Ghost Watershed

Water Monitoring Program

CABiN/STREAM Project

2020

BIOTA CONSULTANTS

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Biota Consultants
2920 Cosgrove Cres.
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Cover photo: Cal Hill – Upper Waiparous Creek

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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* which suggested sampling 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 participated in the first year of the STREAM (Sequencing the Rivers for Environmental Assessment and Monitoring) three-year pilot project. It uses CABiN methods to assess physical and chemical parameters. In addition, water samples are collected for environmental DNA (eDNA) testing to determine presence or absence of aquatic fauna. Four individuals from GWAS participated in a field-based course that covered the CABiN sampling techniques plus modifications for the STREAM component, which were primarily related to minimizing DNA contamination. During the field course, one site was sampled in the Ghost Watershed along Waiparous Creek (WAP01).

GWAS then developed a multi-year water monitoring plan that incorporated the STREAM pilot project. In 2020, the plan focussed mainly on sites above and below creek tributaries (Johnson Creek and Meadow Creek) and other possible point source sites (cadet camp) which could affect water quality as a result of land use activities. Single sites also were sampled on Waiparous Creek above its confluence with the Ghost River, and near its headwaters where there is less human influence. An additional paired site was later chosen on the Ghost River, above and below the confluence with Lesueur Creek, following the onset of a forest fire in the upper Ghost River drainage.

Field sampling occurred between September 1st and October 5th 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 and through subsequent lab analyses.

The stream channel was variable at the eight sites sampled on Waiparous Creek. Most notable among the physical attributes was the size of the substrate in the creek bed above and below confluences. Cobble-sized and boulder-sized substrate tended to be more abundant

downstream than upstream whereas pebble-sized substrate was more abundant upstream. Accordingly, higher maximum and average stream flows were recorded below the confluences, which likely would result in smaller sized substrate being transported downstream.

Average embeddedness of the dominant substrate at the Waiparous Creek sites was consistently 25% except for the most downstream site (WAP08), which was 50% embedded, and the most upstream site (WAP09), which was 0% embedded. This also suggests finer particles are transported downstream. Embeddedness was higher on the Ghost River downstream of Lesueur Creek, suggesting sediment input from the creek.

At the time of sampling, water quality was within the parameters acceptable for benthic macroinvetebrates and fish. The chemical and physical attributes were well below exceedance levels. Total suspended solids and turbidity were very low. These likely would be higher earlier in the year after rainfall events. Dissolved oxygen values were within acceptable limits. There was daily variation as the temperature rose throughout the day, resulting in a slight decrease in dissolved oxygen by the afternoon.

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 organic pollution was unlikely, and water quality was excellent. Differences above and below point sources (tributaries or the cadet camp) suggest a slight decline in quality below the point sources.

The EPT ratio indicates high water quality, with EPT species at much higher abundance than the pollution-tolerant chironomid family. The percent of the more tolerant Hydropsychidae within the Trichoptera and Baetidae within the Ephemeroptera was generally low. Although Waiparous Creek downstream of Johnson Creek had a higher percentage of tolerant species than upstream, the high EPT ratio suggests no concerns.

The results of the 2020 field sampling provide a baseline for comparison in future years. With more data, trends may become apparent. No major differences were found between downstream and upstream paired sites, suggesting the point sources were not having a marked effect on water quality at the time of sampling.

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1.0 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*, 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 learned of the STREAM (Sequencing the Rivers for Environmental Assessment and Monitoring) three-year pilot project. STREAM 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 environmental DNA (eDNA) testing to determine presence or absence of aquatic fauna.

Living Lakes Canada provides training to groups and organizations using the CABiN wadeable stream protocol (Environment Canada 2012). Participants must become certified to take part in the STREAM project. Initially, they must enrol in the CABiN program through the University of New Brunswick and take two modules entitled *Introduction to CABiN* and *CABiN Field Sampling Methods*. Following this, they may take the field course. On July 16th and 17th, 2019, four individuals from GWAS (three Directors of the Board and the Executive Director) participated in the field-based course to become certified Field Technicians. One of the directors undertook further modules to become a certified Project Manager. As part of the field course, one site on Waiparous Creek was sampled on July 18 (WAP01) (Figure 1).

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. The purpose, objectives and scope of the plan are described, along with outcomes and deliverables, program phases, governance and management, budget, time lines, potential risks, and risk mitigation. The plan 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.

2.0 Methods

2.1 Planning Stage

Sampling in 2020 focussed 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. Preliminary sample site locations were predetermined based on these objectives. Input was sought from stakeholders in the watershed (i.e., Alberta Environment and Parks, Alberta Forestry and Agriculture) as well as from GWAS members and directors familiar with the area. Prior to sampling the sites, a ground reconnaissance was conducted to evaluate suitability.

2.2 Field Sampling

The STREAM protocol followed CABiN sampling techniques with several modifications to minimize DNA contamination and to ensure proper preservation of DNA material in the samples (STREAM 2020). The protocol for decontaminating equipment between sites, and for the collecting and storage of the benthic macroinvertebrate DNA samples, was strictly followed.

In 2020, field sampling occurred between September 1st and October 5th during mainly sunny stable weather conditions. Site name codes, date of sampling and geographic locations are presented in Table 1, and locations are mapped in Figure 1. Air and water temperatures at the time of sampling are provided. When sampling paired sites, the downstream site was sampled before the upstream site to ensure that the downstream site was not disturbed by upstream activities.

When whirling disease is present within a watershed, caution is required if equipment is used at different whirling disease decontamination risk zones (i.e., moving from a red zone to a yellow zone). The Ghost Watershed is entirely within the red zone (Government of Alberta 2020); however, this was initially misinterpreted. It was assumed that the upper Waiparous (WAP09) was within a zone of less risk, therefore prior to sampling, the equipment was decontaminated with Quat Plus according to the Alberta government protocol (Alberta Environment and Parks 2016). This proved not to have been necessary.

Site WAP09 was selected as 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.

Table 1. Location of Waiparous Creek (WAP) and Ghost River (GHO) 2020 sites, plus sampling date, time of day, and conditions.

Code/ Date	Latitude	Longitude	Elevation (m)	Comments
WAP02 Sept. 1	51° 23'40"	115°05'09"	1554	Waiparous Creek below confluence with Johnson Creek Afternoon Sunny, air temperature 22.5°C, water temperature 15.0°C
WAP03 Sept. 3	51° 23'33"	115° 05'21"	1560	Waiparous Creek above confluence with Johnson Creek Morning Sunny, air temperature 17.5°C, water temperature 12.8°C
WAP04 Sept. 9	51° 22'26"	115° 00'05"	1441	Waiparous Creek below confluence with Meadow Creek Morning Sunny, air temperature 14.5°C, water temperature 8.5°C
WAP05 Sept. 9	51° 22'34"	115° 00'09"	1446	Waiparous Creek above confluence with Meadow Creek Afternoon Sunny, air temperature 23.5°C, water temperature 12.2°C
WAP06 Sept. 10	51° 19'10"	114° 55'26"	1347	Waiparous Creek below cadet camp Morning Sunny, air temperature 16.1°C, water temperature 8.3°C
WAP07 Sept. 10	51° 19'27"	114° 55'47"	1352	Waiparous Creek above cadet camp Afternoon Sunny, air temperature 25.0°C, water temperature 12.5°C
WAP08 Sept. 17	51° 16'59"	114° 50'16"	1283	Waiparous Creek 75 m downstream of bridge in village of Waiparous, above confluence of the creek with Ghost River Morning Sunny, air temperature 12.0°C, water temperature 8.0°C
GHO01 Sept. 23	51° 15'50"	114° 57'40"	1351	Ghost River below confluence with Lesueur Creek Morning Cloud/sun, air temperature 20.0°C, water temperature 7.9°C
GHO02 Sept. 23	51° 15'46"	114° 57'52"	1351	Ghost River above confluence with Lesueur Creek Afternoon Sunny, air temperature 21.0°C, water temperature 9.7°C
WAP09 Oct. 5	51° 23'35"	115° 11'55"	1700	Upper Waiparous Creek (Ghost PLUZ map Site 129); off trail west of Waiparous Valley Road Afternoon Sunny, air temperature 19.0°C, water temperature 8.2°C

NOTE: Site WAP01 was sampled in 2019 as an additional training exercise under the supervision of LLC and WWF staff.

Code/ Date	Latitude	Longitude	Elevation (m)	Comments
WAP01 July 18, 2019	51° 24'17"	115° 02'59"	1510 m	Waiparous Creek ~500 m west of Mockingbird Camp on Waiparous Valley Road Sunny/scattered rain showers, air temperature 7.0°C, water temperature 10.6°C

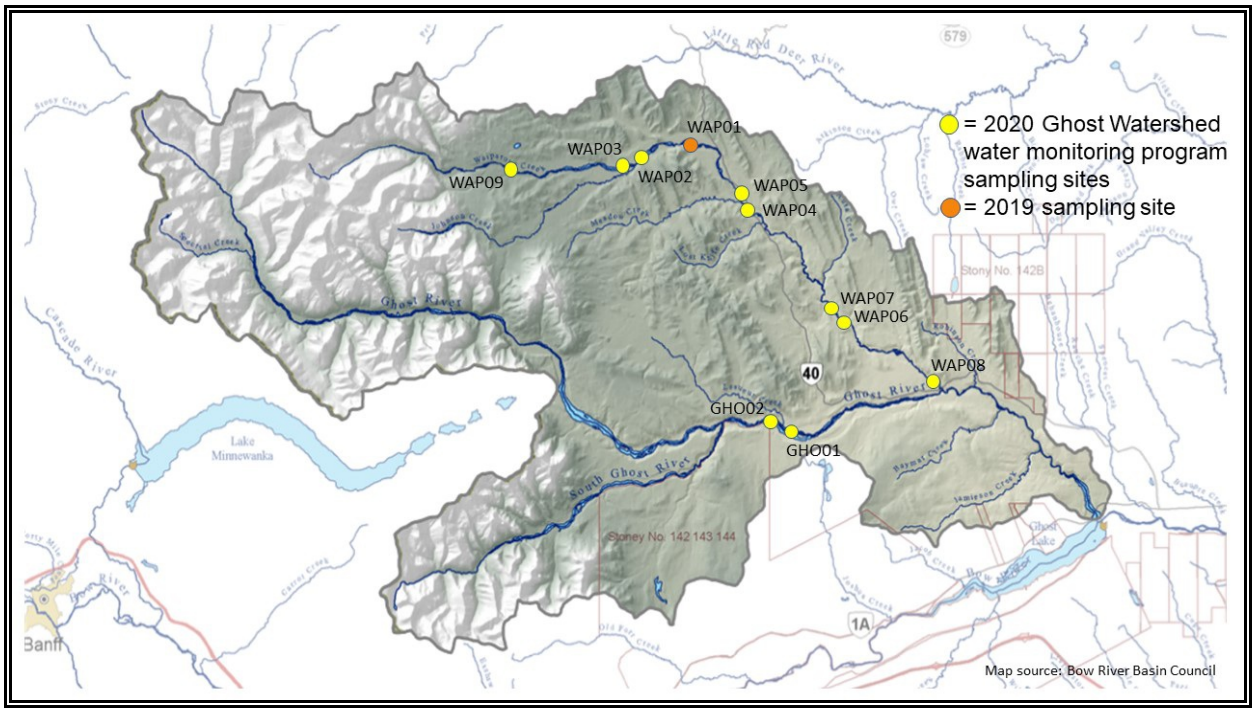

Figure 1. Sampling locations in 2020 within the Ghost River watershed, plus location of WAP01 sampled in 2019 during training.

2.2.1 Biological Sampling

Field sampling began with three separate kicknet samples at each site, starting at the downstream end of the reach. These were used for eDNA analysis. Triplicate samples were required for quality assurance/quality control (QA/QC). A fourth kicknet sample was collected upstream of these to determine the benthic macroinvertebrate community structure. Each three-minute sample was collected in a zigzag pattern across the stream from bank to bank using a specially designed triangular-shaped 400 micron mesh net. Successive kicknet samples were upstream of the previous sample in an undisturbed portion of the reach.

The kicknet samples were brought to the streamside for processing, which followed the CABiN laboratory methods (Environment Canada 2014) with modifications required by STREAM:

- eDNA samples were placed in new sterilized 500 ml plastic containers in a 95% ethanol (Histoprep) solution;
- Morphologic samples were placed in used sterilized 500 ml plastic containers in a 95% ethanol (Histoprep) solution;
- For QA/QC, a substrate sample was collected in one of ten of the morphologic samples using the bucket swirling technique;

- All samples were sealed with parafilm beneath the lid as well as with a strip of duct tape around the outside of the lid and jar;
- All the containers were labelled and placed in a cooler with ice while in the field, and later transferred to a deep freezer at -20°C.

Samples were submitted to the preselected certified laboratories.[1]

2.2.2 Physical Attributes

A detailed description of the benthic macroinvertebrate habitat was documented on waterproof CABiN field sheets (Appendix A). Specific site information included:

- Ecoregion in which the site occurred,
- Streamside vegetation,
- Surrounding land use (upstream),
- Photographs (page 1 of field sheet, upstream, downstream, across site, exposed substrate, under water substrate),
- Whether riffle, rapid, run or pool was present,
- Canopy cover,
- Air temperature.

Stream characteristics included:

- Water temperature,
- Bankfull width,
- Wetted stream width,
- Bankfull-wetted depth,
- Stream velocity (using velocity tube[2]),
- Stream slope,
- Presence of macrophytes,
- Periphyton coverage,
- Substrate characteristics,
- Degree of embeddedness.

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[1] Chemical: Bureau Veritas Laboratories, Calgary, Alberta
eDNA: Hajibabaei Lab at Centre for Biodiversity Genomics (University of Guelph)
Morphologic: Cordillera Consulting Inc., Summerland, British Columbia

[2] A 4.5 cm diameter transparent tube, similar to a velocity rod, where stagnating water depth is taken from the front (upstream side) of the tube, and the depth/flowing water depth is taken inside the tube where the water level does not fluctuate.

2.2.3 Water Chemistry

A YSI-DSS multimeter was rented from Oak Environmental Inc., who calibrated it prior to field use. Water properties were either recorded on-site using the multimeter* or samples were collected in plastic containers provided by Bureau Veritas that were specific to the test**:

- pH*
- Dissolved oxygen (mg/L)*
- Specific conductance (µS/cm)*
- Turbidity (NTU)*
- Total suspended solids (TSS)**
- Nitrogen (nitrate and nitrite)**
- Phosphorous (total)**
- Major ions (alkalinity)**

2.2.4 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 ten sampling sites located on Waiparous Creek and the Ghost River are presented in Table 2. This information was collected in the fall, under conditions of low stream flow and stable weather. The stream channels in these major water courses are known to change as a result of major high water events, such as the floods that occurred in 2005 and 2013 (ALCES and GWAS 2018).

Table 2. Physical characteristics of the sampling sites. (Note: the four paired sites have been denoted for easy comparison.)

Attributes	**Site and Sampling Date**									
	Sept. 1	Sept. 3	Sept. 9	Sept. 9	Sept. 10	Sept. 10	Sept. 17	Sept. 23	Sept. 23	Oct. 5
	WAP02	WAP03	WAP04	WAP05	WAP06	WAP07	WAP08	GHO01	GHO02	WAP09
Elevation (m)	1554	1560	1441	1446	1347	1352	1283	1351	1351	1700
Bankfull width (m)	17.0	15.0	30.0	35.7	17.2	18.0	18.4	18.8	22.6	13.1
Wetted width (m)	9.6	6.9	9.75	14.0	15.7	9.2	14.7	13.4	18.1	7.53
Bankfull wetted depth (cm)	26.5	56.0	21.4	35.0	46.0	30.0	53.0	75.8	45.3	54.4
Maximum channel depth (cm)	27.0	22.0	47.0	24.0	30.5	42.5	34.5	42.3	41.2	15.6
Avg channel depth (cm)	17.7	16.4	25.1	17.4	20.1	26.4	26.1	25.9	24.3	12.2
Maximum velocity (m^3/s)	1.2528	1.1293	1.4691	1.3652	1.1719	1.9308	1.2528	2.0491	1.6867	0.8859
Avg velocity (m^3/s)	0.8760	0.8650	1.0802	1.0772	1.0030	1.1237	1.0240	1.3580	1.5251	0.5114
Slope (m/m)	0.0138	0.015	0.0036	0.0006	0.00175	0.002	0.012	0.00767	0.0057	0.00175
Substrate embeddedness (%)	25	25	25	25	25	25	50	25	0	0
Dominant substrate (cm)	6.4-12.8	3.2-6.4	6.4-12.8	3.2-6.4	3.2-6.4	1.6-3.2	6.4-12.8	3.2-6.4	1.6-3.2	3.2-6.4
2nd dominant substrate (cm)	12.8-25.6	6.4-12.8	3.2-6.4	1.6-3.2	1.6-3.2	3.2-6.4	3.2-6.4	6.4-12.8	3.2-6.4	6.4-12.8
Surrounding material (cm)	0.2-1.6	0.2-1.6	0.2-1.6	0.2-1.6	0.2-1.6	0.2-1.6	0.2-1.6	0.1-0.2	0.1-0.2	0.2-1.6
Geometric median particle size (cm)	10.3	5.9	7.0	4.6	4.1	4.0	6.9	5.1	3.2	6.6
% Sand	0	0	0	0	0	0	0	0	0	0
% Gravel	1	1	2	1	7	9	6	3	7	6
% Pebble	23	56	42	70	73	63	36	60	83	42
% Cobble	68	41	52	29	20	28	52	36	10	45
% Boulder	8	2	4	0	0	0	6	1	0	3

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.

3.1.1 Waiparous Creek

The stream channel was variable at the eight sites sampled on Waiparous Creek (Table 2). The bankfull width at high water ranged from 13.1 m at WAP09, located in upper Waiparous Creek, to 35.7 m at WAP05, located above the confluence of Meadow Creek. The wetted width at the time of sampling ranged from 6.9 m at WAP03, located below the confluence of Meadow Creek, to 15.7 m at WAP06, located below the sewage treatment facility of the Rocky Mountain Cadet Training Centre (RMCTC). The average channel depth ranged from 12.2 cm at WAP09 (the most upstream site), to 26.4 cm at WAP07, located above the RMCTC. The average stream velocity ranged from 0.5114 m^3/s at WAP09 to 1.1237 m^3/s at WAP07.

At the paired sites, an attempt was made to select reach locations with similar stream channel characteristics above and below the point source. However, due to the heterogeneous nature of the sites, stream characteristics varied (Table 2).

The size of the substrate in the creek bed was notably different between sites above and below confluences. Downstream of Johnson Creek tributary (WAP02), the geometric mean particle size was 10.3 cm, compared to 5.9 cm upstream (WAP03). This appears to be related to the difference in the cobble-sized and boulder-sized substrate. Cobble-sized substrate (6.4–25.6 cm) was 68% downstream versus 41% upstream, and boulder-sized substrate (>25.6 cm) was 8% downstream versus 2% upstream. Pebble-sized substrate (1.6–6.4 cm) was less abundant downstream (23% versus 56%). Similar differences were noted at the Meadow Creek sites (WAP04 and WAP05) (Table 2). This suggests that below the tributaries, the finer substrates are transported downstream. Accordingly, slightly higher maximum and average stream flows were recorded below the confluences. In addition, during snow melt or heavy precipitation events, the tributaries may contribute to increased water volume and stream flow below the confluences.

Substrate embeddedness refers to how deeply the dominant substrate is buried in the surrounding finer particles. The more embedded the substrate, the fewer interstitial spaces for macroinvertebrates to occupy. In areas modified by stream side activities (anthropogenic land uses), increased erosion can result in the accumulation of fine material in the interstitial spaces. Embedded substrates provide less desirable habitat for macroinvertebrates, which can reduce productivity (Environment Canada 2012).

Five categories of substrate embeddedness were used.[3] At six of the eight sites sampled, embeddedness was 25%. The most downstream site (WAP08), near the confluence with the Ghost River, had 50% embeddedness. The most upstream site on Waiparous Creek (WAP09) had 0% embeddedness. WAP09 also had the lowest average and maximum velocity flow rate (0.5114 m^3/s and 0.8859 m^3/s, respectively) and the lowest average and maximum depth (12.2 cm and 15.6 cm, respectively).

3.1.2 Ghost River

There were no initial plans to sample the Ghost River in 2020. However, due to a forest fire near the headwaters in early September, it was decided to sample a site in order to obtain baseline information. Paired sites below and above the confluence with Lesueur Creek were chosen.

The flood plain of this major river drainage is very wide and the stream channel is known to shift following major flooding events. This results in stream braiding. As with Waiparous Creek, the physical characteristics of the river made it challenging to select reaches that were similar on either side of the confluence. The bankfull and wetted width (18.8 m and 13.4 m, respectively) of the downstream site (GHO01) was narrower than that of the upstream site (GHO02) (22.6 m and 18.1 m, respectively) (Table 2). However, the maximum and average channel depth was only slightly higher at GHO01. The sites also varied in velocity. The maximum velocity was higher at GHO01, but the average velocity was lower (Table 2).

The embeddedness of the substrate at GHO01 was 25%, whereas at GHO02 it was 0% (Table 2), suggesting sediment input from Lesueur Creek. Small-sized substrate was less abundant below the confluence with Lesueur Creek. As with sites above and below confluences on Waiparous Creek, the percent of cobble-sized substrate was higher downstream (36% versus 10%).

3.2 Water Attributes and Chemical Analysis

The chemical attributes of each site (i.e., anions, nutrients and pH) along with the physical attributes (i.e., total suspended solids, turbidity, dissolved oxygen and specific conductance) are presented in Table 3.

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

Table 3. Chemical and physical attributes of water samples at each site. (Note: the four paired sites have been denoted for easy comparison.)

Tests	Site and Sampling Date									
	Sept. 1	Sept. 3	Sept. 9	Sept. 9	Sept. 10	Sept. 10	Sept. 17	Sept. 23	Sept. 23	Oct. 5
	WAP02	WAP03	WAP04	WAP05	WAP06	WAP07	WAP08	GHO01	GHO02	WAP09
pH	8.36	8.39	8.37	8.38	8.38	8.47	8.41	8.31	8.27	8.26
Total Suspended Solids (TSS) (mg/L)	1.2	2.0	<1.0	1.3	1.3	<1.0	<1.0	<1.0	<1.0	<1.0
Turbidity (field*, lab**) (NTU)	<0.10**	<0.10**	0.24*	1.00*	0.26*	0.22*	0.18*	0.20*	0.21*	0.19**
Specific Conductance (µS/cm)	316.6	320.2	325.2	319.5	345.4	340.3	355.5	317.2	316.4	322.5
Dissolved Oxygen (mg/L)	8.97	8.77	10.02	9.22	10.24	9.30	10.38	10.19	9.82	11.18
Water Temperature (°C)	12.1	12.8	8.5	12.2	8.3	12.5	8.0	7.9	9.7	8.2
Air Temperature (°C)	19.0	17.5	14.5	23.5	16.1	25.0	12.0	20.0	21.0	19.0
Anions										
Alkalinity (Total as $CaCO_3$) (mg/L)	150	140	150	150	160	160	210	150	140	120
Alkalinity (PP as $CaCO_3$) (mg/L)	<1.0	1.4	<1.0	1.1	1.1	1.8	<1.0	<1.0	<1.0	<1.0
Bicarbonate (HCO_3) (mg/L)	180	160	190	180	200	190	250	180	170	150
Carbonate (CO_3) (mg/L)	<1.0	1.7	<1.0	1.3	1.3	2.2	<1.0	<1.0	<1.0	<1.0
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)	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030	<0.0030
Dissolved Nitrogen (N) (mg/L)	0.26	0.25	0.14	0.14	0.14	0.16	0.17	0.21	0.20	0.20
Dissolved Total Kjeldahl Nitrogen (mg/L)	0.14	0.19	0.056	0.064	0.064	0.085	0.087	<0.050	<0.050	0.057
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 (N) (mg/L)	0.12	0.068	0.086	0.077	0.080	0.074	0.088	0.21	0.20	0.14

The chemical analysis suggests that the water quality at the time of sampling was within the parameters acceptable for benthic macroinvetebrates and fish (Government of Alberta 2018). The water quality exceedance criteria, including a brief narrative, are presented in Table 4.

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 anions to alkalinity and inorganic carbon.

Table 4. 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 4. 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)

3.2.1 Alkalinity, Inorganic Carbon, Hardness and pH

Alkalinity, as expressed by the total $CaCO_{3,}$ ranged from 120 mg/L at the most upstream site sampled on Waiparous Creek (WAP09) to 210 mg/L at the most downstream site (WAP08). This is well above the minimum 20 mg/L level indicated in Table 4.

Alkalinity in relation to inorganic carbon influences the "hardness" and pH of water. It is defined as the quantitative capacity of aqueous media to react with hydroxyl ions, and represents the acid neutralizing capacity of an aqueous system (CCME 2008). It is the equivalent sum of the bases in a solution that are titratable with strong acid. Alkalinity is closely related to the amount of bicarbonate, carbonate and carbonic acid present in water (CCME 2008). These inorganic carbon compounds influence the hardness of water (McNeely *et al.* 1979, as cited in CCME 2008).

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 $CaCO_3$

The presence of other constituents, such as iron, manganese and aluminum, may contribute to hardness, but are usually at insignificant concentrations in surface waters (Wetzel 1975, as cited in CCME 1999). Hardness is usually expressed as an equivalent of $CaCO_3$ and varies according to local conditions. It is used as an indication of water type, buffering capacity and productivity (Casiday and Frey 1998). In the presence of carbonate bedrock, waters are usually hard and tend to have large concentrations of bicarbonate (HCO_3) and a high pH (McNeely *et al.* 1979, as cited in CCME 2008; Borgmann 1983, as cited in CCME 1999). At pH 7 to 8, HCO_3 predominates and constitutes 60 to 90% of total inorganic carbon (Loewenthal and Marais 1976, as cited in CCME 2008). The pH of our samples ranged from 8.26 to 8.47, with HCO_3 ranging from 150 to 250 mg/L. During low discharge periods (i.e., late summer to early fall), the concentrations of total inorganic carbon in surface water are usually higher because of greater groundwater influx (McNeely *et al.* 1979, as cited in CCME 2008).

In natural waters, the $CaCO_3$ - HCO_3 system is part of the carbon cycle of the biosphere. These two anions and carbonic acid (H_2CO_3) are maintained in equilibrium (Loewenthal and Marais 1976, as cited in CCME 2008). Their relative amounts in water are related to the pH (Wetzel 1975, as cited in CCME 2008), and their interaction in water causes the equilibrium between hydrogen (H^+) and hydroxide (OH) ions to be displaced, which is indicated by the establishment of a specific pH (Loewenthal and Marais 1976, as cited in CCME 2008).

As the $CaCO_3$ concentration declines in a water body, there is less buffering capacity. Water bodies with low concentrations of calcium carbonate are more susceptible to an increase in hydrogen ion concentration from an acid input and subsequent changes in the physical and chemical properties of the system (Borgmann 1983, as cited in CCME 2008; O'Donnel *et al.* 1985, as cited in CCME 2008). There is evidence that hard water mediates the toxicity of many metals to aquatic life, because of carbonate complexation and calcium antagonism (US EPA 1973, as cited in CCME 2008). A description of how pH affects aquatic insects is presented in the CABiN field manual (Environment Canada 2012).

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). The principal factors that influence the conductivity of an aqueous solution include the nature and concentration of the solutes present, the degree to which they dissociate into ions, the amount of electrical charge on each ion, ion mobility, and the temperature of the solution (FEI 2014a). These conductive ions come from dissolved salts and inorganic materials such as alkalis, chlorides, sulfides and carbonate compounds (Miller *et al.* 1988). The more ions that are present, the higher the conductivity of water. Solutions of most inorganic acids, bases and salts are relatively good conductors. Alternatively, organic compounds that do not dissociate in aqueous solution conduct either no or very little current (McNeely *et al.* 1979, as cited in CCME 2008; Hem 1985).

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). Seasonal variations in conductivity, while affected by temperature, are also affected by the flow of water. In some watersheds, spring runoff creates the highest surface flow volume. Conductivity decreases with increased surface flow and increases with reduced surface flow and an increase in groundwater contribution, which generally has higher ion concentrations. As a result, conductivity can be lower in the spring than in the winter despite the differences in temperature (Perlman 2014, as cited in FEI 2014a). For these reasons, the CABiN protocol requires sampling in late August through September, when the weather is most stable and stream flow is most consistent between years (Environment Canada 2012).

There is no set standard for the conductivity of water (Table 4) 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).

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 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) ranged from <1.0 to 2.0 mg/L (Table 3), which is very low, and well below the exceedence criteria. The following discussion on TSS and sedimentation relates to the physical characteristics of the streambed substrate and embeddedness, as presented in Table 1.

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).

Particles that do not settle to the bottom, being either too small or too light, are called colloidal or nonsettleable solids (Cooke n.d., as cited in 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;
- Inhibit egg development (Alabaster and Lloyd 1984, as cited in CCME 1999).

Some suspended solids settle to the bottom of a water body over a period of time (Kentucky Water Watch n.d.). Heavier particles, such as sand and fine gravel, settle out more quickly than finer particles, such as silt and clay. The latter often settle out when they enter an area of low or no water flow (e.g., Ghost reservoir). Although this settling improves water clarity, the increased silt can smother benthic organisms and fish eggs (EPA 2012, as cited in FEI 2014b).

Settleable solids are also known as bedded sediments or bedload (EPA 2012, as cited in FEI 2014b). These sediments can vary from larger sand and gravel to fine silt and clay, depending on the flow rate of water. Sometimes these sediments can move downstream without rejoining the suspended solids concentration. When settleable solids are moved along the bottom of a body of water by a strong flow, it is called bedload transport (Wood 2014).

3.2.3.2 Turbidity

Turbidity is often reported in nephelometric turbidity units (NTU) and is a measure of relative water clarity. The turbidity of our samples ranged from <0.01 NTU to 1.0 NTU (Table 3), which is considered very low (Table 4).

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).

Turbidity is an expression of the optical property of water, when incident light is scattered and absorbed rather than transmitted in straight lines through the sample (Vanous *et al.* 1982, as cited in CCME 2008). This is due to the shape, size, refractive index and chemical composition of the particulates in aqueous systems, which affect the light-scattering properties (Vanous *et al.* 1982, as cited in CCME 2008). The more particles present, the more light will be scattered. 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).

Water temperature and O_2 concentrations vary daily and seasonally, depending on numerous factors:

- The species of phytoplankton present,
- Light penetration,
- Nutrient availability,
- Air temperature,
- Salinity,
- Water movement,
- Partial pressure of atmospheric oxygen in contact with the water,
- Thickness of the surface film,
- Bio-depletion rates (by aquatic organisms and with oxidation and decomposition processes) (Hart 1974, as cited in CCME 1999; Mullins 1977, as cited in CCME 1999; McNeely *et al.* 1979, as cited in CCME 1999).

The DO values in our samples were within acceptable limits, ranging from 8.77 to 11.18 mg/L (Table 3). Daily variation was noted. For three of the paired sites, sampling was done downstream in the morning and upstream in the afternoon. The air and water temperatures were higher by the afternoon and, correspondingly, the DO in the water decreased slightly.

The sensitivity of aquatic organisms to low concentrations of DO differs among species, life stages (e.g., eggs, larvae, adults) and activities (e.g., food consumption, growth, reproduction) (Alabaster and Lloyd 1984, as cited in CCME 1999). Depending on the intensity and duration of low DO concentrations, a shift in species diversity may occur, causing a change in the benthic macroinvertebrate community and population structure (Environment Canada 2012).

Coldwater fish such as trout and salmon are particularly sensitive to DO levels. If DO is less than 3 mg/L, adult salmonids will begin to die. At 6 mg/L, growth will be impaired, reducing survival rates. Levels below 9 mg/L will impair hatching of salmon and trout eggs. When DO falls below 6 mg/L, egg mortality is likely (US EPA 1986, as cited in Carter 2005).

3.3 Benthic Macroinvertebrate Taxonomy

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 (CCME 2008). Some species adapt to these changes, whereas other species cannot. This causes changes in the productivity of the aquatic environment, as well as spatial and temporal changes in species composition and abundance (CCME 2008), which can influence the community structure and population dynamics.

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 by anthropogenic (using test sites) or natural influences (using reference condition sites). 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 for the 2020 sampling period are presented in the 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. Appendix D contains the taxonomic data at the family level. Appendix E contains the metric indices for the entire taxonomic data to the genus/species level.

For the purpose of this report, the metric indices are the most relevant. There is debate within the scientific community regarding the level of accuracy required to evaluate changes in the community structure of benthic macroinvertebrates within the aquatic ecosystem due to natural variability. There are pros and cons using the metrics at the higher taxonomic level of the family versus the lowest possible level of genus and species (Appendix E) (Jones 2008). For this report, the level of sufficiency uses data at the genus/species level.

Within CABiN, the metrics are generally classified into four main groups: measurements of richness, measurements of abundance or composition, functional measurements, and biotic indices. To better understand the taxonomic data analysis provided by Cordillera Consulting Inc., a description of these groups is presented below. This information was adapted from the University of New Brunswick, Project Manager Course, Module 3 (2019).[4] All of the metric results are presented in Appendix E, and key results are summarized below.

3.3.1 Richness Measurements

The number of species per sample is a measure of richness. The more species present in a sample, the richer the sample. Species richness as a measure on its own does not take into account the number of individuals of each species present. It gives as much weight to those species represented by very few individuals as to those represented by many individuals.

Richness can also be expressed numerically based on the functional feeding group (i.e., predators, shredder-herbivores, collector-gatherers, scrapers, collector-filterers, omnivores, parasites, piercer-herbivores, gatherers or unclassified types).

[4] Barbour *et al.* (1999) provides additional information on this subject:
Table 7-1 "Definitions of best candidate benthic metrics and predicted direction of metric response to increasing perturbation."
Table 7-2 "Definitions of additional potential benthic metrics and predicted direction of metric response to increasing perturbation."
These tables are summarized by the US EPA, Water Research Centre
https://water-research.net/index.php/macroinvertebrates

Richness measurements include:

- Total number of taxa: number present at a selected taxonomic level.

- EPT taxa: number present within each group; high numbers of EPTs generally indicate "good" water quality, as they are sensitive to habitat disturbance.

- EPT individuals: the sum of all Ephemeroptera, Plecoptera and Trichoptera taxa which respond to most types of anthropogenic disturbance. A decline in abundance or richness of EPT individuals would suggest an environmental disturbance. These are compared to the Chironomidae taxa, expressed as a ratio using abundance or composition values (see Section 3.3.2. below).

- Diversity/evenness measurements: the abundance and distribution among the taxa present (i.e., Simpson's Diversity/Evenness Index and Shannon-Weiner Index); these measurements provide a summary of the distribution of the taxa. Diverse communities are indicators of "good" water quality.

  Simpson's Index (D): measures the probability that two individuals randomly selected from a sample will belong to the same species, and is essentially a calculation of the data's evenness (Cordillera Consulting 2015). With this index, zero (0) represents infinite diversity and 1 indicates no diversity. Therefore, the higher the value of D, the lower the diversity. Since this is not intuitive, D is often subtracted from 1 to give Simpson's Index of Diversity (1 - D).

  Simpson's Index of Diversity (1 - D): the transformation measurement of evenness where the value of D ranges between 0 and 1. The diversity index of 1 represents infinite diversity and uneven community structure, whereas 0 indicates no diversity.

  Simpson's Diversity Index takes into account the number of species present, as well as the relative abundance of each species. As species richness and evenness increase, diversity increases (Barcelona Field Studies Centre 2022). The formula is:

$$D = 1 - \left(\frac{\sum n(n-1)}{N(N-1)} \right)$$

  where:

  n = the total number of organisms of a particular species

  N = the total number of organisms of all species

Simpson's Reciprocal Index (1 / D): 1 is the lowest possible figure, representing a community containing only one species. The higher the value, the greater the diversity. The maximum value is the number of species (or other category being used) in the sample. For example if there are five species in the sample, then the maximum value would be five.

Shannon-Weiner Index: measures uncertainty. The degree of uncertainty (entropy or degree of surprise) of predicting that a species is present in a random sample is related to the diversity of the community. If a community has low diversity (e.g., dominated by one species), the uncertainty of prediction is low since the dominant species is highly likely to be sampled. However, if diversity is high, uncertainty is high (Kiernan 2021), and it is more difficult to predict what species will be in a sample or how many species exist in the set of data. The higher the entropy, the higher the Shannon-Weiner value (Cordillera Consulting 2015). The Shannon-Weiner Index is most often calculated as follows:

$$H' = \sum_{i=1}^{s} pi \ln pi$$

where: *pi* is the proportion of individuals that belong to species *i* and *S* is the number of species in the sample. Although the natural logarithm is shown in this equation, the index could be calculated using the logarithm base of 10 and 2 for purposes of historical comparison (Cordillera Consulting 2015). These have become the most popular log bases in applications that use the Shannon entropy (Wikipedia 2021).

The results of the Simpson's Index of Diversity indicate the community composition of the sites is diverse, with values ranging from a low of 0.64 at site WAP06 to a high of 0.88 at sites WAP02 and WAP04 (Figure 2). Similar results are indicated by the Shannon-Weiner Index (Appendix E).

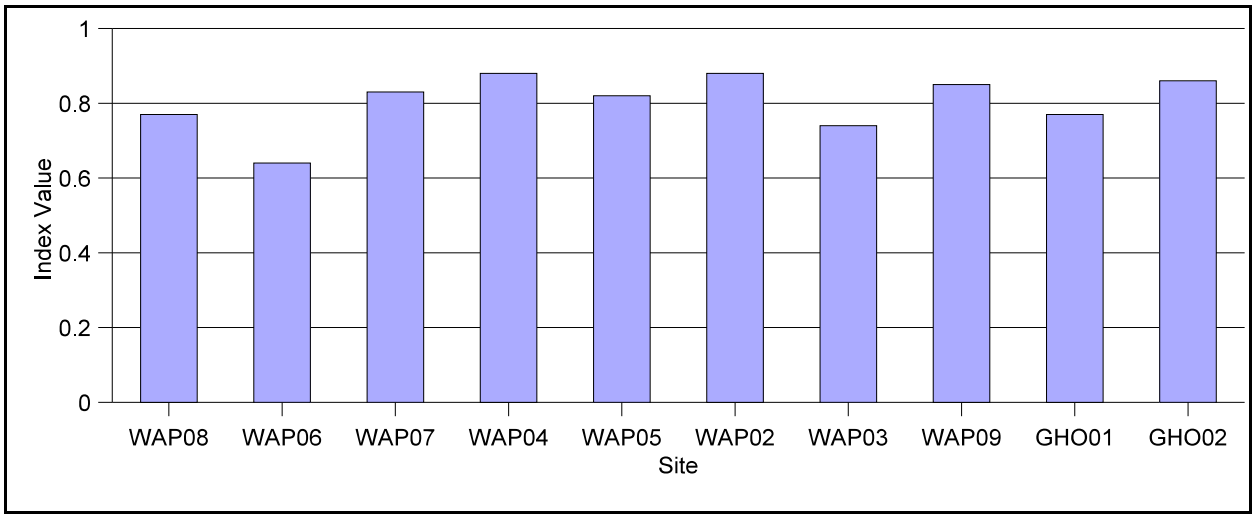


Figure 2. Simpson's Index of Diversity (1-D) for each site, ordered from downstream to upstream for each water course.

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. Potential measures 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.
- % TAXON (any particular taxon): percentage of any particular taxon within the total sample.

- % of dominant taxa: using up to the top five taxa (usually three) that dominate the community. As diversity declines, a few taxa dominate the community. Opportunistic taxa are less particular about their habitat, and replace taxa that require specific foods or physical habitat.

The following graphs illustrate the relationship between the Ephemeroptera, Plecoptera, Trichoptera and Diptera at each site. Of the EPT species, the Trichoptera comprised the lowest number (Figure 3). At all sites, the EPT taxa were far more abundant than the Diptera taxa (Figure 4), and of the Diptera, the chironomid family generally comprised over half (Figure 5). At the paired sites, chironomids were more abundant downstream of Meadow and Johnson creeks, and upstream of Lesueur Creek. Of the Waiparous Creek sites, they were most abundant at the most downstream site (WAP08), but still in low relative numbers (Figure 4).

The EPT ratio was very high at all sites along Waiparous Creek, although slightly lower at the most downstream site (WAP08) (Figure 6). (Note that the y-axis starts at 0.7 versus 0.) These values suggest good water quality. At the most upstream site (WAP09), no chironomids were recorded, resulting in a ratio of 1. The EPT ratio at the two sites sampled on the Ghost River was also high (Figure 6).

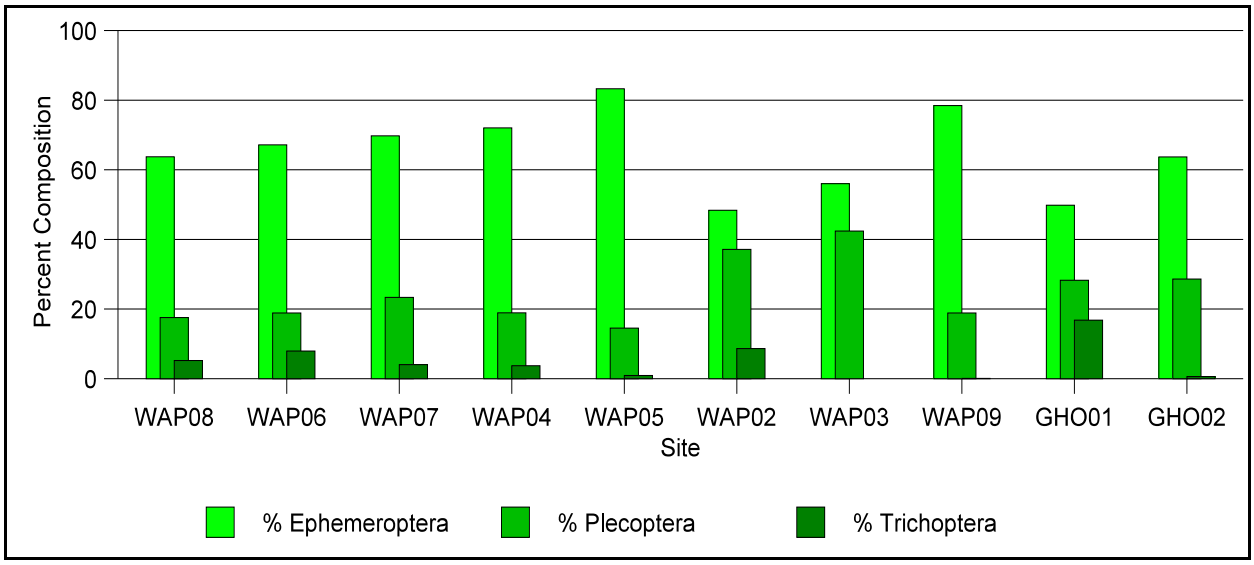


Figure 3. Percent composition of EPT orders at each site, ordered from downstream to upstream for each water course.

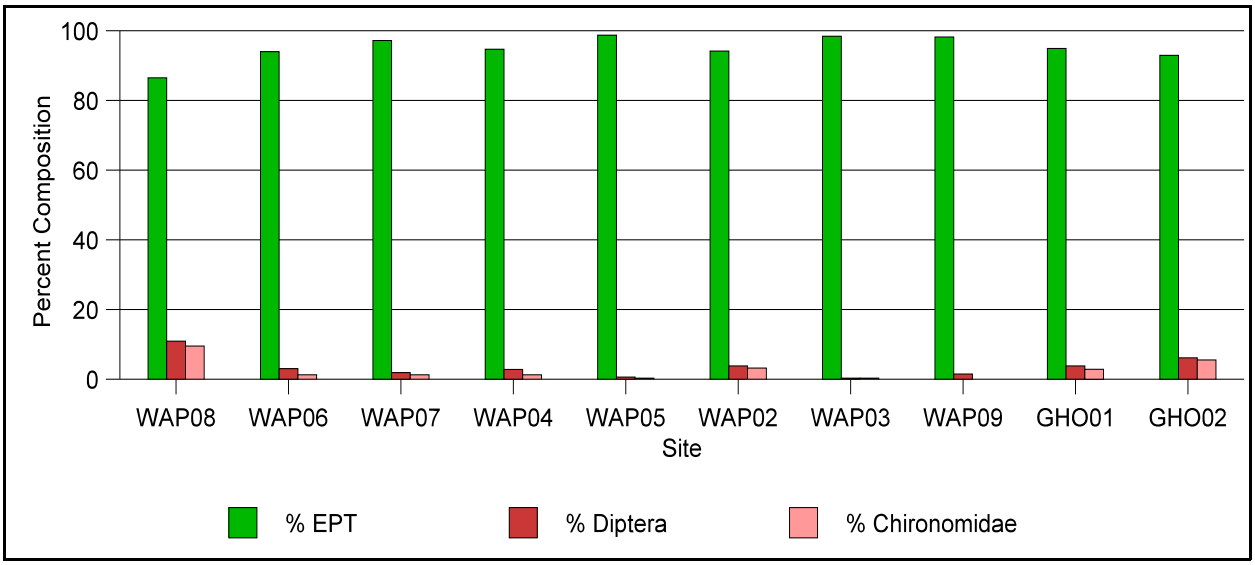


Figure 4. Percent composition of EPT orders, Diptera order and chironomid family at each site, ordered from downstream to upstream for each water course.

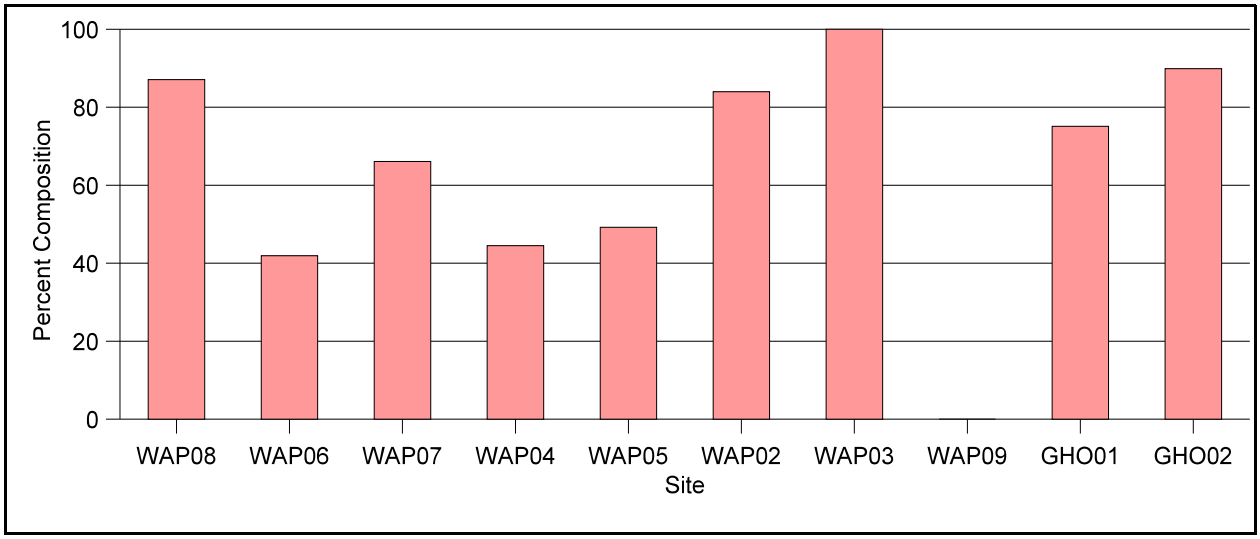


Figure 5. Percent of Diptera that were chironomid flies at each site, ordered from downstream to upstream for each water course.

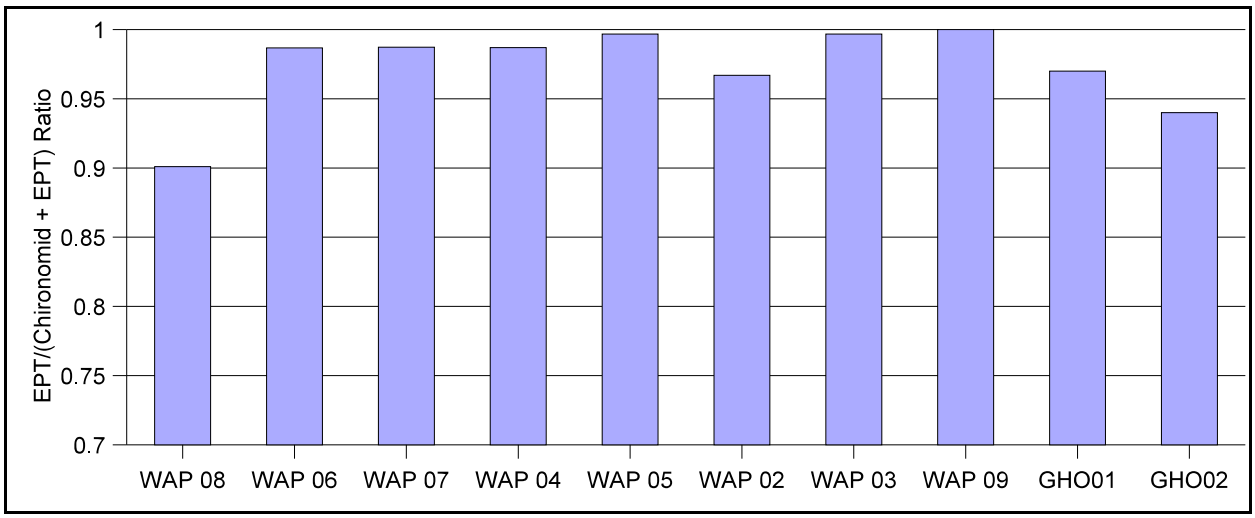


Figure 6. EPT/(chironomid + EPT) ratio using abundance of individuals at each site, ordered from downstream to upstream for each water course.

The abundance of Hydropsychidae within the Trichoptera was variable among sites (Figure 7), whereas the abundance of Baetidae within the Ephemeroptera was more consistent, and generally less than 30% (Figure 8). The low proportion of these tolerant families is encouraging. WAP09, the most upstream site, is an outlier, where all Trichoptera were in the family Hydropsychidae; however, Trichoptera comprised only 0.9% of the total taxa. In addition, the other metrics for this site do not suggest there is any reason for concern. The percent of Hydropsychidae and Baetidae was slightly higher at WAP08 than most other sites.

When comparing the paired sites, there was no clear pattern in taxa abundance. Baetidae were more abundant downstream of Meadow and Johnson creeks, whereas Hydropsychidae were more abundant upstream of the cadet camp and Meadow Creek, but were less abundant upstream of Johnson Creek and Lesueur Creek (Figures 7 and 8). Only Johnson Creek consistently had a higher proportion of tolerant species at the downstream site, including chironomids (Figure 4. The EPT ratio was also slightly lower downstream, but not low enough to suggest any concerns.

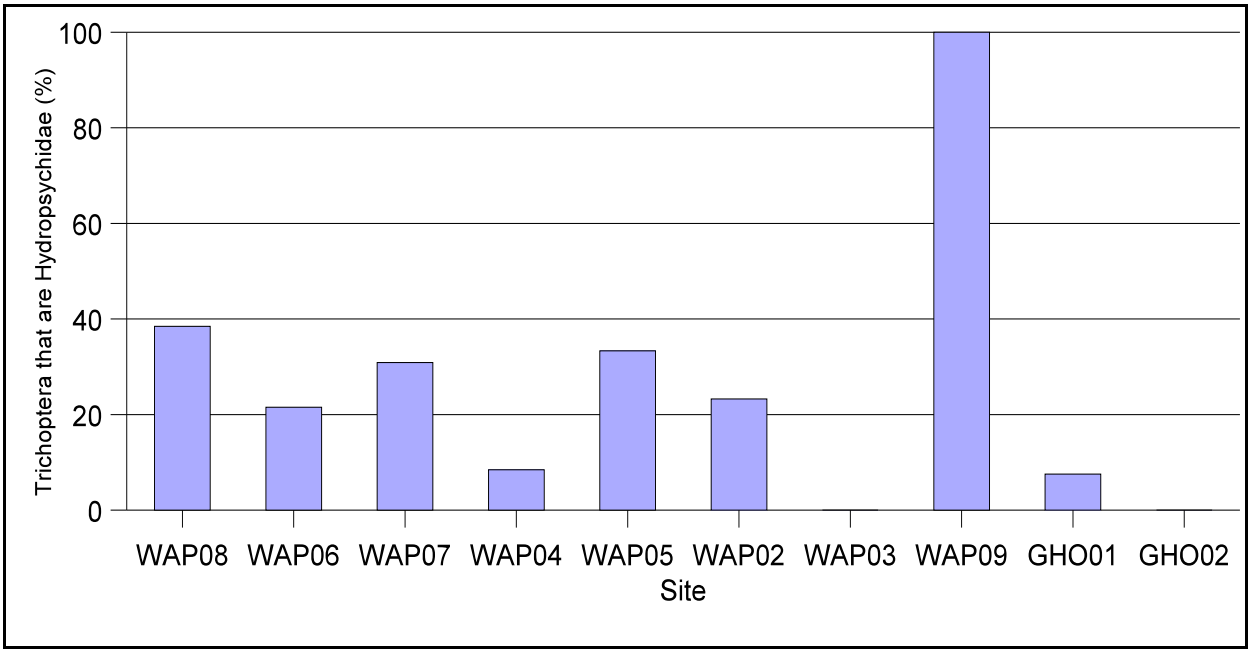


Figure 7. Percent of Trichoptera that were Hydropsychidae at each site, ordered from downstream to upstream for each water course.

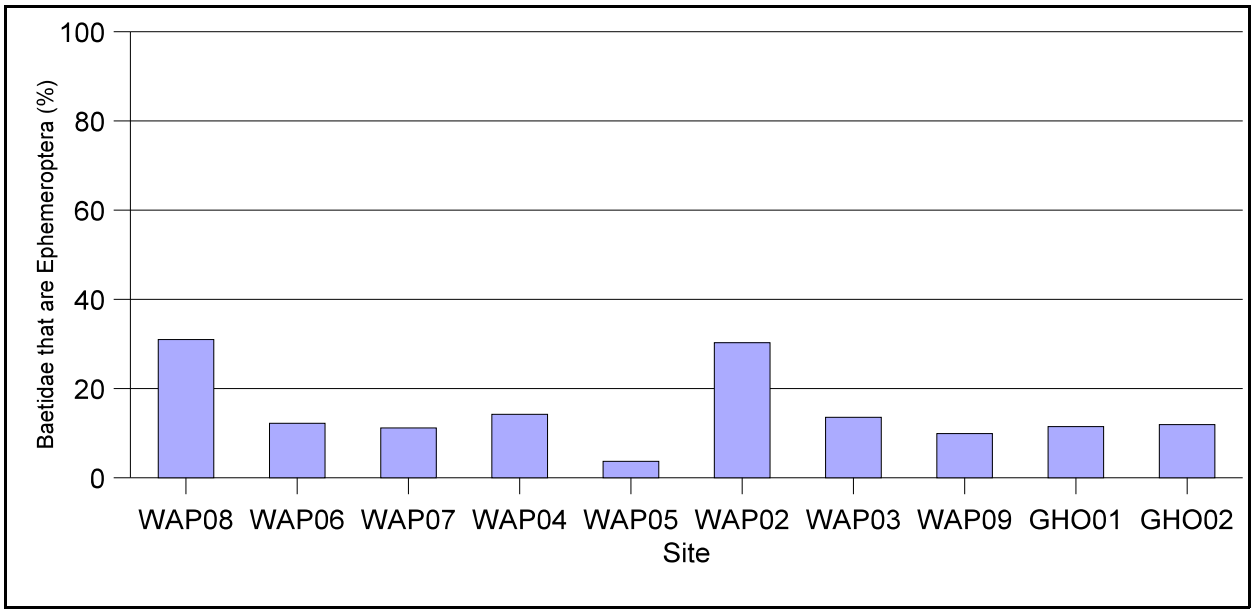


Figure 8. Percent of Ephemeroptera that were Baetidae at each site, ordered from downstream to upstream for each water course.

3.3.3 Functional Measurements

- % Functional feeding group: percent of taxa that are predators, shredders, gatherers, scrapers or filterers. A healthy stream or river has a variety of invertebrates feeding and moving in different ways. Without stable food dynamics, an imbalance in functional feeding groups will result, indicating stressed conditions due to disturbance. Usually scrapers and shredders decrease while filterers and gatherers increase.

- Number of clingers: number of taxa that cling to rock surfaces. These taxa have physical adaptations that allow them to hold onto smooth substrates in fast water. They typically inhabit the open inter-spacial zone between rock and cobble substrates on the stream bottom. They are therefore sensitive to fine sediments that fill these spaces (e.g., simullid blackflies, ephemerellid mayflies, periodid stoneflies).

- Long-lived taxa: number of taxa that require more than one year to complete their life-cycle. They are referred to as multivoltine (multiple year life-cycle) compared to univoltine (single year life). If the streambed dries up, certain life stages of some long-lived taxa (e.g., perlid stoneflies, Megaloptera) can survive being exposed.

- Intolerant taxa: Number of taxa intolerant or highly sensitive to pollution. They are the first to disappear as human disturbance increases (e.g., apatanid caddisflies, pteronarcys stoneflies).

- Tolerant individuals (%): Proportion of pollution-tolerant individuals in the sample. As disturbance increases, tolerant species represent a larger proportion of the sample (e.g., chironomids, amphipods).

3.3.4 Biotic Indices

3.3.4.1 Hilsenhoff Family Biotic Index (FBI)

The Hilsenhoff Family Biotic Index (FBI) estimates overall tolerance to organic pollution of each family based on their proportion (abundance) within the community. Biotic tolerance values are assigned to each family based on their response to organic pollution (Table 5). Sensitive taxa have low scores and tolerant taxa have high scores, ranging from 0 to 10. An increase in the index value suggests decreased water quality due to organic pollution.

The formula is:

$$FBI = \sum (ni * ti) / N$$

where:

ni is the number of individuals of the "*i*"th taxa within a family,

ti is the tolerance index value of that taxa,

N is the total number of individuals in the sample assigned a Hilsenhoff Family "Biotic Tolerance value"; some taxa are not included.

Table 5. Hilsenhoff Family Biotic Index categories.

Family Biotic Index	Water Quality	Degree of Organic Pollution
0.00–3.75	Excellent	Organic pollution unlikely
3.76–4.25	Very Good	Possible slight organic pollution
4.26–5.00	Good	Some organic pollution probable
5.01–5.75	Fair	Fairly substantial pollution likely
5.76–6.50	Fairly Poor	Substantial pollution likely
6.51–7.25	Poor	Very substantial pollution likely
7.26–10.00	Very Poor	Severe organic pollution likely

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, with the FBI usually indicating 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, Hilsenhoff recommends "for greatest sensitivity, everything should be identified to species" (Hilsenhoff 1987).

Jones (2008), in his review of taxonomic sufficiency, concurs. He refers to Yoder and Rankin (1995) when he says that the species level "ensures that summaries of biotic composition are not distorted and that maximal information content is available for, and no limits are imposed on, statistical analyses."

The Hilsenhoff Biotic Index (HBI) estimates a score using taxa at the genus/species level (Appendix E). In this case, the formula is the same except *ni* is the number of individuals of the "*i*"th taxa at the most accurate (lowest) taxonomic level, and the index categories are adjusted (Hilsenhoff 1988) (Table 6). The formula is:

$$HBI = \sum (ni * ti) / N$$

where:

ni is the number of individuals of the group "*i*",

ti is the tolerance index value of that taxa,

N is the total number of individuals in the sample assigned a "Biotic Tolerance value"; some taxa are not included.

Table 6. Hilsenhoff Biotic Index 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

All of the sites sampled in 2020 fall into the excellent category; results range from 0.59 at site WAP05 to 3.43 at site WAP08 (Figure 9). At all paired sites, the index is slightly higher at the downstream sites, suggesting a possible influence by the tributaries, and by the cadet camp in the case of WAP06. The Hilsenhoff Biotic Index at the most downstream site, WAP08, is the highest, although the index suggests that organic pollution is still unlikely.

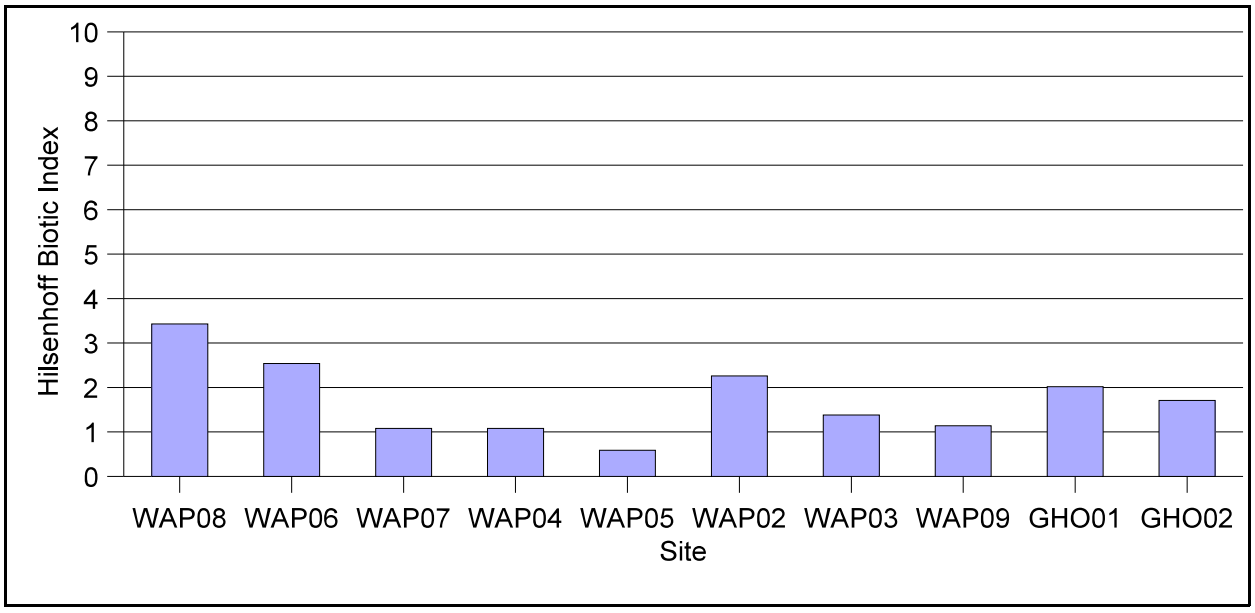


Figure 9. Hilsenhoff Biotic Index for each site, ordered from downstream to upstream for each water course.

3.3.4.2 Biotic Condition Index

The Biotic Condition Index (BCI) is not a metric used in any CABiN taxonomic analyses. It is briefly described in this report since it has been cited in many studies on sedimentation of streams/rivers in the intermountain region of the USA. It is one method of measuring the effects of land use practices, such as logging, road construction, livestock grazing and mining, on aquatic ecosystems in mountainous environments (Platts *et al.* 1983).

The BCI was developed by Winget and Mangum (1979, as cited in Platts *et al.* 1983) to evaluate the effect of sediment on benthic macroinvertebrates and fish habitat, including suitable spawning beds (redds). It incorporates stream habitat (gradient, substrate composition), water quality (alkalinity, sulfate), and environmental tolerances of aquatic macroinvertebrate species (Platts *et al.* 1983). The index has been widely used by the United States Forest Service (USFS) and Bureau of Land Management (BLM) throughout the western United States.

The BCI is a function of a Predicted Community Tolerance Quotient (CTQp) divided by the Actual Community Tolerance Quotient (CTQa). The tolerance quotient (TQ) is the product of values derived from a taxon's tolerance to levels of alkalinity and sulfate, plus its selectivity for or against fine substrate materials and low stream gradients (Platts *et al.* 1983). Taxa are assigned a tolerant quotient from 2 to 108. Taxa assigned low tolerance quotients are found only in high quality, unpolluted water, and taxa assigned high tolerant quotients are found in

severely polluted waters. The CTQd is a dominance-weighted community tolerance quotient (CTQd). The TQs have been determined for 54 taxa, and values have been assigned to an additional 317 taxa (Appendix 7 in Platts *et al.* 1983). Appendix 8 in Platts *et al.* (1983) provides Predicted Community Tolerance Quotients (CTQp) for various combinations of stream gradient, substrate, total alkalinity and sulfate.

The CTQp is the mean of the TQs for a predicted macroinvertebrate community. To obtain a CTQp for a particular stream segment, the site is classified according to the criteria mentioned above (Appendix 8 in Platts *et al*. 1983). A CTQ is simply the mean of the TQs of the macroinvertebrates collected from any site on any given date. The Biotic Condition Index is calculated as:

BCI = CTQp/CTQa X 100 (Values are expressed as percent of expected value)

3.4 STREAM eDNA Results

3.4.1 eDNA and Morphological Identification

The eDNA results complement the results of the morphological identification. An additional 189 species were identified, 60 of which were terrestrial species. The remainder were within 44 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 29 genera that were not detected by eDNA, along with one family of freshwater clams (Pisidiidae), one class of crustacea (Ostracoda) and one class of flatworm (Turbellaria).

There are a number of possible explanations for taxa to be identified in the morphological samples but not in the eDNA samples. If the taxa are not in the eDNA reference database, they will not be detected. If the distribution of the taxa is patchy, the random samples from each method will not match perfectly. In addition, taxa that are low in abundance (approximately <99% of the biomass) are not always identified by DNA metabarcoding (M. Wright, pers. comm.).

The majority of the eDNA detections were to the species level, and the remainder were to the genus level. Morphological identifications were rarely to the species level, usually to the genus level, often to the family level, and sometimes only to the order, class or phylum level. Most direct comparisons, therefore, could only be made at higher taxonomic levels (Table 7). The more detailed combined presence/absence results of each method are presented in

Appendix F. 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 7. Comparison of results of eDNA and morphological identification for non-terrestrial taxa 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] 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									
	GHO01	GHO02	WAP02	WAP03	WAP04	WAP05	WAP06	WAP07	WAP08	WAP09
Class: Insecta										
Order: Coleoptera										
Dytiscidae										
Liodessus	eDNA				eDNA	eDNA	eDNA	eDNA	Both	
Order: Diptera										
Ceratopogonidae		Morph	eDNA							
Chironomidae	Both	Both	Both	Both	Both	Both	Both	Both	Both	eDNA
Micropsectra	Morph	Morph					eDNA		eDNA	eDNA
Orthocladiinae	Both	Both	Both	Both	Both	Both	Both	Both	Both	
Eukiefferiella	Both	Both	Both	Both			eDNA	Morph	eDNA	
Krenosmittia			eDNA		Morph					
Limnophyes		Morph								eDNA
Orthocladius complex	Both	Morph	Both		Morph		Morph		Both	
Tvetenia		Morph	Both		Both	Both	eDNA	Both	eDNA	
Empididae							Morph		Both	eDNA
Neoplasta							Morph		eDNA	
Simuliidae	Both	eDNA	Both	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	
Simulium	eDNA	eDNA	Both	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	
Tipulidae	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Both	Morph
Order: Ephemeroptera										
Ameletidae										
Ameletus	Both	Both	Both	Both	Both	Both	Both	eDNA	Both	Both
Baetidae	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both
Acentrella	eDNA	eDNA	Both	Both	eDNA	eDNA	Both	Both	Both	
Baetis	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both
Ephemerellidae	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both
Drunella	Both	Both	Both	Both	Both	Both	Both	Both	eDNA	Both
D. doddsii	Both	Both	Both	Both	Both	Both	Both	Both	eDNA	Both
Ephemerella	Both	eDNA	eDNA		eDNA		Both	eDNA	Morph	

Taxa	Site									
	GHO01	GHO02	WAP02	WAP03	WAP04	WAP05	WAP06	WAP07	WAP08	WAP09
Heptageniidae	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both
Cinygmula	Both	Both	Both	Both	eDNA	Both	eDNA	Both	eDNA	Both
Epeorus	eDNA	eDNA	Both	Both	Both	Both	eDNA	Both	eDNA	Both
Rhithrogena	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both
Leptophlebiidae	eDNA	eDNA				eDNA	Both	eDNA	eDNA	
Order: Hemiptera										
Corixidae										
Callicorixa		Morph								eDNA
Sigara	eDNA	eDNA					Both	eDNA		
Order: Plecoptera	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both
Capniidae	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both
Chloroperlidae	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both
Plumiperla	eDNA	Both	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA
Sweltsa	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both
Leuctridae	Both	Both	Both		Both	eDNA	Both	Both	Both	Morph
Nemouridae	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both
Visoka cataractae							Morph			eDNA
Zapada	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both
Z. cinctipes	Both	Both	Both	Both	Both	Both	Both	Both	Both	eDNA
Z. columbiana	Both	Both	eDNA	eDNA	eDNA	eDNA	eDNA			eDNA
Z. oregonensis	Morph	Morph				eDNA		eDNA		Morph
Perlidae	Both	Both	Both	Both	Both	Both	Both	Both	Both	eDNA
Doroneuria		eDNA	Both	eDNA	eDNA	Both	eDNA	Both	Morph	
Hesperoperla	Both	eDNA	Both	eDNA	Both	Both	Both	Both	eDNA	
Perlodidae	eDNA	Both	Both	Both	Both	Both	Both	Both	Both	eDNA
Isogenoides		eDNA	eDNA	eDNA	Both	eDNA	Both	Both	Both	
Kogotus	eDNA	eDNA	eDNA		eDNA		Both		eDNA	
Taeniopterygidae	Both	Both	Both	Both	Both	Both	Both	Both	Both	Both
Taenionema	Both	eDNA				eDNA	Morph	Both	Both	Morph
Order: Trichoptera	Both	Both	Both	eDNA	Both	Both	Both	Both	Both	Both
Brachycentridae	Both	Both	Both	eDNA	Both	Both	Both	Both	Both	
Brachycentrus	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	Both	eDNA	eDNA	
Glossosomatidae	Both	Both	Morph	eDNA	Both	Both	Both	Both	Both	
Glossosoma	Both	eDNA		eDNA	Both	Both	Both	Both	Both	
Hydropsychidae	Both	eDNA	Both	eDNA	Both	Both	Both	Both	Both	Both
Arctopsyche	eDNA		Both	eDNA	Both	Both	Both	Both	Both	eDNA
Parapsyche elsis	Both	eDNA		eDNA					eDNA	eDNA
Lepidostomatidae										
Lepidostoma	eDNA	eDNA			eDNA	eDNA	Both	eDNA	Both	
Limnephilidae	eDNA	eDNA			eDNA		eDNA	Both		eDNA
Rhyacophilidae										
Rhyacophila	eDNA		Both	eDNA	Both	eDNA	eDNA	eDNA	eDNA	eDNA
R. brunnea/vemna			eDNA		Both			eDNA	eDNA	
R. hyalinata group			Both	eDNA		eDNA				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 good species richness that is slightly higher at the most downstream sites.

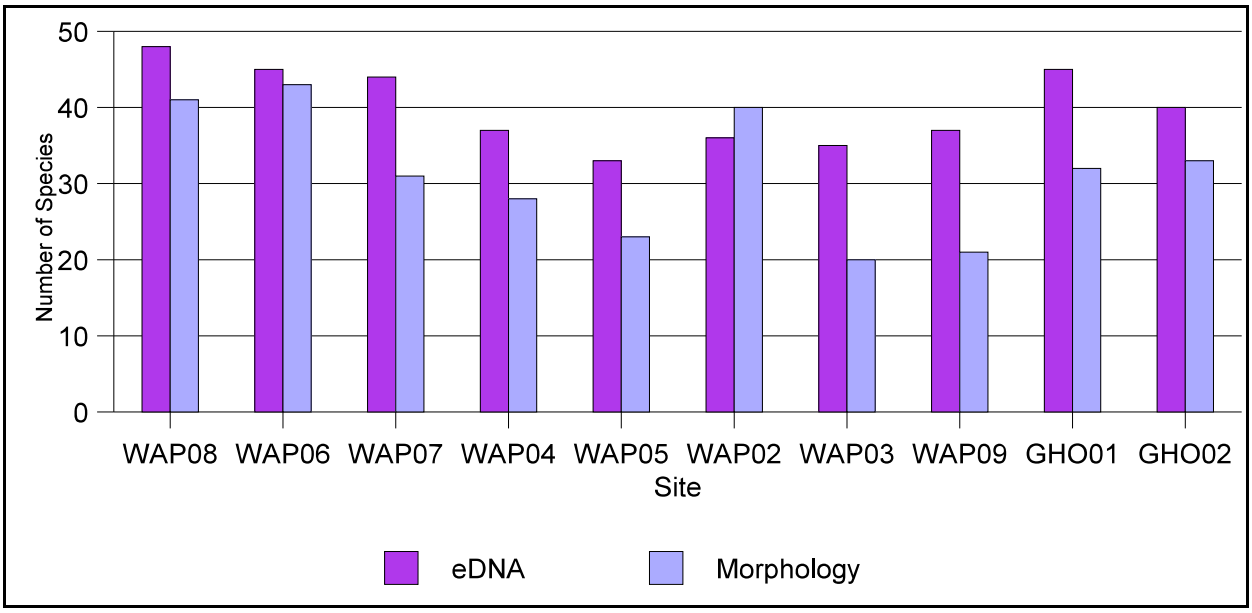


Figure 10. Species richness based on species taxonomically assigned by eDNA with high confidence, and taxa identified morphologically.

3.4.2 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 ten sites. This suggests that the sampling locations may be outside of the confirmed zone for whirling disease (Hajibabaei Lab 2021).

4.0 Conclusions and Recommendations

4.1 Comparison of All Sites

The results of the samples at the ten sites, both on-site and from the laboratory analyses, indicate high water quality. The chemical and physical attributes were well below exceedance levels. TSS and turbidity were extremely low. Water quality parameters were all within acceptable limits for benthic macroinvetebrates and fish.

The Simpson's Index of Diversity and the Shannon-Weiner Index indicate that the sites were highly diverse in their benthic macroinvertebrate community composition. The Hilsenhoff Biotic Index suggests that organic pollution was unlikely, rating water quality as excellent.

The EPT ratio suggests high water quality at all of the sites, with EPT species at much higher abundance than the pollution-tolerant chironomid family. The low percent of the more tolerant Hydropsychidae within the Trichoptera and Baetidae within the Ephemeroptera suggests no concerns.

The most downstream site on Waiparous Creek (WAP08) had the lowest EPT ratio of the sites, and had a higher proportion of tolerant species than most of the other sites. However, these were not sizable enough to suggest water quality concerns. Similarly, the Hilsenhoff Biotic Index was the highest at this site but was still at the level suggesting organic pollution was unlikely.

The results of the 2020 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 of Paired Sites

No major differences were found between paired downstream and upstream sites, suggesting the point sources were not having a marked effect on water quality at the time of sampling.

There was little variation in the physical properties between each paired site. Most notable was the greater amount of pebble-sized substrate upstream of confluences and the greater amount of larger sized substrate downstream. This is likely a result of the smaller sized substrate being transported downstream by the higher maximum and average stream flows below the confluences. Embeddedness was higher on the Ghost River downstream of Lesueur Creek, suggesting sediment input from the creek.

Differences in the Hilsenhoff Biotic Index upstream and downstream of point sources suggest a slight decline in quality at the downstream sites, although the index still suggests that organic pollution was unlikely and water quality was excellent. This should be monitored to determine if these differences persist and become a concern.

Differences in the taxa between the paired sites were subtle and variable, suggesting no immediate concerns. Waiparous Creek downstream of Johnson Creek had a higher percentage of tolerant species than upstream, but the EPT ratio was high enough to suggest no concerns. However, continued monitoring is advisable.

4.3 General Recommendations

- Adequate annual funding for this program should be maintained.

- The *GWAS Water Monitoring Program Plan 2020* should continue to be followed, allowing flexibility if circumstances materialize that suggest a deviation.

- The 2020 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., WAP02, WAP08.

- 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 (water chemical and benthic macroinvertebrate analysis) should continue to be used.

6.0 Literature Cited

ALCES and GWAS (ALCES Landscape and Land-use Ltd. and Ghost Watershed Alliance Society). 2018. Ghost River State of the Watershed Report 2018. ALCES Landscape and Land-use Ltd., Calgary and Ghost Watershed Alliance Society, Cochrane, Alberta. 197 p.

Alberta Environment and Parks. 2016. Interim guidelines for the disinfection of fisheries equipment to reduce the spread of whirling disease in southern Alberta. Alberta Environment and Parks Support and Emergency Response Team (ASERT). AEP Fish and Wildlife 2016 No. 5. 17 p. https://open.alberta.ca/dataset/9b5a368f-a179-41f4-b159-30f820a133cb/resource/ba480b8c-257b-404c-be66-dc6a72bbc0da/download/2016-interim-guidelines-for-disinfection-of-fisheries-equipment-to-reduce-spread.pdf (Accessed March 28, 2022)

Alberta Environmental Protection. 1997. Alberta Water Quality Guideline for the Protection of Freshwater Aquatic Life – Dissolved Oxygen. Standards and Guidelines Branch, Environmental Assessment Division, Environmental Regulatory Service, Edmonton. Pub. No.:T/391. 73 p. https://open.alberta.ca/dataset/82793404-d376-4b9e-a399-94da6e279b0a/resource/f223f816-1268-4f4e-9698-78824bb8a5fe/download/7254.pdf (Accessed March 28, 2022)

Barbour, M.T., J. Gerritsen, B.D. Snyder and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish. Second Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC. EPA 841-B-99-002. https://www.epa.gov/sites/production/files/2019-02/documents/rapid-bioassessment-streams-rivers-1999.pdf (Accessed March 28, 2022)

Barcelona Field Studies Centre. 2022. Simpson's Diversity Index. https://geographyfieldwork.com/Simpson%27sDiversityIndex.htm (Accessed March 28, 2022)

Carter, K. 2005. The effects of dissolved oxygen on steelhead trout, coho salmon, and chinook salmon biology and function by life stage. California Regional Water Quality Control Board, North Coast Region. 9 p. http://www.swrcb.ca.gov/northcoast/water_issues/programs/tmdls/shasta_river/060707/29appendixbetheeffectsofdissolvedoxygenonsteelheadtroutcohosalmonandchinooksalmonbiologyandfunction.pdf (Accessed March 28, 2022)

Clean Water Team. 2004. Electrical conductivity/salinity fact sheet. FS-3.1.3.0(EC). in: The Clean Water Team Guidance Compendium for Watershed Monitoring and Assessment, Version 2.0. Division of Water Quality, California State Water Resources Control Board (SWRCB), Sacramento, California. https://www.waterboards.ca.gov/water_issues//programs/swamp/docs/cwt/guidance/3130en.pdf (Accessed March 28, 2022)

Cordillera Consulting Inc. 2015. Common metric formulas for the analysis of benthic invertebrate data. Summerland, B.C. 4 p.

CCME (Canadian Council of Ministers of the Environment). 1999. Canadian Environmental Quality Guidelines. Canadian Council of Ministers of the Environment, Winnipeg, Manitoba. 2005 - Update 5.0. Publication No. 1299.

CCME (Canadian Council of Ministers of the Environment). 2008. Canadian Water Quality Guidelines. Prepared by the Task Force on Water Quality Guidelines of the Canadian Council of Ministers of the Environment. https://www.ccme.ca/files/Resources/supporting_scientific_documents/cwqg_pn_1040.pdf (Accessed March 10, 2021)

Casiday, R. and R. Frey. 1998. Water hardness: Inorganic reactions experiment. Department of Chemistry, Washington University, Missouri. http://www.chemistry.wustl.edu/~edudev/LabTutorials/Water/FreshWater/hardness.html (Accessed May 9, 2021)

Environment Canada. 2012. Canadian Aquatic Biomonitoring Network Field Manual – Wadeable Streams. Cat. No: En84-87/2012E-PDF. 57 p.

Environment Canada. 2014. Canadian Aquatic Biomonitoring Network Laboratory Methods – Processing, Taxonomy, and Quality Control of Benthic Macroinvertebrate Samples. Cat. No: En84-86/2014E-PDF. 36 p.

FEI (Fondriest Environmental, Inc.). 2013. Dissolved oxygen. Fundamentals of Environmental Measurements. 19 Nov. 2013. https://www.fondriest.com/environmental-measurements/parameters/water-quality/dissolved-oxygen/ (Accessed March 28, 2022)

FEI (Fondriest Environmental, Inc.). 2014a. Conductivity, salinity and total dissolved solids. Fundamentals of Environmental Measurements. 3 March 2014. https://www.fondriest.com/environmental-measurements/parameters/water-quality/conductivity-salinity-tds/ (Accessed March 28, 2022)

FEI (Fondriest Environmental, Inc.). 2014b. Turbidity, total suspended solids and water clarity. Fundamentals of Environmental Measurements. 13 June 2014. https://www.fondriest.com/environmental-measurements/parameters/water-quality/turbidity-total-suspended-solids-water-clarity/ (Accessed March 28, 2022)

Government of Alberta. 2018. Environmental Quality Guidelines for Alberta Surface Waters. Water Policy Branch, Alberta Environment and Parks. Edmonton, Alberta. AEP, Water Policy, 2014, No. 1. 53 p. https://open.alberta.ca/dataset/5298aadb-f5cc-4160-8620-ad139bb985d8/resource/38ed9bb1-233f-4e28-b344-808670b20dae/download/environmentalqualitysurfacewaters-mar28-2018.pdf (Accessed March 28, 2022)

Government of Alberta. 2020. Whirling disease decontamination risk zone. Whirling Disease Program, Resource Stewardship, Alberta Environment and Parks. Map. https://open.alberta.ca/dataset/c240b099-18cb-4037-91fa-4038de4012f7/resource/ac3a4e79-8fba-4a8c-ae91-7662134d7407/download/aep-whirling-disease-decontamination-risk-zone-map-2020-08.pdf (Accessed March 28, 2022)

Hajibabaei Lab. 2021. Preliminary DNA data – Bow River, AB, Ghost Watershed Alliance Society, May 2021. STREAM: Centre for Biodiversity Genomics, University of Guelph, WWF Canada, Environment and Climate Change Canada, Living Lakes Canada. 21 pp.

Hem, J.D. 1985. Study and Interpretation of the Chemical Characteristics of Natural Water. Third Edition. Department of the Interior, US Geological Survey. U.S. Geological Survey Water-Supply Paper 2254. 263 p. http://pubs.usgs.gov/wsp/wsp2254/pdf/wsp2254a.pdf (Accessed March 28, 2022)

Hilsenhoff, W. L. 1987. An improved biotic index of organic stream pollution. The Great Lakes Entomologist 20:31-39.

Hilsenhoff, W. L. 1988. Rapid field assessment of organic pollution with a family-level biotic index. J. N. Am. Benthol. Soc. 7:65-68. http://www.jstor.org/stable/1467832 (Accessed March 28, 2022).

Jones, F.C . 2008. Taxonomic sufficiency: The influence of taxonomic resolution on freshwater bioassessments using benthic macroinvertebrates. Environ. Rev. 16: 45–69.

Kentucky Water Watch. n.d. Total suspended solids and water quality. River Assessment Monitoring Project. 1996 RAMP Studies Lower Cumberland and Tradewater River Watersheds in Western Kentucky. http://www.state.ky.us/nrepc/water/ramp/rmtss.htm (Accessed March 28, 2022)

Kiernan, D. 2021. Natural Resources Biometrics. SUNY College of Environmental Science and Forestry. LibreTexts. https://stats.libretexts.org/@go/page/2932 (Accessed on March 28, 2022)

LCRA (Lower Colorado River Authority). 2014. Water quality indicators. https://www.lcra.org/water/quality/colorado-river-watch-network/water-quality-indicators/ (Accessed on March 28, 2022)

Miller, R. L., W. L. Bradford and N. E. Peters. 1988. Specific conductance: Theoretical considerations and application to analytical quality control. U.S. Geological Survey Water-Supply Paper 2311. 16 p. http://pubs.usgs.gov/wsp/2311/report.pdf (Accessed March 28, 2022)

Murphy, S. 2007. General information on solids. USGS Water Quality Monitoring. Boulder, Colorado. http://bcn.boulder.co.us/basin/data/NEW/info/TSS.html (Accessed March 28, 2022)

Platts, W.S., W.F. Megahan and G. W. Minshall. 1983. Methods for Evaluating Stream, Riparian, and Biotic Conditions. U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. Ogden, Utah. Gen. Tech. Rep. INT-138. 70 p. https://www.fs.fed.us/rm/pubs_int/int_gtr138.pdf (Accessed March 28, 2022).

STREAM (Sequencing the Rivers for Environmental Assessment and Monitoring). 2020. Procedure for collecting benthic macroinvertebrate DNA samples in wadeable streams. Unpublished draft document (March 2020 v1.1). 5 p.

USGS (United States Geological Survey). 2021. Hardness of water. USGS Water Science School. https://www.usgs.gov/special-topic/water-science-school/science/hardness-water?qt-science_center_objects=0#qt-science_center_objects (Accessed March 28, 2022).

Wikipedia. 2021. Diversity index. https://en.wikipedia.org/wiki/Diversity_index (Accessed March 28, 2022)

Wood, M.S. 2014. Estimating suspended sediment in rivers using acoustic Doppler meters. U.S. Geological Survey Fact Sheet 2014-3038. 4 p. https://pubs.usgs.gov/fs/2014/3038/ (Accessed March 28, 2022)

7.0 Personal Communications

Wright, Michael — Laboratory Manager, Hajibabaei Lab, Centre for Biodiversity Genomics, Biodiversity Institute of Ontario, University of Guelph. (Feb. 3, 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**

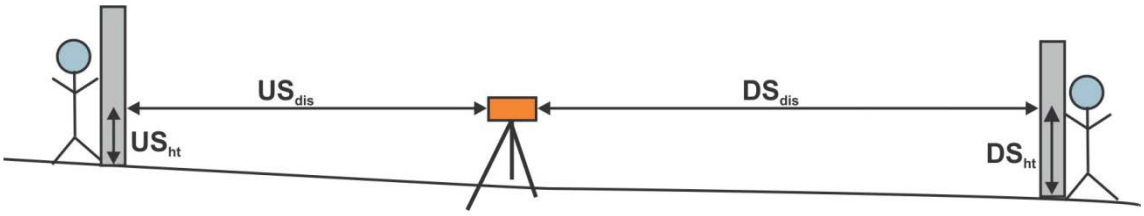
Scale: _____ (Note: small scale map recommended if field measurement is not possible - i.e. 1:20,000).
contour interval (vertical distance) _____ (m),
distance between contour intervals (horizontal distance) _____ (m)
slope = vertical distance/horizontal distance =_____

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: _____

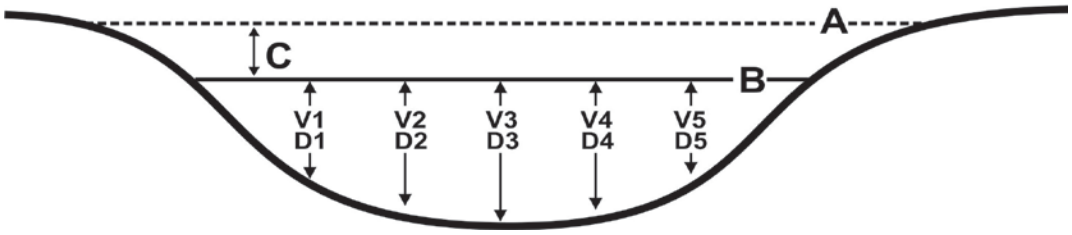
Sampling Date: (DD/MM/YYYY) _____

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) = √ [2(ΔD/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 ($\mathbf{D_1}$) (cm)							
Depth of Stagnation ($\mathbf{D_2}$) (cm)							
Change in depth ($\mathbf{\Delta D=D_2-D_1}$) (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
	Oreoleptidae	Oreoleptid flies
	Psychodidae	Moth 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
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

Order	Family	Common Name
	Hydropsychidae	Net-spinning caddisflies
	Hydroptilidae	Microcaddisflies
	Lepidostomatidae	Bizarre caddisflies
	Limnephilidae	Tube-case caddisflies
	Rhyacophilidae	Free-living caddisflies
	Uenoidae	Stonecase caddisflies
Oribatida		Oribatid mites
	Phthiracaridae	Oribatid mites
	Steganacaridae	Oribatid mites
Trombidiformes		Mites
	Hygrobatidae	Water mites
	Lebertiidae	Water mites
	Sperchontidae	Water mites
	Stygothrombidiidae	Water mites
	Torrenticolidae	Torrent mites
Veneroida		Bivalve molluscs
	Pisidiidae	Pill clams, pea clams
Lumbriculida		Microdrile oligochaetes (worms)
	Lumbriculidae	Aquatic worms
Plectida		Nematodes
	Plectidae	Freshwater nematodes

Appendix C

Fauna Identified Using Morphological Characteristics

Taxa	Site									
	WAP02	WAP03	WAP04	WAP05	WAP06	WAP07	WAP08	WAP09	GHO01	GHO02
Phylum: Arthropoda										
Subphylum: Hexapoda										
| Class: Insecta										
| Order: Ephemeroptera										
| Family: Ameletidae										
Ameletus	3	20	17	33	8	0	9	180	20	188
| Family: Baetidae	1	0	50	0	8	0	4	0	5	12
Acentrella	3	20	0	0	8	17	79	0	0	0
Baetis	53	30	417	100	154	250	200	260	45	76
Baetis fuscatus gr.	2	10	17	33	15	0	0	0	5	0
Baetis rhodani group	14	60	67	33	69	150	13	0	35	59
| Family: Ephemerellidae	10	35	117	67	46	83	0	20	10	18
Drunella	1	0	0	0	0	0	0	0	0	0
Drunella doddsii	5	10	150	117	15	150	0	60	35	35
Ephemerella	0	0	0	0	69	0	30	0	5	0
| Family: Heptageniidae	134	490	2783	3600	1062	2000	470	1950	260	553
Cinygmula	6	10	0	33	0	17	0	20	20	59
Epeorus	1	30	17	17	0	17	0	10	0	0
Rhithrogena	8	170	233	467	615	1050	148	120	345	235
| Family: Leptophlebiidae	0	0	0	0	8	0	0	0	0	0
| Order: Plecoptera	2	0	50	0	0	0	1	10	0	6
| Family: Capniidae	5	70	17	67	46	83	4	40	10	41
| Family: Chloroperlidae	30	20	33	17	8	0	17	10	45	41
Haploperla	0	0	0	0	0	0	0	20	0	0
Neaviperla	0	0	0	0	0	0	0	0	0	6
Plumiperla	0	0	0	0	0	0	0	0	0	24

Taxa	Site									
	WAP02	**WAP03**	**WAP04**	**WAP05**	**WAP06**	**WAP07**	**WAP08**	**WAP09**	**GHO01**	**GHO02**
Sweltsa	15	40	117	267	69	183	39	20	10	18
| Family: Leuctridae	2	0	17	0	15	17	4	30	0	0
| Family: Nemouridae	10	20	83	0	0	0	13	10	25	53
Visoka cataractae	0	0	0	0	8	0	0	0	0	0
Zapada	16	30	0	0	0	0	4	100	0	24
Zapada oregonensis group	0	0	0	0	0	0	0	20	10	41
Zapada cinctipes	21	10	133	17	177	167	22	0	130	71
Zapada columbiana	0	0	0	0	0	0	0	0	10	24
| Family: Perlidae	13	0	33	0	15	17	4	0	0	18
Doroneuria	3	0	0	17	0	17	4	0	0	0
Hesperoperla	1	0	17	17	15	33	0	0	5	0
| Family: Perlodidae	4	10	0	0	15	50	13	0	0	6
Isogenoides	0	0	33	0	31	17	4	0	0	0
Kogotus	0	0	0	0	8	0	0	0	0	0
Skwala	0	0	0	17	0	0	0	0	0	0
| Family: Taeniopterygidae	63	470	483	367	123	650	105	200	190	182
Taenionema	0	0	0	0	54	17	30	170	10	0
| Order: Trichoptera	4	0	0	0	0	0	13	0	0	0
| Family: Brachycentridae	26	0	150	17	15	33	9	0	175	6
Brachycentrus americanus	0	0	0	0	8	0	0	0	0	0
| Family: Glossosomatidae	2	0	0	0	8	0	6	0	10	6
Glossosoma	0	0	17	17	62	100	4	0	60	0
| Family: Hydropsychidae	3	0	0	0	38	50	26	30	5	0
Arctopsyche	7	0	17	17	15	17	4	0	0	0
Parapsyche elsis	0	0	0	0	0	0	0	0	15	0
| Family: Lepidostomatidae										
Lepidostoma	0	0	0	0	100	0	22	0	0	0
| Family: Limnephilidae	0	0	0	0	0	17	0	0	0	0
| Family: Rhyacophilidae										
Rhyacophila										

Taxa	Site									
	WAP02	**WAP03**	**WAP04**	**WAP05**	**WAP06**	**WAP07**	**WAP08**	**WAP09**	**GHO01**	**GHO02**
Rhyacophila brunnea/vemna group	0	0	17	0	0	0	0	0	0	0
Rhyacophila hyalinata group	1	0	0	0	0	0	0	0	0	0
| Order: Coleoptera										
| Family: Dytiscidae										
Liodessus	0	0	0	0	0	0	9	0	0	0
| Family: Elmidae	1	0	17	0	15	33	4	0	0	0
Heterlimnius	2	0	83	17	15	0	0	0	0	0
| Order: Diptera										
| Family: Athericidae										
Atherix	0	0	0	0	0	0	4	0	0	0
| Family: Ceratopogonidae	0	0	0	0	0	0	0	0	0	6
| Family: Chironomidae	2	0	0	0	15	0	13	0	20	47
| Subfamily: Chironominae										
| Tribe: Chironomini										
Microtendipes	0	0	0	0	0	0	9	0	0	0
| Tribe: Tanytarsini	0	0	0	0	0	0	4	0	0	0
Cladotanytarsus	0	0	0	0	0	0	4	0	0	0
Constempellina sp. C	0	0	0	0	8	0	0	0	0	0
Micropsectra	0	0	0	0	0	0	0	0	5	6
Sublettea	0	0	0	0	0	0	4	0	0	0
| Subfamily: Diamesinae										
| Tribe: Diamesini										
Pagastia	1	0	0	0	0	0	0	0	5	6
| Subfamily: Orthocladiinae	0	0	0	0	0	0	9	0	0	0
Brillia	3	0	0	0	0	0	0	0	0	0
Eukiefferiella	2	5	0	0	0	33	0	0	10	12
Heterotrissocladius	0	0	0	0	0	0	4	0	0	0
Hydrobaenus	0	0	0	0	0	0	4	0	0	6
Krenosmittia	0	0	17	0	0	0	0	0	0	0

Taxa	Site									
	WAP02	**WAP03**	**WAP04**	**WAP05**	**WAP06**	**WAP07**	**WAP08**	**WAP09**	**GHO01**	**GHO02**
Limnophyes	0	0	0	0	0	0	0	0	0	6
Orthocladiinae 004 (Like *Heleniella*)	0	0	0	0	0	17	0	0	0	0
Orthocladius complex	1	0	33	0	8	0	91	0	5	6
Parametriocnemus	0	0	0	0	8	0	0	0	0	0
Thienemanniella	0	0	0	0	0	0	0	0	0	6
Tvetenia	7	0	17	17	0	17	0	0	0	12
| Family: Empididae	0	0	0	0	0	0	13	0	0	0
Neoplasta	0	0	0	0	8	0	0	0	0	0
| Family: Oreoleptidae										
Oreoleptis	1	0	0	0	0	0	0	30	0	0
| Family: Psychodidae										
Pericoma/Telmatoscopus	0	0	17	0	15	17	0	0	0	0
| Family: Simuliidae	0	0	0	0	0	0	0	0	5	0
Simulium	2	0	0	0	0	0	0	0	0	0
| Family: Tipulidae	0	0	0	0	0	0	0	10	0	6
Dicranota	0	0	0	0	0	0	0	0	5	0
Hexatoma	0	0	67	17	31	17	4	10	5	0
| Order: Hemiptera										
| Family: Corixidae										
Callicorixa	0	0	0	0	0	0	0	0	0	6
Palmacorixa	0	0	0	0	8	17	0	0	0	0
Sigara	0	0	0	0	8	0	0	0	0	0
| Order: Thysanoptera	1	0	0	0	0	0	0	0	0	0
Subphylum: Chelicerata										
| Class: Arachnida										
| Order: Trombidiformes	0	5	0	0	0	0	0	0	0	0
| Family: Hygrobatidae										
Atractides	2	0	0	17	8	0	4	0	0	6

Taxa	Site									
	WAP02	**WAP03**	**WAP04**	**WAP05**	**WAP06**	**WAP07**	**WAP08**	**WAP09**	**GHO01**	**GHO02**
Hygrobates	2	0	0	0	0	0	0	0	0	0
| Family: Lebertiidae										
Lebertia	1	5	0	0	15	0	9	0	0	0
| Family: Sperchontidae										
Sperchon	0	0	0	0	8	0	13	0	0	0
| Family: Torrenticolidae										
Testudacarus	0	0	33	0	0	0	0	0	0	0
Suborder: Prostigmata										
| Family: Stygothrombidiidae										
Stygothrombium	0	0	0	0	8	0	0	0	0	0
Phylum: Mollusca										
| Class: Bivalvia										
| Order: Veneroida										
| Family: Pisidiidae	0	0	0	0	0	0	0	0	5	0
| Class: Gastropoda	0	0	0	0	8	0	0	0	0	0
Phylum: Annelida										
Subphylum: Clitellata										
| Class: Oligochaeta										
| Order: Lumbriculida										
| Family: Lumbriculidae	1	0	0	0	0	0	0	0	5	6
Rhynchelmis	0	10	0	0	0	0	0	10	10	0
Totals:	**498**	**1580**	**5369**	**5405**	**3093**	**5353**	**1503**	**3340**	**1575**	**1939**

Taxa present but not included:

Phylum: Arthropoda
Subphylum: Crustacea

Taxa	Site									
	WAP02	WAP03	WAP04	WAP05	WAP06	WAP07	WAP08	WAP09	GHO01	GHO02
| Class: Ostracoda	1	0	0	0	8	17	4	10	5	6
Phylum: Annelida										
Subphylum: Clitellata										
| Class: Oligochaeta										
| Order: Tubificida										
| Family: Lumbricidae	0	0	0	0	0	0	0	0	5	0
Phylum: Nemata	1	0	17	0	8	17	0	0	5	0
Phylum: Platyhelminthes										
| Class: Turbellaria	0	0	17	0	8	17	1	10	0	6
Totals:	**2**	**0**	**34**	**0**	**24**	**51**	**5**	**20**	**15**	**12**

Appendix D

Fauna Identified at the Family Level Using Morphological Characteristics

Taxa	Site									
	WAP02	WAP03	WAP04	WAP05	WAP06	WAP07	WAP08	WAP09	GHO01	GHO02
Phylum: Arthropoda										
Subphylum: Hexapoda										
| Class: Insecta										
| Order: Ephemeroptera										
| Family: Ameletidae	3	20	17	33	8	0	9	180	20	188
| Family: Baetidae	73	120	551	166	254	417	296	260	90	147
| Family: Ephemerellidae	16	45	267	184	130	233	30	80	50	53
| Family: Heptageniidae	149	700	3033	4117	1677	3084	618	2100	625	847
| Family: Leptophlebiidae	0	0	0	0	8	0	0	0	0	0
| Order: Plecoptera	2	0	50	0	0	0	1	10	0	6
| Family: Capniidae	5	70	17	67	46	83	4	40	10	41
| Family: Chloroperlidae	45	60	150	284	77	183	56	50	55	89
| Family: Leuctridae	2	0	17	0	15	17	4	30	0	0
| Family: Nemouridae	47	60	216	17	185	167	39	130	175	213
| Family: Perlidae	17	0	50	34	30	67	8	0	5	18
| Family: Perlodidae	4	10	33	17	54	67	17	0	0	6
| Family: Taeniopterygidae	63	470	483	367	177	667	135	370	200	182
| Order: Trichoptera	4	0	0	0	0	0	13	0	0	0
| Family: Brachycentridae	26	0	150	17	23	33	9	0	175	6
| Family: Glossosomatidae	2	0	17	17	70	100	10	0	70	6
| Family: Hydropsychidae	10	0	17	17	53	67	30	30	20	0
| Family: Lepidostomatidae	0	0	0	0	100	0	22	0	0	0
| Family: Limnephilidae	0	0	0	0	0	17	0	0	0	0
| Family: Rhyacophilidae	1	0	17	0	0	0	0	0	0	0

Taxa	Site									
	WAP02	WAP03	WAP04	WAP05	WAP06	WAP07	WAP08	WAP09	GHO01	GHO02
| Order: Coleoptera										
| Family: Dytiscidae	0	0	0	0	0	0	9	0	0	0
| Family: Elmidae	3	0	100	17	30	33	4	0	0	0
| Order: Diptera										
| Family: Athericidae	0	0	0	0	0	0	4	0	0	0
| Family: Ceratopogonidae	0	0	0	0	0	0	0	0	0	6
| Family: Chironomidae	16	5	67	17	39	67	142	0	45	107
| Family: Empididae	0	0	0	0	8	0	13	0	0	0
| Family: Oreoleptidae	1	0	0	0	0	0	0	30	0	0
| Family: Psychodidae	0	0	17	0	15	17	0	0	0	0
| Family: Simuliidae	2	0	0	0	0	0	0	0	5	0
| Family: Tipulidae	0	0	67	17	31	17	4	20	10	6
| Order: Hemiptera										
| Family: Corixidae	0	0	0	0	16	17	0	0	0	6
| Order: Thysanoptera	1	0	0	0	0	0	0	0	0	0
Subphylum: Chelicerata										
| Class: Arachnida										
| Order: Trombidiformes	0	5	0	0	0	0	0	0	0	0
| Family: Hygrobatidae	4	0	0	17	8	0	4	0	0	6
| Family: Lebertiidae	1	5	0	0	15	0	9	0	0	0
| Family: Sperchontidae	0	0	0	0	8	0	13	0	0	0
| Family: Torrenticolidae	0	0	33	0	0	0	0	0	0	0
Suborder: Prostigmata										
| Family: Stygothrombidiidae	0	0	0	0	8	0	0	0	0	0
Phylum: Mollusca										
| Class: Bivalvia										

Taxa	Site									
	WAP02	**WAP03**	**WAP04**	**WAP05**	**WAP06**	**WAP07**	**WAP08**	**WAP09**	**GHO01**	**GHO02**
| Order: Veneroida										
| Family: Pisidiidae	0	0	0	0	0	0	0	0	5	0
| Class: Gastropoda	0	0	0	0	8	0	0	0	0	0
Phylum: Annelida										
Subphylum: Clitellata										
| Class: Oligochaeta										
| Order: Lumbriculida										
| Family: Lumbriculidae	1	10	0	0	0	0	0	10	15	6
Totals:	**498**	**1580**	**5369**	**5405**	**3093**	**5353**	**1503**	**3340**	**1575**	**1939**
<u>Taxa present but not included</u>:										
Phylum: Arthropoda										
Subphylum: Crustacea										
| Class: Ostracoda	1	0	0	0	8	17	4	10	5	6
Phylum: Annelida										
Subphylum: Clitellata										
| Class: Oligochaeta										
| Order: Tubificida										
| Family: Lumbricidae	0	0	0	0	0	0	0	0	5	0
Phylum: Nemata	1	0	17	0	8	17	0	0	5	0
Phylum: Platyhelminthes										
| Class: Turbellaria	0	0	17	0	8	17	1	10	0	6
Totals:	**2**	**0**	**34**	**0**	**24**	**51**	**5**	**20**	**15**	**12**

Appendix E

Metric Indices of the Aquatic Fauna

(Genus/Species Level)

Metric	Site									
	WAP02	**WAP03**	**WAP04**	**WAP05**	**WAP06**	**WAP07**	**WAP08**	**WAP09**	**GHO01**	**GHO02**
Richness Measures										
Species Richness	40	20	28	23	43	31	41	21	32	33
EPT Richness	27	17	21	19	29	24	25	17	22	20
Ephemeroptera Richness	11	9	7	8	9	8	7	7	8	7
Plecoptera Richness	11	8	10	8	13	11	13	9	9	11
Trichoptera Richness	5	0	4	3	7	5	5	1	5	2
Chironomidae Richness	5	1	3	1	3	3	8	0	4	8
Oligochaeta Richness	1	1	0	0	0	0	0	1	2	1
Abundance Measures										
Corrected Abundance	498	1580	5369	5405	3093	5353	1494	3340	1575	1939
EPT Abundance	469	1555	5085	5337	2907	5202	1292	3280	1495	1802
Dominance Measures										
1st Dominant Taxon	*Rhithrogena*	*Rhithrogena*	*Rhithrogena*	*Rhithrogena*	Heptageniidae	*Rhithrogena*	Heptageniidae	*Rhithrogena*	*Rhithrogena*	*Rhithrogena*
1st Dominant Abundance	79	567	2827	3719	1062	2987	470	1680	345	677
2nd Dominant Taxon	Taeniopterygidae	Taeniopterygidae	Taeniopterygidae	Taeniopterygidae	*Rhithrogena*	Taeniopterygidae	*Baetis*	*Cinygmula*	Heptageniidae	*Ameletus*
2nd Dominant Abundance	64	470	508	367	615	650	203	280	260	188
3rd Dominant Taxon	*Cinygmula*	*Epeorus*	*Baetis rhodani* group	*Sweltsa*	*Baetis rhodani* group	*Baetis*	*Rhithrogena*	*Baetis*	Taeniopterygidae	Taeniopterygidae
3rd Dominant Abundance	60	100	439	267	202	250	148	260	190	184
% 1 Dominant Taxon	15.96%	35.86%	52.65%	68.80%	34.34%	55.81%	31.46%	50.30%	21.90%	34.92%
% 2 Dominant Taxon	12.79%	29.75%	9.46%	6.79%	19.88%	12.14%	13.57%	8.38%	16.51%	9.70%
% 3 Dominant Taxon	11.97%	6.33%	8.19%	4.94%	6.53%	4.67%	9.91%	7.78%	12.06%	9.49%
Percent Dominance	40.71%	71.94%	70.30%	80.53%	60.75%	72.62%	54.94%	66.47%	50.48%	54.10%

Metric	Site									
	WAP02	WAP03	WAP04	WAP05	WAP06	WAP07	WAP08	WAP09	GHO01	GHO02
Community Composition										
% Ephemeroptera	48.39%	56.01%	72.04%	83.26%	67.15%	69.76%	63.72%	78.44%	49.84%	63.69%
% Plecoptera	37.15%	42.41%	18.92%	14.54%	18.88%	23.37%	17.54%	18.86%	28.25%	28.62%
% Trichoptera	8.63%		3.74%	0.94%	7.95%	4.05%	5.22%	0.90%	16.83%	0.62%
% EPT	94.18%	98.42%	94.71%	98.74%	93.99%	97.18%	86.48%	98.20%	94.92%	92.93%
% Diptera	3.82%	0.32%	2.81%	0.63%	3.01%	1.89%	10.91%	1.50%	3.81%	6.14%
% Oligochaeta	0.20%	0.63%						0.30%	0.95%	0.31%
% Baetidae	14.66%	7.59%	10.26%	3.07%	8.21%	7.79%	19.75%	7.78%	5.71%	7.58%
% Chironomidae	3.21%	0.32%	1.25%	0.31%	1.26%	1.25%	9.50%		2.86%	5.52%
% Odonata										
Functional Group Composition										
% Predators	16.37%	5.06%	7.06%	7.14%	8.73%	6.87%	9.49%	2.72%	5.40%	6.24%
% Shredder-Herbivores	24.44%	37.97%	14.36%	8.34%	15.16%	17.13%	11.87%	12.47%	23.81%	23.04%
% Collector-Gatherers	21.34%	12.34%	18.98%	7.72%	15.65%	13.71%	31.17%	15.87%	12.83%	24.73%
% Scrapers	30.36%	44.30%	56.81%	76.48%	56.74%	59.48%	41.69%	62.87%	44.13%	43.99%
% Macrophyte-Herbivore										
% Collector-Filterer	6.82%		2.79%	0.31%	1.71%	1.55%	3.77%	0.90%	12.06%	0.31%
% Omnivore	0.46%	0.32%			2.00%	0.93%	2.01%	5.17%	1.78%	1.10%
% Parasite										
% Piercer-Herbivore										
% Gatherer										
% Unclassified	0.20%					0.32%				0.58%
Functional Group Richness										
Predators Richness	11	4	9	8	16	9	14	4	6	7
Shredder-Herbivores Richness	7	5	5	3	6	4	7	7	5	7
Collector-Gatherers Richness	13	7	10	7	12	9	12	5	10	13
Scrapers Richness	4	3	3	4	5	4	3	3	5	3
MH Richness	0	0	0	0	0	0	0	0	0	0
CF Richness	3	0	1	1	2	2	4	1	4	1
OM Richness	1	1	0	0	2	2	1	1	2	1

Metric	Site									
	WAP02	WAP03	WAP04	WAP05	WAP06	WAP07	WAP08	WAP09	GHO01	GHO02
PA Richness	0	0	0	0	0	0	0	0	0	0
Piercer-Herbivore Richness	0	0	0	0	0	0	0	0	0	0
Gatherer Richness	0	0	0	0	0	0	0	0	0	0
Unclassified	1	0	0	0	0	1	0	0	0	1
Diversity/Evenness Measures										
Shannon-Weiner H' (log 10)	1.26	0.86	0.86	0.61	1.08	0.82	1.12	0.84	1.10	1.09
Shannon-Weiner H' (log 2)	4.18	2.85	2.85	2.01	3.57	2.72	3.73	2.79	3.67	3.63
Shannon-Weiner H' (log e)	2.90	1.98	1.98	1.40	2.48	1.89	2.59	1.93	2.54	2.51
Simpson's Index (D)	0.08	0.23	0.30	0.48	0.17	0.33	0.14	0.28	0.12	0.16
Simpson's Index of Diversity (1 - D)	0.92	0.77	0.70	0.52	0.83	0.67	0.86	0.72	0.88	0.84
Simpson's Reciprocal Index (1/D)	12.40	4.38	3.35	2.06	5.85	3.00	7.04	3.59	8.51	6.40
Biotic Indices										
Hilsenhoff Biotic Index	2.26	1.38	1.08	0.59	2.54	1.08	3.43	1.14	2.02	1.71

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.

Taxa	Site									
	GHO01	GHO02	WAP02	WAP03	WAP04	WAP05	WAP06	WAP07	WAP08	WAP09
INSECTS										
Order: Coleoptera										
Dytiscidae										
Liodessus									Morph	
L. affinus	eDNA				eDNA	eDNA	eDNA	eDNA		
L. obscurellus	eDNA				eDNA	eDNA	eDNA	eDNA	eDNA	
Elmidae			Morph		Morph		Morph	Morph	Morph	
Heterlimnius			Morph		Morph	Morph	Morph			
Order: Diptera										
Athericidae										
Atherix									Morph	
Ceratopogonidae		Morph								
Dasyhelea modesta			eDNA							
Forcipomyia bipunctata			eDNA							
Chironomidae	Morph	Morph	Morph				Morph		Morph	
Ablabesmyia aspera									eDNA	
Chironomus atrella		eDNA								
Cladotanytarsus									Morph	
Conchapelopia pallens	eDNA									
Constempellina							Morph			
Micropsectra	Morph	Morph								
M. lacustris							eDNA			eDNA
M. logani							eDNA		eDNA	
M. nigripila										eDNA
M. subletteorum										eDNA
Microtendipes									Morph	
Pagastia	Morph	Morph	Morph							
Polypedilum bullum							eDNA	eDNA	eDNA	
P. tuberculum						eDNA			eDNA	
Rheopelopia ornata	eDNA								eDNA	
Sublettea									Morph	
Tanytarsus mendax	eDNA							eDNA		
T. volgensis		eDNA								
Orthocladiinae								Morph	Morph	
Brillia			Morph							
Corynoneura artica										eDNA

Taxa	Site									
	GHO01	GHO02	WAP02	WAP03	WAP04	WAP05	WAP06	WAP07	WAP08	WAP09
Cricotopus sylvestris							eDNA			
Eukiefferiella	Morph	Morph	Morph	Morph				Morph		
E. claripennis	eDNA	eDNA	eDNA	eDNA			eDNA		eDNA	
Heterotrissocladius									Morph	
Hydrobaenus									Morph	
Krenosmittia					Morph					
K. halvorseni			eDNA							
Limnophyes		Morph								
L. ninae										eDNA
Nanocladius anderseni									eDNA	
Orthocladius	Morph	Morph	Morph		Morph		Morph		Morph	
O. glabripennis	eDNA		eDNA						eDNA	
Parametriocnemus							Morph			
Paraphaenocladius impensus		eDNA					eDNA			
Synorthocladius semivirens									eDNA	
Thienemanniella		Morph								
Tvetenia		Morph	Morph		Morph	Morph		Morph		
T. paucunca			eDNA		eDNA	eDNA	eDNA	eDNA	eDNA	
Empididae									Morph	
Clinocera lineata										eDNA
Neoplasta							Morph			
N. megorchis									eDNA	
Oreoleptidae										
Oreoleptis			Morph							Morph
Psychodidae										
Pericoma/Telmatoscopus					Morph		Morph	Morph		
Simuliidae	Morph									
Simulium			Morph							
S. apricarium	eDNA			eDNA						
S. arcticum	eDNA	eDNA	eDNA	eDNA	eDNA		eDNA		eDNA	
S. defoliarti									eDNA	
S. joculator								eDNA		
S. negativum									eDNA	
S. tuberosum			eDNA		eDNA	eDNA			eDNA	
Tipulidae										
Dicranota	Morph									Morph
Hexatoma	Morph				Morph	Morph	Morph	Morph	Morph	Morph
Tipula mainensis									eDNA	
Order: Ephemeroptera										
Ameletidae										
Ameletus	Morph	Morph	Morph	Morph	Morph	Morph	Morph		Morph	Morph
A. bellulus			eDNA	eDNA				eDNA		
A. celer	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA			eDNA	eDNA
A. subnotatus							eDNA	eDNA		

Taxa	Site									
	GHO01	GHO02	WAP02	WAP03	WAP04	WAP05	WAP06	WAP07	WAP08	WAP09
Baetidae	Morph	Morph	Morph		Morph		Morph		Morph	
Acentrella			Morph	Morph			Morph	Morph	Morph	
A. insignificans	eDNA					eDNA	eDNA		eDNA	
A. turbida	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	
Baetis	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph
B. bicaudatus	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA
B. fuscatus group	Morph		Morph	Morph	Morph	Morph	Morph			
B. phoebus	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	
B. rhodani group	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph	
B. tricaudatus	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA
Diphetor hageni	eDNA								eDNA	
Caenidae										
Caenis amica					eDNA					
Ephemerellidae	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph		Morph
Drunella	Morph									
D. coloradensis	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA
D. doddsii	Both	Both	Both	Both	Both	Both	Both	Both	eDNA	Both
D. flavilinea								eDNA		
D. grandis	eDNA	eDNA					eDNA			
Ephemerella	Morph						Morph		Morph	
E. tibialis	eDNA	eDNA	eDNA		eDNA		eDNA	eDNA		
Heptageniidae	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph
Cinygmula	Both	Both	Both	Both	eDNA	Both	eDNA	Both	eDNA	Both
C. mimus								eDNA	eDNA	
Ecdyonurus simplicioides								eDNA	eDNA	
Epeorus			Morph	Morph	Morph	Morph		Morph		Morph
E. albertae								eDNA	eDNA	
E. deceptivus	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA		eDNA
E. grandis				eDNA		eDNA				eDNA
E. longimanus	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA		eDNA	eDNA	
Heptagenia solitaria									eDNA	
Rhithrogena	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph
R. impersonata	eDNA	eDNA		eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	
R. robusta	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA
Leptophlebiidae							Morph			
Paraleptophlebia heteronea	eDNA	eDNA				eDNA	eDNA	eDNA	eDNA	
P. memorialis									eDNA	
Siphlonuridae										
Siphlonurus occidentalis							eDNA			eDNA
Order: Hemiptera										
Corixidae										
Callicorixa		Morph								
C. alaskensis										eDNA
C. audeni										eDNA

Taxa	Site									
	GHO01	GHO02	WAP02	WAP03	WAP04	WAP05	WAP06	WAP07	WAP08	WAP09
Palmacorixa							Morph	Morph		
Sigara							Morph			
S. conocephala	eDNA	eDNA					eDNA	eDNA		
S. decoratella	eDNA									
Order: Plecoptera		Morph	Morph		Morph					Morph
Capniidae	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph
Capnia coloradensis					eDNA		eDNA		eDNA	eDNA
C. confusa	eDNA				eDNA	eDNA	eDNA		eDNA	eDNA
C. gracilaria	eDNA	eDNA	eDNA		eDNA	eDNA	eDNA		eDNA	eDNA
C. petila		eDNA				eDNA	eDNA			eDNA
Eucapnopsis brevicauda	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA
Utacapnia columbiana	eDNA								eDNA	eDNA
U. logana	eDNA				eDNA	eDNA			eDNA	
Chloroperlidae	Morph	Morph	Morph	Morph	Morph	Morph	Morph		Morph	Morph
Alloperla serrata				eDNA						eDNA
A. severa	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA		eDNA	eDNA	
Haploperla										Morph
Neaviperla		Morph								
Paraperla frontalis										eDNA
Plumiperla		Morph								
P. diversa	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA
Suwallia teleckojensis	eDNA	eDNA		eDNA						eDNA
Sweltsa	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph
S. borealis	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA		eDNA
S. coloradensis	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	
S. naica								eDNA	eDNA	
Leuctridae	Morph	Morph	Morph		Morph		Morph	Morph	Morph	Morph
Paraleuctra occidentalis	eDNA	eDNA	eDNA		eDNA	eDNA	eDNA	eDNA	eDNA	
Nemouridae	Morph	Morph	Morph	Morph	Morph				Morph	Morph
Prostoia besametsa	eDNA	eDNA		eDNA	eDNA	eDNA		eDNA	eDNA	eDNA
Visoka cataractae							Morph			eDNA
Zapada		Morph	Morph	Morph					Morph	Morph
Z. cinctipes	Both	Both	Both	Both	Both	Both	Both	Both	Both	eDNA
Z. columbiana	Both	Both	eDNA	eDNA	eDNA	eDNA	eDNA			eDNA
Z. haysi	eDNA	eDNA	eDNA	eDNA			eDNA			eDNA
Z. oregonensis	Morph	Morph				eDNA		eDNA		Morph
Perlidae		Morph	Morph	Morph			Morph	Morph	Morph	
Acroneuria lycorias								eDNA	eDNA	eDNA
Doroneuria			Morph			Morph		Morph	Morph	
D. theodora		eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA		
Hesperoperla	Morph		Morph		Morph	Morph	Morph	Morph		
H. pacifica	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	
Perlodidae		Morph	Morph	Morph			Morph	Morph	Morph	
Isogenoides					Morph		Morph	Morph	Morph	
I. frontalis		eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	

Taxa	Site									
	GHO01	GHO02	WAP02	WAP03	WAP04	WAP05	WAP06	WAP07	WAP08	WAP09
Isoperla petersoni	eDNA	eDNA					eDNA			
I. sobria		eDNA								
Kogotus							Morph			
K. modestus	eDNA	eDNA	eDNA		eDNA		eDNA		eDNA	
Megarcys signata			eDNA	eDNA	eDNA	eDNA	eDNA	eDNA		eDNA
M. subtruncata				eDNA						eDNA
M. watertoni				eDNA					eDNA	eDNA
Setvena bradleyi										eDNA
Skwala						Morph				
Pteronarcyidae										
Pteronarcella badia		eDNA								
Taeniopterygidae	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph	Morph
Doddsia occidentalis	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA
Taenionema	Morph						Morph	Morph	Morph	Morph
T. pacificum	eDNA	eDNA				eDNA			eDNA	
T. pallidum								eDNA		
Order: Trichoptera			Morph						Morph	
Brachycentridae	Morph	Morph	Morph		Morph	Morph	Morph	Morph	Morph	
Brachycentrus americanus	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	Both	eDNA	eDNA	
Glossosomatidae	Morph	Morph	Morph				Morph	Morph	Morph	
Glossosoma	Morph				Morph	Morph	Morph	Morph	Morph	
G. alascense	eDNA			eDNA		eDNA	eDNA	eDNA		
G. pyroxum	eDNA	eDNA		eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	
G. traviatum							eDNA	eDNA	eDNA	
G. velonum							eDNA			
G. wenatchee	eDNA	eDNA		eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	
Hydropsychidae	Morph		Morph				Morph	Morph	Morph	Morph
Arctopsyche			Morph		Morph	Morph	Morph	Morph	Morph	
A. grandis	eDNA		eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA
A. inermis								eDNA	eDNA	
Ceratopsyche alhedra								eDNA		
C. alternans					eDNA	eDNA				
C. bronta									eDNA	
C. cockerelli								eDNA		
C. oslari							eDNA		eDNA	
C. slossonae						eDNA	eDNA	eDNA	eDNA	
Hydropsyche oslari									eDNA	
Parapsyche elsis	Both	eDNA		eDNA					eDNA	eDNA
Hydroptilidae										
Agraylea saltesea		eDNA		eDNA						eDNA
Lepidostomatidae										
Lepidostoma							Morph		Morph	
L. cascadense	eDNA	eDNA			eDNA	eDNA		eDNA		
L. pluviale		eDNA			eDNA		eDNA	eDNA	eDNA	
L. unicolor	eDNA	eDNA								

Taxa	Site									
	GHO01	GHO02	WAP02	WAP03	WAP04	WAP05	WAP06	WAP07	WAP08	WAP09
Limnephilidae								Morph		
Ecclisomyia conspersa		eDNA								eDNA
Hesperophylax magnus					eDNA					
Onocosmoecus unicolor	eDNA						eDNA	eDNA		
Rhyacophilidae										
Rhyacophila angelita	eDNA						eDNA	eDNA		
R. brunnea			eDNA		eDNA			eDNA	eDNA	
R. brunnea/vemna group					Morph					
R. hyalinata			Both	eDNA		eDNA				eDNA
R. narvae	eDNA									
R. pellisa					eDNA	eDNA				eDNA
R. vaccua										eDNA
Uenoidae										
Neophylax splendens		eDNA					eDNA			
ARACHNIDS										
Order: Oribatida										
Phthiracaridae										
Phthiracarus longulus	eDNA									
Steganacaridae										
Atropacarus striculus	eDNA			eDNA						
Order: Trombidiformes			Morph							
Hygrobatidae										
Atractides		Morph	Morph			Morph	Morph		Morph	
Hygrobates			Morph							
Lebertiidae										
Lebertia			Morph	Morph			Morph		Morph	
Sperchontidae										
Sperchon							Morph		Morph	
Stygothrombidiidae										
Stygothrombium							Morph			
Torrenticolidae										
Testudacarus					Morph					
BIVALVE MOLLUSCS										
Order: Veneroida										
Pisidiidae	Morph									
OLIGOCHAETE WORMS										
Order: Lumbriculida										
Lumbriculidae	Morph	Morph	Morph							
Rhynchelmis	Morph			Morph						Morph
NEMATODES										
Order: Plectida										
Plectidae										
Plectus aquatilis								eDNA		