

Ghost Watershed
Water Monitoring Program
CABiN/STREAM Project
2022



BIOTA CONSULTANTS

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CABiN/STREAM Project
2022

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Cover photo credit: Sharlene Fritz – Measuring velocity at Meadow Creek site

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Executive Summary

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

In 2019, GWAS began participation in the STREAM (Sequencing the Rivers for Environmental Assessment and Monitoring) three-year pilot project, which uses CABiN methods to collect samples to identify benthic macro-invertebrates using environmental DNA (eDNA). GWAS then developed a multi-year water monitoring plan that incorporated the STREAM pilot project.

The water monitoring program began in the fall of 2020 when Biota Consultants was contracted to oversee the sampling of ten sites, eight along Waiparous Creek (WAP02 to WAP09) and two on the Ghost River (GHO01 and GHO02). In the second year (2021) of the program, the focus was the Ghost River (GHO03 to GHO07), but included one site on Johnson Creek (JOH01), whose headwaters had been affected by the Devil's Head/Black Rock wildfire (CWF-156-2020) in fall, 2020. In addition, sites WAP02 and WAP03, that were sampled in 2020 below and above the confluence with Johnson Creek, were resampled.

In 2022, the focus of the sampling program was tributaries of Waiparous Creek. This included resampling the Johnson Creek site from 2021, as well as sites WAP02 and WAP03. Human disturbance to the channel precluded sampling WAP02 at exactly the same location as in previous years. The 2022 location is referred to as WAP02a.

Field sampling occurred between August 30th and September 12th. The reach at four of the seven sites was long enough to allow triplicate kicknet samples for use in the eDNA analysis, plus a fourth kicknet sample for morphological analysis. This provided data on benthic macro-invertebrate abundance, required to determine the EPT ratio, among other metrics. The reach on Lookout Creek allowed only a single kicknet (for morphological analysis), whereas two kicknets were possible on Aura Creek and Margaret Creek, one for morphological analysis and one for eDNA analysis.

Based on lab analyses and direct measurements, water quality was within the parameters acceptable for benthic macroinvertebrates and fish. The chemical and physical attributes were well below exceedance levels. Total suspended solids and turbidity were very low except at Lookout Creek.

The Simpson's Index of Diversity and the Shannon-Weiner Index indicate the sites were diverse in their community composition. The Hilsenhoff Biotic Index suggests there was possible slight organic pollution at four of the tributary sites (rating of very good), whereas organic pollution was unlikely (rating of excellent) at Margaret Creek and the two Waiparous Creek sites.

The EPT ratio indicates high water quality at most of the sites, with EPT species more abundant than the pollution-tolerant chironomid family. An exception was JOH01 where the ratio was 0.51 indicating degraded conditions. This may be related to the high off-highway vehicle activity upstream of the site.

The more tolerant Hydropsychidae within the Trichoptera was found only at JOH01, WAP02a and WAP03, and was highest at WAP03. Baetidae is a more tolerant family within the Ephemeroptera. It was identified at all sites but in low abundance except at the Meadow, Margaret and Lookout creek sites.

The proportion of functional feeding groups (FFGs) varied among the sites, reflecting the habitat and adjacent riparian vegetation. Scrapers dominated in Aura Creek and Waiparous Creek, collector-gatherers were prominent in Meadow Creek and Johnson Creek, and shredders were highest at the Margaret Creek site.

The EPT ratio at JOH01 was the lowest of all sites. However, benthic macroinvertebrate diversity was high. The Hilsenhoff Biotic Index rated water quality as very good, and physical and chemical attributes of the water were below the exceedance criteria. Although only the EPT ratio suggested poor water quality, further investigation along Johnson Creek is advised.

The stream channel differed at WAP02/02a and WAP03 from 2020 to 2022 as a result of natural processes and human-caused disturbances. The Hilsenhoff Biotic Index was within the excellent water quality category in all three years, and the EPT ratio differed only slightly. The proportion of functional feeding groups varied among the years. The changes in the stream channel may explain these differences.

The results of the 2022 field sampling provide a baseline for comparison in future years. With more data, trends may become apparent. If issues with water quality are suggested, sampling effort may become more focussed.

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1.0 Introduction

1.1 Background

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

In 2019, GWAS began participating in a three-year environmental DNA (eDNA) project called STREAM (Sequencing the Rivers for Environmental Assessment and Monitoring), 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 and diversity. Through STREAM, rather than quantifying abundance, water samples are submitted for eDNA testing to determine presence or absence of benthic macroinvertebrate species.

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

The water monitoring program began in the fall of 2020 when ten sites were sampled, eight along Waiparous Creek (WAP02 to WAP09) and two on the Ghost River (GHO01 and GHO02). In this first year of the plan, the focus was mainly on sites above and below creek tributaries and

other possible point source sites which might affect water quality as a result of land use activities (see Biota Consultants 2022a). In the second year (2021), the focus was the Ghost River (sites GHO03 to GHO07), with a slight modification due to the Devil's Head/Black Rock wildfire (CWF-156-2020)¹, which occurred in the fall of 2020 (see Biota Consultants 2022b). Since the fire encroached on the southwest fork of the headwaters of Johnson Creek in the Waiparous Creek sub-basin, it was decided to sample this creek above its confluence with Waiparous Creek (JOH01). In addition, the paired sites on Waiparous Creek that were sampled in 2020 below and above the confluence with Johnson Creek, WAP02 and WAP03, were resampled.

1.2 Field Plan

The focus of the water monitoring program in 2022 was on tributaries to Waiparous Creek: Aura Creek (AUR01), Meadow Creek (MEA01), Lookout Creek (LOO01) and Margaret Creek (MAR01). In addition, JOH01, WAP02 and WAP03 were resampled to provide further monitoring data after the Devil's Head/Black Rock wildfire.

2.0 Methods

2.1 Field Sampling

The field sampling followed the same CABI¹N and STREAM protocols as in 2020, described in *Ghost Watershed Water Monitoring Program CABI¹N/STREAM Project 2020* (Biota Consultants 2022a), with a few exceptions. Normally three kicknet samples are collected for the STREAM eDNA analysis; however, this was only possible at four of the seven sites where the reach was long enough to fit these plus one kicknet sample for the morphological analysis. It was possible to fit a single kicknet sample for eDNA at the sites on Aura and Margaret creeks, but not on Lookout Creek. Therefore, only morphological data are available for the Lookout Creek site.

Field sampling occurred between August 30th and September 12th when there was low stream flow and mainly stable sunny weather conditions. The CABI¹N Field Sheet is included in Appendix A. Site locations are mapped in Figure 1, and site name codes, date of sampling, and geographic locations are presented in Table 1. Air and water temperatures at the time of sampling are provided. At WAP02, sampling could not be done in the exact location as the previous two years (see section 3.1.6). The site in 2022 is therefore referred to as WAP02a.

¹ Code assigned by Alberta Agriculture and Forestry.

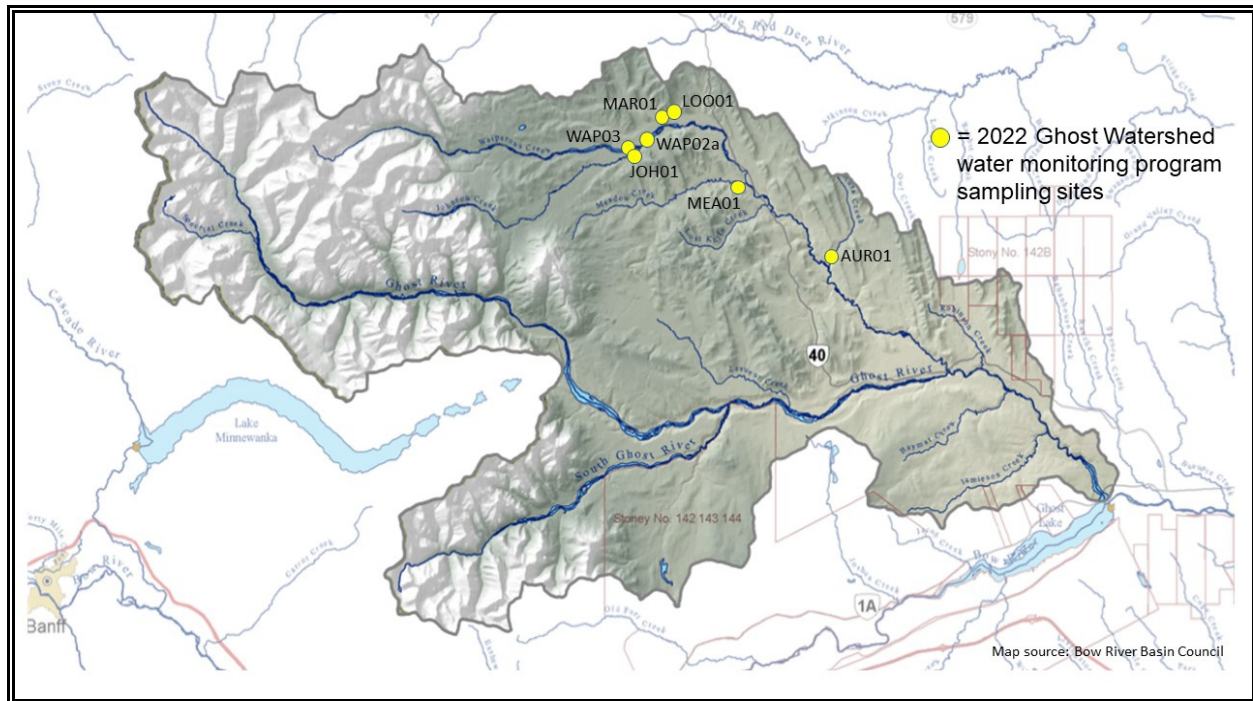


Figure 1. Sampling locations in 2022 within the Ghost River watershed.

Table 1. Location of sites sampled in 2022, plus sampling date, time of day, and conditions.

Code/ Date	Latitude	Longitude	Elevation (m)	Comments
JOH01 Aug. 30	51.3916°	-115.0895°	1569	Johnson Creek above confluence with Waiparous Creek Morning Sunny, air temperature 21.0°C, water temperature 9.8°C
MEA01 Aug. 30	51.3746°	-115.0027°	1446	Meadow Creek above confluence with Waiparous Creek Afternoon Sunny, air temperature 28.5°C, water temperature 15.0°C
WAP02a Sept. 7	51.3944°	-115.0860°	1559	Waiparous Creek below confluence with Johnson Creek Morning/afternoon Sun and cloud, air temperature 21.5°C, water temperature 12.7°C
WAP03 Sept. 7	51.3925°	-115.0892°	1565	Waiparous Creek above confluence with Johnson Creek Afternoon Sun and cloud, air temperature 23.0°C, water temperature 15.7°C
LOO01 Sept. 9	51.4060°	-115.0623°	1534	Lookout Creek above confluence with Waiparous Creek Morning Sun and cloud, air temperature 7.5°C, water temperature 7.8°C
MAR01 Sept. 9	51.4034°	-115.0726°	1541	Margaret Creek above confluence with Waiparous Creek Afternoon Sun and cloud, air temperature 10.5°C, water temperature 9.1°C
AUR01 Sept. 12	51.3341°	-114.9350°	1379	Aura Creek above confluence with Waiparous Creek Afternoon Sun and cloud, air temperature 14.0°C, water temperature 8.1°C

When sampling the paired sites on Waiparous Creek, the downstream site (WAP02a) was sampled prior to the upstream site (WAP03) to ensure that the downstream site was not disturbed by upstream activities.

Biological sampling followed the CABiN/STREAM protocols used in 2020 (Biota Consultants 2022a), with two minor modifications. As in 2021, Absolute Zero RV waterline antifreeze was used to preserve the eDNA samples instead of 95% ethanol. In addition, the sample jars were sealed by winding a strip of parafilm tightly around the outside of the jar and lid. The description of physical attributes of each site and the collection of water chemistry data followed the same protocols described by Biota Consultants (2022a).

2.2 Data Entry

All of the data, except the benthic macroinvertebrate community structure information, were entered into the CABiN database by the Project Manager. To reduce potential errors, the morphologic consultant (Cordillera Consulting Inc.) uploaded the benthic macroinvertebrate community data. The STREAM eDNA data will be uploaded by ECCC staff into the shared STREAM study within the CABiN database.

3.0 Results and Discussion

3.1 Physical Characteristics

The physical characteristics of the seven sample sites are presented in Table 2, ordered from downstream to upstream within the watershed. Substrate embeddedness refers to how deeply the dominant substrate is buried in the surrounding finer particles. Five categories of substrate embeddedness² were used. In areas modified by streamside activities (anthropogenic land uses), increased erosion can result in the accumulation of fine material in the interstitial spaces. The more embedded the substrate, the fewer interstitial spaces for macroinvertebrates to occupy, which can reduce productivity (Environment Canada 2012).

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

Table 2. Physical characteristics of the sample sites.

Attributes	Site						
	AUR01	MEA01	LOO01	MAR01	JOH01	WAP02a	WAP03
Elevation (m)	1379	1446	1534	1541	1569	1554	1560
Bankfull width (m)	4.7	5.6	7.01	8	6.2	15.3	18.5
Wetted width (m)	2.19	4.82	1.63	3.38	5.4	7.78	4.18
Bankfull wetted depth (cm)	35	69.5	63.5	58.6	7.4	32.7	55
Maximum channel depth (cm)	7.2	20.4	12	14.6	23.8	42.6	32.8
Average channel depth (cm)	5.9	17.5	9.4	10.6	21.8	23.8	26.8
Maximum velocity (m/s)	0.4202	1.084	0.7672	0.5603	0.7799	1.0759	1.2838
Average velocity (m/s)	0.2007	0.504	0.4751	0.3585	0.6798	0.7192	1.0052
Slope (m/m)	0.0269	0.013	0.0142	0.0175	0.0085	0.0233	0.0087
Substrate embeddedness (%)	25	25	25	0	25	25	0
Dominant substrate (cm)	3.2-6.4	3.2-6.4	1.6-3.2	6.4-12.8	6.4-12.8	6.4-12.8	6.4-12.8
2 nd dominant substrate (cm)	1.6-3.2	6.4-12.8	3.2-6.4	3.2-6.4	3.2-6.4	3.2-6.4	12.8-25.6
Surrounding material (cm)	0.2-1.6	0.2-1.6	0.1-0.2	0.2-1.6	0.1-0.2	0.2-1.6	0.2-1.6
Geometric median particle size (cm)	3.1	5.8	2.9	5.2	7.1	5.3	10
% Sand	0	0	0	0	0	0	0
% Gravel	20	3	12	5	1	2	1
% Pebble	63	49	78	52	44	50	21
% Cobble	16	31	10	43	50	46	67
% Boulder	1	5	0	0	5	0	11
% Bedrock	0	11	0	0	0	2	0

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

3.1.1 Aura Creek

The Aura Creek site was situated just upstream of the Waiparous Creek flood plain. Aura Creek is a small tributary of Waiparous Creek and had one of the lowest wetted widths of all sites sampled, at 2.19 m, with an average channel depth of only 5.9 cm. It also had the lowest velocity of all sites sampled (Table 2).

3.1.2 Meadow Creek

The Meadow Creek site was situated just above its confluence with Waiparous Creek. There was a random campsite above the north bank beside an undesignated off-highway vehicle (OHV) trail. The higher proportion of bedrock in the stream set this site apart from other sites (Table 2).

3.1.3 Lookout Creek

The Lookout Creek site was situated approximately 1 km upstream from its confluence with Waiparous Creek. It was difficult to select an appropriate site to sample due to numerous old

beaver dams with deeply incised channels and muddy banks. The reach chosen was relatively small in length, with the narrowest wetted width of all the sites at 1.63 m, and the smallest dominant substrate class, in the small pebble category (1.6-3.2 cm) (Table 2).

3.1.4 Margaret Creek

The Margaret Creek site was approximately 160 m upstream from its confluence with Waiparous Creek and 110 m upstream from the bridge on Waiparous Valley Road. It was another relatively small and shallow creek (Table 2).

3.1.5 Johnson Creek

The Johnson Creek sampling location was approximately 220 m upstream from its confluence with Waiparous Creek. It was first sampled in 2021; however, the exact location of the tape, when it was stretched across the creek to determine the physical attributes, was not identical, which will have contributed to the variation in these data between years (Table 3). Average velocity was higher in 2022, but median particle size was smaller, with more pebbles and fewer cobbles.

Table 3. Comparison of physical attributes at the Johnson Creek site (JOH01) in 2021 and 2022.

Attributes	Site and Date of Sampling	
	JOH01	
	Sept. 7, 2021	Aug. 30, 2022
Elevation (m)	1569	1569
Bankfull width (m)	6.66	6.2
Wetted width (m)	5.64	5.4
Bankfull wetted depth (cm)	23	7.4
Maximum channel depth (cm)	23.5	23.8
Avg channel depth (cm)	18.5	21.8
Maximum velocity (m/s)	0.8287	0.7799
Avg velocity (m/s)	0.5658	0.6798
Slope (m/m)	0.0085	0.0085
Substrate embeddedness (%)	25	25
Dominant substrate (cm)	6.4-12.8	6.4-12.8
2nd dominant substrate (cm)	12.8-25.6	3.2-6.4
Surrounding material (cm)	0.2-1.6	0.1-0.2
Geometric median particle size (cm)	9.6	7.1
% Sand	0	0
% Gravel	0	1
% Pebble	29	44
% Cobble	67	50
% Boulder	4	5
% Bedrock	0	0

3.1.6 Waiparous Creek

2022 was the third consecutive year that sites WAP02 and WAP03 were sampled, below and above the confluence with Johnson Creek, respectively. In 2020, an attempt was made to select reach locations at these two sites with similar stream channel characteristics. However, the heterogeneous nature of the stream channel made this impossible (Table 4). The fluvial action altering the stream channel between years likely contributed to the variation in the data at each site. An additional factor at WAP02 was human interference. This site bordered random campsites. In all three years, alteration of stream flow occurred from placement of rock dams at the edge of the creek. However, in 2022, rock dams also had been created across the creek. The flow at the location of the sampling site in 2020 and 2021 was too highly altered, no longer providing riffle habitat. Therefore, sampling was relocated upstream, with the first kick net location corresponding to the fourth location the previous two years. Since this is a slightly different site, it is referred to as WAP02a.

Table 4. Comparison of physical attributes at Waiparous Creek sites, WAP02/WAP02a and WAP03, in 2020, 2021 and 2022.

Attributes	Site and Date of Sampling					
	WAP02		WAP02a	WAP03		
	Sept. 1 2020	Sept. 2 2021	Sept. 7 2022	Sept. 3 2020	Sept. 2 2021	Sept. 7 2022
Elevation (m)	1554	1554	1554	1560	1560	1560
Bankfull width (m)	17	12.02	15.3	15	21.9	18.5
Wetted width (m)	9.6	10.35	7.78	6.9	8.9	4.18
Bankfull wetted depth (cm)	26.5	17	32.7	56	57	55
Maximum channel depth (cm)	27	21.2	42.6	22	24.2	32.8
Avg channel depth (cm)	17.4	17.2	23.8	16.4	15.2	26.8
Maximum velocity (m/s)	1.253	1.3065	1.0759	1.129	1.085	1.2838
Avg velocity (m/s)	0.876	0.863	0.7192	0.865	0.7305	1.0052
Slope (m/m)	0.014	0.011	0.0233	0.015	0.0087	0.0087
Substrate embeddedness (%)	25	25	25	25	25	0
Dominant substrate (cm)	6.4-12.8	3.2-6.4	6.4-12.8	3.2-6.4	3.2-6.4	6.4-12.8
2nd dominant substrate (cm)	12.8-25.6	6.4-12.8	3.2-6.4	6.4-12.8	6.4-12.8	12.8-25.6
Surrounding material (cm)	0.2-1.6	0.1-0.2	0.2-1.6	0.2-1.6	0.2-1.6	0.2-1.6
Geometric median particle size (cm)	10.3	6.9	5.3	5.9	5.8	10
% Sand	0	0	0	0	0	0
% Gravel	1	0	2	1	1	1
% Pebble	23	49	50	56	57	21
% Cobble	68	51	46	41	37	67
% Boulder	8	0	0	2	2	11
% Bedrock	0	0	2	0	3	0

The depth at the slightly upstream location of WAP02a was greater than the location of WAP02 in 2020 and 2021, plus slope was greater but velocity was less (Table 4). The geometric median particle size of the substrate was reduced each year, corresponding to the decline in velocity.

Continued fluvial action at WAP03 resulted in variations in wetted width, with a narrower, deeper channel in 2022 (Table 4). Correspondingly, the average velocity increased from a low of 0.7305 m/s in 2021 to 1.0052 m/s in 2022. With this higher velocity, the geometric median particle size increased from 5.9 cm and 5.8 cm in the previous two years to 10.0 cm in 2022, and substrate embeddedness declined from the 25% category to the 0% category (average 0.125).

In 2020 and 2021, the velocity was higher at WAP02 than WAP03, possibly due to the extra volume of water from Johnson Creek. The lower velocity at WAP02a is likely due to the two rock dams that were upstream at the time of sampling. The geometric median particle size of the substrate was higher at WAP02 than WAP03 in the previous two years but was the reverse in 2022 at WAP02a. This also could be a result of the two rock dams, reducing velocity and allowing the smaller substrate to accumulate.

3.2 Land Use

Forest habitat was present at all sites and was the dominant adjacent land use at all but the Waiparous Creek sites, where the dominant activities in the surrounding area were random camping, day-use and off-highway vehicle (OHV) use (Table 5). OHV use occurred upstream of all sites to varying degrees. The commercial recreation use upstream of Margaret Creek was Camp Howard.

3.3 Water Attributes and Chemical Analysis

The chemical attributes (i.e., pH, dissolved oxygen, anions, nutrients) along with the physical attributes (i.e., total suspended solids, turbidity, specific conductance, temperature) are presented for each site in Table 6. The chemical analysis suggests that the water quality at the time of sampling was within the parameters acceptable for benthic macroinvertebrates and fish (Government of Alberta 2018). The water quality exceedance criteria, including a brief narrative, are presented in Table 7.

Table 5. Land uses adjacent and upstream of each site. Xs in bold indicate dominant land use(s).

Site	Land Use Location	Land Use							
		Forest	Grazing	Logging	OHV	Day-use	Camping	Shooting	Commercial Recreation
AUR01	Adjacent	x							
	Upstream	x	x	x	x				
MEA01	Adjacent	x	x		x		x	x	
	Upstream	x	x	x	x				
LOO01	Adjacent	x							
	Upstream	x			x		x	x	
MAR01	Adjacent	x				x			
	Upstream	x			x		x		x
JOH01	Adjacent	x				x			
	Upstream	x			x				
WAP02a & WAP03	Adjacent	x			x	x	x		
	Upstream	x			x	x	x		

Table 6. Chemical and physical attributes of water samples at each site.

Tests	Site						
	AUR01	MEA01	LOO01	MAR01	JOH01	WAP02a	WAP03
Total Suspended Solids (mg/L)	<1.0	2	5.8	<1.0	<1.0	<1.0	<1.0
Turbidity (lab) (NTU)	2.5	1.1	16	1.7	0.4	0.3	0.3
Specific Conductance (μS/cm)	459.4	396.1	226.9	289.3	323.1	333.7	339
Air Temperature (°C)	14	28.5	7.5	10.5	21	21.5	23
Water Temperature (°C)	8.1	15	7.8	9.1	9.8	12.7	15.7
Dissolved Oxygen (mg/L)	9.53	8.64	8.82	9.42	9.61	8.49	8.05
pH	8.24	8.45	7.47	8.25	8.23	8.04	8.27
Anions							
Alkalinity (Total as CaCO ₃) (mg/L)	260	230	130	160	170	160	140
Alkalinity (PP as CaCO ₃) (mg/L)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Bicarbonate (HCO ₃) (mg/L)	320	280	150	190	210	200	180
Carbonate (CO ₃) (mg/L)	<1.0	<1.0	<1.0	<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
Nutrients							
Total Phosphorus (P) (mg/L)	<0.0030	<0.0030	0.004	<0.0030	<0.0030	<0.0030	<0.0030
Dissolved Nitrogen (N) (mg/L)	0.36	0.18	0.26	0.09	0.29	0.23	0.14
Dissolved Nitrite (NO ₂) (mg/L)	<0.033	<0.033	<0.033	<0.033	<0.033	<0.033	<0.033
Dissolved Nitrate plus Nitrite (N)	0.01	0.024	0.01	0.014	0.24	0.19	0.11

Note: Lab analyses by Bureau Veritas Laboratories, Calgary, Alberta.

Table 7. 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 7. 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	<p><u>During clear flows or for clear waters:</u> 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).</p> <p><u>During high flow or for turbid waters:</u> 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.</p>
Specific Conductance	-	-	
Turbidity (NTU)	Narrative	Narrative	<p><u>For clear waters:</u> 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).</p> <p><u>For high flow or turbid waters:</u> 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.</p>

Source: Government of Alberta (2018)

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

3.3.1 Alkalinity, Inorganic Carbon, Hardness and pH

A full description of alkalinity, inorganic carbon, hardness and pH is given in the report on the 2020 monitoring program (Biota Consultants 2022a).

Alkalinity, as expressed by the total CaCO_3 , was lowest at LOO01 (130 mg/L) and highest at AUR01 (260 mg/L) (Table 6). These values are well above the minimum 20 mg/L level indicated in Table 7. 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 sites LOO01, MAR01, JOH01, WAP03 and WAP02 would be classified as hard, whereas water at sites AUR01 and MEA01 would be classified as very 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 pH of the samples varied from 7.47 to 8.45, which is in the safe range for acute toxicity according to Government of Alberta (2018) criteria (Table 7). The lowest pH value was at LOO01 which also had the lowest total CaCO_3 and bicarbonate (HCO_3^-) (Table 6).

3.3.2 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 ($\mu\text{S}/\text{cm}$). Specific conductance is measured at, or corrected to, 25°C (Miller *et al.* 1988). Since conductivity increases with temperature, reporting conductivity at the reference temperature of 25°C allows data to be easily compared (FEI 2014a). The lowest conductivity value was at LOO01 (226.9 $\mu\text{S}/\text{cm}$), while the highest value occurred at AUR01 (459.4 $\mu\text{S}/\text{cm}$) (Table 6).

There is no set standard for the conductivity of water (Table 7) because conductivity can differ regionally and between neighbouring streams if there is enough difference in the surrounding geology, or if one source has a separate inflow (FEI 2014a). Freshwater that runs through granite bedrock will have a very low conductivity value. Clay- and limestone-derived soils can contribute to higher conductivity values in freshwater systems (LCRA 2014). Despite the lack of standards and the fact that the surrounding environment can affect conductivity, there are approximate values that can be expected based on the source of the water (American Public Health Assoc. *et al.* 1999, as cited in FEI 2014a; Clean Water Team 2004).

A full discussion on specific conductance is provided in Biota Consultants (2022a). 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, 2021 and 2022 data provide baseline measurements for comparison in the future.

3.3.3 Total Suspended Solids, Turbidity and Dissolved Oxygen

3.3.3.1 Total Suspended Solids

Total suspended solids (TSS) were <1 mg/L at all sites except MEA01 and LOO01, where they were 2.0 mg/L and 5.8 mg/L, respectively (Table 6), but still below the exceedance criteria. In the case of Lookout Creek, the higher level is probably related to the old beaver dams and exposed soils along the banks of the incised channels immediately upstream of the sampling site.

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

Suspended solids can adversely affect aquatic organisms in several ways:

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

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

3.3.3.2 Turbidity

Turbidity is often reported as nephelometric turbidity units (NTU) and is a measure of relative water clarity. The majority of sites had turbidity values between 0.3 NTU to 2.5 NTU (Table 6), which is considered very low (Table 7). The exception was LOO01 at 16.0 NTU, which again may be explained by its location below old beaver dams and exposed banks.

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

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

3.3.3.3 Dissolved Oxygen and Temperature

The DO values in our samples were within acceptable limits, ranging from 8.05 to 9.61 mg/L (Table 6). Dissolved oxygen (DO) is the concentration of free oxygen (O₂) present in water or other liquids and is usually measured in mg/L. An O₂ level that is too low or too high can affect water quality, harming aquatic life (Alberta Environmental Protection 1997). The amount of O₂ dissolved in water primarily depends on temperature, atmospheric (barometric) pressure and turbulence (e.g., rapids, waterfalls, waves), although salinity also has an effect (FEI 2013). Temperature is the main factor, as cold water can hold more oxygen (Environment Canada 2012). Therefore, water temperature and the amount of DO are important in assessing water quality due to their influence on organisms within a body of water. Please see Biota Consultants (2022a) for a further discussion on factors influencing DO and the effects of DO on aquatic fauna.

3.3.4 Comparison of Waiparous Creek and Johnson Creek Sites Between Successive Years

A comparison among years of the physical and chemical attributes of the water samples at sites JOH01, WAP02/WAP02a and WAP03 is presented in Table 8. There were slight variations between years at each site, but all were below the exceedance criteria. This variation is expected in a natural environment.

The outflow from Johnson Creek may have influenced the chemical attributes of Waiparous Creek below the confluence. The higher dissolved nitrogen at WAP02a (0.23 mg/L) versus WAP03 (0.14 mg/L) possibly was a result of the even higher dissolved nitrogen at JOH01 (0.29 mg/L). The higher alkalinity and bicarbonate values at JOH01 (170 mg/L and 210 mg/L, respectively) may explain the slightly higher alkalinity of WAP02a versus WAP03, although these differences are within the range of natural variation.

Table 8. Comparison among years of physical and chemical attributes of water samples at Johnson Creek site and Waiparous Creek sites, WAP02/WAP02a and WAP03.

Tests	Site and Date of Sampling							
	JOH01		WAP02		WAP02a	WAP03		
	Sept. 7 2021	Aug. 30 2022	Sept. 1 2020	Sept. 2 2021	Sept. 7 2022	Sept. 3 2020	Sept. 2 2021	Sept. 7 2022
pH	8.21	8.23	8.18	8.29	8.04	8.38	8.38	8.27
Total Suspended Solids (mg/L)	<1.0	<1.0	1.2	<1.0	<1.0	2	<1.0	<1.0
Turbidity (NTU)	0.11	0.4	<0.10	0.22	0.3	<0.10	0.11	0.2
Specific Conductance (µS/cm)	335.8	323.1	316.6	336.8	333.7	320.2	336.7	339.0
Dissolved Oxygen (mg/L)	10.24	9.61	8.97	9.92	8.04	8.77	9.28	8.27
Water Temperature (°C)	6.2	9.8	15	7.4	12.7	12.8	10.2	15.7
Air Temperature (°C)	16	21	22.5	10.5	21.5	17.5	19	23
<u>Anions</u>								
Alkalinity (Total as CaCO ₃) (mg/L)	150	170	150	140	160	140	130	140
Alkalinity (PP as CaCO ₃) (mg/L)	<1.0	<1.0	<1.0	<1.0	<1.0	1.4	<1.0	<1.0
Bicarbonate (HCO ₃) (mg/L)	190	210	180	170	200	160	160	180
Carbonate (CO ₃) (mg/L)	<1.0	<1.0	<1.0	<1.0	<1.0	1.7	<1.0	<1.0
Hydroxide (OH) (mg/L)	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
<u>Nutrients</u>								
Total Phosphorus (P) (mg/L)	<0.003	<0.0030	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
Dissolved Nitrogen (N) (mg/L)	0.16	0.29	0.26	0.13	0.23	0.25	0.1	0.14
Dissolved Nitrite (NO ₂) (mg/L)	<0.010	<0.033	<0.010	<0.010	<0.033	<0.010	<0.010	<0.033
Dissolved Nitrate plus Nitrite (N)	0.17	0.24	0.14	0.13	0.19	0.19	0.13	0.11

3.4 Benthic Macroinvertebrate Morphological Analysis

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

The orders Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies) (EPT) are taxa sensitive to pollution or degraded aquatic environments. The EPT index is the proportion of these taxa in the benthic invertebrate community. In contrast, the family Chironomidae (non-biting midges) in the order Diptera are tolerant of degraded waterbodies. Therefore, determining the ratio of chironomids to EPT species can be a good indicator of water quality. Monitoring the ratio over time can be used to determine whether the community is changing, either because of anthropogenic influences (using test sites) or naturally-caused 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 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 identified based on morphological characteristics. Appendix D contains this taxonomic data at the family level. Appendix E contains the metric indices for the entire 2022 taxonomic data to the genus/species level based on morphological identification.

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

3.4.1 Richness Measurements

The number of different species present is a measure of richness. This can be the total number of species at a site, or the number within a taxon(s), or the number within a functional group (i.e., predators, shredder-herbivores, collector-gatherers, scrapers, collector-filterers, omnivores, parasites, piercer-herbivores or unclassified types). Species richness does not take

into account the number of individuals of each species present. Rather, it gives as much weight to those species represented by very few individuals as to those represented by many individuals.

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

The 2022 results of the Simpson’s Index of Diversity indicate the community composition of most sites sampled are highly diverse (Figure 2). AUR01 had the lowest diversity with a value of 0.68. Similarly, values for the Shannon-Weiner Diversity Index were lowest for Aura Creek (1.89) and highest for Lookout Creek (2.83). Simpson’s Index of Diversity was similar between years at JOH01 and WAP03, with a dip in 2021 at WAP02 (Figure 2).

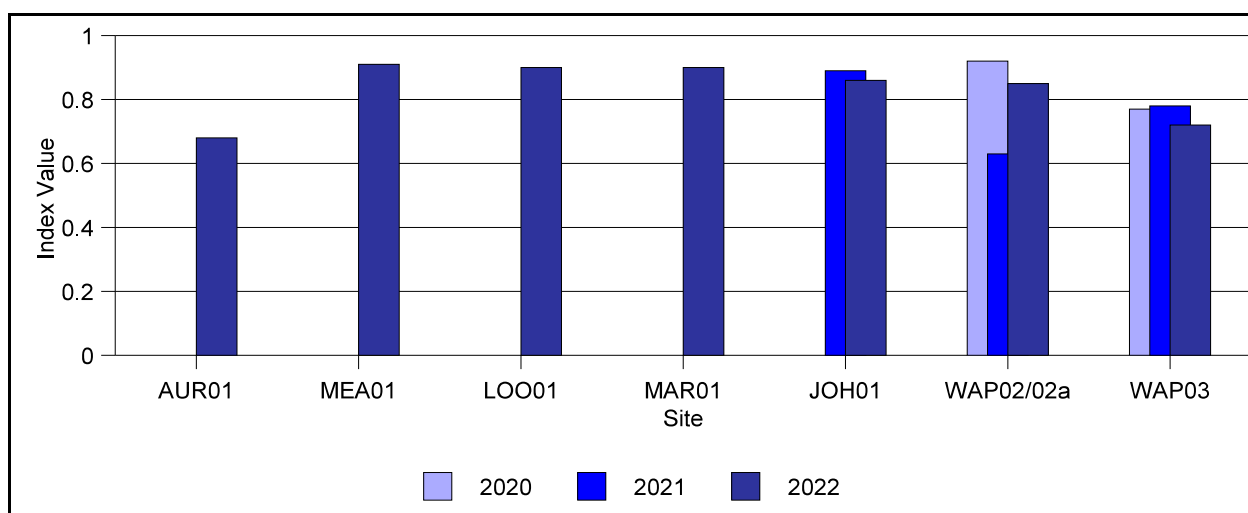


Figure 2. Simpson’s Index of Diversity (1-D) for each site.

3.4.2 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 of the population. Shifts within the population can alter the structure at various trophic levels, as certain species increase or decrease due to changes in the aquatic environment. The abundance and compositional measures presented include:

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

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

The following graphs illustrate the relationship between the Ephemeroptera, Plecoptera, Trichoptera (Figure 3) and Diptera at each site (Figure 4). Of the EPT species, the Ephemeroptera dominated all sites except the JOH01 site, where Plecoptera dominated at 28.8%. At MAR01, the percent of Ephemeroptera and Plecoptera was almost identical (38.1% versus 38.9%, respectively). Trichoptera were very low in abundance (<1%) at four of the tributary creeks (MEA01, LOO01, MAR01 and AUR01).

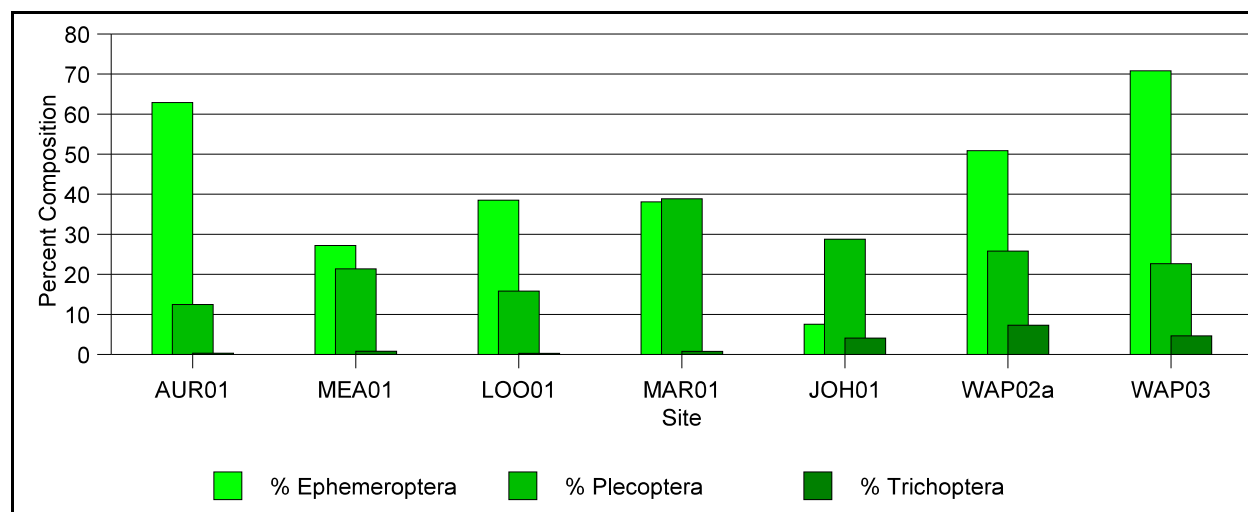


Figure 3. Percent composition of EPT orders at each site.

The EPT species were more prevalent than the Diptera species at all sites except JOH01 (Figure 4). In addition, the chironomid family comprised 96.4% of the taxa within the Diptera at JOH01 (Figure 5). Similarly in 2021, Diptera were prominent at JOH01 and chironomids comprised 94.0% of the Diptera. At the other sites, the percentage of chironomid flies ranged from 54.9% at MEA01 to 93.0% at WAP02a (Figure 5). At WAP03, the percentage of chironomids within the Diptera order was 83.3%; however, the percentage of Diptera within the community composition was only 1.9% (Figure 4). In the previous two years, Diptera and chironomids were also low at WAP02 and WAP03 compared to the EPT.

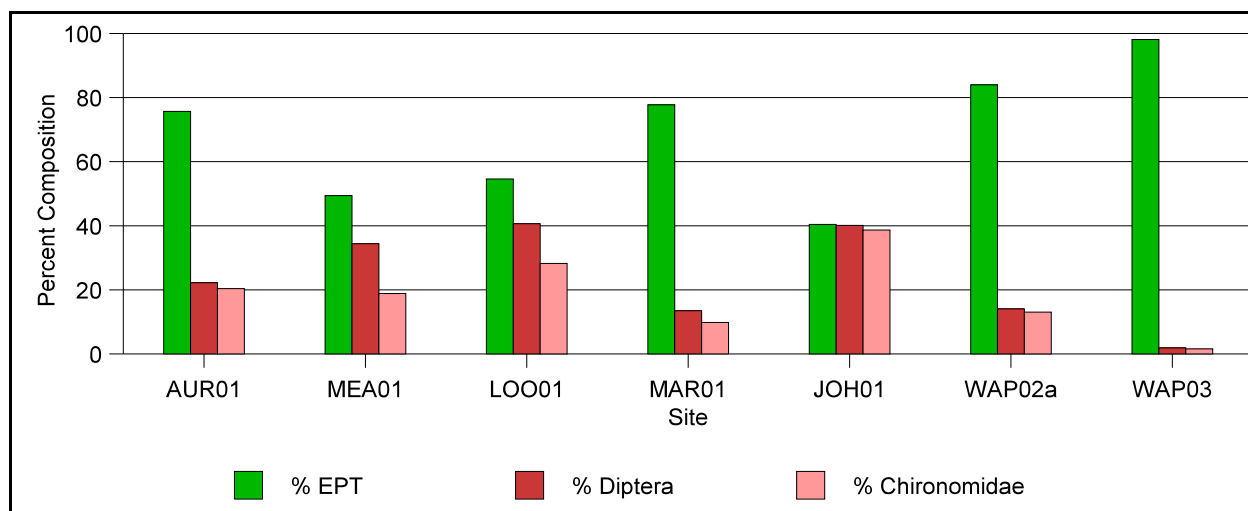


Figure 4. Percent composition of EPT orders, Diptera order and chironomid family at each site.

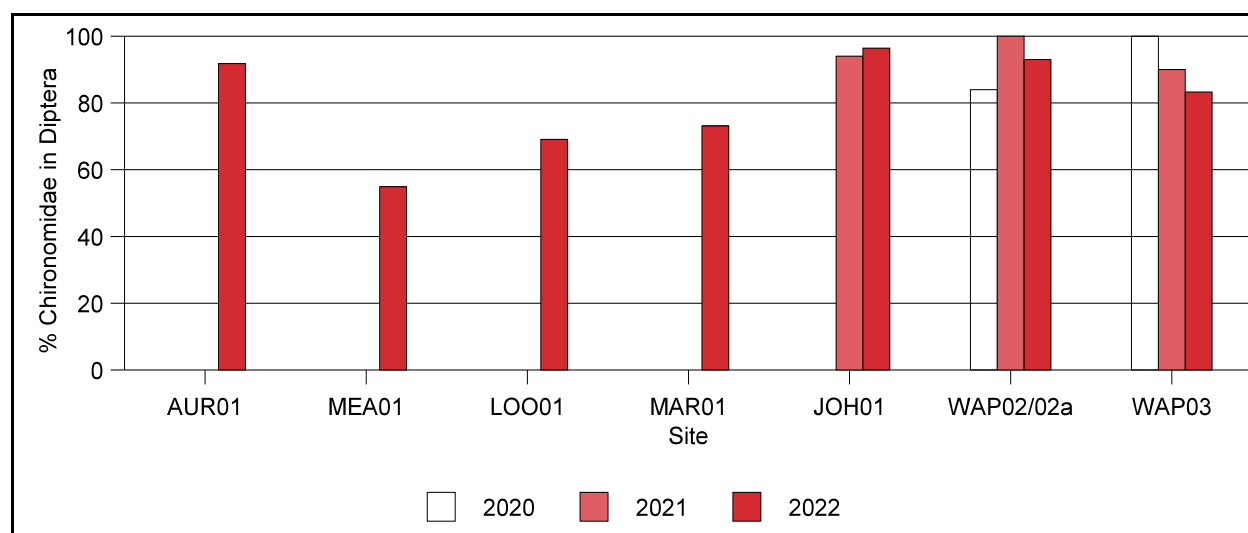


Figure 5. Percent of Diptera that were chironomid flies at each site.

The EPT ratio puts this into perspective (Figure 6). The JOH01 site had the lowest value and declined slightly from 0.59 in 2021 to 0.51 in 2022. Lookout Creek was the next lowest at 0.66. The high values at WAP02/02a and WAP03 suggest good water quality. In all three years, values were slightly lower below the confluence with Johnson Creek, although not enough to clearly suggest that the inflow from Johnson Creek influenced these values.

Based on the first three years of the water monitoring program, JOH01 is of most concern with respect to the benthic macroinvertebrate community. The percent of Ephemeroptera at the JOH01 site in 2021 (19.2%) and 2022 (7.6%) and was the lowest of all twenty sites studied within the past three years. JOH01 also had the lowest total EPT% composition, at 42.5% in 2021 and 40.4% in 2022. This suggests low water quality compared to the other sites. The Devil’s Head/Black Rock fire may be at least partially responsible for this, but no data were collected prior to the fire for comparison. There is no clear relationship between land use and water quality results at the sites sampled in 2022; however, there is high OHV activity on some upstream sections of Johnson Creek. This includes “Johnson Bog”, a wetland area that became a mud bowl.

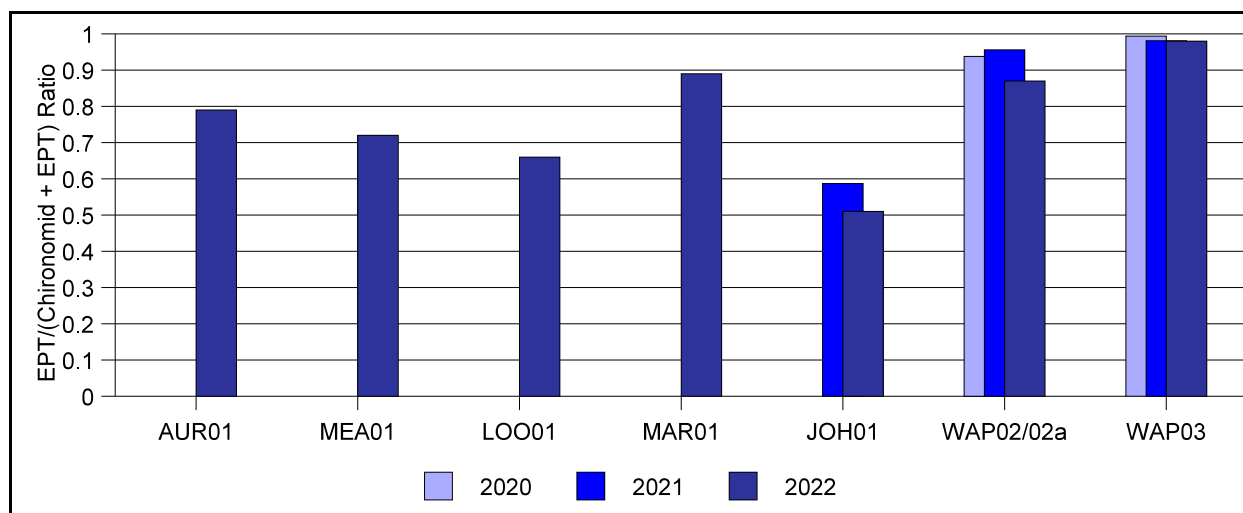


Figure 6. EPT/(chironomid + EPT) ratio for each site using percent community composition.

The percentage of Trichoptera within the community at each site was very low, ranging from 0.3% to 7.3% (Figure 3). Hydropsychidae, a family within Trichoptera that is more tolerant to adverse conditions, was found only at JOH01, WAP02a and WAP03, where it was variable in abundance and most prevalent at WAP03 (Figure 7). At the paired sites on Waiparous Creek, Hydropsychidae comprised a higher proportion of the Trichoptera at WAP02 in 2021 than in 2020, but was lower at WAP02a in 2022 (Figure 7). No Trichoptera were detected by morphological identification at WAP03 in 2020, but in 2021 and 2022, 59.5% and 80.0% of the Trichoptera identified were Hydropsychidae, respectively.

The percent of Baetidae, a family within Ephemeroptera that is more tolerant to adverse conditions, also was variable among the sites and between years at sites JOH01, WAP02/02a and WAP03 (Figure 8). It was markedly higher at MEA01, MAR01 and LOO01. The decline in

percent Baetidae at JOH01 in 2022 corresponded with a decline in all Ephemeroptera at the site. At WAP02 and WAP03, percent Baetidae dropped between 2020 and 2021 but was higher in 2022 at WAP02a and WAP03.

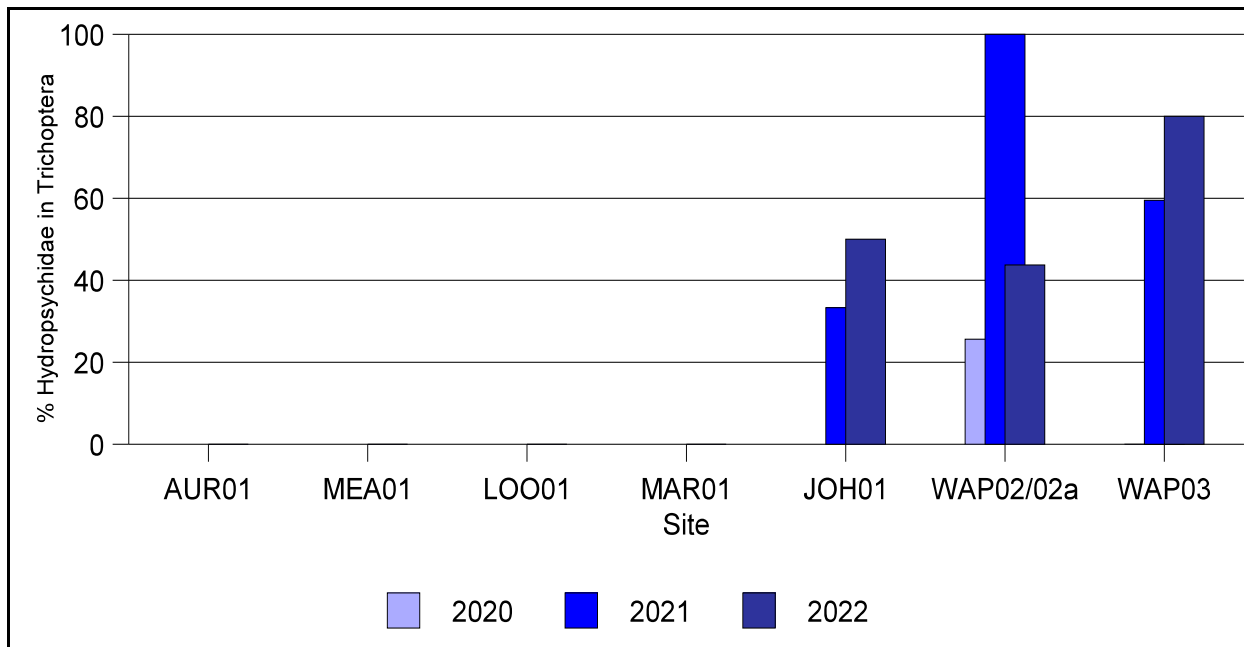


Figure 7. Percent of Trichoptera that were Hydropsychidae at each site.

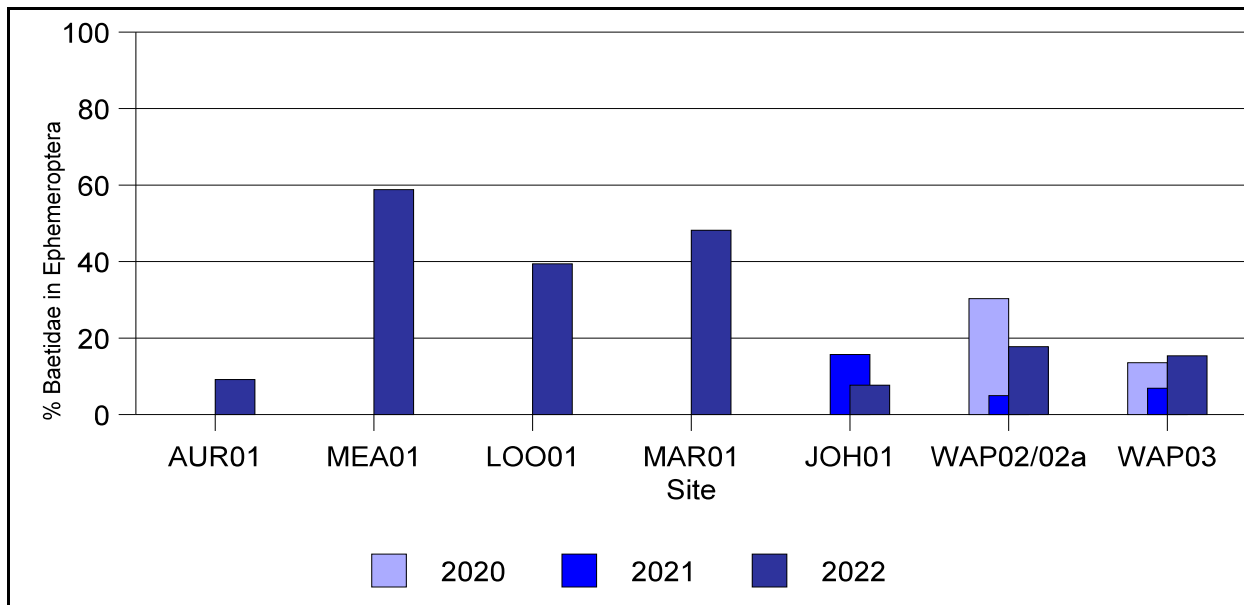


Figure 8. Percent of Ephemeroptera that were Baetidae at each site.

3.4.3 Functional Feeding Groups

A functional feeding group (FFG) is a classification based on the benthic macroinvertebrate's primary method of obtaining food, and therefore can include several different taxa. There are five main groups (Cummins 1973, 2021):

- shredders, which eat leaf litter, rooted aquatic vascular plants or other coarse particulate organic matter (CPOM; >1 mm);
- scrapers/grazers, which eat algae and other associated material;
- collector-gatherers, which eat fine particulate organic matter (FPOM; ≤ 1 mm) on or in the stream sediments;
- collector-filterers, which filter fine particulate organic matter from the water column; and
- predators, which prey on live invertebrates.

Comparing FFGs in a stream is a way to simplify analyses without the need to identify all specimens to lower taxon levels (Cummins 2021). The FFGs present depends on the type of available food, which varies with stream characteristics and adjacent riparian vegetation. Their abundance will differ along the upstream to downstream continuum, with a higher proportion of shredders upstream versus downstream, and lower proportion of collectors (Vannote *et al.* 1980). Min *et al.* (2019) discovered that FFG distribution was largely influenced by stream width and slope.

The presence of certain groups, or the ratio of certain groups with respect to other groups, has been shown to be related to stream health. In general, specialists (e.g., many of the shredder species) are presumed to be more sensitive and therefore associated with healthy streams, whereas generalists (e.g., many of the collector species), with their broader diet, are presumed to be more tolerant to disturbance (Cummins and Klug 1979; Barbour *et al.* 1999). Cummins (2021) determined ratios of the relative numbers of FFGs that can be used as surrogates for stream ecological conditions. For example, a 2:1 ratio of collector-filterers to collector-gatherers suggests abnormal turbidity, with an unusually high concentration of FPOM. Fu *et al.* (2014), Bhawsar *et al.* (2015) and Birara *et al.* (2020) discovered that streams with the same FFGs had similar land use patterns in their catchment areas.

3.4.3.1 Functional Feeding Groups at Tributary Streams

The graphical illustration of the FFGs in the tributary streams is presented in Figure 9. There was no obvious trend along the upstream/downstream continuum. The scrapers were noticeably more abundant in Aura Creek although algae were not perceived to be any greater than at any of the other creeks with the exception of Lookout Creek, where there was no obvious colour on the rocks. Collector-gatherers were prominent in Meadow Creek and

Johnson Creek, suggesting greater sediment. Shredders were highest at the Margaret Creek site. Shrubs were the dominant streamside vegetation here, which may have provided more leaf debris, but shrubs were also dominant along Lookout Creek. It is premature to draw conclusions on the increase in the shredders at Johnson Creek between 2021 and 2022, although it may suggest a slight increase in water quality between years.

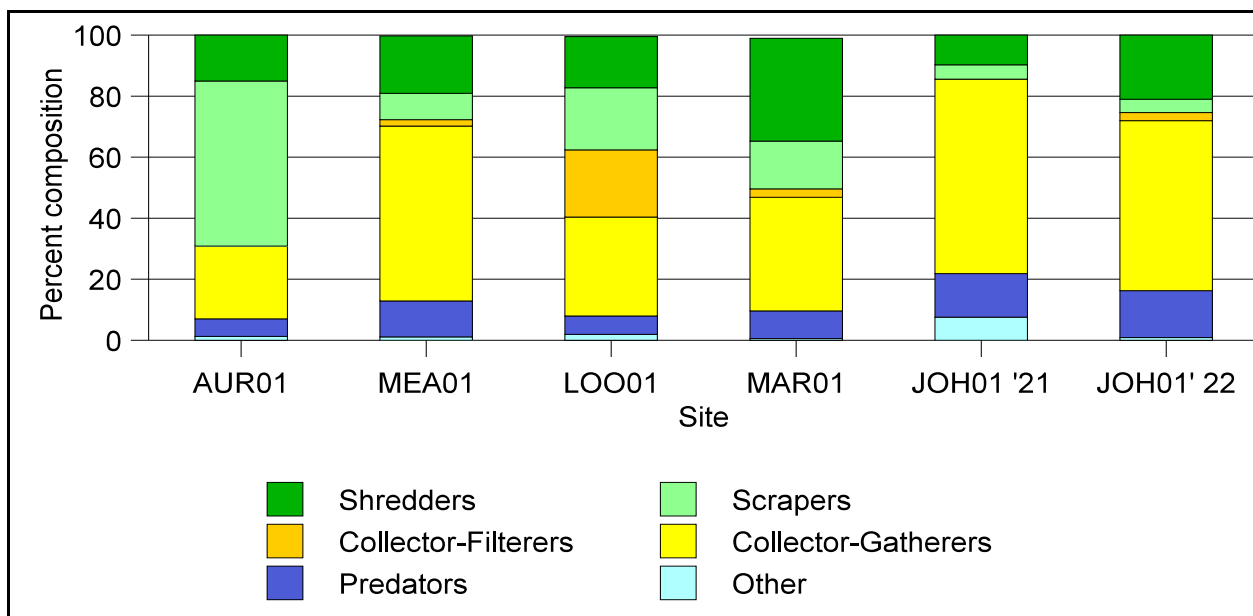


Figure 9. Percent of functional feeding groups at the tributary creeks.

3.4.3.2 Functional Feeding Groups at WAP02/02a and WAP03

The three years of data at WAP02/02a and WAP03 show the variability in FFG communities over time (Figures 10 and 11). Some declined in 2021 and then recovered in 2022, whereas others increased and then dropped down again. Most notable in variation among years at WAP02 were the scrapers, shredders and collectors. While the scrapers increased and then declined, the shredders, collector-filterers and collector-gatherers declined and then increased. The shredders and predators varied the most at WAP03 (Figure 11). The shredders steadily declined while the predators steadily increased.

At both sites, natural alterations in the stream channel was evident between successive years, which may have contributed to variations in the FFG composition. In addition, the human disturbance in the stream channel at and near WAP02/02a may have had an influence, as well as the fact that WAP02a is slightly upstream of WAP02. This variability probably has more impact on the benthic macroinvertebrate population trends and community shifts than the influence of Johnson Creek.

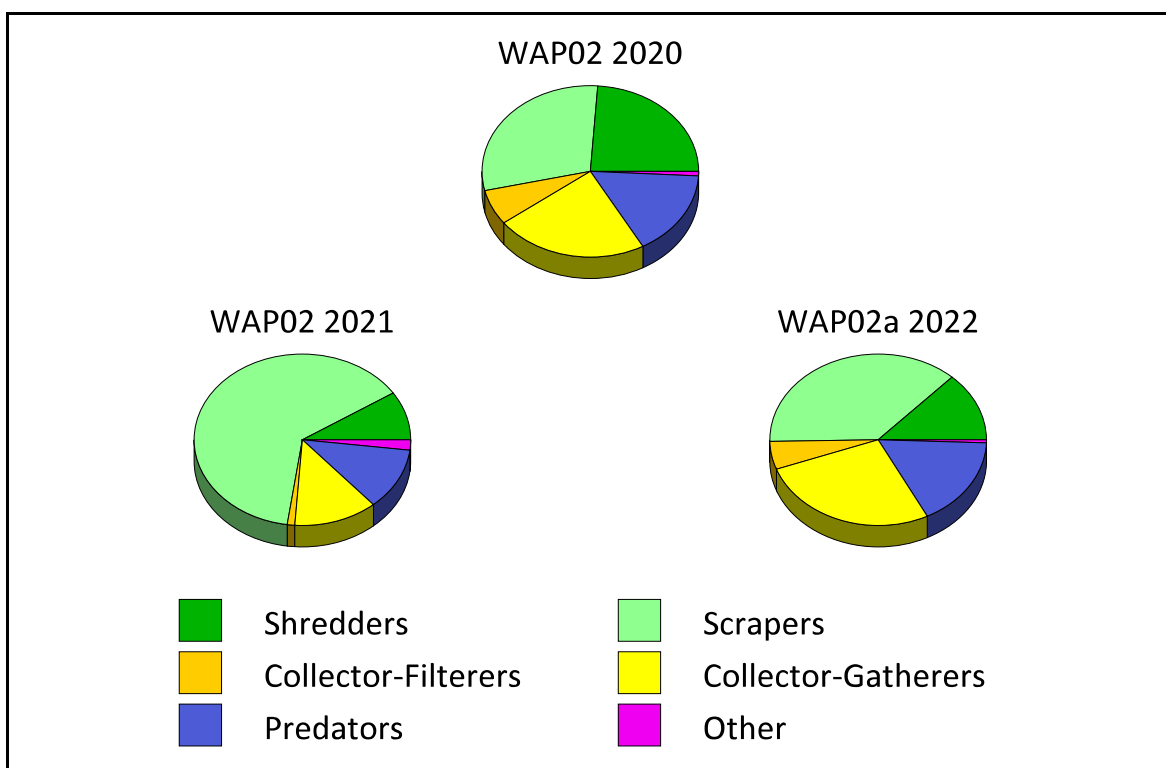


Figure 10. Percent of functional feeding groups at WAP02/02a from 2020 to 2022.

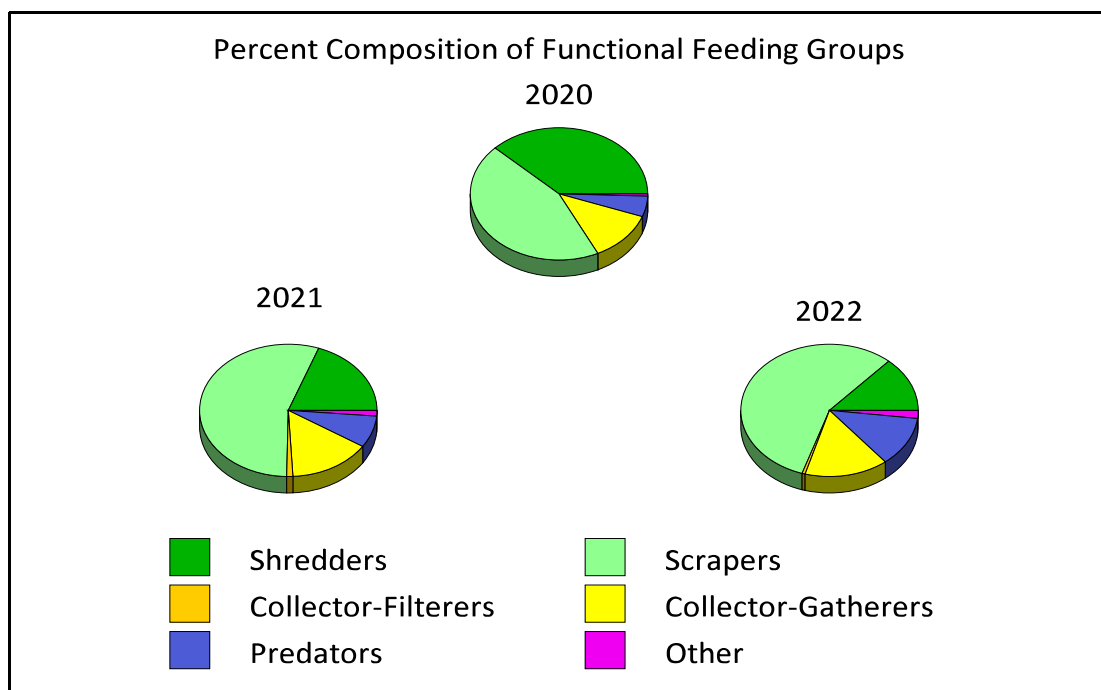


Figure 11. Percent of functional feeding groups at WAP03 from 2020 to 2022.

3.4.4 Hilsenhoff Biotic Index

The Hilsenhoff Biotic Index (HBI) estimates organic pollution using the proportion (abundance) of taxa at the genus/species level (Appendix E). Biotic tolerance values are assigned to each taxa based on their response to organic pollution. Index scores range from 0 to 10 (Table 9). Sensitive taxa have low scores and tolerant taxa have high scores, therefore an increase in the index suggests decreased water quality due to organic pollution (Hilsenhoff 1987).

Table 9. Hilsenhoff Biotic Index (HBI) categories.

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

The water quality at four of the tributary sites was rated as very good, with possible slight organic pollution (Figure 12). One tributary site (MAR01) was rated as excellent, as were the two Waiparous Creek sites.

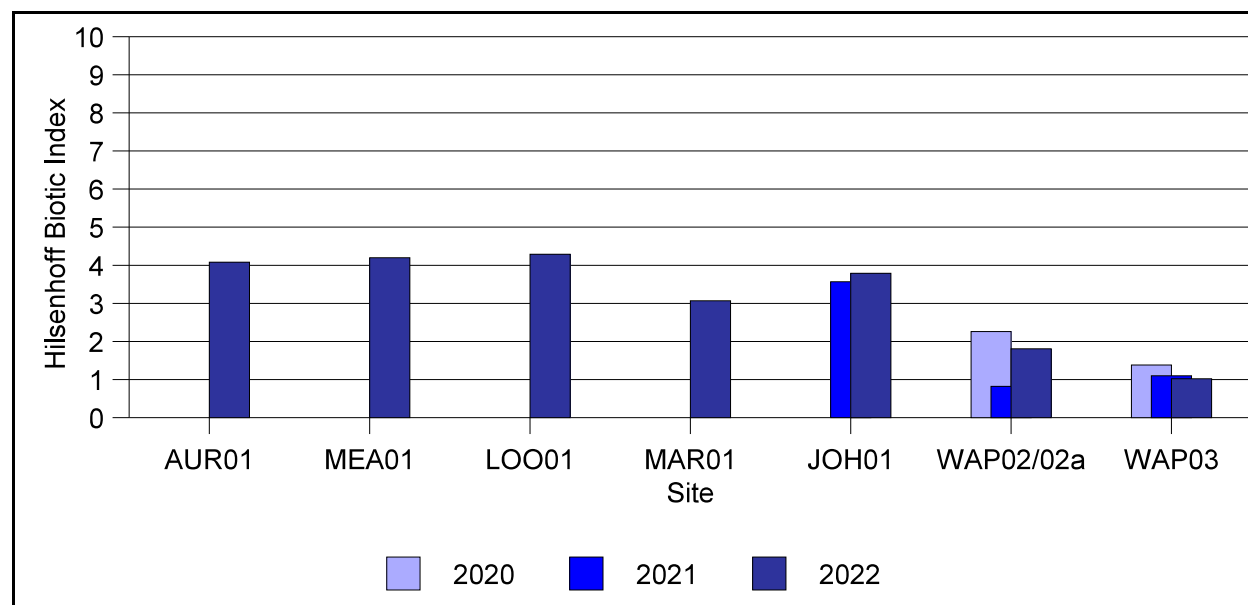


Figure 12. Hilsenhoff Biotic Index for each site.

3.5 STREAM eDNA Results

3.5.1 eDNA and Morphological Identification

The eDNA results complement the results of the morphological identification. An additional 141 species were identified, 51 of which were terrestrial species. The remainder were within 70 different genera. It was expected that more taxa would be identified by eDNA, partly because at four of the sites, three kicknet samples were collected versus one, and partly because the method does not require a recognizable specimen. DNA trapped in the sediment, and DNA in gut contents and animal waste is also detected (M. Wright, pers. comm.). The morphological identification included 15 genera that were not detected by eDNA, along with one order (Amphipoda), two classes (Copepoda, Turbellaria) and two phylums (Tardigrada and Nemata).

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

Other possible reasons include:

- The sequences in the reference database are from different species within the genus than those present in their sample, and are genetically distinct enough from each other that the species in their sample is not identified;
- The DNA primers that are used, which target the specific DNA region to be sequenced and compared, were not compatible with the species in their sample (three different primers are used in the workflow to overcome this known issue, but there are still sometimes taxa that are not compatible);
- The taxa may be too rare within the sample to be;
- Smaller or rarer taxa that make up less than 1% of the sample biomass are less likely to be identified by DNA metabarcoding than abundant or large taxa;
- The taxa may not be in the sample (since the samples collected for morphology and eDNA are different subsamples of the watercourse, and distribution of the taxa may be patchy).

The majority of the eDNA detections were to the species level, with only eight at just the genus level. Morphological identifications were seldom to the species level, usually to the genus level, often to the family level and, in rare cases, only to the order, class or phylum level. Most direct comparisons, therefore, could only be made at higher taxonomic levels (Table 10). The more detailed combined presence/absence results of each method are presented in Appendix 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.

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. AUR01 had the lowest richness based on eDNA but the second lowest, second to WAP03, based on morphological identification. The species richness at the Lookout Creek site based on morphological identification was 39, comparable to nearby Margaret Creek.

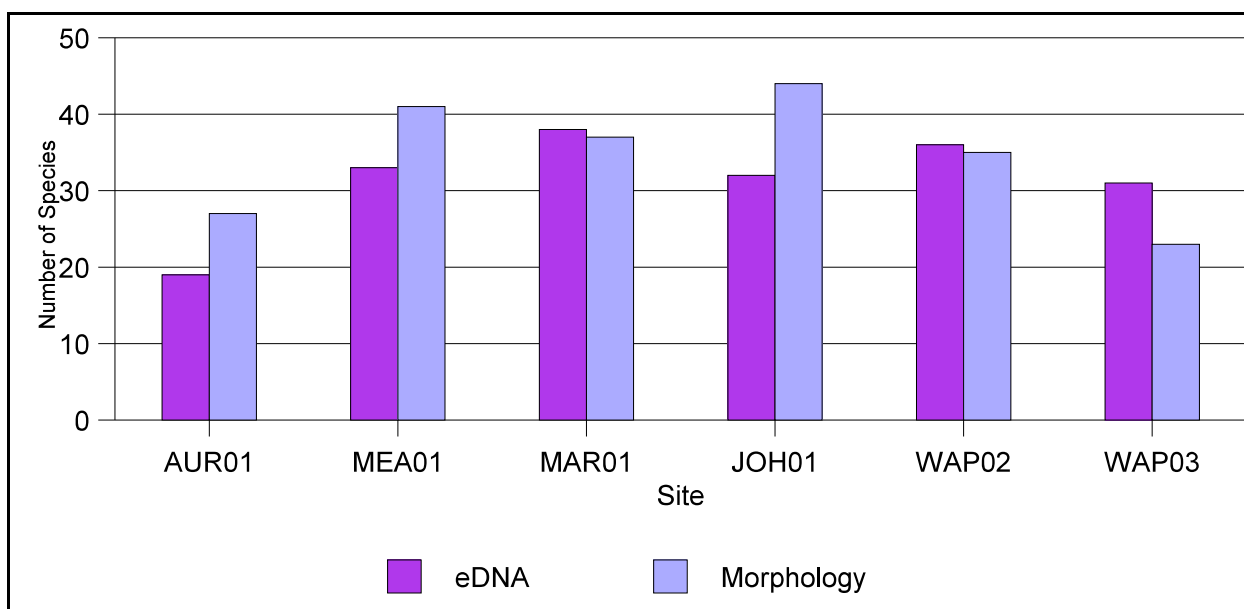


Figure 13. Species richness based on species taxonomically assigned by eDNA with high confidence based on normalized sequence data, and taxa identified morphologically.

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

Taxa	Site					
	AUR01	MEA01	MAR01	JOH01	WAP02a	WAP03
Class: Insecta						
Order: Diptera						
Chironomidae	Both	Both	Both	Both	Both	Both
Chironominae						
Chironomini	eDNA	Both	eDNA	Morph	eDNA	eDNA
<i>Microtendipes</i>		Both		Morph		
Tanytarsini	Both	Both	Both	Both	Both	Both
<i>Micropsectra</i>	Both	Both	Both	Morph	Both	
Diamesinae		Both		Both	eDNA	
Orthocladiinae	Both	Both	Both	Both	Both	Both
<i>Corynoneura</i>	eDNA		Morph			Morph
<i>Eukiefferiella</i>	Morph	eDNA			Morph	Both
<i>Orthocladus</i>	Both	Both	Both	Both	Both	Morph
<i>Tvetenia</i>	Morph	Both	Morph	Both	Morph	
Tanypodinae	Morph	eDNA	eDNA	eDNA		
Empididae	Morph	Both	Morph	Both		
<i>Neoplasta</i>		Both		Morph		
<i>Oreogeton</i>	Morph	Both				
Psychodidae		Both	Morph			
Simuliidae						
<i>Simulium</i>		Both	Both	eDNA	Both	
Tipulidae	Both	Both	Both	Both	Both	Morph
<i>Tipula</i>	eDNA	eDNA	eDNA	Both	eDNA	
Order: Ephemeroptera						
Ameletidae						
<i>Ameletus</i>	eDNA	eDNA	Both	Both	Both	Both
Baetidae	Both	Both	Both	Both	Both	Both
<i>Acentrella</i>	Morph	Both	Morph	eDNA	Both	Both
<i>Baetis</i>	Both	Both	Both	eDNA	Both	Both
Ephemerellidae	Both	Both	eDNA	Both	Both	Both
<i>Drunella</i>	eDNA	Both	eDNA	eDNA	Both	Both
<i>Drunella doddsii</i>		eDNA		eDNA	Both	Both
<i>Ephemerella</i>		eDNA	eDNA	eDNA	Both	eDNA

Taxa	Site					
	AUR01	MEA01	MAR01	JOH01	WAP02a	WAP03
Heptageniidae	Both	Both	Both	Both	Both	Both
<i>Cinygmula</i>	eDNA	eDNA	eDNA	Both	eDNA	eDNA
<i>Epeorus</i>		eDNA		eDNA	Both	Both
<i>Rhithrogena</i>		eDNA	eDNA	Both	Both	Both
Leptophlebiidae		eDNA	Both	Both	eDNA	
Order: Plecoptera	Both	Both	Both	Both	Both	Both
Capniidae	Both	eDNA	eDNA	Both	Both	Both
Chloroperlidae	Both	Both	Both	Both	Both	Both
<i>Sweltsa</i>	Both	Both	Both	Both	Both	Both
Leuctridae	eDNA	eDNA	Both	Both	eDNA	
Nemouridae	Both	Both	Both	Both	Both	Both
<i>Visoka cataractae</i>				Both		
<i>Zapada</i>	Both	Both	Both	Both	Both	Both
<i>Zapada cinctipes</i>	Both	Both	Both	Both	Both	Both
<i>Zapada columbiana</i>				Both	eDNA	Both
Perlidae		Both	Both	Both	Both	Both
<i>Hesperoperla</i>		Both	Both	Both	Both	eDNA
Perlodidae	Both	Both		Both	Both	Both
<i>Isogenoides</i>					eDNA	Both
<i>Kogotus</i>	Both	Both		eDNA		eDNA
Pteronarcyidae						
<i>Pteronarcella</i>					eDNA	Both
Taeniopterygidae					Both	Both
Order: Trichoptera	Both	Both	Morph	Both	Both	Both
Brachycentridae		Morph	Morph	Both	Morph	Morph
<i>Brachycentrus americanus</i>			Morph	eDNA		Morph
Hydropsychidae		eDNA		Both	Both	Both
<i>Arctopsyche</i>		eDNA		Both	Both	Both
Rhyacophilidae						
<i>Rhyacophila</i>	Both	Morph		Morph		Both
Class: Arachnida						
Order: Trombidiformes	Morph	Both	Both	Morph	Both	eDNA
Torrenticolidae						
<i>Testudacarus</i>		Both	eDNA	Morph	eDNA	eDNA
Class: Hydrozoa						
Order: Anthoathecata		Morph		eDNA	eDNA	
Class: Ostracoda	Morph	Both		Morph	Morph	
Class: Oligochaeta						
Order: Tubificida						
Enchytraeidae		Both				
Lumbricidae		Both	eDNA	Both	eDNA	

3.5.2 Whirling Disease

Whirling disease has previously been detected in the Ghost River watershed (Government of Alberta 2020). However, the DNA of *Tubifex tubifex* (sludge worm), the intermediate host of the microscopic parasite that causes the disease, was not found at any of the sites in this study until 2022, when it was detected at AUR01 (Hajibabaei Lab 2023). Subsequently, the sample taken at AUR01 for morphological identification was rechecked by Cordillera Consulting. A single specimen was noted that appeared to be *Tubifex*. Only the back half was present which had body hairs and pectinate hair chaetae that are *Tubifex* characters (S. Finlayson, pers. comm.).

Whirling disease is spread when infected organisms, or contaminated equipment, water, plants or soil, are moved to a body of water (Canadian Food Inspection Agency 2016). There was not necessarily additional activity upstream of AUR01 that would explain sludge worm being detected at this site versus several of the other sites sampled in 2022 or previous years. However, there is high potential to spread the disease in any of the areas where logging or substantial recreational use occurs, resulting in more activity and more sedimentation providing habitat for sludge worms.

4.0 Conclusions and Recommendations

4.1 Comparison of All Sites

The analyses of chemical and physical attributes of water samples at the seven sites indicate high water quality. TSS and turbidity were extremely low at all sites except Lookout Creek, but all were well below the exceedance criteria. Water quality parameters were all within acceptable limits for benthic macroinvertebrates and fish.

There was evidence that Johnson Creek might have influenced the chemical attributes of Waiparous Creek below the confluence. The higher dissolved nitrogen at WAP02a versus WAP03 possibly was a result of the even higher dissolved nitrogen at JOH01. Similarly, the higher alkalinity and bicarbonate values at JOH01 may explain the slightly higher alkalinity of WAP02a versus WAP03, but may just be natural variation. Johnson Creek appeared to have less of an influence on the physical characteristics of WAP02a than it had on WAP02 in the previous two years, probably due to the two new man-made rock dams upstream of WAP02a.

The Simpson's Index of Diversity and the Shannon-Weiner Index indicate that the sites were diverse in their benthic macroinvertebrate community composition, with the lowest diversity at AUR01. Similarly, richness was low at this site. The Hilsenhoff Biotic Index suggests high water quality at all sites with respect to organic pollution.

The EPT ratio suggests high water quality at most of the sites, with EPT species in much greater abundance than the pollution-tolerant chironomid family. The main exception was JOH01 where the ratio was 0.51, potentially raising concerns.

The more tolerant Hydropsychidae within the Trichoptera was found only at JOH01, WAP02a and WAP03, and was highest at WAP03. Baetidae were identified at all sites but in low abundance except at the Meadow, Margaret and Lookout creek sites.

The proportion of FFGs varied among the sites, reflecting the habitat and adjacent riparian vegetation. Scrapers dominated in Aura Creek and Waiparous Creek, suggesting more algae; collector-gatherers were prominent in Meadow Creek and Johnson Creek, suggesting greater sediment; shredders were highest at the Margaret Creek site, suggesting greater leaf litter.

The results of the 2022 field sampling provide a baseline for comparison in future years. With more data, trends may become apparent. If issues with water quality are suggested, sampling effort may become more focussed.

4.2 Comparison Between Years of Johnson Creek Site

The physical characteristics varied between years possibly largely as a result of the measurements not being in exactly the same location. The average velocity was higher in 2022, but median particle size was smaller.

There were slight variations in physical and chemical attributes of the water samples, but all were below the exceedance criteria. The Hilsenhoff Biotic Index was slightly higher in 2022, but water quality was rated as very good in both years, indicating possible slight organic pollution.

Diversity indices indicate highly diverse community composition in both years. However, the EPT ratio declined from 0.59 to 0.51, plus the percent of Hydropsychidae within the Trichoptera went up while the percent of Baetidae within the Ephemeroptera went down. The proportion of shredders increased while that of the collector-gatherers declined slightly.

While some health indicators suggest concerns with water quality at Johnson Creek, most notably the EPT ratio, others do not. Also, some suggest a decline in quality between years (EPT, Hydropsychidae) whereas others suggest the opposite, such as the increase in shredders. The low EPT ratio may be related to the high OHV activity upstream from the site. Further monitoring over the years should help to establish the health of this site.

4.3 Comparison Among Years of Waiparous Creek Sites

The physical characteristics varied among years largely as a result of natural variability in the stream channel from fluvial events, and, in the case of WAP02/02a, human alteration of the stream channel. Notable differences were observed in the depth, velocity and median particle size of the substrate. In addition, embeddedness declined at WAP03 in 2022 versus the previous two years. There were slight variations in physical and chemical attributes of the water samples, but all were below the exceedance criteria. The Hilsenhoff Biotic Index has consistently been in the “excellent” category, indicating organic pollution is unlikely.

The diversity indices indicate high diversity in benthic macroinvertebrates over the three years. The EPT ratio also has been high, suggesting good water quality. The percent of Hydropsychidae within the Trichoptera and percent of Baetidae within the Ephemeroptera has been variable at both sites, but the proportion of Baetidae has been low.

The proportion of FFGs varied among the years. At WAP02/02a, the greatest variability was among the scrapers, shredders and collectors. The shredders and predators varied the most at WAP03. The variation in the stream channel may explain these differences.

4.4 General Recommendations

- Adequate annual funding for this program should be maintained.
- The *GWAS Water Monitoring Program Plan* should continue to be followed, allowing flexibility if circumstances materialize that suggest a deviation.
- The sites sampled from 2020 to 2022 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., JOH01. (If monitoring in successive years, three years may be considered adequate, but CABI_N does not specify a frequency.)
- Additional sampling sites should be established on Johnson Creek, ideally including a site above all/most OHV activity.
- Prior to conducting the field sampling, the survey team should read and fully understand the methodology presented in the *CABI_N 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 CABI_N-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.
- To maintain consistency, the same laboratories that were originally selected and used in from 2020 to 2022 (water chemical and benthic macroinvertebrate analysis) should continue to be used.

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6.0 Personal Communications

- | | |
|------------------|------------------------------------------------------------------------------------------------------------------------------------------------|
| Finlayson, Scott | President and Head Taxonomist, Cordillera Consulting Inc., Summerland, BC. (June 28, 2023) |
| Wright, Michael | Laboratory Manager, Hajibabaei Lab, Centre for Biodiversity Genomics, Biodiversity Institute of Ontario, University of Guelph. (Aug. 10, 2022) |

Appendix A
CABiN Field Sheet

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)

☐ Forest ☐ Field/Pasture ☐ Agriculture ☐ Residential/Urban
☐ Logging ☐ Mining ☐ Commercial/Industrial ☐ Other _____

Information Source: _____

Dominant Surrounding Land Use: (check one)

☐ Forest ☐ Field/Pasture ☐ Agriculture ☐ Residential/Urban
☐ Logging ☐ Mining ☐ Commercial/Industrial ☐ Other _____

Information Source: _____

Location Data

Latitude: _____ N Longitude: - _____ W (DMS or DD)

Elevation: _____ (fast 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: _____ (°C) Water Temp: _____ (°C) pH: _____

Specific Conductance: _____ (µs/cm) DO: _____ (mg/L) Turbidity: _____ (NTU)

Check if water samples were collected for the following analyses:

- ☐ TSS (Total Suspended Solids)
- ☐ Nitrogen (i.e. Total, Nitrate, Nitrite, Dissolved, and/or Ammonia)
- ☐ Phosphorus (Total, Ortho, and/or Dissolved)
- ☐ Major Ions (i.e. Alkalinity, Hardness, Chloride, and/or Sulphate) ☐ Other _____

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

CHANNEL DATA

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

☐ **Calculated from map**

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

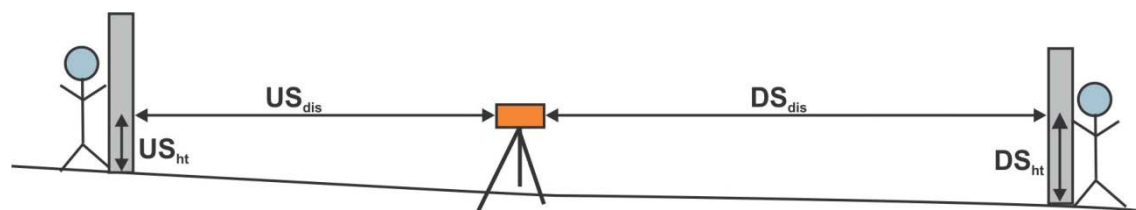
OR

☐ **Measured in field**

Circle device used and fill out table according to device:

a. Survey Equipment b. Hand Level & Measuring Tape

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



Field Crew: _____ Site Code: _____

Sampling Date: (DD/MM/YYYY) _____

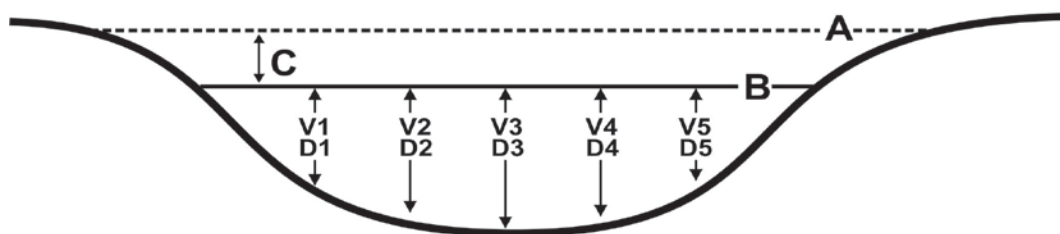
Widths and Depth

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

A - Bankfull Width: _____ (m)

B - Wetted Stream Width: _____ (m)

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



Note:

Wetted widths > 5 m, measure a minimum of 5-6 equidistant locations;

Wetted widths < 5 m, measure 3-4 equidistant locations.

Velocity and Depth

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

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

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

☐ **Direct velocity measurements:** ☐ Marsh-McBirney ☐ Sontek or ☐ Other _____

	1	2	3	4	5	6	AVG
Distance from Shore (m)							
Depth (D) (cm)							
Velocity Head Rod (ruler)							
Flowing water Depth (D ₁) (cm)							
Depth of Stagnation (D ₂) (cm)							
Change in depth (ΔD=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.
- Embeddedness 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
	Chrysomelidae	Leaf beetles*
	Elmidae	Riffle beetles
Diptera		Flies
	Ceratopogonidae	Biting midges
	Chironomidae	Non-biting midges
	Empididae	Dagger flies, balloon flies
	Limoniidae	Craneflies
	Psychodidae	Moth flies, sand flies
	Simuliidae	Black flies
	Tipulidae	Craneflies
Ephemeroptera		Mayflies
	Ameletidae	Combmouthed minnow mayflies
	Baetidae	Small minnow mayflies
	Ephemerellidae	Spiny crawler mayflies
	Heptageniidae	Flat-headed mayflies
	Leptophlebiidae	Prong-gilled mayflies
	Siphonuridae	Primitive minnow mayflies
Megaloptera		
	Corydalidae	Dobsonflies and fishflies†
	Sialidae	Alderflies
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
	Hydropsychidae	Net-spinning caddisflies
	Rhyacophilidae	Free-living caddisflies
Trombidiformes		Mites
	Aturidae	Water mites

Order	Family	Common Name
	Feltriidae	Water mites
	Hydryphantidae	Water mites
	Hygrobatidae	Water mites
	Lebertiidae	Water mites
	Sperchontidae	Water mites
	Stygothrombidiidae	Water mites
	Torrenticolidae	Torrent mites
Entomobryomorpha		Springtails
	Entomobryidae	Slender springtails
Anthoathecata		Athecate hydroids
	Hydriidae	Hydra
	Pennariidae	Hydrozoans
Amphipoda		Amphipods
Decapoda		Decapods
	Cambaridae	Freshwater crayfish
Podocopida		Ostracods, seed shrimp
	Candonidae	Freshwater ostracods
Tubificida		Annelid worms
	Enchytraeidae	Microdrile oligochaetes (worms)
	Lumbricidae	Earthworm
	Naididae	Clitellate oligochaete worms

* Genus *Donacia* identified – aquatic leaf beetle

† Genus *Nigronia* identified – dark fishfly

Appendix C
Benthic Macroinvertebrates Identified Using Morphological Characteristics

Taxa	Site						
	AUR01	MEA001	LOO01	MAR01	JOH01	WAP02a	WAP03
Phylum: Arthropoda							
Subphylum: Hexapoda							
Class: Insecta							
Order: Ephemeroptera	0	0	67	700	0	0	0
Family: Ameletidae	0	0	0	0	0	0	0
<i>Ameletus</i>	0	0	83	140	40	25	8
Family: Baetidae	43	93	267	700	20	14	16
<i>Acentrella</i>	14	7	0	40	0	33	28
<i>Baetis</i>	214	313	633	300	0	36	56
<i>Baetis fuscatus</i> gr.	0	7	33	20	0	0	0
<i>Baetis rhodani</i> group	0	0	0	20	0	0	40
Family: Ephemerellidae	143	60	50	0	40	3	0
<i>Drunella</i>	0	7	0	0	0	3	0
<i>Drunella doddsii</i>	0	0	0	0	0	8	36
<i>Ephemerella</i>	0	0	0	0	0	3	0
Family: Heptageniidae	2543	227	1250	920	130	289	456
<i>Cinygmula</i>	0	0	0	0	10	0	0
<i>Epeorus</i>	0	0	0	0	0	3	28
<i>Rhithrogena</i>	0	0	0	0	10	50	244
Family: Leptophlebiidae	0	0	50	100	10	0	0
 Order: Plecoptera	 0	 7	 117	 80	 100	 3	 4
Family: Capniidae	57	0	183	0	10	31	48
Family: Chloroperlidae	29	0	0	160	40	58	48
<i>Sweltsa</i>	14	7	0	100	20	31	24
Family: Leuctridae	0	0	333	20	10	0	0

Taxa	Site						
	AUR01	MEA001	LOO01	MAR01	JOH01	WAP02a	WAP03
Family: Nemouridae	43	0	83	540	310	11	0
<i>Malenka</i>	29	0	0	0	0	0	0
<i>Visoka cataractae</i>	0	0	0	0	30	0	0
<i>Zapada</i>	14	107	67	300	20	8	0
<i>Zapada cinctipes</i>	329	360	150	1700	240	39	12
<i>Zapada columbiana</i>	0	0	0	0	20	0	4
Family: Perlidae	0	33	0	60	70	17	20
<i>Hesperoperla</i>	0	27	0	40	90	8	0
Family: Perlodidae	43	13	50	0	30	3	0
<i>Isogenoides</i>	0	0	0	0	0	0	8
<i>Kogotus</i>	29	7	0	0	0	0	0
<i>Megarcys</i>	0	0	17	0	0	0	0
Family: Pteronarcyidae	0	0	0	0	0	0	0
<i>Pteronarcella</i>	0	0	0	0	0	0	16
Family: Taeniopterygidae	0	0	0	0	0	28	108
 Order: Trichoptera	 0	 7	 0	 0	 0	 3	 0
Family: Brachycentridae	0	7	17	40	0	36	8
<i>Brachycentrus americanus</i>	0	0	0	20	0	0	0
<i>Micrasema</i>	0	0	0	0	10	0	0
Family: Hydropsychidae	0	0	0	0	40	6	12
<i>Arctopsyche</i>	0	0	0	0	30	22	36
Family: Rhyacophilidae	0	0	0	0	0	0	0
<i>Rhyacophila</i>	0	0	0	0	10	0	0
<i>Rhyacophila betteni</i> group	14	0	0	0	0	0	0
<i>Rhyacophila brunnea/vemna</i> group	0	7	0	0	10	0	0
<i>Rhyacophila vofixa</i> group	0	0	0	0	0	0	4
<i>Rhyacophila atrata</i> complex	0	0	0	0	10	0	0
<i>Rhyacophila narvae</i>	0	0	0	0	30	0	0

Taxa	Site						
	AUR01	MEA001	LOO01	MAR01	JOH01	WAP02a	WAP03
Order: Coleoptera	0	0	0	0	0	0	0
Family: Elmidae	0	100	0	320	190	0	0
<i>Heterlimnius</i>	57	146	17	180	410	9	0
Order: Diptera	0	0	33	0	0	0	0
Family: Ceratopogonidae	0	0	0	0	0	0	0
<i>Mallochohelea</i>	0	7	0	80	20	0	0
Family: Chironomidae	29	27	67	60	70	8	4
Subfamily: Chironominae	0	0	0	0	0	0	0
Tribe: Chironomini	0	0	0	0	0	0	0
<i>Microtendipes</i>	0	13	0	0	30	0	0
Tribe: Tanytarsini	0	0	0	0	0	0	0
<i>Constempellina</i> sp. C	29	7	133	0	20	0	0
<i>Micropsectra</i>	57	67	117	40	130	14	0
<i>Paratanytarsus</i>	0	0	950	120	0	0	0
<i>Rheotanytarsus</i>	0	20	0	0	20	0	0
<i>Stempellina</i>	0	0	0	0	10	0	0
<i>Stempellinella</i>	0	0	0	20	0	0	0
Subfamily: Diamesinae	0	0	0	0	0	0	0
Tribe: Diamesini	0	0	0	0	0	0	0
<i>Pagastia</i>	0	0	0	0	60	0	0
<i>Potthastia</i>	0	0	0	0	0	0	0
<i>Potthastia longimana</i> group	0	7	0	0	0	0	0
Subfamily: Orthoclaadiinae	0	0	0	0	0	0	0
<i>Brillia</i>	229	7	50	40	10	3	0
<i>Eukiefferiella</i>	57	0	100	0	0	3	4
<i>Hydrobaenus</i>	0	0	0	0	0	3	0
<i>Krenosmittia</i>	0	0	17	0	0	0	0
<i>Orthocladus</i> complex	443	280	33	380	930	72	12

Taxa	Site						
	AUR01	MEA001	LOO01	MAR01	JOH01	WAP02a	WAP03
<i>Parametriocnemus</i>	14	20	200	40	0	3	0
<i>Rheocricotopus</i>	0	27	17	20	10	0	0
<i>Tvetenia</i>	86	20	17	40	40	14	0
Subfamily: Tanypodinae	0	0	0	0	0	0	0
Tribe: Pentaneurini	0	0	0	0	0	0	0
<i>Thienemannimyia</i> group	14	0	67	0	0	0	0
Tribe: Procladiini	0	0	0	0	0	0	0
<i>Procladius</i>	0	0	17	0	0	0	0
Family: Empididae	43	0	34	20	0	0	0
<i>Neoplasta</i>	0	27	0	0	10	0	0
<i>Oreogeton</i>	0	7	0	0	0	0	0
<i>Roederiodes</i>	29	0	0	0	0	0	0
Family: Psychodidae	0	0	0	0	0	0	0
<i>Pericoma/Telmatoscopus</i>	0	340	183	80	0	0	0
Family: Simuliidae	0	0	317	0	0	0	0
<i>Simulium</i>	0	13	67	20	0	3	0
Family: Tipulidae	0	13	83	0	0	0	0
<i>Antocha</i>	0	0	0	20	0	0	0
<i>Dicranota</i>	14	0	83	60	0	0	0
<i>Hexatoma</i>	0	0	0	0	10	6	4
<i>Tipula</i>	0	0	0	0	10	0	0
Subphylum: Chelicerata							
Class: Arachnida							
Order: Trombidiformes	0	20	0	0	10	0	0
Family: Aturidae	0	0	0	0	0	0	0
<i>Aturus</i>	0	13	0	0	0	0	0
Family: Feltriidae	0	0	0	0	0	0	0
<i>Feltria</i>	0	7	0	0	20	0	0

Taxa	Site						
	AUR01	MEA001	LOO01	MAR01	JOH01	WAP02a	WAP03
Family: Hydryphantidae	0	0	0	0	0	0	0
<i>Protzia</i>	0	0	0	0	0	3	0
Family: Hygrobatidae	0	0	0	0	0	0	0
<i>Atractides</i>	0	0	17	0	10	3	0
<i>Hygrobates</i>	0	13	50	20	0	0	0
Family: Lebertiidae	0	0	0	0	0	0	0
<i>Lebertia</i>	29	67	50	60	20	3	0
Family: Sperchontidae	0	0	0	0	0	0	0
<i>Sperchon</i>	14	0	0	0	0	0	0
Family: Torrenticolidae	0	0	0	0	0	0	0
<i>Testudacarus</i>	0	33	0	0	50	0	0
<i>Torrenticola</i>	0	13	0	60	10	0	0
 Suborder: Prostigmata	0	0	0	0	0	0	0
Family: Stygothrombidiidae	0	0	0	0	0	0	0
<i>Stygothrombium</i>	0	0	0	40	0	0	0
 Class: Malacostraca							
Order: Amphipoda	0	0	0	0	0	3	0
 Phylum: Annelida							
Subphylum: Clitellata							
Class: Oligochaeta							
Order: Tubificida	0	0	0	0	0	0	0
Family: Enchytraeidae	0	0	0	0	0	0	0
<i>Enchytraeus</i>	0	20	0	0	0	0	0
Family: Naididae	0	0	0	0	0	0	0
<i>Nais</i>	0	0	133	0	0	0	0
Subfamily: Tubificinae without hair chaetae	0	0	33	0	0	0	0

Taxa	Site						
	AUR01	MEA001	LOO01	MAR01	JOH01	WAP02a	WAP03
Phylum: Cnidaria							
Class: Hydrozoa							
Order: Anthoathecatae	0	0	0	0	0	0	0
Family: Hydridae	0	0	0	0	0	0	0
<i>Hydra</i>	0	7	0	0	0	0	0
Phylum: Tardigrada	0	0	0	40	0	0	0
Totals:	4702	2637	6335	7760	3490	921	1288
<u>Taxa present but not included:</u>							
Phylum: Arthropoda							
Subphylum: Crustacea							
Class: Ostracoda	14	7	17	0	10	3	0
Class: Maxillipoda	0	0	0	0	0	0	0
Class: Copepoda	0	7	17	0	10	3	0
Phylum: Annelida							
Subphylum: Clitellata							
Class: Oligochaeta							
Order: Tubificida	0	0	0	0	0	0	0
Family: Lumbricidae	0	7	0	0	50	0	0
Phylum: Nemata	14	7	17	20	0	3	4
Phylum: Platyhelminthes							
Class: Turbellaria	0	7	0	0	0	0	0
Totals:	28	35	51	20	70	9	4

Appendix D
Benthic Macroinvertebrates Identified at the Family Level Using Morphological Characteristics

Taxa	Site						
	AUR01	MEA001	LOO01	MAR01	JOH01	WAP02a	WAP03
Phylum: Arthropoda							
Subphylum: Hexapoda							
Class: Insecta							
Order: Ephemeroptera	0	0	67	700	0	0	0
Family: Ameletidae	0	0	83	140	40	25	8
Family: Baetidae	271	420	933	1080	20	83	140
Family: Ephemerellidae	143	67	50	0	40	17	36
Family: Heptageniidae	2543	227	1250	920	150	342	728
Family: Leptophlebiidae	0	0	50	100	10	0	0
Order: Plecoptera	0	7	117	80	100	3	4
Family: Capniidae	57	0	183	0	10	31	48
Family: Chloroperlidae	43	7	0	260	60	89	72
Family: Leuctridae	0	0	333	20	10	0	0
Family: Nemouridae	415	467	300	2540	620	58	16
Family: Perlidae	0	60	0	100	160	25	20
Family: Perlodidae	72	20	67	0	30	3	8
Family: Pteronarcyidae	0	0	0	0	0	0	16
Family: Taeniopterygidae	0	0	0	0	0	28	108
Order: Trichoptera	0	7	0	0	0	3	0
Family: Brachycentridae	0	7	17	60	10	36	8
Family: Hydropsychidae	0	0	0	0	70	28	48
Family: Rhyacophilidae	14	7	0	0	60	0	4
Order: Coleoptera	0	0	0	0	0	0	0
Family: Elmidae	57	246	17	500	600	9	0

Taxa	Site						
	AUR01	MEA001	LOO01	MAR01	JOH01	WAP02a	WAP03
Order: Diptera	0	0	33	0	0	0	0
Family: Ceratopogonidae	0	7	0	80	20	0	0
Family: Chironomidae	958	495	1785	760	1330	120	20
Family: Empididae	72	34	34	20	10	0	0
Family: Psychodidae	0	340	183	80	0	0	0
Family: Simuliidae	0	13	384	20	0	3	0
Family: Tipulidae	14	13	166	80	20	6	4
Subphylum: Chelicerata							
Class: Arachnida							
Order: Trombidiformes	0	20	0	0	10	0	0
Family: Aturidae	0	13	0	0	0	0	0
Family: Feltriidae	0	7	0	0	20	0	0
Family: Hydryphantidae	0	0	0	0	0	3	0
Family: Hygrobatidae	0	13	67	20	10	3	0
Family: Lebertiidae	29	67	50	60	20	3	0
Family: Sperchontidae	14	0	0	0	0	0	0
Family: Torrenticolidae	0	46	0	60	60	0	0
Suborder: Prostigmata							
Family: Stygothrombidiidae	0	0	0	40	0	0	0
Class: Malacostraca	0	0	0	0	0	0	0
Order: Amphipoda	0	0	0	0	0	3	0
Phylum: Annelida	0	0	0	0	0	0	0
Subphylum: Clitellata	0	0	0	0	0	0	0
Class: Oligochaeta	0	0	0	0	0	0	0
Order: Tubificida	0	0	0	0	0	0	0

Taxa	Site						
	AUR01	MEA001	LOO01	MAR01	JOH01	WAP02a	WAP03
Family: Enchytraeidae	0	20	0	0	0	0	0
Family: Naididae	0	0	166	0	0	0	0
Phylum: Cnidaria	0	0	0	0	0	0	0
Class: Hydrozoa	0	0	0	0	0	0	0
Order: Anthoathecatae	0	0	0	0	0	0	0
Family: Hydridae	0	7	0	0	0	0	0
Phylum: Tardigrada	0	0	0	40	0	0	0
Totals:	9397	4891	12440	14874	6357	1915	2660
<u>Taxa present but not included:</u>							
Phylum: Arthropoda	0	0	0	0	0	0	0
Subphylum: Crustacea	0	0	0	0	0	0	0
Class: Ostracoda	14	7	17	0	10	3	0
Class: Maxillipoda	0	0	0	0	0	0	0
Class: Copepoda	0	7	17	0	10	3	0
Phylum: Annelida	0	0	0	0	0	0	0
Subphylum: Clitellata	0	0	0	0	0	0	0
Class: Oligochaeta	0	0	0	0	0	0	0
Order: Tubificida	0	0	0	0	0	0	0
Family: Lumbricidae	0	7	0	0	50	0	0
Phylum: Nemata	14	7	17	20	0	3	4
Phylum: Platyhelminthes	0	0	0	0	0	0	0
Class: Turbellaria	0	7	0	0	0	0	0
Totals:	28	35	51	20	70	9	4

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

Metric	Site						
	AUR01	MEA001	LOO01	MAR01	JOH01	WAP02a	WAP03
Richness Measures							
Species Richness	27	41	39	37	44	35	23
EPT Richness	13	15	14	17	23	21	19
Ephemeroptera Richness	4	6	6	6	6	8	7
Plecoptera Richness	8	6	7	9	11	10	9
Trichoptera Richness	1	3	1	2	6	3	3
Chironomidae Richness	8	10	12	8	10	7	3
Oligochaeta Richness		1	2				
Abundance Measures							
Corrected Abundance	4702	2624	6318	7720	3440	918	1288
EPT Abundance	3558	1296	3450	6000	1390	771	1264
Dominance Measures							
1st Dominant Taxon	Heptageniidae	<i>Baetis</i> Heptageniidae		<i>Zapada cinctipes</i>	<i>Orthocladius</i> complex	<i>Rhithrogena</i>	<i>Rhithrogena</i>
1st Dominant Abundance	2543	402	1285	1700	982	323	653
2nd Dominant Taxon	<i>Orthocladius</i> complex	<i>Zapada cinctipes</i>	<i>Paratanytarsus</i> Heptageniidae		<i>Heterlimnius</i>	<i>Orthocladius</i> complex	Taeniopterygidae
2nd Dominant Abundance	457	365	987	1208	550	77	110
3rd Dominant Taxon	<i>Zapada cinctipes</i>	<i>Pericoma/Telmatoscopus</i>	<i>Baetis</i>	<i>Baetis fuscatus</i> group	<i>Zapada cinctipes</i>	Chloroperlidae	<i>Epeorus</i>
3rd Dominant Abundance	367	340	912	634	534	59	75
% 1 Dominant Taxon	54.08	15.32	20.35	22.02	28.54	35.15	50.7
% 2 Dominant Taxon	9.72	13.89	15.62	15.64	15.99	8.4	8.5
% 3 Dominant Taxon	7.81	12.96	14.43	8.21	15.52	6.4	5.82

Metric	Site						
	AUR01	MEA001	LOO01	MAR01	JOH01	WAP02a	WAP03
Percent Dominance	71.6	42.17	50.4	45.88	60.05	49.95	65.02
Community Composition							
% Ephemeroptera	62.89	27.21	38.51	38.08	7.56	50.87	70.81
% Plecoptera	12.48	21.38	15.83	38.86	28.78	25.82	22.67
% Trichoptera	0.3	0.8	0.27	0.78	4.07	7.3	4.66
% EPT	75.67	49.39	54.61	77.72	40.41	83.99	98.14
% Diptera	22.2	34.38	40.65	13.47	40.12	14.05	1.86
% Oligochaeta		0.76	2.63				
% Baetidae	5.76	16.01	15.19	18.36	0.58	9.04	10.87
% Chironomidae	20.37	18.86	28.25	9.84	38.66	13.07	1.55
% Odonata							
% Chironomidae within Diptera	91.76	54.88	69.05	73.08	96.38	93.02	83.33
% Hydropsychidae within Trichoptera	0	0	0	0	50	43.75	80
% Baetidae within Ephemeroptera	9.16	58.82	39.43	48.21	7.69	17.77	15.35
EPT ratio	0.79	0.72	0.66	0.89	0.51	0.87	0.98
Functional Group Composition							
% Predators	5.79	11.78	6.02	9.07	15.35	17.05	12.22
% Shredder-Herbivores	15.06	18.8	16.76	33.72	21	13.26	13.54
% Collector-Gatherers	23.81	57.32	32.46	37.25	55.7	26.97	15.22
% Scrapers	54.08	8.65	20.35	15.64	4.36	37.25	56.52
% Macrophyte-Herbivore					0.29		
% Collector-Filterer		2.09	21.97	2.75	2.7	5.12	0.62
% Omnivore	1.25	1.09	1.92	0.54	0.6	0.35	1.57
% Parasite							
% Piercer-Herbivore							
% Gatherer							
% Unclassified		0.27	0.52	1.04			0.31

Metric	Site						
	AUR01	MEA001	LOO01	MAR01	JOH01	WAP02a	WAP03
Functional Group Richness							
Predators Richness	11	16	9	11	18	10	7
Shredder-Herbivores Richness	5	4	7	5	7	6	4
Collector-Gatherers Richness	9	14	15	13	11	13	6
Scrapers Richness	1	1	1	1	2	2	2
MH Richness					1		
CF Richness		4	4	4	3	3	1
OM Richness	1	1	2	2	2	1	2
PA Richness							
Piercer-Herbivore Richness							
Gatherer Richness							
Unclassified		1	1	1			1
Voltinism Composition							
% Univoltine	9.02	14.16	5.62	25.44	16.68	8.88	4.36
% Semivoltine	0.3	0.27		1.3	3.88	3.42	2.2
% Multivoltine	5.41	15.82	15.49	0.26		5.04	4.91
Voltinism Richness							
Univoltine	3	2	4	4	2	5	3
Semivoltine	1	1		1	3	1	2
Multivoltine	1	2	2	1		2	1
Diversity/Evenness Measures							
Shannon-Weiner H' (log 10)	0.82	1.2	1.23	1.2	1.16	1.15	0.87
Shannon-Weiner H' (log 2)	2.72	4	4.09	3.97	3.86	3.81	2.91
Shannon-Weiner H' (log e)	1.89	2.77	2.83	2.76	2.67	2.64	2.01
Simpson's Index (D)	0.32	0.09	0.1	0.1	0.14	0.15	0.28
Simpson's Index of Diversity (1 - D)	0.68	0.91	0.9	0.9	0.86	0.85	0.72
Simpson's Reciprocal Index (1/D)	3.17	10.82	10.04	9.79	7.27	6.75	3.6
Biotic Indices							
Hilsenhoff Biotic Index	4.08	4.2	4.29	3.07	3.79	1.81	1.02

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

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

Taxa	Site					
	AUR01	MEA01	MAR01	JOH01	WAP02a	WAP03
INSECTS						
Order: Coleoptera						
Chrysomelidae						
<i>Donacia</i>					eDNA	eDNA
<i>Donacia clavipes</i>			eDNA			
Elmidae		Morph	Morph	Morph		
<i>Heterlimnius</i>	Morph	Morph	Morph	Morph	Morph	
Order: Diptera						
Ceratopogonidae						
<i>Mallochohelea</i>		Morph	Morph	Morph		
Chironomidae	Morph	Morph	Morph	Morph	Morph	Morph
Chironominae						
Chironomini						
<i>Microtendipes</i>		Both		Morph		
<i>Paracladopelma winnelli</i>		eDNA				
<i>Polypedilum</i>	eDNA				eDNA	eDNA
<i>Polypedilum albicorne</i>			eDNA			
Tanytarsini						
<i>Constempellina</i> sp. C	Morph	Morph		Morph		
<i>Micropsectra</i>	Both	Both	Both	Morph	Both	
<i>Microspectra logani</i>				eDNA		
<i>Paratanytarsus</i>			Morph			
<i>Rheotanytarsus</i>		Morph		Morph		
<i>Stempellina</i>				Morph		
<i>Stempellinella</i>			Morph			
Diamesinae						
<i>Diamesa</i>					eDNA	
<i>Pagastia</i>				Morph		
<i>Pagastia orthogonia</i>		eDNA		eDNA		
<i>Potthastia longimana</i> group		Morph				
Orthocladiinae						
<i>Brillia</i>	Morph	Morph	Morph	Morph	Morph	
<i>Chaetocladius</i>		eDNA				
<i>Corynoneura</i>	eDNA		Morph			Morph
<i>Cricotopus</i>		eDNA		eDNA	eDNA	eDNA
<i>Cricotopus trifascia</i>			eDNA			

Taxa	Site					
	AUR01	MEA01	MAR01	JOH01	WAP02a	WAP03
<i>Eukiefferiella</i>	Morph				Morph	Morph
<i>Eukiefferiella claripennis</i>		eDNA				eDNA
<i>Hydrobaenus</i>					Morph	
<i>Orthocladius</i> complex	Morph	Morph	Morph	Morph	Morph	Morph
<i>Orthocladius</i>		eDNA	eDNA			
<i>Orthocladius glabripennis</i>				eDNA	eDNA	
<i>Orthocladius oblidens</i>	eDNA					
<i>Rheocricotopus</i>		Morph	Morph	Morph		
<i>Thienemanniella xena</i>			eDNA			
<i>Tvetenia</i>	Morph	Both	Morph	Morph	Morph	
<i>Tvetenia paucunca</i>				eDNA		
Tanypodinae						
<i>Conchapelopia pallens</i>		eDNA	eDNA	eDNA		
<i>Thienemannimyia</i> group	Morph					
Empididae	Morph		Morph			
<i>Metachela</i>		eDNA				
<i>Metachela collusor</i>				eDNA		
<i>Neoplasta</i>		Morph		Morph		
<i>Neoplasta megorchis</i>		eDNA				
<i>Oreogeton</i>		Morph				
<i>Oreogeton scopifer</i>		eDNA				
<i>Roederiodes</i>	Morph					
Limoniidae						
<i>Symplecta cana</i>						eDNA
Psychodidae						
<i>Pericoma/Telmatoscopus</i>		Morph	Morph			
<i>Pneumia</i>		eDNA				
Simuliidae						
<i>Simulium</i>		Morph	Morph		Both	
<i>Simulium arcticum</i>		eDNA		eDNA		
<i>Simulium defoliarti</i>		eDNA	eDNA			
<i>Simulium tuberosum</i>		eDNA		eDNA		
Tipulidae		Morph				
<i>Antocha</i>			Morph			
<i>Dicranota</i>	Morph		Morph			
<i>Hexatoma</i>				Morph	Morph	Morph
<i>Rhabdomastix</i>				Morph		
<i>Tipula</i>	eDNA	eDNA	eDNA	Both		
<i>Tipula macrolabis</i>					eDNA	
Order: Ephemeroptera						
Ameletidae						
<i>Ameletus</i>		eDNA	Morph	Morph	Morph	Morph
<i>Ameletus bellulus</i>			eDNA	eDNA	eDNA	
<i>Ameletus celer</i>	eDNA				eDNA	eDNA

Taxa	Site					
	AUR01	MEA01	MAR01	JOH01	WAP02a	WAP03
Baetidae	Morph	Morph	Morph	Morph	Morph	Morph
<i>Acentrella</i>	Morph	Morph	Morph	eDNA	Morph	Morph
<i>Acentrella turbida</i>		eDNA			eDNA	eDNA
<i>Baetis</i>	Morph	Morph	Morph		Morph	Morph
<i>Baetis bicaudatus</i>		eDNA	eDNA		eDNA	eDNA
<i>Baetis fuscatus</i> group		Morph	Morph			
<i>Baetis phoebus</i>		eDNA	eDNA	eDNA	eDNA	eDNA
<i>Baetis rhodani</i> group			Morph			Morph
<i>Baetis tricaudatus</i>	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA
<i>Dipheter hageni</i>	eDNA	eDNA	eDNA	eDNA	eDNA	
Ephemerellidae	Morph	Morph		Morph	Morph	
<i>Drunella</i>		Morph			Morph	
<i>Drunella coloradensis</i>	eDNA	eDNA	eDNA	eDNA	eDNA	
<i>Drunella doddsii</i>		eDNA		eDNA	Both	Both
<i>Drunella grandis</i>		eDNA	eDNA	eDNA	eDNA	
<i>Ephemerella</i>					Morph	
<i>Ephemerella subvaria</i>						eDNA
<i>Ephemerella tibialis</i>		eDNA	eDNA	eDNA	eDNA	eDNA
Heptageniidae	Morph	Morph	Morph	Morph	Morph	Morph
<i>Cinygmula</i>	eDNA			Morph		
<i>Cinygmula</i> spJMW3		eDNA	eDNA	eDNA	eDNA	eDNA
<i>Ecdyonurus simplicioides</i>	eDNA					
<i>Epeorus</i>					Morph	Morph
<i>Epeorus deceptivus</i>		eDNA		eDNA	eDNA	eDNA
<i>Epeorus grandis</i>						eDNA
<i>Epeorus longimanus</i>		eDNA				eDNA
<i>Rhithrogena</i>				Morph	Morph	Morph
<i>Rhithrogena impersonata</i>					eDNA	eDNA
<i>Rhithrogena robusta</i>		eDNA	eDNA	eDNA	eDNA	eDNA
Leptophlebiidae			Morph	Morph		
<i>Paraleptophlebia heteronea</i>		eDNA	eDNA	eDNA	eDNA	
Siphonuridae						
<i>Siphonurus alternatus</i>		eDNA			eDNA	
<i>Siphonurus occidentalis</i>				eDNA	eDNA	
Order: Megaloptera						
Corydalidae						
<i>Nigronia</i>		eDNA				
<i>Nigronia serricornis</i>					eDNA	eDNA
Sialidae						
<i>Sialis</i>	eDNA					
Order: Plecoptera		Morph	Morph	Morph	Morph	Morph
Capniidae	Morph			Morph	Morph	Morph
<i>Capnia</i>		eDNA				
<i>Capnia coloradensis</i>						eDNA
<i>Capnia gracilaria</i>	eDNA			eDNA	eDNA	

Taxa	Site					
	AUR01	MEA01	MAR01	JOH01	WAP02a	WAP03
<i>Capnia petila</i>				eDNA		
<i>Eucapnopsis brevicauda</i>	eDNA	eDNA	eDNA		eDNA	eDNA
<i>Mesocapnia</i>					eDNA	
<i>Utacapnia</i>		eDNA				
<i>Utacapnia columbiana</i>			eDNA			
<i>Utacapnia logana</i>	eDNA		eDNA		eDNA	
Chloroperlidae	Morph		Morph	Morph	Morph	Morph
<i>Alloperla serrata</i>						eDNA
<i>Plumiperla diversa</i>					eDNA	eDNA
<i>Suwallia</i>					eDNA	
<i>Suwallia teleckojensis</i>						eDNA
<i>Sweltsa</i>	Morph	Morph	Morph	Morph	Morph	Morph
<i>Sweltsa borealis</i>	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA
<i>Sweltsa coloradensis</i>	eDNA	eDNA	eDNA	eDNA	eDNA	eDNA
Leuctridae			Morph	Morph		
<i>Paraleuctra occidentalis</i>	eDNA	eDNA	eDNA	eDNA	eDNA	
Nemouridae	Morph		Morph	Morph	Morph	
<i>Malenka</i>	Morph					
<i>Nemoura arctica</i>	eDNA					
<i>Podmosta decepta</i>			eDNA			
<i>Podmosta delicatula</i>			eDNA			
<i>Prostoia besametsa</i>		eDNA	eDNA		eDNA	
<i>Visoka cataractae</i>				Both		
<i>Zapada</i>	Morph	Morph	Morph	Morph	Morph	
<i>Zapada cinctipes</i>	Both	Both	Both	Both	Both	Both
<i>Zapada columbiana</i>				Both	eDNA	Both
<i>Zapada oregonensis</i>		eDNA	eDNA	eDNA		
Perlidae		Morph	Morph	Morph	Morph	Morph
<i>Doroneuria</i>		eDNA				
<i>Doroneuria theodora</i>				eDNA	eDNA	
<i>Hesperoperla</i>		Morph	Morph	Morph	Morph	
<i>Hesperoperla pacifica</i>		eDNA	eDNA	eDNA	eDNA	eDNA
Perlodidae	Morph	Morph		Morph	Morph	
<i>Isogenoides</i>						Morph
<i>Isogenoides frontalis</i>					eDNA	eDNA
<i>Isoperla fulva</i>					eDNA	eDNA
<i>Kogotus</i>	Morph	Both				
<i>Kogotus modestus</i>	eDNA			eDNA		eDNA
<i>Megarcys signata</i>					eDNA	eDNA
<i>Megarcys subtruncata</i>						eDNA
<i>Setvena bradleyi</i>						eDNA
Pteronarcyidae						
<i>Pteronarcella</i>						Morph
<i>Pteronarcella badia</i>					eDNA	eDNA

Taxa	Site					
	AUR01	MEA01	MAR01	JOH01	WAP02a	WAP03
Taeniopterygidae					Morph	Morph
<i>Doddsia occidentalis</i>					eDNA	eDNA
<i>Taenionema pacificum</i>					eDNA	eDNA
<i>Taenionema pallidum</i>			eDNA			
Order: Trichoptera		Morph			Morph	
Brachycentridae		Morph	Morph		Morph	Morph
<i>Brachycentrus americanus</i>			Morph	eDNA		Morph
<i>Micrasema</i>				Morph		
Hydropsychidae				Morph	Morph	Morph
<i>Arctopsyche</i>				Morph	Morph	Morph
<i>Arctopsyche grandis</i>		eDNA		eDNA	eDNA	eDNA
Rhyacophilidae						
<i>Rhyacophila</i>				Morph		
<i>Rhyacophila atrata</i> complex				Morph		
<i>Rhyacophila betteni</i> group	Morph					
<i>Rhyacophila brunnea/vemna</i> group		Morph		Morph		
<i>Rhyacophila hyalinata</i>	eDNA					eDNA
<i>Rhyacophila narvae</i>				Morph		
<i>Rhyacophila vaccua</i>	eDNA					
<i>Rhyacophila vofixa</i> group						Morph
ARACHNIDS						
Order: Oribatida						
Steganacaridae						
<i>Atropacarus striculus</i>				eDNA		
Order: Trombidiformes		Morph		Morph		
Aturidae						
<i>Aturus</i>		Morph				
Feltriidae						
<i>Feltria</i>		Morph		Morph		
Hydryphantidae						
<i>Protzia</i>					Morph	
Hygrobatidae						
<i>Atractides</i>				Morph	Morph	
<i>Hygrobates</i>		Morph	Morph			
Lebertiidae						
<i>Lebertia</i>	Morph	Morph	Morph	Morph	Morph	
Sperchontidae						
<i>Sperchon</i>	Morph					
Stygothrombidiidae						
<i>Stygothrombium</i>			Morph			
Torrenticolidae						
<i>Testudacarus</i>		Morph	eDNA	Morph	eDNA	eDNA
<i>Testudacarus minimus</i>		eDNA				
<i>Torrenticola</i>		Morph	Morph	Morph		

Taxa	Site					
	AUR01	MEA01	MAR01	JOH01	WAP02a	WAP03
SPRINGTAILS						
Order: Entomobryomorpha						
Entomobryidae						
<i>Entomobrya gisini</i>		eDNA				
HYDROZOANS						
Order: Anthoathecata						
Hydridae						
<i>Hydra</i>		Morph				
Pennariidae						
<i>Pennaria</i>				eDNA	eDNA	
COPEPODS		Morph		Morph	Morph	
MALACOSTRACANS						
Order: Amphipoda					Morph	
Order: Decapoda						
Cambaridae						
<i>Cambarus bartonii</i>						eDNA
OSTRACODS	Morph	Morph		Morph	Morph	
Order: Podocopida						
Candonidae						
<i>Candona candida</i>		eDNA				
OLIGOCHAETE WORMS						
Order: Tubificida						
Enchytraeidae						
<i>Enchytraeus</i>		Morph				
<i>Fridericia ratzeli</i>		eDNA				
Lumbricidae		Morph		Morph		
<i>Eiseniella tetraedra</i>		eDNA	eDNA	eDNA	eDNA	
Naididae						
<i>Allonais</i>		eDNA	eDNA	eDNA		
<i>Nais communis</i>				eDNA		
<i>Tubifex tubifex</i>	eDNA					
FLAT WORMS (Turbellaria)		Morph				