxon	35.30%	22.21%	33.36%	44.24%	59.63%	42.54%	23.33%	21.68%
% 2 Dominant Taxon	20.84%	17.57%	18.44%	20.68%	4.46%	16.34%	19.82%	16.38%
% 3 Dominant Taxon	19.05%	13.41%	11.67%	11.84%	4.46%	9.01%	6.95%	10.41%
Percent Dominance	75.19%	53.19%	63.47%	76.76%	68.55%	67.90%	50.10%	48.47%

Metric	Site							
	GHO03	GHO04	GHO05	GHO06	WAP02	WAP03	JOH01	GHO07
Community Composition								
% Ephemeroptera	81.17%	48.51%	54.79%	32.84%	71.86%	68.94%	19.22%	47.48%
% Plecoptera	5.03%	10.11%	27.10%	12.19%	16.30%	25.28%	18.35%	22.46%
% Trichoptera	7.60%	19.96%	8.12%		7.14%	3.66%	4.92%	9.24%
% EPT	93.80%	78.58%	90.00%	45.03%	95.30%	97.88%	42.49%	79.18%
% Diptera	5.96%	17.85%	9.73%	50.98%	4.41%	2.12%	31.74%	15.09%
% Oligochaeta	0.24%	0.59%	0.27%	2.46%				0.31%
% Baetidae	39.89%	16.35%	0.27%		3.55%	4.75%	3.02%	27.57%
% Chironomidae	5.48%	17.55%	8.92%	49.45%	4.41%	1.91%	29.84%	10.93%
% Odonata								
Functional Group Composition								
% Predators	3.79%	8.74%	2.42%	3.06%	11.57%	7.76%	14.31%	30.32%
% Shredder-Herbivores	0.92%	5.64%	25.48%	12.19%	9.19%	19.45%	9.74%	3.33%
% Collector-Gatherers	47.32%	34.66%	22.97%	55.18%	12.72%	15.00%	63.71%	39.06%
% Scrapers	43.34%	47.33%	43.66%	27.98%	63.84%	55.34%	4.68%	18.92%
% Macrophyte-Herbivore							0.27%	
% Collector-Filterer	2.99%	2.62%	1.61%		0.88%	1.30%		6.39%
% Omnivore	0.60%	1.01%	3.59%	1.59%		1.15%	4.00%	1.99%
% Parasite								
% Piercer-Herbivore								
% Gatherer								
% Unclassified	1.04%		0.27%		1.79%		3.29%	
Functional Group Richness								
Predators Richness	6	10	6	4	7	8	13	12
Shredder-Herbivores Richness	3	4	4	2	5	6	3	4
Collector-Gatherers Richness	9	13	13	9	10	10	11	13
Scrapers Richness	3	3	3	2	3	3	1	3
MH Richness							1	
CF Richness	3	2	2		1	2		2

Metric	Site							
	GHO03	GHO04	GHO05	GHO06	WAP02	WAP03	JOH01	GHO07
OM Richness	1	1	2	1		3	3	2
PA Richness								
Piercer-Herbivore Richness								
Gatherer Richness								
Unclassified	1		1		1		1	
Voltinism Composition								
% Univoltine	4.36%	2.52%	16.83%	3.66%	3.26%	6.04%	6.34%	9.05%
% Semivoltine	1.94%	2.09%	1.34%		3.26%	2.92%	0.55%	3.66%
% Multivoltine	21.08%	13.41%	0.27%	0.60%	1.18%		2.75%	5.57%
Voltinism Richness								
Univoltine	4	3	3	2	2	3	3	4
Semivoltine	1	1	2		2	2	1	2
Multivoltine	2	1	1	1	1		1	1
Diversity/Evenness Measures								
Shannon-Weiner H' (log 10)	0.87	1.13	0.99	0.78	0.81	0.93	1.17	1.21
Shannon-Weiner H' (log 2)	2.88	3.74	3.28	2.60	2.69	3.10	3.88	4.03
Shannon-Weiner H' (log e)	2.00	2.59	2.27	1.80	1.87	2.15	2.69	2.79
Simpson's Index (D)	0.21	0.12	0.17	0.26	0.37	0.22	0.11	0.10
Simpson's Index of Diversity (1 - D)	0.79	0.88	0.83	0.74	0.63	0.78	0.89	0.90
Simpson's Reciprocal Index (1/D)	4.74	8.55	5.81	3.84	2.73	4.46	8.76	9.95
Biotic Indices								
Hilsenhoff Biotic Index	4.04	2.81	2.54	4.36	0.82	1.10	3.57	3.60

Appendix F
Combined Presence/Absence Results of STREAM eDNA Analysis and Morphological Identification

Note: The lowest taxonomic level detected by each method is indicated. Terrestrial species are excluded. Suffix “idae” = family level, “inae” = subfamily level, “ini” = tribe level

Taxa	Site							
	GHO03	GHO04	GHO05	GHO06	GHO07	WAP02	WAP03	JOH01
INSECTS								
Order: Coleoptera								
Dytiscidae								
Boreonectes griseostriatus		eDNA						
Liodessus affinus		eDNA						
L. obscurellus					eDNA			
Elmidae					Morph			Morph
Heterlimnius		Morph				Morph		Morph
Order: Diptera								
Athericidae								
Atherix					Morph			
Ceratopogonidae								
Culicoides				Morph				
Chironomidae	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph
Chironominae								
Chironomini								
Polypedilum albicorne								eDNA
Tanytarsini			Morph					
Constempellina								Morph
Micropsectra	Morph	Morph			Morph	Morph	Morph	Morph
M. logani		eDNA			eDNA	eDNA	eDNA	
M. subletteorum		eDNA						
Stempellinella	Morph	Morph						
Diamesinae								
Diamesa bertrami				eDNA				
Pagastia		Morph	Morph		Morph			Morph
P. orthogonia		eDNA						eDNA
Potthastia gaedii group					Morph			
P. gaedii					eDNA			
Pseudodiamesa			Morph					
Orthocladiinae								
Corynoneura			Morph	Morph			Morph	
Cricotopus sylvestris		eDNA						
Eukiefferiella	Morph	Morph	Morph		Morph		Morph	Morph
E. claripennis	eDNA	eDNA		eDNA	eDNA			
Heleniella					Morph			

Taxa	Site							
	GHO03	GHO04	GHO05	GHO06	GHO07	WAP02	WAP03	JOH01
Orthocladius complex	Morph	Morph	Morph	Morph	Morph	Morph		Morph
O. glabripennis		eDNA						eDNA
O. oblidens		eDNA						
Parametriocnemus						Morph		
P. boreoalpinus			eDNA					
Parorthocladius			Morph	Morph		Morph		
Rheocricotopus			Morph	Morph				Morph
Tvetenia	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph
T. paucunca		eDNA			eDNA			
Tanypodinae								
Conchapelopia pallens		eDNA						
C. telema					eDNA			
Empididae			Morph	Morph	Morph			Morph
Metachela collusor		eDNA			eDNA			
Neoplasta		Morph		Morph	Morph			Morph
N. megorchis	eDNA							
Simuliidae			Morph					
Helodon alpestris				eDNA				
Simulium	Morph							
S. arcticum	eDNA	eDNA				eDNA		
S. defoliarti	eDNA	eDNA			eDNA	eDNA		eDNA
S. negativum					eDNA			
S. tuberosum		eDNA						eDNA
Tipulidae								
Dicranota	Morph							
Hexatoma			Morph		Morph		Morph	
Rhabdomastix								Morph
Tipula besselsoides	eDNA	eDNA	eDNA	eDNA				
Order: Ephemeroptera								
Ameletidae								
Ameletus		Morph	Morph	Morph			Morph	Morph
A. bellulus		eDNA						eDNA
A. celer	eDNA		eDNA	eDNA		eDNA	eDNA	eDNA
A. subnotatus		eDNA						
Baetidae	Morph	Morph			Morph		Morph	
Acentrella		Morph			Morph			
A. turbida		eDNA			eDNA	eDNA	eDNA	
Baetis	Morph	Morph	Morph		Morph	Morph	Morph	Morph
B. bicaudatus	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA
B. fuscatus group							Morph	
B. phoebus		eDNA				eDNA	eDNA	
B. rhodani group	Morph	Morph			Morph	Morph	Morph	Morph
B. tricaudatus	eDNA	eDNA		eDNA	eDNA	eDNA	eDNA	eDNA
Diphetor hageni		eDNA						eDNA

Taxa	Site							
	GHO03	GHO04	GHO05	GHO06	GHO07	WAP02	WAP03	JOH01
Caenidae								
Caenis diminuta			eDNA				eDNA	
Ephemerellidae	Morph	Morph		Morph	Morph	Morph	Morph	Morph
Drunella	Morph							
D. coloradensis	eDNA	eDNA	Both	eDNA		eDNA	eDNA	eDNA
D. doddsii	Both	Both	Both	eDNA	Both	Both	Both	eDNA
D. flavilinea					eDNA			
D. grandis	eDNA	eDNA			eDNA			eDNA
D. spinifera		Morph						Morph
Ephemerella excrucians complex	Morph	Morph		Morph				
E. tibialis	eDNA	eDNA	eDNA		eDNA	eDNA		eDNA
Heptageniidae	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph
Cinygmula	eDNA	Both	eDNA	Both	eDNA	eDNA	Both	eDNA
C. subaequalis		eDNA						
Epeorus				Morph		Morph	Morph	
E. albertae					eDNA			
E. deceptivus	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA
E. grandis				eDNA				
E. longimanus	eDNA	eDNA			eDNA	eDNA		
Heptagenia pulla								eDNA
Rhithrogena	Morph	Morph	Morph		Morph	Morph	Morph	
R. impersonata	eDNA	eDNA			eDNA	eDNA		
R. robusta	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	
Leptophlebiidae		Morph	Morph				Morph	Morph
Paraleptophlebia heteronea	eDNA	eDNA			eDNA	eDNA		eDNA
P. memorialis		eDNA						
Siphlonuridae								
Siphlonurus occidentalis								eDNA
Order: Hemiptera								
Corixidae								
Callicorixa audeni		eDNA						
Sigara decoratella		eDNA						
Order: Odonata								
Coenagrionidae								
Enallagma annexum		eDNA						
Ischnura kellicotti		eDNA						
Gomphidae								
Ophiogomphus arizonicus					eDNA			
Order: Plecoptera				Morph	Morph		Morph	Morph
Capniidae			Morph	Morph	Morph	Morph	Morph	Morph
Capnia confusa		eDNA			eDNA	eDNA		
C. gracilaria						eDNA		eDNA
C. petila				eDNA				eDNA
Eucapnopsis brevicauda	eDNA	eDNA			eDNA	eDNA	eDNA	
Utacapnia columbiana					eDNA			

Taxa	Site							
	GHO03	GHO04	GHO05	GHO06	GHO07	WAP02	WAP03	JOH01
Chloroperlidae	Morph	Morph			Morph	Morph	Morph	Morph
Alloperla serrata		eDNA				eDNA	eDNA	eDNA
A. severa		eDNA			eDNA			
Haploperla			Morph					
Neaviperla	Morph		Morph					
Plumiperla diversa	eDNA	eDNA	eDNA	eDNA		eDNA	eDNA	
Suwallia			Morph					
S. teleckojensis	eDNA		eDNA			eDNA		
Sweltsa	Morph	Morph	Morph		Morph	Morph	Morph	Morph
S. borealis	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA
S. coloradensis	eDNA	eDNA			eDNA	eDNA	eDNA	eDNA
Triznaka signata		eDNA						
Leuctridae							Morph	
Paraleuctra occidentalis	eDNA	eDNA			eDNA	eDNA		eDNA
Nemouridae	Morph	Morph						
Podmosta delicatula								eDNA
Prostoia besametsa				eDNA				
Visoka cataractae			eDNA					eDNA
Zapada		Morph	Morph	Morph	Morph	Morph	Morph	Morph
Z. cinctipes	Both	eDNA	eDNA	eDNA	Both	Both	Both	Both
Z. columbiana	eDNA		Both	eDNA		Both	Both	eDNA
Z. frigida								eDNA
Z. haysi	eDNA	eDNA	eDNA	eDNA		eDNA		eDNA
Z. oregonensis		eDNA						eDNA
Perlidae	Morph	Morph			Morph	Morph		Morph
Doroneuria					Morph	Morph		
D. theodora	eDNA	eDNA	eDNA		eDNA	eDNA		eDNA
Hesperoperla	Morph	Morph			Morph		Morph	Morph
H. pacifica	eDNA	eDNA			eDNA	eDNA	eDNA	eDNA
Perlodidae						Morph		
Isogenoides						Morph	Morph	
I. frontalis		eDNA			eDNA	eDNA	eDNA	
Isoperla fulva		eDNA			eDNA			
I. petersoni	eDNA		eDNA			eDNA		
I. sobria				eDNA			eDNA	
Kogotus modestus		eDNA				eDNA		
Megarcys signata		eDNA	eDNA			eDNA	eDNA	
M. subtruncata			eDNA	eDNA				
M. watertoni			eDNA	eDNA				
Setvena bradleyi				eDNA				
Pteronarcyidae								
Pteronarcella							Morph	
P. badia		eDNA			eDNA	eDNA		
Taeniopterygidae	Morph	Morph	Morph		Morph	Morph	Morph	
Doddsia occidentalis	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA

Taxa	Site							
	GHO03	GHO04	GHO05	GHO06	GHO07	WAP02	WAP03	JOH01
Taenionema pacificum		eDNA			eDNA	eDNA		
Order: Trichoptera						Morph		Morph
Brachycentridae					Morph		Morph	
Brachycentrus americanus		eDNA			Morph		Morph	Both
Micrasema								Morph
Glossosomatidae	Morph	Morph	Morph		Morph	Morph		
Glossosoma		Morph	Morph		Morph			
G. pyroxum	eDNA	eDNA						
G. verdonum			eDNA					
Hydropsychidae	Morph	Morph	Morph		Morph	Morph	Morph	
Arctopsyche		Morph			Morph	Morph	Morph	Morph
A. grandis	eDNA	eDNA			eDNA	eDNA	eDNA	eDNA
A. inermis		eDNA			eDNA		eDNA	
A. ladogensis								eDNA
Ceratopsyche cockerelli		eDNA		eDNA	eDNA	eDNA	eDNA	eDNA
C. oslari		eDNA			eDNA			
C. slossonae		eDNA			eDNA	eDNA		
Hydropsyche					Morph			
H. bronta								eDNA
Parapsyche	Morph							
P. elsis	eDNA		eDNA	eDNA		eDNA		
Lepidostomatidae								
Lepidostoma		Morph						
L. cascadense	eDNA					eDNA		
L. pluviale		eDNA						
L. roafi		eDNA						
Limnephilidae			Morph				Morph	
Ecclisomyia conspersa			eDNA					
E. maculosa								eDNA
Rhyacophilidae								
Rhyacophila		Morph					Morph	
R. angelita						eDNA		
R. betteni group							Morph	
R. brunnea								eDNA
R. brunnea/vemna group								Morph
R. narvae	Morph							
R. vao		eDNA						
ARACHNIDS								
Order: Oribatida								
Steganacaridae								
Atropacarus striculus								eDNA
Order: Trombidiformes								
Lebertiidae								
Lebertia		Morph		Morph	Morph			Morph

Taxa	Site							
	GHO03	GHO04	GHO05	GHO06	GHO07	WAP02	WAP03	JOH01
Sperchontidae								
Sperchon		Morph			Morph			
Torrenticolidae					Morph			Both
Testudacarus					Morph			Morph
T. minimus								eDNA
Torrenticola								Morph
HYDROZOANS								
Order: Anthoathecata								
Hydridae								
Hydra vulgaris		eDNA						
MALACOSTRACANS								
Order: Amphipoda								
Hyalellidae								
Hyalella azteca								eDNA
OSTRACODS	Morph	Morph				Morph		Morph
Order: Podocopida								
Candonidae								
Candona candida					eDNA			eDNA
Cyprididae								
Cypridopsis vidua								eDNA
BIVALVE MOLLUSCS								
Order: Veneroida								
Montacutidae								
Kurtiella bidentata		eDNA			eDNA			
OLIGOCHAETE WORMS								
Order: Haplotaxida								
Naididae	Morph		Morph		Morph			
Nais	Morph							
N. bretscheri		eDNA						
Order: Lumbriculida								
Lumbriculidae				Morph				
Rhynchelmis				Morph				