# Ghost Watershed
# Water Monitoring Program
# CABiN/STREAM Project
# 2023


**BIOTA CONSULTANTS**

A division of Aspen Croft Holdings Ltd.


**March 2024**

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Cover photo credit: Cal Hill – Johnson Creek, looking downstream from JOH01 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 as well as the traditional CABiN sampling for morphological analysis. The latter provided data on benthic macro-invertebrate abundance, required to determine the EPT ratio, among other metrics.

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. Instead a site slightly upstream was sampled and referred to as WAP02a.

In 2023, key sites were resampled, and additional sites were added. Field sampling occurred between August 29$^{th}$ and September 7$^{th}$. WAP02a was resampled, and site WAP03a was established slightly upstream of WAP03 since natural fluvial action and low stream flow had altered the latter, eliminating riffle habitat.

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 within the guidelines for the protection of freshwater aquatic life. The Hilsenhoff Biotic Index suggests there was possible slight organic pollution at almost all sites (water quality rating of very good), whereas some organic pollution was probable at the downstream Lesueur Creek site (LES02) (water quality rating of good).

The Simpson's Index of Diversity and the Shannon-Weiner Index indicate the sites were diverse in their community composition. The EPT ratio indicates high water quality at most of the sites, with EPT species more abundant than the pollution-tolerant chironomid family. Exceptions were LES02 and the Johnson Creek sites (JOH01 and JOH02) where the ratio was 0.49, 0.67 and 0.59, respectively. The ratio at the Ghost River site (GHO06) had rebounded since 2021, going from 0.48 to 0.83.

The proportion of functional feeding groups (FFGs) varied among the sites, reflecting the habitat and adjacent riparian vegetation. Shredders and predators dominated the lower Lesueur Creek site (LES02), whereas collector-gatherers dominated the upper site (LES01). At GHO06, collector gatherers had declined sharply since 2021. Scrapers and shredders were now most prominent. Scrapers continued to dominate at the Aura Creek and Waiparous Creek sites, although collector-gatherers were also abundant at WAP03a. Collector-gatherers and shredders were prominent at the Johnson Creek sites.

The eDNA results added 85 aquatic and semi-aquatic species to the species list for the sites, as well as providing richness measurements. Species richness was highest at the Waiparous Creek sites, GH06 and JOH02, and lowest at LES01 and JOH01.

The lower Lesueur Creek site and the Johnson Creek sites were potentially of most concern based on several of the results, with some discrepancies. These sites had the lowest EPT ratios, but also the highest diversity scores. Water quality with respect to organic pollution was lowest at LES02, but still rated as good. JOH02 had a high proportion of the more tolerant Baetidae (within the order Ephemeroptera), and LES02 had a high proportion of the more tolerant Hydropsychidae (within the order Trichoptera), although there were only three specimens. Collector-gatherers were the dominant functional feed group at JOH02, which as generalists are presumed to be more tolerant to disturbance. In contrast, shredders were prominent at LES02, which as specialists are presumed to be more sensitive. Both shredders and collector-gatherers dominated JOH01. Further sampling of these two creeks is recommended to better determine their health.

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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 the 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 as part of the field course in July, 2019 (WAP01). 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 that 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 2022, tributaries to Waiparous Creek were sampled: Aura Creek (AUR01), Meadow Creek (MEA01), Lookout Creek (LOO01) and Margaret Creek (MAR01). In addition, Johnson Creek (JOH01) was resampled. The paired sites on Waiparous Creek that were sampled in 2020 below and above the confluence with Johnson Creek, WAP02 and WAP03, were resampled in 2021 and 2022.

## 1.2 Field Plan

The focus of the water monitoring program in 2023 was to add to the inventory of sites and to resample key sites to provide further monitoring data and information on variability. Key sites included AUR01, WAP02/02a and WAP03, and GHO06 on the Ghost River above the Devil's Head/Black Rock wildfire. In 2021, some water quality parameters at GHO06 suggested poor water quality despite this site being close to the headwaters and in a relatively undisturbed area. In addition, JOH01 was resampled and a site upstream, JOH02, was created to learn more about the water quality along the length of the creek. Two sites along Lesueur Creek, a tributary of the Ghost River, were added to the inventory of monitoring sites.

[1] Code assigned by Alberta Agriculture and Forestry.

## 2.0 Methods

### 2.1 Field Sampling

The field sampling followed the same CABiN and STREAM protocols as in 2020, described in *Ghost Watershed Water Monitoring Program CABiN/STREAM Project 2020* (Biota Consultants 2022a), with a few exceptions. The STREAM protocol suggests obtaining three kicknet samples for the eDNA analysis; however, to reduce field time, only one kicknet sample was collected for this purpose, along with one for the morphological analysis.

A YSI-DSS multimeter again was rented from Oak Environmental Inc., whose staff calibrated it prior to field use. It was used to measure water temperature, dissolved oxygen, specific conductance and pH. Other water properties were measured in the lab by Bureau Veritas in Calgary, Alberta.

Field sampling occurred between August 29th and September 7th when there was low stream flow and mainly stable, sunny weather conditions. The CABiN 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.

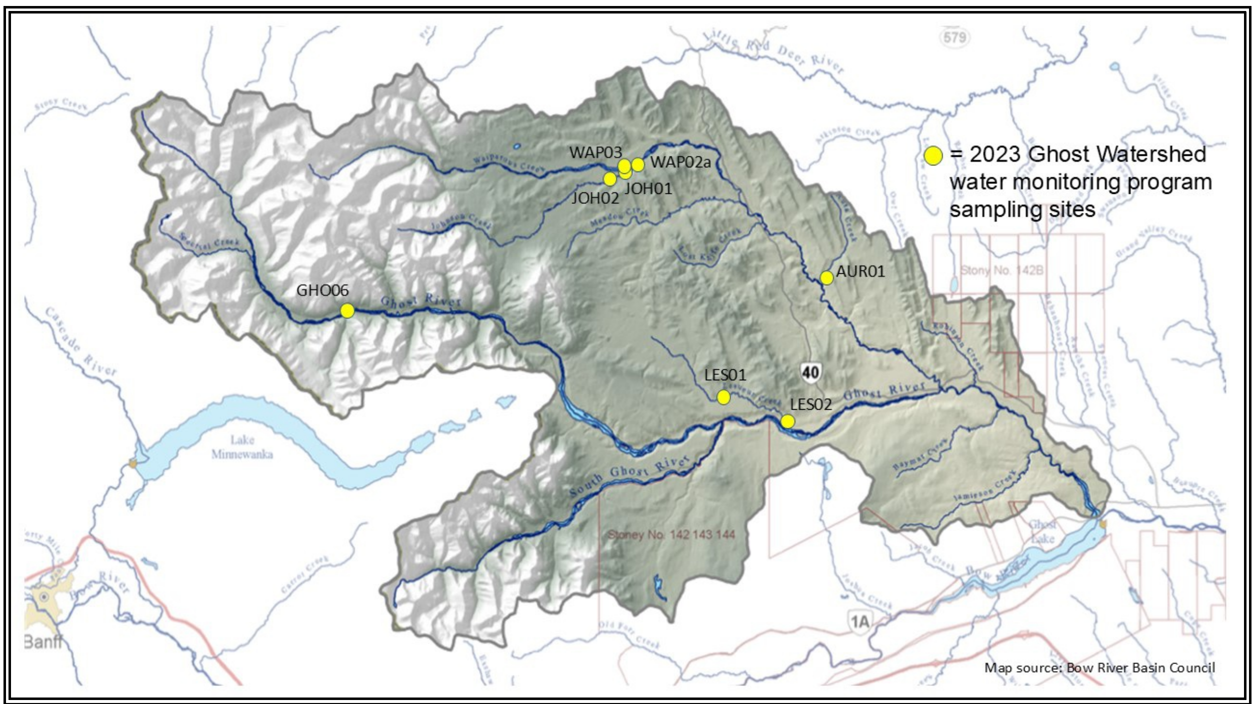


Figure 1. Sampling locations in 2023 within the Ghost River watershed.

Table 1. Location of sites sampled in 2023, plus sampling date, time of day, and conditions.

| Code/ Date | Latitude | Longitude | Elevation (m) | Comments |
|---|---|---|---|---|
| AUR01 Aug. 29 | 51.334136° | -114.935031° | 1379 | Aura Creek above confluence with Waiparous Creek<br>Sampled in afternoon<br>Sunny, air temperature 25.0°C, water temperature 13.4°C |
| WAP02a Aug. 30 | 51.394025° | -115.086074° | 1559 | Waiparous Creek below confluence with Johnson Creek<br>Sampled in morning<br>Sun and cloud, air temperature 22.5°C, water temperature 12.2°C |
| WAP03a Aug. 30 | 51.392402° | -115.089771° | 1565 | Waiparous Creek above confluence with Johnson Creek<br>Sampled in afternoon<br>Sun and cloud, air temperature 25.5°C, water temperature 15.9°C |
| JOH01 Aug. 31 | 51.391433° | -115.089451° | 1569 | Johnson Creek above confluence with Waiparous Creek<br>Sampled in morning<br>Cloudy/hazy, air temperature 14.0°C, water temperature 8.2°C |
| JOH02 Aug. 31 | 51.386647° | -115.106648° | 1588 | Johnson Creek above former “Johnson bog”<br>Sampled in afternoon<br>Cloudy/hazy, air temperature 17.5°C, water temperature 8.4°C |
| LES01 Sept. 6 | 51.279274° | -115.018454° | 1458 | Approximately halfway up Lesueur Creek<br>Sampled in morning<br>Sun, cloud, rain showers, air temperature 11.0°C, water temperature 11.7°C |
| LES02 Sept. 6 | 51.265376° | -114.967536° | 1370 | Lesueur Creek above confluence with Ghost River<br>Sampled in afternoon<br>Sun and cloud, air temperature 14.0°C, water temperature 11.3°C |
| GHO06 Sept. 7 | 51.320065° | -115.320123° | 1732 | Ghost River upstream of 2021 Devil’s Head/Blackrock wildfire<br>Sampled in afternoon<br>Sun and cloud, air temperature 15.5°C, water temperature 8.2°C |

In 2022, WAP02 could not be sampled in the exact location as the previous two years and the slightly upstream site was referred to as WAP02a. In 2023, this was the case with WAP03 (see section 3.1.5) and the site sampled was labelled WAP03a.

When sampling the paired sites on Waiparous Creek and the two sites on Johnson Creek, the downstream site (i.e., WAP02a, JOH01) was sampled prior to the upstream site (i.e., WAP03a, JOH02) to ensure 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 was begun 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 linked to the shared STREAM study within the CABiN database.

## 3.0 Results and Discussion

### 3.1 Physical Characteristics

The physical characteristics of the eight 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 embeddedness[2] 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).

#### 3.1.1 Lesueur Creek

The lowermost Lesueur Creek site (LES02) was situated approximately 95 m downstream of the TransAlta Road bridge and 500 m above the confluence with the Ghost River. There is a random campsite on the north side of the bridge beside the creek. LES02 had the lowest average channel depth of all sites sampled over the four years of the project, at only 4.1 cm, plus high embeddedness (Table 2).

LES01 was approximately 5 km upstream of LES02 and 40 m west of a linear pipeline clearing used by off-highway vehicles (OHV) to cross the creek. This clearing also bisects a random camping area above the creek on the south side. LES01 had one of the lowest wetted widths of all sites sampled, at 3.7 m, with an average channel depth of only 5.8 cm (Table 2). It also had comparitively low velocity.

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[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 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| Elevation (m) | 1370 | 1458 | 1732 | 1379 | 1569 | 1588 | 1559 | 1565 |
| Bankfull width (m) | 7.8 | 10.2 | 15.5 | 3.3 | 7.0 | 8.0 | 14.5 | 11.5 |
| Wetted width (m) | 4.9 | 3.7 | 11.4 | 2.0 | 5.8 | 5.5 | 14.2 | 7.6 |
| Bankfull wetted depth (cm) | 21.0 | 22.5 | 18.5 | 17.5 | 12.0 | 24.3 | 21.2 | 34.5 |
| Maximum channel depth (cm) | 5.7 | 7.5 | 18.0 | 7.5 | 26.3 | 18.0 | 28.1 | 23.4 |
| Average channel depth (cm) | 4.1 | 5.8 | 13.0 | 4.5 | 22.1 | 15.6 | 16.8 | 18.2 |
| Maximum velocity (m/s) | 0.6106 | 0.3132 | 0.9291 | 0.3431 | 0.9078 | 1.4353 | 1.0293 | 1.0575 |
| Average velocity (m/s) | 0.3699 | 0.2474 | 0.5272 | 0.2341 | 0.6712 | 1.0042 | 0.5841 | 0.8191 |
| Slope (m/m) | 0.0079 | 0.0041 | 0.0057 | 0.0275 | 0.0085 | 0.0071 | 0.0008 | 0.0133 |
| Substrate embeddedness (%) | 50 | 25 | 0 | 25 | 25 | 0 | 0 | 0 |
| Dominant substrate (cm) | 6.4-12.8 | 6.4-12.8 | 3.2-6.4 | 3.2-6.4 | 6.4-12.8 | 3.2-6.4 | 3.2-6.4 | 6.4-12.8 |
| $2^{nd}$ dominant substrate (cm) | 3.2-6.4 | 3.2-6.4 | 6.4-12.8 | 1.6-3.2 | 3.2-6.4 | 1.6-3.2 | 6.4-12.8 | 3.2-6.4 |
| Surrounding material (cm) | 0.2-1.6 | 0.2-1.6 | 0.2-1.6 | 0.2-1.6 | 02.-1.6 | 0.2-1.6 | 0.2-1.6 | 0.2-1.6 |
| Geometric median particle size (cm) | 6.5 | 6.2 | 5.7 | 3.9 | 8.5 | 3.6 | 5.0 | 7.7 |
| % Sand | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| % Gravel | 0 | 0 | 2 | 5 | 2 | 7 | 3 | 0 |
| % Pebble | 44 | 51 | 65 | 71 | 33 | 82 | 62 | 39 |
| % Cobble | 56 | 49 | 33 | 24 | 59 | 11 | 35 | 58 |
| % Boulder | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 3 |
| % Bedrock | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

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

### 3.1.2 Ghost River

The site sampled on the Ghost River is currently the uppermost site (GHO06) on the river and was sampled previously in 2021. The location of the tape, when it was stretched across the creek to determine the physical attributes, was not exactly the same in 2023, which will have contributed to the variation in data between years (Table 3). GHO06 had the lowest channel depth and velocity of the Ghost River sites, which were lower in 2023 (13.0 cm; 0.5272 m/s) compared to 2021 (19.1 cm; 0.7227 m/s). Another notable difference was embeddedness, which was less in 2023 (Table 3).

Table 3. Comparison of physical attributes at the Ghost River site (GHO06) in 2021 and 2023.

| **Attributes** | **Site and Date of Sampling** | |
|---|---|---|
| | **GHO06** | |
| | **Aug. 30, 2021** | **Sept. 7, 2023** |
| Elevation (m) | 1732 | 1732 |
| Bankfull width (m) | 16.4 | 15.5 |
| Wetted width (m) | 10.5 | 11.4 |
| Bankfull wetted depth (cm) | 23.5 | 18.5 |
| Maximum channel depth (cm) | 29.7 | 18.0 |
| Avg channel depth (cm) | 19.1 | 13.0 |
| Maximum velocity (m/s) | 0.9078 | 0.9291 |
| Avg velocity (m/s) | 0.7227 | 0.5272 |
| Slope (m/m) | 0.0050 | 0.0057 |
| Substrate embeddedness (%) | 25 | 0 |
| Dominant substrate (cm) | 3.2-6.4 | 3.2-6.4 |
| 2nd dominant substrate (cm) | 6.4-12.8 | 6.4-12.8 |
| Surrounding material (cm) | 0.2-1.6 | 0.2-1.6 |
| Geometric median particle size (cm) | 5.4 | 5.7 |
| % Sand | 0 | 0 |
| % Gravel | 3 | 2 |
| % Pebble | 56 | 65 |
| % Cobble | 41 | 33 |
| % Boulder | 0 | 0 |
| % Bedrock | 0 | 0 |

### 3.1.3 Aura Creek

Aura Creek is a small tributary of Waiparous Creek. The Aura Creek site, situated just upstream of the Waiparous Creek flood plain, was sampled previously in 2022. It had one of the lowest wetted widths of all sites sampled over the four years of the project, at 2.0 m in 2023, with an average channel depth of only 4.5 cm (Table 4). It also had comparitively low velocity in both years. As with GHO06, the exact location where the various bank and channel measurements were made differed between years, which will have contributed to some of the variability.

Table 4. Comparison of physical attributes at the Aura Creek site (AUR01) in 2022 and 2023.

| **Attributes** | **Site and Date of Sampling** | |
|---|---|---|
| | **AUR01** | |
| | **Sept. 12, 2022** | **Aug. 29, 2023** |
| Elevation (m) | 1379 | 1379 |
| Bankfull width (m) | 4.7 | 3.3 |
| Wetted width (m) | 2.2 | 2.0 |
| Bankfull wetted depth (cm) | 35.0 | 17.5 |
| Maximum channel depth (cm) | 7.2 | 7.5 |
| Avg channel depth (cm) | 5.9 | 4.5 |
| Maximum velocity (m/s) | 0.4202 | 0.3431 |
| Avg velocity (m/s) | 0.2007 | 0.2341 |
| Slope (m/m) | 0.0269 | 0.0275 |
| Substrate embeddedness (%) | 25 | 25 |
| Dominant substrate (cm) | 3.2-6.4 | 3.2-6.4 |
| 2nd dominant substrate (cm) | 1.6-3.2 | 1.6-3.2 |
| Surrounding material (cm) | 0.2-1.6 | 0.2-1.6 |
| Geometric median particle size (cm) | 3.1 | 3.9 |
| % Sand | 0 | 0 |
| % Gravel | 20 | 5 |
| % Pebble | 63 | 71 |
| % Cobble | 16 | 24 |
| % Boulder | 1 | 0 |
| % Bedrock | 0 | 0 |

### 3.1.4 Johnson Creek

The location of JOH01 was approximately 220 m upstream from its confluence with Waiparous Creek. It also was sampled in 2021 and 2022; however, it should be noted that the exact location where the various bank and channel measurements were made differed slightly between years. Variations in physical attributes tended to be relatively subtle among years (Table 5).

JOH02 was approximately 1.5 km upstream of JOH01. The bankful wetted depth was double that of JOH01 but channel depth was less. It had the highest velocity of all the sites sampled in 2023, and corresponding lower embeddedness than JOH01. Particle size, however, was smaller.

Table 5. Comparison of physical attributes at the Johnson Creek site (JOH01) from 2021 to 2023.

| **Attributes** | **Site and Date of Sampling** | | |
|---|---|---|---|
| | **JOH01** | | |
| | **Sept. 7, 2021** | **Aug. 30, 2022** | **Aug. 31, 2023** |
| Elevation (m) | 1569 | 1569 | 1569 |
| Bankfull width (m) | 6.7 | 6.2 | 7.0 |
| Wetted width (m) | 5.6 | 5.4 | 5.8 |
| Bankfull wetted depth (cm) | 23.0 | 7.4 | 12.0 |
| Maximum channel depth (cm) | 23.5 | 23.8 | 26.3 |
| Avg channel depth (cm) | 18.5 | 21.8 | 22.1 |
| Maximum velocity (m/s) | 0.8287 | 0.7799 | 0.9078 |
| Avg velocity (m/s) | 0.5658 | 0.6798 | 0.6712 |
| Slope (m/m) | 0.0085 | 0.0085 | 0.0085 |
| Substrate embeddedness (%) | 25 | 25 | 25 |
| Dominant substrate (cm) | 6.4-12.8 | 6.4-12.8 | 6.4-12.8 |
| 2nd dominant substrate (cm) | 12.8-25.6 | 3.2-6.4 | 3.2-6.4 |
| Surrounding material (cm) | 0.2-1.6 | 0.1-0.2 | 02.-1.6 |
| Geometric median particle size (cm) | 9.6 | 7.1 | 8.5 |
| % Sand | 0 | 0 | 0 |
| % Gravel | 0 | 1 | 2 |
| % Pebble | 29 | 44 | 33 |
| % Cobble | 67 | 50 | 59 |
| % Boulder | 4 | 5 | 6 |
| % Bedrock | 0 | 0 | 0 |

### 3.1.5 Waiparous Creek

2023 was the fourth consecutive year that sites below and above the confluence with Johnson Creek were sampled. Despite attempts to select reach locations at these two sites with similar stream channel characteristics, the heterogeneous nature of the stream channel, along with annual fluvial action and human interference altering the stream channel, has made this impossible. In 2022, human interference below the confluence with Johnson Creek was so great that the original WAP02 location no longer contained riffle habitat. As a result, the sampling site was moved slightly upstream to WAP02a. These sites bordered random campsites. In all four years, alteration of stream flow occurred from placement of rock dams at the edge of the creek and/or across the creek. The WAP02a site was again sampled in 2023.

Table 6. Comparison of physical attributes at Waiparous Creek sites, WAP02/WAP02a and WAP03/WAP03a, from 2020 to 2023.

| **Attributes** | **Site and Date of Sampling** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **WAP02** | | **WAP02a** | | **WAP03** | | | **WAP03a** |
| | **Sept. 1 2020** | **Sept. 2 2021** | **Sept. 7 2022** | **Aug. 30 2023** | **Sept. 3 2020** | **Sept. 2 2021** | **Sept. 7 2022** | **Aug. 30 2023** |
| Elevation (m) | 1554 | 1554 | 1559 | 1559 | 1560 | 1560 | 1560 | 1565 |
| Bankfull width (m) | 17.0 | 12.0 | 15.3 | 14.5 | 15.0 | 21.9 | 18.5 | 11.5 |
| Wetted width (m) | 9.6 | 10.4 | 7.8 | 14.2 | 6.9 | 8.9 | 4.2 | 7.6 |
| Bankfull wetted depth (cm) | 26.5 | 17.0 | 32.7 | 21.2 | 56.0 | 57.0 | 55.0 | 34.5 |
| Maximum channel depth (cm) | 27.0 | 21.2 | 42.6 | 28.1 | 22.0 | 24.2 | 32.8 | 23.4 |
| Avg channel depth (cm) | 17.4 | 17.2 | 23.8 | 16.8 | 16.4 | 15.2 | 26.8 | 18.2 |
| Maximum velocity (m/s) | 1.2528 | 1.3065 | 1.0759 | 1.0293 | 1.1293 | 1.0850 | 1.2838 | 1.0575 |
| Avg velocity (m/s) | 0.8760 | 0.8630 | 0.7192 | 0.5841 | 0.8650 | 0.7305 | 1.0052 | 0.8191 |
| Slope (m/m) | 0.0138 | 0.0110 | 0.0233 | 0.0008 | 0.0150 | 0.0087 | 0.0087 | 0.0133 |
| Substrate embeddedness (%) | 25 | 25 | 25 | 0 | 25 | 25 | 0 | 0 |
| Dominant substrate (cm) | 6.4-12.8 | 3.2-6.4 | 6.4-12.8 | 3.2-6.4 | 3.2-6.4 | 3.2-6.4 | 6.4-12.8 | 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 | 6.4-12.8 | 12.8-25.6 | 3.2-6.4 |
| 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 | 0.2-1.6 | 0.2-1.6 |
| Geometric median particle size (cm) | 10.3 | 6.9 | 5.3 | 5.0 | 5.9 | 5.8 | 10.0 | 7.7 |
| % Sand | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| % Gravel | 1 | 0 | 2 | 3 | 1 | 1 | 1 | 0 |
| % Pebble | 23 | 49 | 50 | 62 | 56 | 57 | 21 | 39 |
| % Cobble | 68 | 51 | 46 | 35 | 41 | 37 | 67 | 58 |
| % Boulder | 8 | 0 | 0 | 0 | 2 | 2 | 11 | 3 |
| % Bedrock | 0 | 0 | 2 | 0 | 0 | 3 | 0 | 0 |

The velocity at the slightly upstream location of WAP02a was less than the location of WAP02 (Table 6), which is likely due to the full or partial rock dams upstream, two in 2022 and seven in 2023. The geometric median particle size of the substrate was reduced each year, corresponding to the decline in velocity.

In 2023, natural fluvial action and low stream flow had altered the original WAP03 site enough to eliminate riffle habitat, therefore sampling occurred slightly upstream and the site was named WAP03a. As would be expected, physical attributes differed between the two sites (Table 6). Embeddedness was less at WAP03/03a in 2022 and 2023 versus the previous two years, and median particle size was greater.

## 3.2 Land Use

Forest habitat was present at all sites and was the dominant adjacent land use at all but the Waiparous Creek and upper Lesueur Creek sites, where the dominant activities in the surrounding area were random camping, day-use and off-highway vehicle (OHV) use (Table 7). OHV use occurred upstream of all sites to varying degrees with the exception of GHO06. Livestock grazing was a dominant upstream activity at the Lesueur Creek sites.

Table 7. 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** |
| LES02 | Adjacent | **x** | | | x | x | x | | |
| | Upstream | **x** | **x** | x | **x** | x | x | x | x |
| LES01 | Adjacent | x | x | | **x** | **x** | **x** | x | |
| | Upstream | **x** | **x** | x | **x** | x | x | x | x |
| GHO06 | Adjacent | **x** | | | | | | | |
| | Upstream | **x** | | | | | | | |
| AUR01 | Adjacent | **x** | | | x | | | | |
| | Upstream | **x** | x | x | x | | | | |
| JOH01 | Adjacent | **x** | | | x | x | | | |
| | Upstream | **x** | | | x | | | | |
| JOH02 | Adjacent | **x** | | | x | | | | |
| | Upstream | **x** | | | x | | | | |
| WAP02a & WAP03a | Adjacent | x | | | **x** | **x** | **x** | | |
| | Upstream | **x** | | | x | x | x | | |

### 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 8. 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 surface water quality guidelines and criteria, including brief narratives, are presented in Table 9.

Table 8. Chemical and physical attributes of water samples at each site.

| **Tests** | **Site** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **LES02** | **LES01** | **GHO06** | **AUR01** | **JOH01** | **JOH02** | **WAP02a** | **WAP03a** |
| Air Temperature (°C) | 14.0 | 11.0 | 15.5 | 25.0 | 14.0 | 17.5 | 22.5 | 25.5 |
| Water Temperature (°C) | 11.3 | 11.7 | 8.2 | 13.4 | 8.2 | 8.4 | 12.2 | 15.9 |
| Dissolved Oxygen (mg/L) | 9.75 | 9.48 | 9.75 | 9.40 | 9.76 | 9.97 | 9.05 | 8.27 |
| Specific Conductance (µS/cm) | 366.3 | 414.5 | 390.2 | 456.7 | 392.2 | 324.9 | 341.7 | 352.0 |
| pH | 8.20 | 8.37 | 8.09 | 8.35 | 8.17 | 8.18 | 8.24 | 8.18 |
| Total Suspended Solids (mg/L) | 1.6 | <1.0 | <1.0 | 2.2 | 1.5 | 1.1 | <1.0 | <1.0 |
| Turbidity (lab) (NTU) | 3.20 | 1.20 | <0.10 | 4.60 | 0.11 | 0.11 | 0.54 | 0.19 |
| | | | | | | | | |
| Anions | | | | | | | | |
| Alkalinity (Total as $CaCO_3$) (mg/L) | 220 | 260 | 160 | 260 | 140 | 160 | 160 | 140 |
| Alkalinity (PP as $CaCO_3$) (mg/L) | 5.4 | <1.0 | <1.0 | 5.0 | <1.0 | <1.0 | <1.0 | <1.0 |
| Bicarbonate ($HCO_3$) (mg/L) | 260 | 320 | 190 | 310 | 180 | 200 | 190 | 180 |
| Carbonate ($CO_3$) (mg/L) | 6.5 | <1.0 | <1.0 | 6.0 | <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 | <1.0 |
| | | | | | | | | |
| Nutrients | | | | | | | | |
| Total Phosphorus (P) (mg/L) | <0.003 | <0.003 | <0.003 | 0.004 | <0.003 | <0.003 | <0.003 | <0.003 |
| Dissolved Nitrogen (N) (mg/L) | 0.13 | 0.19 | 0.11 | 0.46 | 0.35 | 0.34 | 0.22 | 0.12 |
| Dissolved Nitrate (N) (mg/L) | <0.010 | <0.010 | 0.06 | <0.010 | 0.32 | 0.33 | 0.23 | 0.14 |
| Dissolved Nitrite (N) (mg/L) | <0.010 | <0.010 | <0.010 | <0.010 | <0.010 | <0.010 | <0.010 | <0.010 |
| Dissolved Total Kjeldahl Nitrogen (mg/L) | 0.127 | 0.191 | 0.051 | 0.455 | 0.030 | <0.020 | <0.020 | <0.020 |

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

Table 9. Surface water quality guidelines and criteria for protection of freshwater aquatic life.

| 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) | - | Narrative | 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 9. Continued

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

Source: Government of Alberta (2018)

The water quality guidelines 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 JOH01 and WAP03a (140 mg/L) and highest at LES01 and AUR01 (260 mg/L) (Table 8). These values are well above the minimum 20 mg/L level indicated in Table 9. 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 LES01, LES02 and AUR01 would be classified as very hard according to the USGS Water Science School (2018a) classification, whereas water at the other sites would be classified as hard:

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 8.09 to 8.37, which is in the safe range for acute toxicity according to Government of Alberta (2018) criteria (Table 9).

### 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 (µS/cm). Specific conductance is measured at, or corrected to, 25°C (Miller *et al.* 1988). Since conductivity increases with temperature, reporting conductivity at the reference temperature of 25°C allows data to easily be compared (FEI 2014a). The lowest conductivity value was at JOH02 (324.9 µS/cm), while the highest value occurred at AUR01 (456.7 µS/cm) (Table 8).

There is no set standard for the conductivity of water (Table 9) 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, whereas clay- and limestone-derived soils can contribute to higher conductivity values (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. Freshwater streams vary from 100 to 2,000 µS/cm whereas industrial wastewater is in the order of 10,000 µS/cm (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 change in conductivity, whether due to natural flooding, evaporation or man-made pollution, can be detrimental to water quality, hence to aquatic insects (FEI 2014a). The 2020 to 2023 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 <1.0 mg/L at four of the sites and up to 2.2 mg/L at AUR01 (Table 8), but were still within the guidelines. A TSS measurement of less than 20 mg/L generally appears clear, while levels over 40 mg/L may begin to appear cloudy (Michigan Department of Environmental Quality n.d., as cited in FEI 2014b).

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 (Murphy 2007; EPA 2012, as cited in FEI 2014b). Water clarity is significantly affected, declining as the amount of solids increases. Water temperature then increases, which reduces dissolved oxygen (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. Turbid water can appear cloudy, murky, hazy, muddy, coloured or opaque. At 5 NTU, water appears clear, at 55 NTU it is obviously cloudy, and at 500 NTU, it appears completely opaque (USGS Water Science School 2018b). The majority of sites had turbidity values between <0.1 NTU to 1.2 NTU (Table 8), which are considered very low (Table 9). The highest values were still relatively low at 3.20 NTU (LES02) and 4.60 NTU (AUR01).

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). Coloured dissolved organic matter, also known as humic stain, also can cause turbity. This is produced from decaying underwater vegetation and the release of tannins and other molecules. Water will appear red or brown, depending on the type of flora present. These dissolved substances may be too small to be considered suspended solids, but they contribute to turbidity by affecting water clarity (FEI 2014b).

By blocking sunlight, turbity can inhibit photosynthesis, thereby reducing plant growth, which in turn reduces dissolved oxygen. If the turbidity blocks enough sunlight to kill aquatic vegetation, aquatic organisms that rely on underwater plants also will decline (FEI 2014b).

Turbidity and TSS are related, as both reduce water clarity. However, turbidity is not a direct measurement of 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 dissolved oxygen values were within acceptable limits, ranging from 8.27 to 9.97 mg/L (Table 8). Dissolved oxygen (DO) is the concentration of free oxygen ($O_2$) present in water or other liquids, and is usually measured in mg/L. An $O_2$ level that is too low or too high can affect water quality, harming aquatic life (FEI 2013). The amount of $O_2$ dissolved in water primarily depends on temperature, atmospheric (barometric) pressure and salinity, and can be introduced through turbulence (e.g., rapids, waterfalls, waves) (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 Sites Between Years

A comparison among years of the physical and chemical attributes of the water samples at sites that have been sampled more than once are presented in Tables 10 and 11. There were slight variations between years at each site, but all were within the guidelines. This variation is expected in a natural environment.

Total suspended solids and turbidity were higher at AUR01 in 2023 versus 2022, which may be related to land uses upstream, such as logging. The higher measurements did not affect the level of DO; however, further monitoring would be prudent.

Table 10. Comparison among years of physical and chemical attributes of water samples at Ghost River (GHO06), Aura Creek (AUR01) and Johnson Creek (JOH01) sites.

| **Tests** | **Site and Date of Sampling** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **GHO06** | | **AUR01** | | **JOH01** | | |
| | **Aug. 31 2021** | **Sept. 7 2023** | **Sept. 12 2022** | **Aug. 29 2023** | **Sept. 7 2021** | **Aug. 30 2022** | **Aug. 31 2023** |
| Air Temperature (°C) | 20.0 | 15.5 | 14.0 | 25.0 | 16.0 | 21.0 | 14.0 |
| Water Temperature (°C) | 8.2 | 8.2 | 8.1 | 13.4 | 6.2 | 9.8 | 8.2 |
| Dissolved Oxygen (mg/L) | 9.36 | 9.75 | 9.53 | 9.40 | 10.24 | 9.61 | 9.76 |
| Specific Conductance (µS/cm) | 384.0 | 390.2 | 459.4 | 456.7 | 335.8 | 323.1 | 392.2 |
| pH | 8.20 | 8.09 | 8.24 | 8.35 | 8.21 | 8.23 | 8.17 |
| Total Suspended Solids (mg/L) | <1.0 | <1.0 | <1.0 | 2.2 | <1.0 | <1.0 | 1.5 |
| Turbidity (NTU) | 0.10 | <0.10 | 2.50 | 4.60 | 0.11 | 0.40 | 0.11 |
| | | | | | | | |
| Anions | | | | | | | |
| Alkalinity (Total as $CaCO_3$) (mg/L) | 130 | 160 | 260 | 260 | 150 | 170 | 140 |
| Alkalinity (PP as $CaCO_3$) (mg/L) | <1.0 | <1.0 | <1.0 | 5.0 | <1.0 | <1.0 | <1.0 |
| Bicarbonate ($HCO_3$) (mg/L) | 160 | 190 | 320 | 310 | 190 | 210 | 180 |
| Carbonate ($CO_3$) (mg/L) | <1.0 | <1.0 | <1.0 | 6.0 | <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) | <0.003 | <0.003 | <0.003 | 0.004 | <0.003 | <0.003 | <0.003 |
| Dissolved Nitrogen (N) (mg/L) | 0.17 | 0.11 | 0.36 | 0.46 | 0.16 | 0.29 | 0.35 |
| Dissolved Nitrite ($NO_2$) (mg/L) | <0.010 | <0.010 | <0.010 | <0.010 | <0.010 | <0.010 | <0.010 |
| Dissolved Nitrate plus Nitrite (N) | 0.23 | 0.06 | 0.01 | <0.010 | 0.17 | 0.24 | 0.32 |

Dissolved nitrogen and dissolved nitrate have gradually increased at JOH01 over the three years of sampling (Table 11). The outflow from Johnson Creek may have influenced the chemical attributes of Waiparous Creek below the confluence. The consistently higher dissolved nitrogen at WAP02/02a versus WAP03/03a, and the higher dissolved nitrate in the last two years (Table 10) possibly resulted from the even higher dissolved nitrogen and dissolved nitrate at JOH01, although these differences are within the range of natural variation.

Table 11. Comparison among years of physical and chemical attributes of water samples at the Waiparous Creek sites, WAP02/WAP02a and WAP03/WAP03a.

| **Tests** | **Site and Date of Sampling** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **WAP02** | | **WAP02a** | | **WAP03** | | | **WAP03a** |
| | **Sept. 1 2020** | **Sept. 2 2021** | **Sept. 7 2022** | **Aug. 30 2023** | **Sept. 3 2020** | **Sept. 2 2021** | **Sept. 7 2022** | **Aug. 30 2023** |
| Air Temperature (°C) | 22.5 | 10.5 | 21.5 | 22.5 | 17.5 | 19.0 | 23.0 | 25.5 |
| Water Temperature (°C) | 15.0 | 7.4 | 12.7 | 12.2 | 12.8 | 10.2 | 15.7 | 15.9 |
| Dissolved Oxygen (mg/L) | 8.97 | 9.92 | 8.04 | 9.05 | 8.77 | 9.28 | 8.27 | 8.27 |
| Specific Conductance (µS/cm) | 316.6 | 336.8 | 333.7 | 341.7 | 320.2 | 336.7 | 339.0 | 352.0 |
| pH | 8.18 | 8.29 | 8.04 | 8.24 | 8.38 | 8.38 | 8.27 | 8.18 |
| Total Suspended Solids (mg/L) | 1.2 | <1.0 | <1.0 | <1.0 | 2.0 | <1.0 | <1.0 | <1.0 |
| Turbidity (NTU) | <0.10 | 0.22 | 0.30 | 0.54 | <0.10 | 0.11 | 0.20 | 0.19 |
| | | | | | | | | |
| <u>Anions</u> | | | | | | | | |
| Alkalinity (Total as $CaCO_3$) (mg/L) | 150 | 140 | 160 | 160 | 140 | 130 | 140 | 140 |
| Alkalinity (PP as $CaCO_3$) (mg/L) | <1.0 | <1.0 | <1.0 | <1.0 | 1.4 | <1.0 | <1.0 | <1.0 |
| Bicarbonate ($HCO_3$) (mg/L) | 180 | 170 | 200 | 190 | 160 | 160 | 180 | 180 |
| Carbonate ($CO_3$) (mg/L) | <1.0 | <1.0 | <1.0 | <1.0 | 1.7 | <1.0 | <1.0 | <1.0 |
| Hydroxide (OH) (mg/L) | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 |
| | | | | | | | | |
| <u>Nutrients</u> | | | | | | | | |
| Total Phosphorus (P) (mg/L) | <0.003 | <0.003 | <0.003 | <0.003 | <0.003 | <0.003 | <0.003 | <0.003 |
| Dissolved Nitrogen (N) (mg/L) | 0.26 | 0.13 | 0.23 | 0.22 | 0.25 | 0.10 | 0.14 | 0.12 |
| Dissolved Nitrite ($NO_2$) (mg/L) | <0.010 | <0.010 | <0.010 | <0.010 | <0.010 | <0.010 | <0.010 | <0.010 |
| Dissolved Nitrate plus Nitrite (N) | 0.14 | 0.13 | 0.19 | 0.23 | 0.19 | 0.13 | 0.11 | 0.14 |

### 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 that are 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 2023 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 (e.g., Simpson's Diversity/Evenness Index and Shannon-Weiner Diversity Index). Diverse communities are indicators of "good" water quality.

The Simpson's Index of Diversity indicates the community composition of most sites sampled in 2023 are highly diverse (Figure 2). AUR01 had the lowest diversity with a value of 0.75. Similarly, values for the Shannon-Weiner Diversity Index were lowest for Aura Creek (2.14). Simpson's Index of Diversity was similar among years at JOH01 and WAP03/03a, with a dip in 2021 at WAP02/02a. Of those sites sampled more than once, diversity was highest in 2023 with the exception of WAP02/02a (Figure 2).

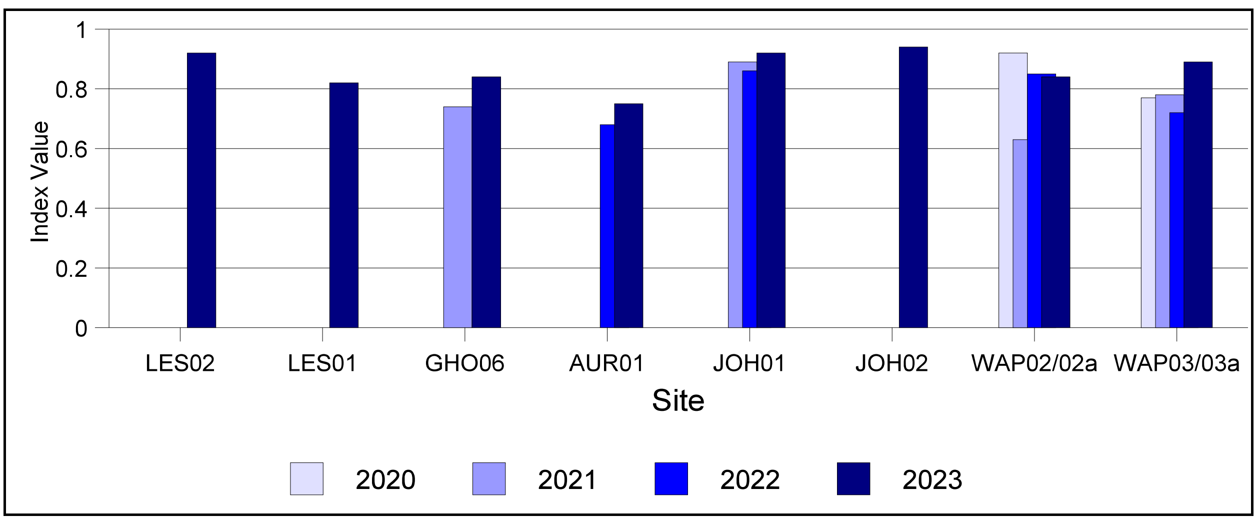


Figure 2. Simpson's Index of Diversity (1-D) for each site.

### 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 at four of the sites (LES01, AUR01, WAP02a, WAP03a), and were more abundant than Plecoptera at two (GHO06, JOH02). Plecoptera dominated at LES02 and exceeded the Ephemeroptera at JOH01, but did not dominate as in 2022 (Biota Consultants 2023). Trichoptera were very low in abundance (<1%) at three of the sites (LES02, GHO06, JOH02), and were not detected in the morphological sample from WAP02a. Note the abundance at LES02 was 0.3%, which is barely noticeable in Figure 3.

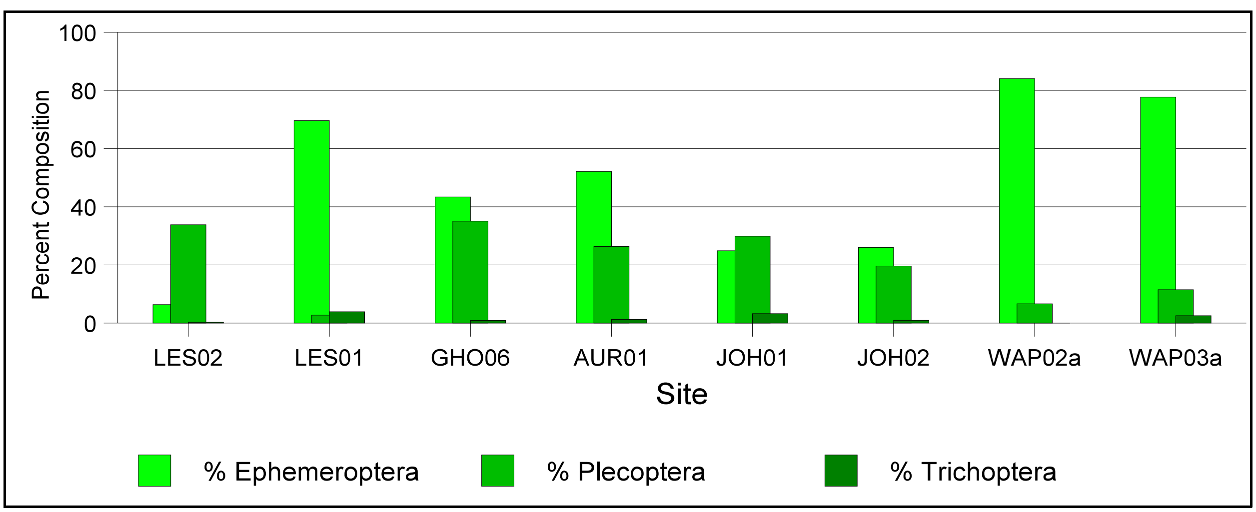


Figure 3. Percent composition of EPT orders at each site.

The EPT species were more prevalent than the Diptera species at all sites except LES02, but were close in abundance at JOH02 (46.5% and 38.0%, respectively) (Figure 4). In the previous three years, the EPT species were also much more abundant than Diptera and chironomids at WAP02/02a and WAP03 (Biota Consultants 2023). Chironomid flies comprised over 80% of the Diptera species except at AUR01, where 72.7% of the Diptera were chironomids (Figure 5). The percentage of chironimids was high in samples from previous years as well, including AUR01 in 2022.

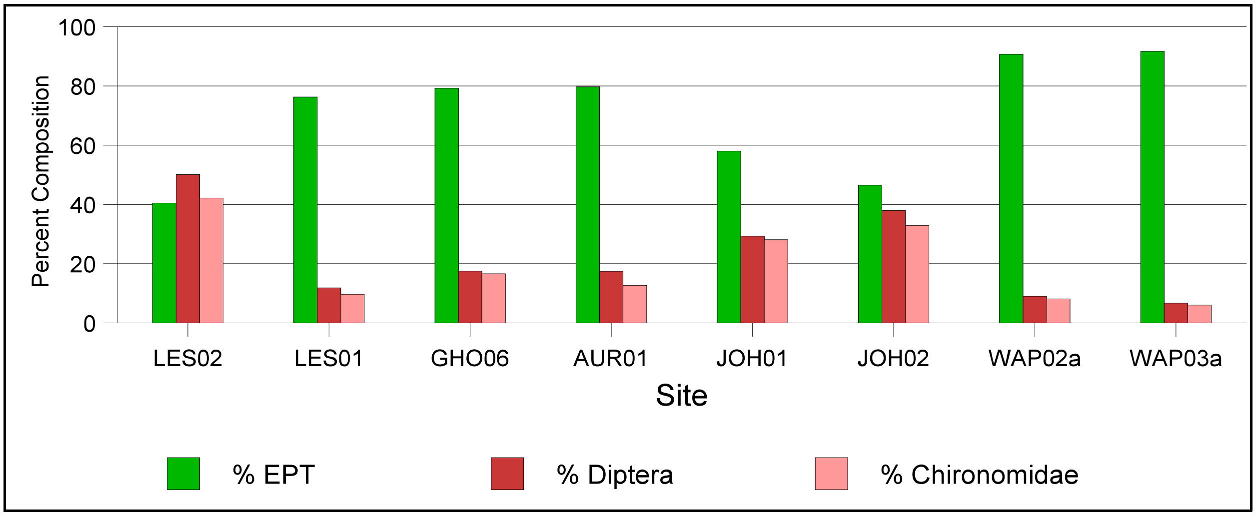


Figure 4. Percent composition of EPT orders, Diptera order and chironomid family at each site.

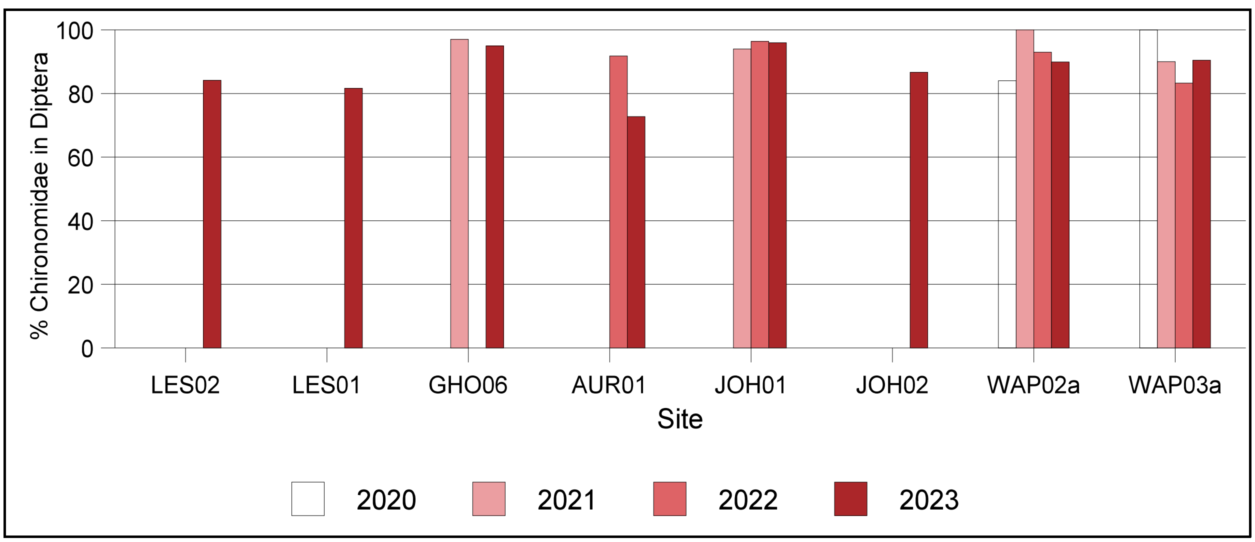


Figure 5. Percent of Diptera that were chironomid flies at each site.

The EPT ratio puts this into perspective (Figure 6). The downstream LES02 site had the lowest value, followed by JOH02 and JOH01. The ratio at JOH01 was slightly higher than the previous two years, at 0.67 (0.51 in 2022). The high values at WAP02/02a and WAP03/03a suggest good water quality. In all four years, values were slightly lower below the confluence with Johnson Creek, although not enough to clearly suggest the inflow from Johnson Creek influenced these values.

The EPT ratio of 0.48 at GHO06 in 2021 was a concern, but not easily explained since there were no obvious anthropogenic disturbances. There was great improvement in 2023 (0.83), which came closer to the very high ratio (0.99) recorded at the same location in 2020 by fRI Research (Alberta Agriculture and Forestry) and the City of Calgary. Further monitoring should establish the norm at this site.

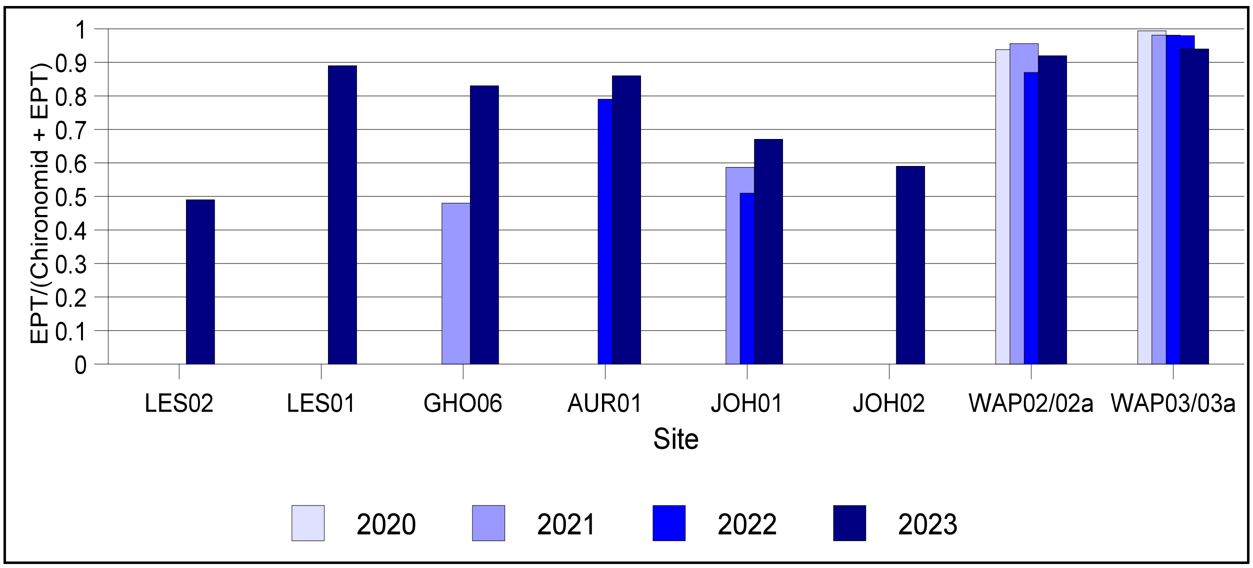


Figure 6. EPT/(chironomid + EPT) ratio for each site using percent community composition.

Based on the first four years of the water monitoring program, LES02 and the two Johnson Creek sites are of most concern with respect to the benthic macroinvertebrate community. The EPT ratio at LES02, and the percent of Ephemeroptera (6.4%), are the lowest of all 23 sites sampled during that time. This may be related to the amount of recreational use upstream of this site, largely including random camping and OHV use, and/or the agricultural use. In addition, regular flushes of sediment were provided from a puddle on the bridge just 95 m upstream whenever it was hit by a vehicle travelling at a high enough speed to make it splash into the creek.

The Devil's Head/Black Rock fire may be at least partially responsible for the low EPT ratio at the Johnson Creek sites, but no data were collected prior to the fire for comparison. There is high OHV activity on some upstream sections of Johnson Creek, but these are below JOH02.

The percentage of Trichoptera within the community at each site was very low, ranging from 0% to 3.9% (Figure 3). Hydropsychidae, a family within Trichoptera that is more tolerant to adverse conditions, was found at all sites except GHO06 and WAP02a (Figure 7). At the paired sites on Waiparous Creek, a variable proportion of the Trichoptera were Hydropsychidae over the four years of sampling (Figure 7). The proportion of Hydropsychidae has been less variable over the three years of sampling at JOH01. No Hydropyschidae were detected at AUR01 in 2022, but 9 of the 36 (25.0%) Trichoptera specimens in 2023 were Hydropyschidae. All three (100%) of the Trichoptera specimens at LES02 were Hydropsychidae.

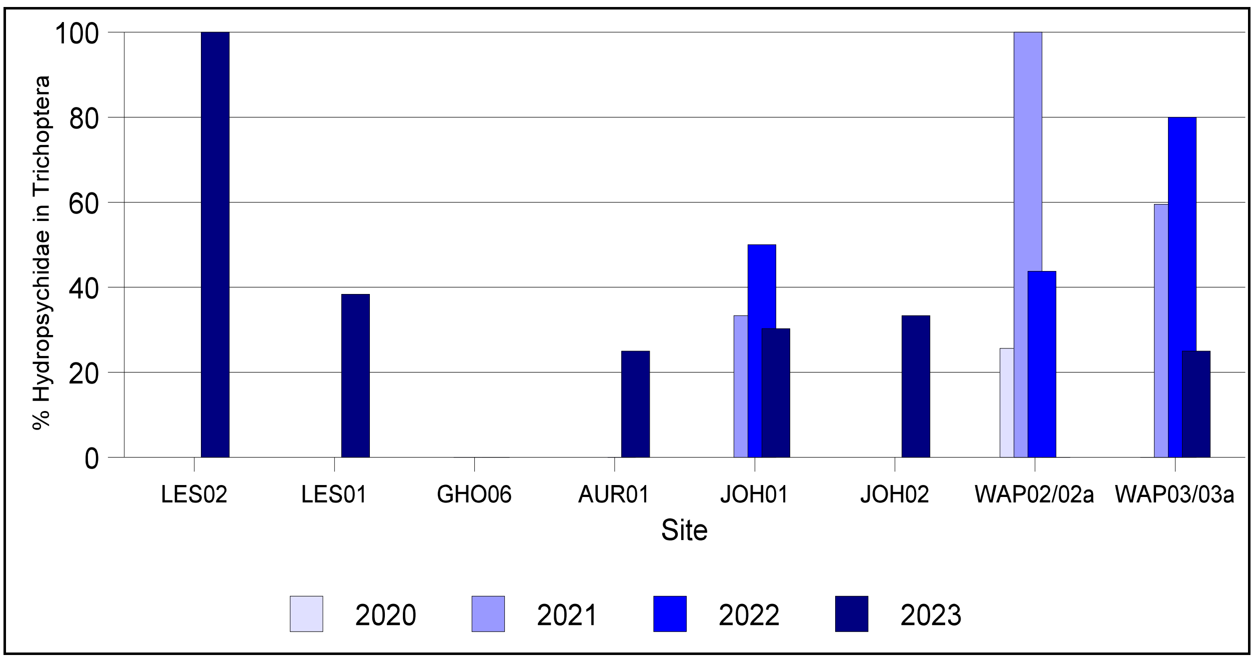


Figure 7. Percent of Trichoptera that were Hydropsychidae at each site.

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 AUR01, JOH01, WAP02/02a and WAP03/03a (Figure 8). It was highest at JOH02. There has been a steady increase in the proportion of Baetidae at WAP02/02a and WAP03/03a. No Baetidae were identifed by morphological analysis in 2021, but a very small proportion (0.7%) were reported in 2023.

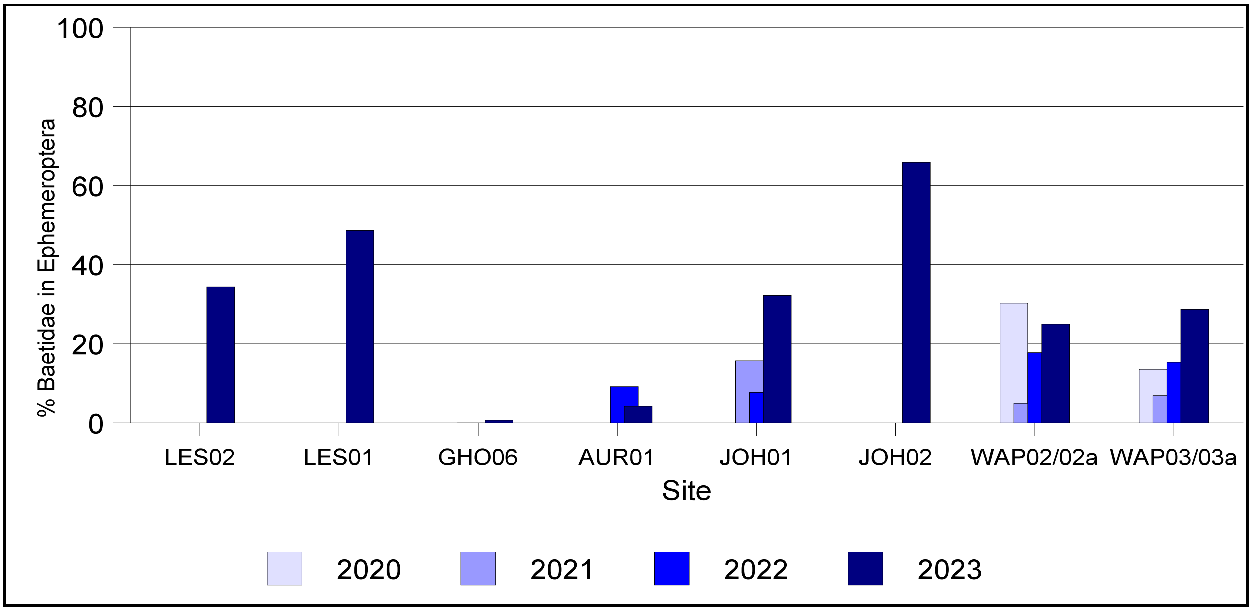


Figure 8. Percent of Ephemeroptera that were Baetidae at each site.

### 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 depend 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.

Caution is advised when comparing FFGs at the same site over multiple years for several reasons. At the family level, there are often different FFGs within the same family, and at the species level, there may be different FFGs at different growth stages. In addition, data reporting changed in 2023 from having blanks for taxa where the FFG could not be specified, to having those taxa reported as "other", i.e., unclassified (S. Finlayson, pers. comm.). As a result, the other category is larger in 2023 versus the previous years.

#### 3.4.3.1 Functional Feeding Groups at Ghost River and Tributary Sites

The graphical illustration of the FFGs at the Ghost River site (GHO06) and Lesueur Creek sites is presented in Figure 9. Collector-gatherers were noticeably more abundant at LES01 versus LES02, suggesting greater sediment, although more sediment was observed at LES02. Percent of shredders was higher at LES02, where there was likely more leaf litter from the higher cover of deciduous trees and shrubs. Collector-gatherers declined at GHO06 between 2021 and 2023, whereas shredders and scrapers increased. It is premature to draw conclusions on the increase in shredders and decline in collector-gatherers, although it may suggest an increase in water quality between years.

#### 3.4.3.2 Functional Feeding Groups at Waiparous Creek Tributary Sites

As in 2022, 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 (Figure 10). In the previous two years, collector-gatherers were prominent at the lower Johnson Creek site (JOH01), suggesting greater sediment, but declined in 2023. They were more abundant at JOH02, although sediment was not as noticeable at this site.

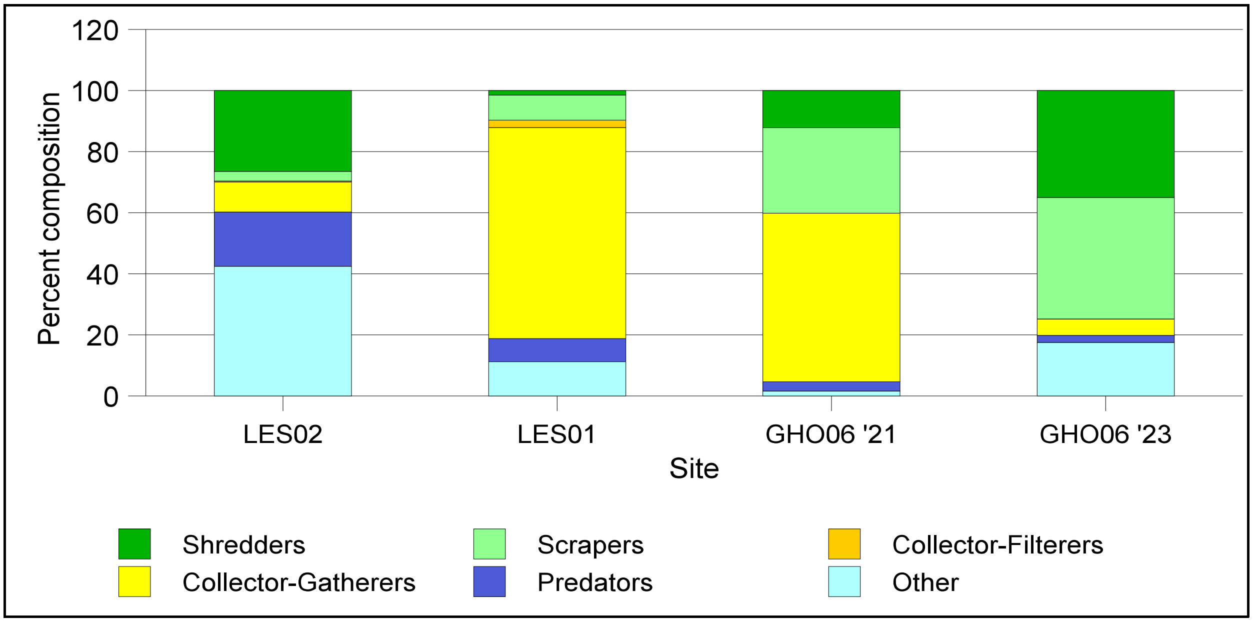


Figure 9. Percent of functional feeding groups at Ghost River and Lesueur Creek sites.

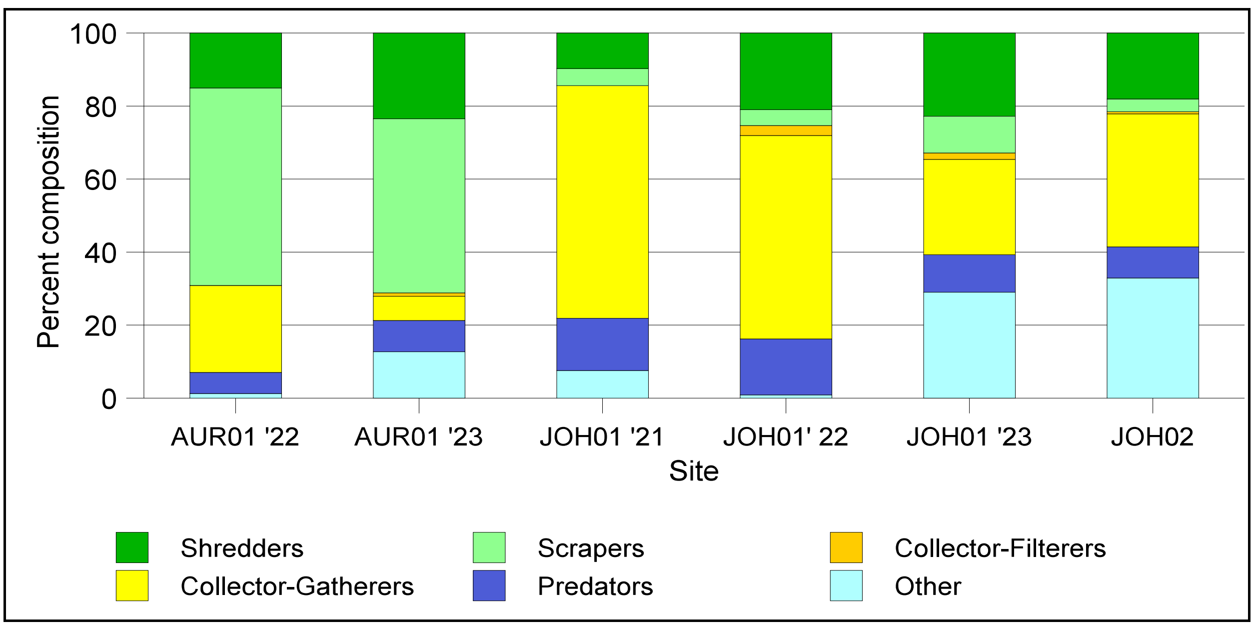


Figure 10. Percent of functional feeding groups at the tributary creeks of Waiparous Creek.

### 3.4.3.3 Functional Feeding Groups at WAP02/WAP02a and WAP03/WAP03a

The four years of data at WAP02/02a and WAP03/03a show the variability in FFG communities over time (Figures 11 and 12). The scrapers at WAP02/02a have become the dominant FFG, whereas the shredders and predators have declined, and the collector-gatherers have remained prominent (Figure 11). Similarly, the scrapers and collector-gatherers have become the dominant FFGs at WAP03/03a, and the shredders have declined since 2020 (Figure 12).

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.

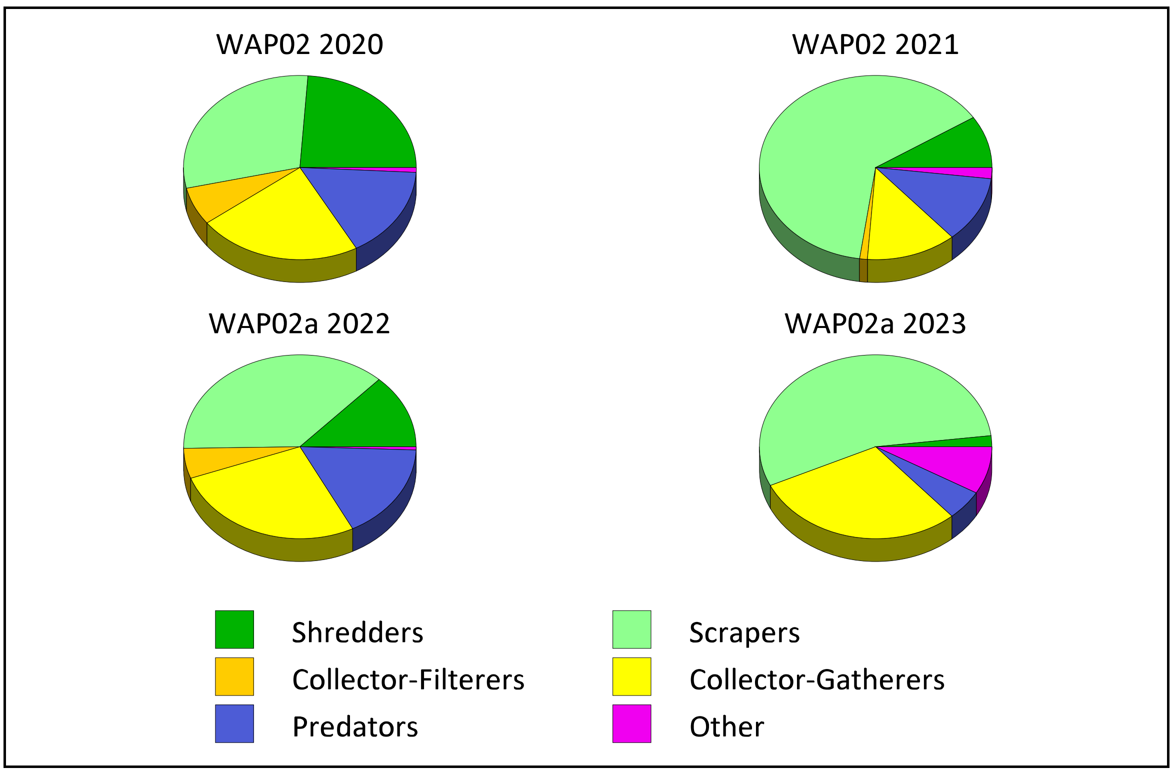


Figure 11. Percent of functional feeding groups at WAP02/WAP02a from 2020 to 2023.

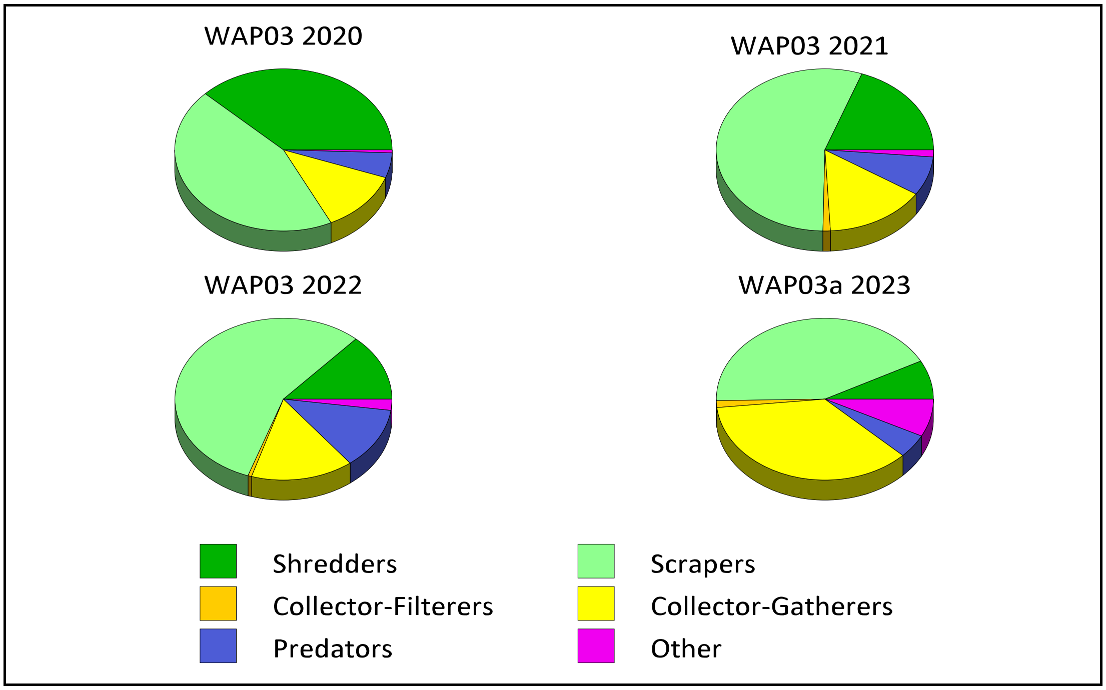


Figure 12. Percent of functional feeding groups at WAP03/WAP03a from 2020 to 2023.

### 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 11). 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 12. 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 all but one of the sites was rated as very good, with possible slight organic pollution (Figure 13). The downstream Lesueur Creek site (LES02) was rated as good with an index of 4.52, suggesting some organic pollution was probable. In previous years, the Waiparous Creek sites have been rated as excellent, suggesting that organic pollution increased in 2023.

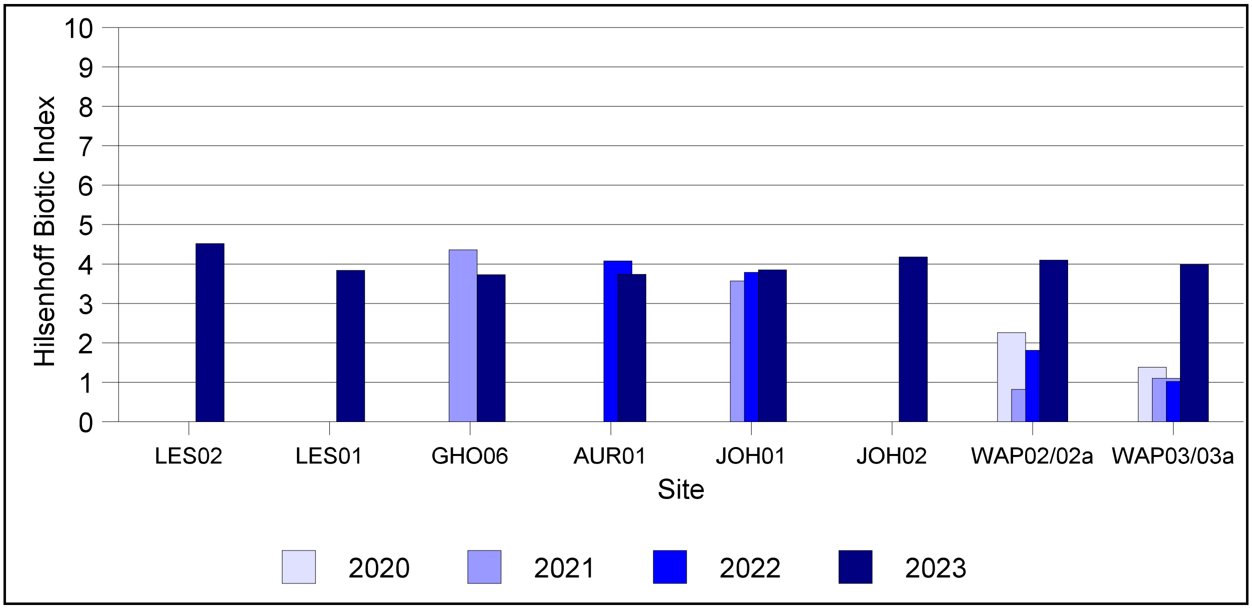


Figure 13. Hilsenhoff Biotic Index for each site.

## 3.5 STREAM eDNA Results

### 3.5.1 eDNA and Morphological Identification

The eDNA results complement the results of the morphological identification. An additional 85 aquatic or semi-aquatic species were identified, along with 17 terrestrial species. The additional aquatic and semi-aquatic taxa were within 69 different genera. It was expected that more taxa would be identified by eDNA since 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.). Fewer additional species were identified than previous years, probably largely because only one kicknet sample was collected versus three. The morphological identification included 47 genera that were not detected by eDNA, along with one family (Limnephilidae), three orders (Collembola, Basommatophora, Lumbriculida), three classes (Arachnida, Copepoda, Turbellaria) and one phylum (Nemata).

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

- The sequences in the reference database are from different species within the genus than those present in the sample, and are genetically distinct enough from each other that the species in the 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 the sample (three different primers are used in the workflow to overcome this known issue, but sometimes there still are taxa that are not compatible);
- The taxa may be too rare within the sample to be identified by DNA metabarcoding;
- 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 seventeen at just the genus level. Morphological identifications were occasionally to the species level, usually to the genus level, often to just 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 12). 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 because of the different techniques used. LES01 and JOH01 had the lowest richness based on eDNA but much higher richness measurements based on morphological identification.

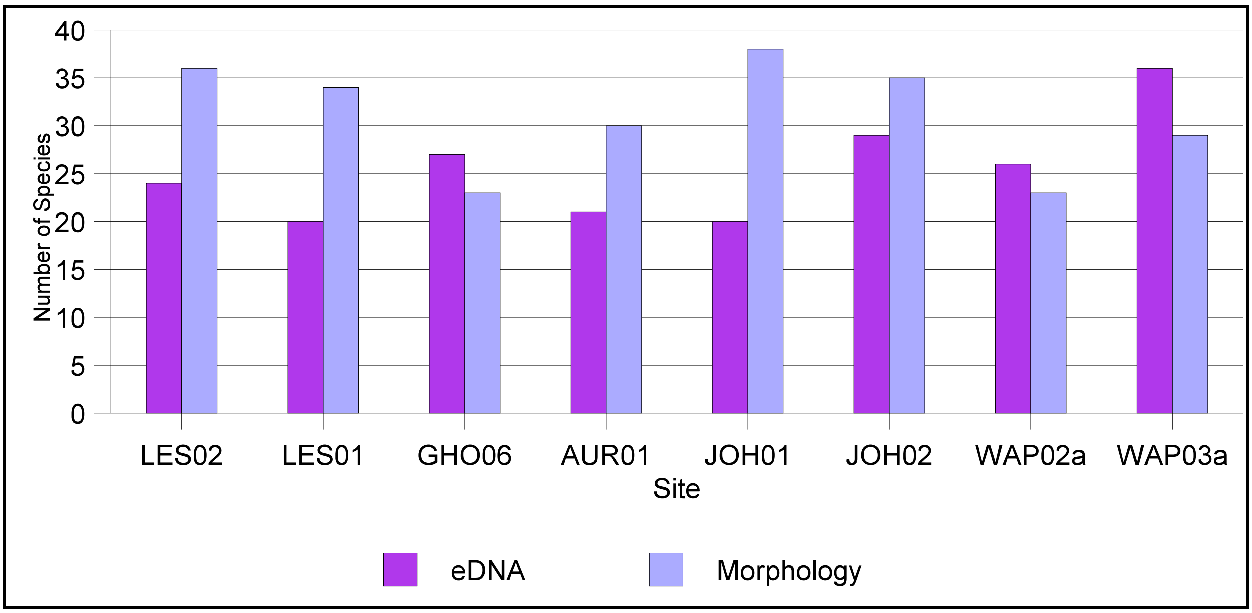


Figure 14. Species richness based on species taxonomically assigned by eDNA with high confidence based on normalized sequence data, and taxa identified morphologically.

Table 13. 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 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| **Class: Insecta** | | | | | | | | |
| **Order: Diptera** | | | | | | | | |
| **Chironomidae** | Both | Both | Both | Both | Both | Both | Both | Both |
| Chironominae | | | | | | | | |
| Chironomini | | | | | | | | |
| *Polypedilum* | Both | | eDNA | | | | Both | |
| Tanytarsini | | | | | | | | |
| *Micropsectra* | | Both | Morph | | Both | Both | Both | Both |
| Diamesinae | | | | | | | | |
| Diamesini | | | | | | | | |
| *Pagastia* | eDNA | | | Morph | Both | Both | | |
| *Potthastia gaedii* | Both | | | | | | | |
| Orthocladiinae | | | | | | | | |
| *Eukiefferiella* | | | Morph | Morph | Morph | Both | | |
| *Hydrobaenus* | Morph | | Both | | | | | |
| *Orthocladius* complex | Morph | | Both | Both | Morph | Morph | Morph | Morph |
| *Parametriocnemus* | eDNA | | | | | Morph | | |
| *Tvetenia* | | | Morph | Both | Both | Both | Morph | Both |
| Tanypodinae | Both | Both | | | | | | |
| Pentaneurini | Both | Both | | | | | | |
| **Empididae** | Morph | Morph | Morph | Both | Morph | Both | | Morph |
| **Simuliidae** | | | | | | | | |
| *Simulium* | | Morph | eDNA | Morph | Morph | Both | | eDNA |
| **Order: Ephemeroptera** | | | | | | | | |
| **Ameletidae** | | | | | | | | |
| *Ameletus* | Morph | | Both | | Morph | Both | Both | Both |
| **Baetidae** | Both | Both | Both | Both | Both | Both | Both | Both |
| *Acentrella* | | | | | Both | Both | Both | Both |
| *Baetis* | Both | | eDNA | Both | Both | Both | Both | Both |
| *Baetis bicaudatus* | | | eDNA | | Both | eDNA | Both | Both |
| *Diphetor hageni* | eDNA | eDNA | | eDNA | Both | eDNA | | |
| **Ephemerellidae** | eDNA | Both | Both | Both | Both | Both | Both | Both |
| *Drunella* | eDNA | Both | eDNA | eDNA | Both | eDNA | Both | Both |
| *Drunella doddsii* | | | | | | | Both | Both |
| *Ephemerella* | eDNA | Morph | eDNA | | Both | eDNA | Both | Both |

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| **Heptageniidae** | Both | Both | Both | Both | Morph | Both | Both | Both |
| *Cinygmula* | eDNA | eDNA | Both | Both | eDNA | eDNA | Both | Both |
| *Epeorus* | | eDNA | Both | | | | Both | Both |
| *Rhithrogena* | | | eDNA | | Morph | Both | Both | Both |
| **Leptophlebiidae** | Both | Both | | | Both | Both | Both | |
| **Order: Plecoptera** | Both | Both | Both | Both | Both | Both | Both | Both |
| **Capniidae** | Both | eDNA | Both | Morph | | Both | eDNA | eDNA |
| **Chloroperlidae** | | Both | eDNA | Both | Both | Both | Both | Both |
| *Plumiperla* | | | | | | | eDNA | Both |
| *Sweltsa* | | Both | | Both | Both | Both | Both | eDNA |
| **Nemouridae** | Both | Both | Both | Both | Both | Both | Both | Both |
| *Visoka cataractae* | | | | | Morph | eDNA | | |
| *Zapada* | Both | Both | Both | Both | Both | Both | Both | Both |
| *Zapada cinctipes* | Both | Both | Both | Both | Both | Both | Both | Both |
| *Zapada columbiana* | | | eDNA | | eDNA | Both | | eDNA |
| **Perlidae** | Both | Both | | Both | Both | Both | Both | Both |
| *Hesperoperla* | Both | Both | | eDNA | Both | Both | Both | eDNA |
| **Perlodidae** | Morph | Morph | eDNA | Both | Morph | | eDNA | Both |
| *Isogenoides* | | | | | | | eDNA | Both |
| *Kogotus* | Morph | | | Both | Morph | | | eDNA |
| **Pteronarcyidae** | | | | | | | | |
| *Pteronarcella* | | | eDNA | | | | eDNA | Morph |
| **Taeniopterygidae** | Morph | | Both | | | | Both | Both |
| **Order: Trichoptera** | Morph | Both | Morph | Morph | Both | Both | | Both |
| **Hydropsychidae** | Morph | Both | | Morph | Both | Morph | | eDNA |
| *Arctopsyche* | | | | | Both | Morph | | eDNA |
| *Hydropsyche* | | Both | | | | | | |
| **Lepidostomatidae** | | | | | | | | |
| *Lepidostoma* | | | | Morph | | eDNA | | |
| **Class: Bivalvia** | | | | | | | | |
| **Order: Veneroida** | | | | | | | | |
| **Pisidiidae** | | Both | | | | | | |
| *Pisidium* | | Both | eDNA | | | | | |
| **Class: Gastropoda** | Morph | Morph | eDNA | | | Morph | | |
| **Class: Oligachaeta** | | | | | | | | |
| **Order: Tubificida** | | | | | | | | |
| **Enchytraeidae** | Both | | | | | | Morph | eDNA |
| **Lumbricidae** | Both | Both | | | Both | eDNA | Both | eDNA |
| **Naididae** | | | | | | | | |
| Tubificinae | | Morph | | Both | | | | |

### 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* characteristics (S. Finlayson, pers. comm.). Sludge worm again was detected at AUR01 in 2023 by eDNA (Hajibabaei Lab 2024).

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 2024). 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 2023 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 which provides 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 eight sites indicate high water quality. 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 and nitrate at WAP02/02a versus WAP03/03a was possibly a result of the even higher dissolved nitrogen and nitrate at JOH01, although this may just be natural variation.

The Hilsenhoff Biotic Index suggests high water quality at almost all sites with respect to organic pollution, with the downstream Lesueur Creek site (LES02) rated slightly lower as good quality.

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. Richness was lowest at AUR01, LES01 and JOH01.

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 LES02 where the ratio was 0.49, potentially raising concerns. As in the previous two years, the EPT ratio at JOH01 was relatively low, at 0.67, but the upstream site, JOH02, was lower, at 0.59.

The percentage of the more tolerant Hydropsychidae within the Trichoptera was relatively low or none. The exception was LES02; however, there were only three Trichoptera specimens recorded at this site, all of which were Hydropsychidae. Baetidae were identified at all sites but in low to relatively low abundance except at JOH02.

The proportion of FFGs varied among the sites, largely reflecting the habitat and adjacent riparian vegetation. Scrapers dominated in Aura Creek and Waiparous Creek, suggesting more algae; collector-gatherers were prominent LES01, JOH01, WAP02a and WAP03a, suggesting greater sediment; shredders were highest at LES02, GHO06, JOH01 and AUR01, suggesting greater leaf litter.

## 4.2 Comparison Between Years of Ghost River Site

Channel depth was less at GHO06 in 2023 versus 2021, reflecting the summer drought. Velocity also declined. Another notable difference was embeddedness, which was less in 2023.

There were slight variations in physical and chemical attributes of the water samples, but all were within the guidelines. Water quality with respect to organic pollution remained very good between years.

Diversity went up slightly in 2023, and most importantly, the EPT ratio was much improved in 2023, matching more closely that reported at the same site in 2020 by staff from fRI Research (Alberta Agriculture and Forestry) and the City of Calgary. There were no Hydropsychidae reported in either year, and very low numbers of Baetidae reported in 2023. Among the FFGs, shredders and scrapers were more abundant in 2023 and collector-gatherers were substantially fewer, possibly suggesting an increase in water quality.

## 4.3 Comparison Between Years of Aura Creek Site

The physical characteristics of AUR01 were not markedly different between years. There were slight variations in physical and chemical attributes of the water samples. Most notable were total suspended solids and turbidity which were higher in 2023 versus 2022, but not high enough to cause concern. The Hilsenhoff Biotic Index suggested very good water quality in both years.

There was a slight increase in diversity and in the EPT ratio between years, suggesting a healthy ecosystem. Hydropsychidae and Baetidae were relatively low in abundance. The proportion of FFGs differed between years, with shredders slightly more abundant in 2023 and collector-gatherers less abundant, possibly suggesting an increase in water quality.

## 4.4 Comparison Among Years of Johnson Creek Site

There were slight variations in physical and chemical attributes of the water samples at JOH01, but all were within the guidelines. There has been a gradual increase in dissolved nitrogen and dissolved nitrate over the three years of sampling. The Hilsenhoff Biotic Index has varied little, indicating possible slight organic pollution with a rating of very good.

Diversity indices indicate highly diverse community composition in all three years. However, the EPT ratio remained lower than most sites, but did increase in 2023 to 0.67. The percent of Hydropsychidae within the Trichoptera went down while the percent of Baetidae within the Ephemeroptera went up. The proportion of shredders increased slightly while that of the collector-gatherers declined.

While some health indicators suggest concerns with water quality at JOH01, others do not. Although still relatively low, the increase in the EPT ratio is positive. Further monitoring over the years should help to determine the health of this site.

### 4.5 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 average velocity and median particle size of the substrate, both of which declined. Embeddedness was less at WAP03/03a in 2022 and 2023 versus the previous two years, and median particle size was greater.

There were slight variations in physical and chemical attributes of the water samples, but all were within the guidelines. The Hilsenhoff Biotic Index had consistently been in the “excellent” category, indicating organic pollution was unlikely, but dropped to the “very good” category in 2023, suggesting possible slight organic pollution.

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

The proportion of FFGs varied among the years. At WAP02/02a, there was a notable decline in shredders and predators over the four years. At WAP03/03a, the shredders have declined and collector-gatherers have increased. The variation in the stream channels may explain these differences.

### 4.6 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 2023 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., Lesueur Creek, Johnson Creek. (If monitoring in successive years, three years may be considered adequate, but CABiN does not specify a frequency.)

- If possible, at least one additional sampling site should be established on Lesueur Creek above LES01, ideally including a site above all/most OHV activity. A site above the random campsite beside the TransAlta Road, between LES01 and LES02, may help to determine the effect of the campsite and road/bridge on LES02.

- Further sampling should occur at AUR01 to monitor the levels of TSS and turbidity.

- Further sites on Johnson Creek would help to determine the health of the creek, ideally including a site above JOH02, above all/most OHV activity.

- Requests should be made to modify the bridge over Lesueur Creek to prevent the introduction of sediment into the creek by vehicle traffic.

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

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

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

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

- To maintain consistency, the same laboratories that were originally selected and used in from 2020 to 2023 (water chemical and benthic macroinvertebrate analysis) should continue to be used.

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## 6.0 Personal Communications

| | |
|---|---|
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# 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) Information Source: _______________

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

☐ Logging ☐ Mining ☐ Commercial/Industrial ☐ Other ___________

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

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

☐ Logging ☐ Mining ☐ Commercial/Industrial ☐ Other ___________

### Location Data

Latitude: ___________N Longitude: - ____________W (DMS or DD)

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

### Site Location Map Drawing

Note: Indicate north

Field Crew: ______________________________________ Site Code: ______________

Sampling Date: (DD/MM/YYYY) __________________________

**Photos**

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

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

## REACH DATA *(represents 6 times bankfull width)*

1. Habitat Types: *(check those present)*

☐ Riffle ☐ Rapids ☐ Straight run ☐ Pool/Back Eddy

2. Canopy Coverage: *(stand in middle of stream and look up, check one)*

☐ 0 % ☐ 1-25 % ☐ 26-50 % ☐ 51-75 % ☐ 76-100 %

3. Macrophyte Coverage: *(not algae or moss, check one)*

☐ 0 % ☐ 1-25 % ☐ 26-50 % ☐ 51-75 % ☐ 76-100 %

4. Streamside Vegetation: (*check those present)*

☐ ferns/grasses ☐ shrubs ☐ deciduous trees ☐ coniferous trees

5. Dominant Streamside Vegetation: *(check one)*

☐ ferns/grasses ☐ shrubs ☐ deciduous trees ☐ coniferous trees

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

☐ **1** - Rocks are not slippery, no obvious colour (thin layer < 0.5 mm thick)

☐ **2** - Rocks are slightly slippery, yellow-brown to light green colour (0.5-1 mm thick)

☐ **3** - Rocks have a noticeable slippery feel (footing is slippery), with patches of thicker green to brown algae (1-5 mm thick)

☐ **4** - Rocks are very slippery (algae can be removed with thumbnail), numerous large clumps of green to dark brown algae (5 mm -20 mm thick)

☐ **5** - Rocks are mostly obscured by algal mat, extensive green, brown to black algal mass may have long strands (> 20 mm thick)

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

## BENTHIC MACROINVERTEBRATE DATA

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

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

Preservative used: __________________

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

If YES, debris collected for QAQC ☐

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

Field Crew: ____________________________________________ Site Code: ________________

Sampling Date: (DD/MM/YYYY) ______________________________

**WATER CHEMISTRY DATA** **Time:**__________ (24 hr clock) **Time zone**: ________________

Air Temp: __________________(ºC) Water Temp: ___________(ºC) pH: ________________

Specific Conductance: ________(μs/cm) DO: ____________(mg/L) Turbidity: __________(NTU)

Check if water samples were collected for the following analyses:

☐ TSS (Total Suspended Solids)

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

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

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

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

## CHANNEL DATA

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

☐ **Calculated from map**

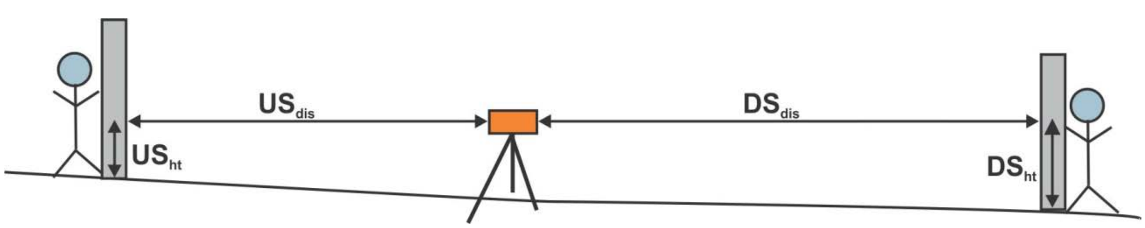
Scale: _________________ (Note: small scale map recommended if field measurement is not possible - i.e. 1:20,000).
contour interval (vertical distance) ____________ (m),
distance between contour intervals (horizontal distance) ____________ (m)
slope = vertical distance/horizontal distance =___________________

**OR**

☐ **Measured in field**

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

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



Field Crew: ______________________________________ Site Code: ______________

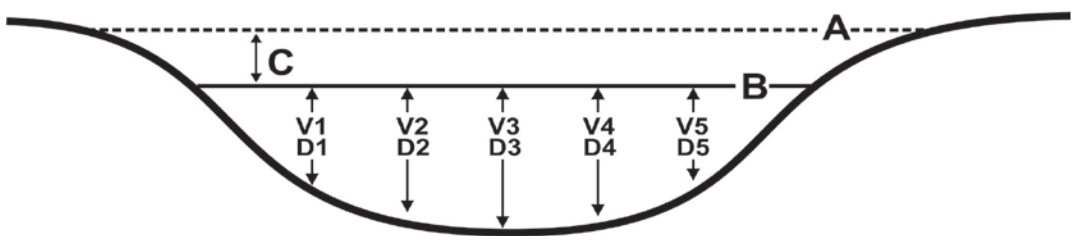
Sampling Date: (DD/MM/YYYY) __________________________

## Widths and Depth

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

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

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




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

## Velocity and Depth

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

☐ **Velocity Head Rod (or ruler)**: Velocity Equation (m/s) = √ [ 2(ΔD/100) * 9.81]

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

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

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



CABIN CANADIAN AQUATIC BIOMONITORING NETWORK

Field Crew: ______________________________________ Site Code: _____________

Sampling Date: (DD/MM/YYYY) __________________________

## SUBSTRATE DATA

### Surrounding/Interstitial Material

Circle the substrate size category for the surrounding material.

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

### 100 Pebble Count & Substrate Embeddedness

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

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

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

Field Crew: ______________________________________ Site Code: ______________

Sampling Date: (DD/MM/YYYY) __________________________

# SITE INSPECTION

Site Inspected by: __________________________________

**Communication Information**

☐ Itinerary left with contact person (include contact numbers)

Contact Person: ____________________________ Time checked-in: ________

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

Phone number: ( ) _____________

**Vehicle Safety**

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

☐ Equipment and chemicals safely secured for transport

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

Notes:

**Shore & Wading Safety**

☐ Wading Task Hazard Analysis read by all field staff

☐ Wading Safe Work Procedures read by all field staff

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

☐ PFD worn

☐ Appropriate footwear, waders, wading belt

☐ Belay used

Notes:

## Appendix B
## Benthic Macroinvertebrate Common Names

| Order | Family | Common Name |
|---|---|---|
| Coleoptera | | Beetles |
| | Elmidae | Riffle beetles |
| | Psephenidae | Water-penny beetles |
| Diptera | | Flies |
| | Ceratopogonidae | Biting midges |
| | Chironomidae | Non-biting midges |
| | Empididae | Dagger flies, balloon flies |
| | 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 |
| | Siphlonuridae | Primitive minnow mayflies |
| 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 |
| | Apataniidae | Early smoky wing sedges |
| | Brachycentridae | Humpless casemaker caddisflies |
| | Hydropsychidae | Net-spinning caddisflies |
| | Hydroptilidae | Microcaddisflies |
| | Lepidostomatidae | Bizarre caddisflies |
| | Limnephilidae | Tube-case 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 |
| | Torrenticolidae | Torrent mites |
| Collembola | | Springtails |
| Podocopida | | Ostracods, seed shrimp |
| | Candonidae | Freshwater ostracods |
| Veneroida | | Bivalve molluscs |
| | Pisidiidae | Pea clams, fingernail clams |
| | Sphaeriidae | Pea clams, fingernail clams |
| Littorinimorpha | | Snails |
| | Amnicolidae | Freshwater snails |
| Basommatophora | | Pulmonate freshwater snails |
| | Lymnaeidae | Pond snails |
| Monostilifera | | Nemertean worms |
| | Tetrastemmatidae | Ribbon worms |
| Lumbriculida | | Microdrile oligochaetes (worms) |
| | Lumbriculidae | Aquatic worms |
| Tubificida | | Annelid worms |
| | Enchytraeidae* | Microdrile oligochaetes (worms) |
| | Lumbricidae† | Earthworms |
| | Naididae | Clitellate oligochaete worms |
| Apochela | | Tartigrades |
| | Milnesiidae | Water bears |

* Species *Enchytraeus buchholzi* identified – Grindal worm
† Species *Eiseniella tetraedra* identified – a semi-aquatic worm

## Appendix C
## Benthic Macroinvertebrates Identified Using Morphological Characteristics

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| **Phylum: Arthropoda** | | | | | | | | |
| **Subphylum: Hexapoda** | | | | | | | | |
| **Class: Insecta** | | | | | | | | |
| **Order: Ephemeroptera** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Family: Ameletidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ameletus* | 10 | 0 | 64 | 0 | 71 | 40 | 30 | 24 |
| **Family: Baetidae** | 19 | 733 | 9 | 9 | 129 | 460 | 43 | 24 |
| *Acentrella* | 0 | 0 | 0 | 0 | 43 | 60 | 83 | 76 |
| *Baetis* | 3 | 0 | 0 | 27 | 29 | 200 | 4 | 32 |
| *Baetis bicaudatus* | 0 | 0 | 0 | 0 | 14 | 0 | 74 | 4 |
| *Baetis rhodani group* | 0 | 13 | 0 | 27 | 143 | 360 | 96 | 144 |
| *Diphetor hageni* | 0 | 0 | 0 | 0 | 29 | 0 | 0 | 0 |
| **Family: Ephemerellidae** | 0 | 27 | 36 | 64 | 43 | 40 | 26 | 32 |
| *Drunella* | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 8 |
| *Drunella doddsii* | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 52 |
| *Drunella spinifera* | 0 | 7 | 0 | 0 | 14 | 0 | 0 | 0 |
| *Ephemerella* | 0 | 47 | 0 | 0 | 14 | 0 | 26 | 48 |
| **Family: Heptageniidae** | 13 | 87 | 309 | 1318 | 457 | 140 | 465 | 312 |
| *Cinygmula* | 0 | 0 | 545 | 45 | 0 | 0 | 13 | 4 |
| *Epeorus* | 0 | 0 | 364 | 0 | 0 | 0 | 17 | 48 |
| *Rhithrogena* | 0 | 0 | 0 | 0 | 29 | 60 | 291 | 168 |
| **Family: Leptophlebiidae** | 19 | 553 | 0 | 0 | 186 | 280 | 9 | 0 |
| *Neoleptophlebia* | 0 | 67 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | |
| **Order: Plecoptera** | 3 | 0 | 0 | 0 | 29 | 0 | 0 | 4 |
| **Family: Capniidae** | 6 | 0 | 18 | 9 | 0 | 40 | 0 | 0 |

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| **Family: Chloroperlidae** | 0 | 0 | 0 | 18 | 14 | 20 | 26 | 12 |
| *Plumiperla* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16 |
| *Sweltsa* | 0 | 7 | 0 | 55 | 57 | 60 | 30 | 0 |
| **Family: Nemouridae** | 84 | 13 | 27 | 518 | 700 | 240 | 0 | 4 |
| *Visoka cataractae* | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 |
| *Zapada* | 3 | 0 | 73 | 0 | 0 | 80 | 0 | 4 |
| *Zapada cinctipes* | 165 | 13 | 9 | 109 | 386 | 660 | 13 | 28 |
| *Zapada columbiana* | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 |
| **Family: Perlidae** | 26 | 13 | 0 | 9 | 100 | 40 | 0 | 12 |
| *Hesperoperla* | 45 | 7 | 0 | 0 | 129 | 80 | 13 | 0 |
| **Family: Perlodidae** | 3 | 7 | 0 | 9 | 0 | 0 | 0 | 0 |
| *Kogotus* | 3 | 0 | 0 | 27 | 14 | 0 | 0 | 0 |
| **Family: Pteronarcyidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Pteronarcella* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| **Family: Taeniopterygidae** | 3 | 0 | 945 | 0 | 0 | 0 | 13 | 60 |
| | | | | | | | | |
| **Order: Trichoptera** | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 |
| **Family: Apataniidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Allomyia* | 0 | 0 | 18 | 0 | 0 | 0 | 0 | 0 |
| *Apatania* | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 |
| **Family: Brachycentridae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Brachycentrus* | 0 | 0 | 0 | 9 | 14 | 0 | 0 | 12 |
| **Family: Hydropsychidae** | 3 | 20 | 0 | 9 | 0 | 0 | 0 | 0 |
| *Arctopsyche* | 0 | 0 | 0 | 0 | 43 | 20 | 0 | 8 |
| *Hydropsyche* | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Family: Hydroptilidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| *Agraylea* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| *Hydroptila* | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 4 |
| **Family: Lepidostomatidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Lepidostoma* | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 |

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| **Family: Limnephilidae** | 0 | 40 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Family: Rhyacophilidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Rhyacophila* | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 |
| *Rhyacophila atrata complex* | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 |
| *Rhyacophila betteni group* | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 |
| *Rhyacophila brunnea/vemna group* | 0 | 0 | 0 | 0 | 57 | 20 | 0 | 0 |
| *Rhyacophila narvae* | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 |
| | | | | | | | | |
| **Order: Diptera** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Family: Ceratopogonidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Dasyhelea* | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Mallochohelea* | 0 | 0 | 0 | 0 | 14 | 120 | 9 | 0 |
| **Family: Chironomidae** | 13 | 0 | 82 | 45 | 0 | 100 | 13 | 8 |
| **Subfamily: Chironominae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Tribe: Chironomini** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Microtendipes* | 0 | 0 | 0 | 0 | 29 | 0 | 0 | 0 |
| *Polypedilum* | 3 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |
| **Tribe: Tanytarsini** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Cladotanytarsus* | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Constempellina sp. C* | 0 | 0 | 0 | 27 | 14 | 560 | 0 | 0 |
| *Micropsectra* | 0 | 27 | 36 | 0 | 143 | 560 | 78 | 52 |
| *Rheotanytarsus* | 0 | 0 | 0 | 9 | 71 | 0 | 4 | 0 |
| *Stempellinella* | 0 | 0 | 0 | 0 | 0 | 20 | 4 | 0 |
| *Tanytarsus* | 55 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Subfamily: Diamesinae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Tribe: Diamesini** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Pagastia* | 0 | 0 | 0 | 9 | 86 | 80 | 0 | 0 |
| *Potthastia gaedii group* | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Subfamily: Orthocladiinae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Brillia* | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| *Corynoneura* | 0 | 0 | 82 | 0 | 0 | 0 | 0 | 4 |
| *Eukiefferiella* | 0 | 0 | 18 | 18 | 71 | 40 | 0 | 0 |
| *Hydrobaenus* | 3 | 0 | 9 | 0 | 0 | 0 | 0 | 0 |
| *Krenosmittia* | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Orthocladius complex* | 142 | 0 | 27 | 191 | 843 | 600 | 9 | 4 |
| *Parametriocnemus* | 0 | 0 | 0 | 0 | 0 | 40 | 0 | 0 |
| *Parorthocladius* | 0 | 0 | 236 | 0 | 0 | 0 | 0 | 0 |
| *Rheocricotopus* | 3 | 0 | 0 | 0 | 43 | 0 | 0 | 4 |
| *Thienemanniella* | 0 | 0 | 9 | 9 | 0 | 0 | 0 | 0 |
| *Tvetenia* | 0 | 0 | 9 | 55 | 57 | 80 | 4 | 4 |
| **Subfamily: Tanypodinae** | 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ablabesmyia* | 0 | 33 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Tribe: Pentaneurini** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Thienemannimyia group* | 142 | 140 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Family: Empididae** | 19 | 0 | 9 | 0 | 0 | 0 | 0 | 0 |
| *Chelifera/ Metachela* | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Clinocera* | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Clinocerinae Unknown Genus A* | 0 | 0 | 18 | 0 | 0 | 0 | 0 | 0 |
| *Neoplasta* | 23 | 20 | 0 | 45 | 0 | 20 | 0 | 0 |
| *Roederiodes* | 3 | 0 | 0 | 55 | 0 | 0 | 0 | 8 |
| *Trichoclinocera* | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 |
| **Family: Psychodidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Pericoma/Telmatoscopus* | 0 | 7 | 0 | 0 | 0 | 60 | 0 | 0 |
| **Family: Simuliidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Simulium* | 0 | 7 | 0 | 9 | 29 | 20 | 0 | 0 |
| **Family: Tipulidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Antocha* | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 |
| *Dicranota* | 3 | 0 | 0 | 18 | 0 | 0 | 0 | 0 |
| *Hexatoma* | 0 | 7 | 0 | 0 | 0 | 80 | 4 | 0 |
| *Rhabdomastix* | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| *Tipula* | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 |
| | | | | | | | | |
| **Order: Coleoptera** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Family: Elmidae** | 0 | 20 | 0 | 9 | 71 | 60 | 0 | 0 |
| *Heterlimnius* | 0 | 0 | 0 | 27 | 472 | 740 | 0 | 8 |
| | | | | | | | | |
| **Order: Collembola** | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 |
| | | | | | | | | |
| **Subphylum: Chelicerata** | | | | | | | | |
| **Class: Arachnida** | | | | | | | | |
| **Order: Trombidiformes** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Family: Aturidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Aturus* | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Brachypoda* | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ljania* | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 |
| **Family: Feltriidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Feltria* | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 |
| **Family: Hydryphantidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Protzia* | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Family: Hygrobatidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Atractides* | 3 | 13 | 0 | 0 | 43 | 20 | 0 | 0 |
| *Corticacarus* | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Family: Lebertiidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Lebertia* | 3 | 20 | 45 | 18 | 0 | 0 | 0 | 8 |
| **Family: Sperchontidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Sperchonopsis* | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Family: Torrenticolidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Testudacarus* | 0 | 7 | 0 | 0 | 14 | 100 | 0 | 4 |
| *Torrenticola* | 3 | 53 | 0 | 0 | 0 | 20 | 0 | 0 |

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| **Phylum: Annelida** | | | | | | | | |
| **Subphylum: Clitellata** | | | | | | | | |
| **Class: Oligochaeta** | | | | | | | | |
| **Order: Lumbriculida** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Family: Lumbriculidae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Rhynchelmis* | 0 | 0 | 55 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | |
| **Order: Tubificida** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Family: Enchytraeidae** | 48 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |
| **Family: Naididae** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Subfamily: Tubificinae** with hair chaetae | 0 | 7 | 0 | 18 | 0 | 0 | 0 | 0 |
| | | | | | | | | |
| **Phylum: Mollusca** | | | | | | | | |
| **Class: Bivalvia** | | | | | | | | |
| **Order: Veneroida** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Family: Pisidiidae** | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Pisidium* | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | |
| **Class: Gastropoda** | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Order: Basommatophora** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Family: Lymnaeidae** | 19 | 80 | 0 | 0 | 0 | 20 | 0 | 0 |
| *Fossaria* | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Totals:** | **1008** | **2203** | **3061** | **2860** | **4828** | **6320** | **1431** | **1256** |

**Taxa present but not included:**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Phylum: Arthropoda** | | | | | | | | |
| **Class: Copepoda** | 3 | 0 | 0 | 0 | 0 | 20 | 0 | 0 |

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| **Subphylum: Crustacea** | | | | | | | | |
| **Class: Ostracoda** | 3 | 7 | 0 | 9 | 14 | 20 | 4 | 0 |
| | | | | | | | | |
| **Phylum: Annelida** | | | | | | | | |
| **Subphylum: Clitellata** | | | | | | | | |
| **Class: Oligochaeta** | | | | | | | | |
| **Order: Tubificida** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Family: Lumbricidae** | 10 | 7 | 0 | 0 | 86 | 0 | 4 | 0 |
| | | | | | | | | |
| **Phylum: Nemata** | 0 | 0 | 9 | 9 | 14 | 20 | 0 | 4 |
| | | | | | | | | |
| **Phylum: Platyhelminthes** | | | | | | | | |
| **Class: Turbellaria** | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 |
| **Totals:** | **16** | **14** | **9** | **18** | **128** | **60** | **8** | **4** |

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

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| **Phylum: Arthropoda** | | | | | | | | |
| **Subphylum: Hexapoda** | | | | | | | | |
| **Class: Insecta** | | | | | | | | |
| **Order: Ephemeroptera** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Family: Ameletidae | 10 | 0 | 64 | 0 | 71 | 40 | 30 | 24 |
| Family: Baetidae | 22 | 746 | 9 | 63 | 387 | 1080 | 300 | 280 |
| Family: Ephemerellidae | 0 | 81 | 36 | 64 | 71 | 40 | 78 | 140 |
| Family: Heptageniidae | 13 | 87 | 1218 | 1363 | 486 | 200 | 786 | 532 |
| Family: Leptophlebiidae | 19 | 620 | 0 | 0 | 186 | 280 | 9 | 0 |
| | | | | | | | | |
| **Order: Plecoptera** | 3 | 0 | 0 | 0 | 29 | 0 | 0 | 4 |
| Family: Capniidae | 6 | 0 | 18 | 9 | 0 | 40 | 0 | 0 |
| Family: Chloroperlidae | 0 | 7 | 0 | 73 | 71 | 80 | 56 | 28 |
| Family: Nemouridae | 252 | 26 | 109 | 627 | 1100 | 1000 | 13 | 36 |
| Family: Perlidae | 71 | 20 | 0 | 9 | 229 | 120 | 13 | 12 |
| Family: Perlodidae | 6 | 7 | 0 | 36 | 14 | 0 | 0 | 0 |
| Family: Pteronarcyidae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| Family: Taeniopterygidae | 3 | 0 | 945 | 0 | 0 | 0 | 13 | 60 |
| | | | | | | | | |
| **Order: Trichoptera** | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 |
| Family: Apataniidae | 0 | 0 | 27 | 0 | 0 | 0 | 0 | 0 |
| Family: Brachycentridae | 0 | 0 | 0 | 9 | 14 | 0 | 0 | 12 |
| Family: Hydropsychidae | 3 | 33 | 0 | 9 | 43 | 20 | 0 | 8 |
| Family: Hydroptilidae | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 12 |
| Family: Lepidostomatidae | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 |
| Family: Limnephilidae | 0 | 40 | 0 | 0 | 0 | 0 | 0 | 0 |
| Family: Rhyacophilidae | 0 | 0 | 0 | 9 | 85 | 40 | 0 | 0 |

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| **Order: Diptera** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Family: Ceratopogonidae | 23 | 0 | 0 | 0 | 14 | 120 | 9 | 0 |
| Family: Chironomidae | 425 | 213 | 508 | 363 | 1357 | 2080 | 116 | 76 |
| Family: Empididae | 51 | 27 | 27 | 100 | 14 | 20 | 0 | 8 |
| Family: Psychodidae | 0 | 7 | 0 | 0 | 0 | 60 | 0 | 0 |
| Family: Simuliidae | 0 | 7 | 0 | 9 | 29 | 20 | 0 | 0 |
| Family: Tipulidae | 6 | 7 | 0 | 27 | 0 | 100 | 4 | 0 |
| **Order: Coleoptera** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Family: Elmidae | 0 | 20 | 0 | 36 | 543 | 800 | 0 | 8 |
| **Order: Collembola** | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 |
| **Subphylum: Chelicerata** | | | | | | | | |
| **Class: Arachnida** | | | | | | | | |
| **Order: Trombidiformes** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Family: Aturidae | 16 | 7 | 0 | 0 | 0 | 20 | 0 | 0 |
| Family: Feltriidae | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 |
| Family: Hydryphantidae | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| Family: Hygrobatidae | 6 | 13 | 0 | 0 | 43 | 20 | 0 | 0 |
| Family: Lebertiidae | 3 | 20 | 45 | 18 | 0 | 0 | 0 | 8 |
| Family: Sperchontidae | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| Family: Torrenticolidae | 3 | 60 | 0 | 0 | 14 | 120 | 0 | 4 |
| **Phylum: Annelida** | | | | | | | | |
| **Subphylum: Clitellata** | | | | | | | | |
| **Class: Oligochaeta** | | | | | | | | |
| **Order: Lumbriculida** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Family: Lumbriculidae | 0 | 0 | 55 | 0 | 0 | 0 | 0 | 0 |

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| **Order: Tubificida** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Family: Enchytraeidae | 48 | 0 | 0 | 0 | 0 | 0 | 4 | 0 |
| Family: Naididae | 0 | 7 | 0 | 18 | 0 | 0 | 0 | 0 |
| **Phylum: Mollusca** | | | | | | | | |
| **Class: Bivalvia** | | | | | | | | |
| **Order: Veneroida** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Family: Pisidiidae | 0 | 14 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Class: Gastropoda** | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Order: Basommatophora** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Family: Lymnaeidae | 19 | 87 | 0 | 0 | 0 | 20 | 0 | 0 |
| **Totals:** | **1008** | **2203** | **3061** | **2860** | **4828** | **6320** | **1431** | **1256** |

**Taxa present but not included:**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Phylum: Arthropoda** | | | | | | | | |
| **Class: Copepoda** | 3 | 0 | 0 | 0 | 0 | 20 | 0 | 0 |
| **Subphylum: Crustacea** | | | | | | | | |
| **Class: Ostracoda** | 3 | 7 | 0 | 9 | 14 | 20 | 4 | 0 |
| **Phylum: Annelida** | | | | | | | | |
| **Subphylum: Clitellata** | | | | | | | | |
| **Class: Oligochaeta** | | | | | | | | |
| **Order: Tubificida** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Family: Lumbricidae | 10 | 7 | 0 | 0 | 86 | 0 | 4 | 0 |

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| **Phylum: Nemata** | 0 | 0 | 9 | 9 | 14 | 20 | 0 | 4 |
| **Phylum: Platyhelminthes** | | | | | | | | |
| **Class: Turbellaria** | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 |
| **Totals:** | **16** | **14** | **9** | **18** | **128** | **60** | **8** | **4** |

## Appendix E

## Metric Indices of the Benthic Macroinvertebrates

**(Genus/Species Level)**

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **LES02** | **LES01** | **GHO06** | **AUR01** | **JOH01** | **JOH02** | **WAP02a** | **WAP03a** |
| **Richness Measures** | | | | | | | | |
| Species Richness | 36 | 34 | 23 | 30 | 38 | 35 | 23 | 29 |
| EPT Richness | 12 | 13 | 11 | 14 | 22 | 15 | 14 | 20 |
| Ephemeroptera Richness | 5 | 6 | 5 | 4 | 10 | 7 | 10 | 9 |
| Plecoptera Richness | 6 | 4 | 4 | 6 | 6 | 5 | 4 | 6 |
| Trichoptera Richness | 1 | 3 | 2 | 4 | 6 | 3 | 0 | 5 |
| Chironomidae Richness | 10 | 4 | 8 | 7 | 9 | 8 | 6 | 5 |
| Oligochaeta Richness | 2 | 2 | 1 | 1 | 1 | 0 | 2 | 0 |
| Non-Chiro. Non-Olig. Richness | 24 | 28 | 14 | 22 | 28 | 27 | 15 | 24 |
| **Abundance Measures** | | | | | | | | |
| Corrected Abundance | 1008 | 2203 | 3061 | 2860 | 4828 | 6320 | 1431 | 1256 |
| EPT Abundance | 408 | 1680 | 2426 | 2280 | 2800 | 2940 | 1298 | 1152 |
| **Dominance Measures** | | | | | | | | |
| 1st Dominant Taxon | *Zapada cinctipes* | Baetidae | Taeniopterygidae | Heptageniidae | *Orthocladius* complex | *Zapada cinctipes* | Heptageniidae | Heptageniidae |
| 1st Dominant Abundance | 165 | 733 | 945 | 1318 | 843 | 660 | 465 | 312 |
| 2nd Dominant Taxon | *Thienemannimyia* group | Leptophlebiidae | *Cinygmula* | Nemouridae | Nemouridae | *Orthocladius* complex | *Rhithrogena* | *Rhithrogena* |
| 2nd Dominant Abundance | 142 | 553 | 545 | 518 | 700 | 600 | 291 | 168 |
| 3rd Dominant Taxon | *Orthocladius* complex | *Thienemannimyia* group | *Epeorus* | *Orthocladius* complex | Heptageniidae | *Micropsectra* | *Baetis rhodani* group | *Baetis rhodani* group |
| 3rd Dominant Abundance | 142 | 140 | 364 | 191 | 457 | 560 | 96 | 144 |

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| % 1 Dominant Taxon | 16.37% | 33.27% | 30.87% | 46.08% | 17.46% | 10.44% | 32.49% | 24.84% |
| % 2 Dominant Taxon | 14.09% | 25.10% | 17.80% | 18.11% | 14.50% | 9.49% | 20.34% | 13.38% |
| % 3 Dominant Taxon | 14.09% | 6.35% | 11.89% | 6.68% | 9.47% | 8.86% | 6.71% | 11.46% |
| Percent Dominance | 44.55% | 64.72% | 60.56% | 70.87% | 41.43% | 28.79% | 59.54% | 49.68% |
| | | | | | | | | |
| **Community Composition** | | | | | | | | |
| % Ephemeroptera | 6.35% | 69.63% | 43.35% | 52.10% | 24.88% | 25.95% | 84.07% | 77.71% |
| % Plecoptera | 33.83% | 2.72% | 35.02% | 26.36% | 29.89% | 19.62% | 6.64% | 11.46% |
| % Trichoptera | 0.30% | 3.90% | 0.88% | 1.26% | 3.23% | 0.95% | 0.00% | 2.55% |
| % EPT | 40.48% | 76.26% | 79.26% | 79.72% | 58.00% | 46.52% | 90.71% | 91.72% |
| % Diptera | 50.10% | 11.85% | 17.48% | 17.45% | 29.29% | 37.97% | 9.01% | 6.69% |
| % Oligochaeta | 4.76% | 0.32% | 1.80% | 0.63% | 0.00% | 0.00% | 0.28% | 0.00% |
| % Baetidae | 2.18% | 33.86% | 0.29% | 2.20% | 8.02% | 17.09% | 20.96% | 22.29% |
| % Chironomidae | 42.16% | 9.67% | 16.60% | 12.69% | 28.11% | 32.91% | 8.11% | 6.05% |
| % Odonata | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | | | | | | | | |
| EPT ratio | 0.49 | 0.89 | 0.83 | 0.86 | 0.67 | 0.59 | 0.92 | 0.94 |
| % Chironomidae within Diptera | 84.16 | 81.61 | 94.95 | 72.75 | 95.97 | 86.67 | 89.92 | 90.48 |
| % Hydropsychidae within Trichoptera | 100.00 | 38.37 | 0.00 | 25.00 | 27.56 | 33.33 | 0.00 | 25.00 |
| % Baetidae within Ephemeroptera | 34.38 | 48.63 | 0.68 | 4.23 | 32.22 | 65.85 | 24.94 | 28.69 |
| | | | | | | | | |
| **Functional Group Composition** | | | | | | | | |
| % Predators | 10.32% | 2.32% | 0.26% | 1.01% | 0.81% | 0.51% | 1.33% | 1.35% |
| % Shredder-Herbivores | 8.13% | 0.18% | 3.85% | 2.48% | 1.59% | 0.82% | 0.42% | 1.91% |
| % Collector-Gatherers | 9.33% | 10.80% | 2.06% | 1.85% | 3.46% | 3.35% | 8.32% | 10.27% |
| % Scrapers | 0.99% | 1.23% | 4.48% | 5.24% | 0.70% | 0.17% | 12.65% | 10.59% |
| % Macrophyte-Herbivore | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| % Collector-Filterer | 1.79% | 0.36% | 0.00% | 0.10% | 0.19% | 0.03% | 0.14% | 0.00% |
| % Omnivore | 0.10% | 0.00% | 0.07% | 0.14% | 0.19% | 0.03% | 0.00% | 0.40% |

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| % Parasite | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| % Piercer-Herbivore | 0.00% | 0.09% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.16% |
| % Gatherer | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| % Unclassified | 0.50% | 0.00% | 0.29% | 0.17% | 0.06% | 0.08% | 0.21% | 0.32% |
| | | | | | | | | |
| **Functional Group Richness** | | | | | | | | |
| Predators Richness | 18 | 16 | 3 | 10 | 15 | 14 | 5 | 7 |
| Shredder-Herbivores Richness | 6 | 2 | 5 | 4 | 3 | 5 | 2 | 4 |
| Collector-Gatherers Richness | 11 | 13 | 11 | 13 | 18 | 18 | 16 | 15 |
| Scrapers Richness | 2 | 4 | 5 | 2 | 2 | 3 | 4 | 4 |
| MH Richness | | | | | | | | |
| CF Richness | 2 | 5 | | 3 | 3 | 2 | 2 | |
| OM Richness | 1 | | 1 | 3 | 3 | 1 | | 3 |
| PA Richness | | | | | | | | |
| Piercer-Herbivore Richness | | 1 | | | | | | 2 |
| Gatherer Richness | | | | | | | | |
| Unclassified | 2 | | 1 | 1 | 2 | 1 | 1 | 3 |
| | | | | | | | | |
| **Voltinism Composition** | | | | | | | | |
| % Univoltine | 19.54% | 3.90% | 2.68% | 4.76% | 9.76% | 11.08% | 6.64% | 13.06% |
| % Semivoltine | 0.00% | 1.23% | 0.00% | 2.24% | 2.94% | 2.22% | 2.10% | 0.00% |
| % Multivoltine | 0.30% | 0.32% | 0.59% | 1.26% | 1.20% | 3.48% | 0.28% | 2.55% |
| | | | | | | | | |
| **Voltinism Richness** | | | | | | | | |
| Univoltine | 4 | 4 | 3 | 3 | 3 | 2 | 4 | 5 |
| Semivoltine | 0 | 2 | 0 | 1 | 2 | 2 | 1 | 0 |
| Multivoltine | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| | | | | | | | | |
| **Diversity/Evenness Measures** | | | | | | | | |

| Taxa | Site | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | **LES02** | **LES01** | **GHO06** | **AUR01** | **JOH01** | **JOH02** | **WAP02a** | **WAP03a** |
| Shannon-Weiner H' (log 10) | 1.27 | 1.04 | 0.99 | 0.93 | 1.3 | 1.36 | 1.04 | 1.2 |
| Shannon-Weiner H' (log 2) | 4.21 | 3.46 | 3.29 | 3.08 | 4.32 | 4.51 | 3.46 | 3.97 |
| Shannon-Weiner H' (log e) | 2.92 | 2.4 | 2.28 | 2.14 | 2.99 | 3.13 | 2.4 | 2.75 |
| Simpson's Index (D) | 0.08 | 0.18 | 0.16 | 0.25 | 0.08 | 0.06 | 0.16 | 0.11 |
| Simpson's Index of Diversity (1 - D) | 0.92 | 0.82 | 0.84 | 0.75 | 0.92 | 0.94 | 0.84 | 0.89 |
| Simpson's Reciprocal Index (1/D) | 11.77 | 5.41 | 6.22 | 3.94 | 12.09 | 16.12 | 6.12 | 9.23 |
| | | | | | | | | |
| **Biotic Indices** | | | | | | | | |
| Hilsenhoff Biotic Index | 4.52 | 3.84 | 3.73 | 3.74 | 3.85 | 4.18 | 4.1 | 3.99 |

## 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 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| **INSECTS** | | | | | | | | |
| **Order: Coleoptera** | | | | | | | | |
| **Elmidae** | | Morph | | Morph | Morph | Morph | | |
| *Heterlimnius* | | | | Morph | Morph | Morph | | |
| **Psephenidae** | | | | | | | | |
| *Psephenus herricki* | eDNA | | | | | | | |
| **Order: Diptera** | | | | | | | | |
| **Ceratopogonidae** | | | | | | | | |
| *Dasyhelea* | Morph | | | | | | | |
| *Mallochohelea* | | | | | Morph | Morph | Morph | |
| **Chironomidae** | Morph | | Morph | Morph | | Morph | Morph | Morph |
| Chironominae | | | | | | | | |
| Chironomini | | | | | | | | |
| *Microtendipes* | | | | | Morph | | | |
| *Polypedilum* | Both | | eDNA | | | | Both | |
| Tanytarsini | | | | | | | | |
| *Cladotanytarsus* | | Morph | | | | | | |
| *Constempellina* sp. C | | | | Morph | Morph | Morph | | |
| *Micropsectra* | | Morph | Morph | | Both | Both | Morph | Both |
| *Microspectra logani* | | eDNA | | | | | eDNA | |
| *Rheotanytarsus* | | | | Morph | Morph | | Morph | |
| *Stempellinella* | | | | | | Morph | Morph | |
| *Tanytarsus* | Morph | | | | | | | |
| *Tanytarsus buckleyi* | eDNA | eDNA | | | | eDNA | | |
| Diamesinae | | | | | | | | |
| Diamesini | | | | | | | | |
| *Diamesa* | | | | | eDNA | | | |
| *Pagastia* | | | | Morph | Morph | Morph | | |
| *Pagastia orthogonia* | eDNA | | | | eDNA | eDNA | | |
| *Potthastia gaedii* | Both | | | | | | | |
| Orthocladiinae | | | | | | | | |
| *Brillia* | Morph | | | | | | | |
| *Chaetocladius* | | | | | | eDNA | | |
| *Corynoneura* | | | Morph | | | | | Morph |
| *Eukiefferiella* | | | Morph | Morph | Morph | Morph | | |
| *Eukiefferiella claripennis* | | | | | | eDNA | | |
| *Hydrobaenus* | Morph | | Both | | | | | |

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| *Krenosmittia* | Morph | | | | | | | |
| *Orthocladius* complex | Morph | | Morph | Both | Morph | Morph | Morph | Morph |
| *Orthocladius carlatus* | | | eDNA | | | | | |
| *Parametriocnemus* | | | | | | Morph | | |
| *Parametriocnemus boreoalpinus* | eDNA | | | | | | | |
| *Paratrichocladius* | | | eDNA | | | | eDNA | |
| *Parorthocladius* | | | Morph | | | | | |
| *Rheocricotopus* | Morph | | | | Morph | | | Morph |
| *Thienemanniella* | | | Morph | Morph | | | | |
| *Tvetenia* | | | Morph | Morph | Both | Both | Morph | Both |
| *Tvetenia paucunca* | | | | eDNA | | | | |
| Tanypodinae | Morph | | | | | | | |
| Ablabesmyia | | Morph | | | | | | |
| Pentaneurini | | | | | | | | |
| *Conchapelopia pallens* | eDNA | eDNA | | | | | | |
| *Thienemannimyia* group | Morph | Morph | | | | | | |
| **Empididae** | Morph | | Morph | | | | | |
| *Chelifera/Metachela* | | Morph | | | | | | |
| *Metachela* | | | | | | eDNA | | |
| *Neoplasta* | Morph | Morph | | Morph | | Morph | | |
| *Neoplasta megorchis* | | | | eDNA | | | | |
| Clinocerinae Unknown Genus A | | | Morph | | | | | |
| *Clinocera* | Morph | | | | | | | |
| *Roederiodes* | Morph | | | Morph | | | | Morph |
| *Trichoclinocera* | | | | | Morph | | | |
| **Psychodidae** | | | | | | | | |
| *Pericoma/Telmatoscopus* | | Morph | | | | Morph | | |
| *Pneumia* | | | | | | eDNA | | |
| **Simuliidae** | | | | | | | | |
| *Helodon alpestris* | | | eDNA | | | | | |
| *Simulium* | | Morph | | Morph | Morph | Morph | | |
| *Simulium arcticum* | | | | | | | | eDNA |
| *Simulium defoliarti* | | | eDNA | | | eDNA | | eDNA |
| *Simulium tuberosum* | | | | | | eDNA | | |
| **Tipulidae** | | Morph | | | | | | |
| *Antocha* | | | | | | Morph | | |
| *Dicranota* | Morph | | | Morph | | | | |
| *Hexatoma* | | Morph | | | | Morph | Morph | |
| *Rhabdomastix* | Morph | | | | | | | |
| *Tipula* | | | | Morph | | | | |
| **Order: Ephemeroptera** | | | | | | | | |
| **Ameletidae** | | | | | | | | |
| *Ameletus* | Morph | | Morph | | Morph | Morph | Morph | Morph |
| *Ameletus bellulus* | | | | | | eDNA | | |
| *Ameletus celer* | | | eDNA | | | | eDNA | eDNA |

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| **Baetidae** | Morph | Morph | Morph | Morph | Morph | Morph | Morph | Morph |
| *Acentrella* | | | | | Morph | Morph | Morph | Morph |
| *Acentrella turbida* | | | | | eDNA | eDNA | eDNA | eDNA |
| *Acerpenna pygmaea* | | | eDNA | eDNA | | | | |
| *Baetis* | Morph | | | Morph | Morph | Morph | Morph | Morph |
| *Baetis bicaudatus* | | | eDNA | | Both | eDNA | Both | Both |
| *Baetis brunneicolor* | | | | eDNA | | | | |
| *Baetis phoebus* | | | | | eDNA | eDNA | eDNA | eDNA |
| *Baetis rhodani* group | | Morph | | Morph | Morph | Morph | Morph | Morph |
| *Baetis tricaudatus* | eDNA | eDNA | | eDNA | eDNA | eDNA | eDNA | eDNA |
| *Diphetor hageni* | eDNA | eDNA | | eDNA | Both | eDNA | | |
| *Plauditus cingulatus* | | | eDNA | | | | | |
| **Ephemerellidae** | | Morph | Morph | Morph | Morph | Morph | Morph | Morph |
| *Attenella attenuata* | eDNA | eDNA | eDNA | | | | | |
| *Drunella* | | | | | | | Morph | Morph |
| *Drunella coloradensis* | | | eDNA | eDNA | | eDNA | eDNA | eDNA |
| *Drunella doddsii* | | | | | | | Both | Both |
| *Drunella grandis* | eDNA | eDNA | | | eDNA | eDNA | | eDNA |
| *Drunella spinifera* | | Morph | | | Morph | | | |
| *Ephemerella* | | Morph | | | Morph | | Morph | Morph |
| *Ephemerella invaria* | eDNA | | eDNA | | | | | |
| *Ephemerella tibialis* | eDNA | | | | eDNA | eDNA | eDNA | eDNA |
| **Heptageniidae** | Morph | Morph | Morph | Morph | Morph | Morph | Morph | Morph |
| *Cinygmula* | | eDNA | Morph | Morph | eDNA | | Morph | Morph |
| *Cinygmula mimus* | | | | eDNA | | | | |
| *Cinygmula* spJMW3 | eDNA | | eDNA | eDNA | | eDNA | eDNA | eDNA |
| *Ecdyonurus simplicioides* | | eDNA | | eDNA | | | | |
| *Epeorus* | | | Morph | | | | Morph | Morph |
| *Epeorus deceptivus* | | eDNA | eDNA | | | | eDNA | eDNA |
| *Epeorus grandis* | | | eDNA | | | | | |
| *Maccaffertium smithae* | eDNA | | | | | | | |
| *Rhithrogena* | | | | | Morph | Both | Morph | Morph |
| *Rhithrogena robusta* | | | eDNA | | | | eDNA | eDNA |
| *Stenacron interpunctatum* | | | | | | | | eDNA |
| **Leptophlebiidae** | Morph | Morph | | | Morph | Morph | Morph | |
| *Leptophlebia nebulosa* | | eDNA | | | | | | |
| *Neoleptophlebia* | | Morph | | | | | | |
| *Paraleptophlebia heteronea* | eDNA | eDNA | | | eDNA | eDNA | eDNA | |
| **Siphlonuridae** | | | | | | | | |
| *Siphlonurus* | eDNA | | | | | | | |
| **Order: Plecoptera** | Morph | | | | Morph | | | Morph |
| **Capniidae** | Morph | | Morph | Morph | | Morph | | |
| *Capnia* | eDNA | | | | | | | |
| *Capnia coloradensis* | | eDNA | | | | | | |
| *Capnia gracilaria* | | | | | | eDNA | | |

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| *Capnia petila* | | | eDNA | | | | eDNA | |
| *Eucapnopsis brevicauda* | | | | | | | eDNA | eDNA |
| *Mesocapnia* | | | eDNA | | | | eDNA | eDNA |
| *Paracapnia angulata* | | | eDNA | | | | | |
| *Utacapnia columbiana* | | eDNA | | | | | | |
| *Utacapnia trava* | | eDNA | | | | | | |
| **Chloroperlidae** | | | | Morph | Morph | Morph | Morph | Morph |
| *Plumiperla* | | | | | | | | Morph |
| *Plumiperla diversa* | | | | | | | eDNA | eDNA |
| *Suwallia* | | | eDNA | | | | eDNA | |
| *Sweltsa* | | Morph | | Morph | Morph | Morph | Morph | |
| *Sweltsa borealis* | | | | eDNA | eDNA | eDNA | eDNA | eDNA |
| *Sweltsa coloradensis* | | eDNA | | | | | eDNA | eDNA |
| **Leuctridae** | | | | | | | | |
| *Paraleuctra occidentalis* | | | | | | | | eDNA |
| **Nemouridae** | Morph | Morph | Morph | Morph | Morph | Morph | | Morph |
| *Nemoura arctica* | | | | eDNA | | | | |
| *Podmosta decepta* | eDNA | | | | | | | |
| *Prostoia besametsa* | | | eDNA | | | | | |
| *Visoka cataractae* | | | | | Morph | eDNA | | |
| *Zapada* | Morph | | Morph | | | Morph | | Morph |
| *Zapada cinctipes* | Both | Both | Both | Both | Both | Both | Both | Both |
| *Zapada columbiana* | | | eDNA | | eDNA | Both | | eDNA |
| *Zapada haysi* | | | | | | | | eDNA |
| *Zapada oregonensis* | | | | | eDNA | | | |
| **Perlidae** | Morph | Morph | | Morph | Morph | Morph | | Morph |
| *Doroneuria theodora* | eDNA | | | eDNA | eDNA | eDNA | eDNA | eDNA |
| *Hesperoperla* | Morph | Morph | | | Morph | Morph | Morph | |
| *Hesperoperla pacifica* | eDNA | eDNA | | eDNA | eDNA | eDNA | eDNA | eDNA |
| **Perlodidae** | Morph | Morph | | Morph | Morph | | | |
| *Isogenoides* | | | | | | | | Morph |
| *Isogenoides frontalis* | | | | | | | eDNA | eDNA |
| *Isoperla petersoni* | | | eDNA | | | | | eDNA |
| *Kogotus* | Morph | | | Morph | Morph | | | |
| *Kogotus modestus* | | | | eDNA | | | | eDNA |
| *Megarcys subtruncata* | | | eDNA | | | | | |
| *Setvena bradleyi* | | | | | | | | eDNA |
| **Pteronarcyidae** | | | | | | | | |
| *Pteronarcella* | | | | | | | | Morph |
| *Pteronarcella badia* | | | eDNA | | | | eDNA | |
| **Taeniopterygidae** | Morph | | Morph | | | | Morph | Morph |
| *Doddsia occidentalis* | | | eDNA | | | | eDNA | eDNA |
| **Order: Trichoptera** | | | | | Morph | | | |
| **Apataniidae** | | | | | | | | |
| *Allomyia* | | | Morph | | | | | |

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| *Apatania* | | | Morph | | | | | |
| **Brachycentridae** | | | | | | | | |
| *Brachycentrus* | | | | Morph | Morph | | | Morph |
| **Hydropsychidae** | Morph | Morph | | Morph | | | | |
| *Arctopsyche* | | | | | Morph | Morph | | |
| *Arctopsyche grandis* | | | | | eDNA | | | eDNA |
| *Hydropsyche* | | Morph | | | | | | |
| *Hydropsyche bronta* | | eDNA | | | | | | |
| **Hydroptilidae** | | | | | | | | Morph |
| *Agraylea* | | | | | | | | Morph |
| *Hydroptila* | | Morph | | | | | | Morph |
| **Lepidostomatidae** | | | | | | | | |
| *Lepidostoma* | | | | Morph | | | | |
| *Lepidostoma cascadense* | | | | | | eDNA | | |
| **Limnephilidae** | | Morph | | | | | | |
| **Rhyacophilidae** | | | | | | | | |
| *Rhyacophila* | | | | | | Morph | | |
| *Rhyacophila atrata* complex | | | | | Morph | | | |
| *Rhyacophila betteni* group | | | | Morph | | | | |
| *Rhyacophila brunnea/vemna* | | | | | Morph | Morph | | |
| *Rhyacophila narvae* | | | | | Morph | | | |
| **ARACHNIDS** | | | | | | | | |
| **Order: Trombidiformes** | | | | | | | | |
| **Aturidae** | | | | | | | | |
| *Aturus* | Morph | | | | | | | |
| *Brachypoda* | | Morph | | | | | | |
| *Ljania* | | | | | | Morph | | |
| **Feltriidae** | | | | | | | | |
| *Feltria* | | | | | Morph | | | |
| **Hydryphantidae** | | | | | | | | |
| *Protzia* | | Morph | | | | | | |
| **Hygrobatidae** | | | | | | | | |
| *Atractides* | Morph | Morph | | | Morph | Morph | | |
| *Corticacarus* | Morph | | | | | | | |
| **Lebertiidae** | | | | | | | | |
| *Lebertia* | Morph | Morph | Morph | Morph | | | | Morph |
| Sperchontidae | | | | | | | | |
| *Sperchonopsis* | | Morph | | | | | | |
| Stygothrombidiidae | | | | | | | | |
| *Stygothrombium* | | | Morph | | | | | |
| **Torrenticolidae** | | | | | | | | |
| *Testudacarus* | | Morph | | | Morph | Morph | | Morph |
| *Torrenticola* | Morph | Morph | | | | Morph | | |
| **SPRINGTAILS** | | | | | | | | |
| **Order: Collembola** | | | | Morph | | | | |

| Taxa | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | LES02 | LES01 | GHO06 | AUR01 | JOH01 | JOH02 | WAP02a | WAP03a |
| **COPEPODS** | Morph | | | | | Morph | | |
| **OSTRACODS** | Morph | Morph | | Morph | Morph | Morph | Morph | |
| **Order: Podocopida** | | | | | | | | |
| **Candonidae** | | | | | | | | |
| *Candona candida* | | | | eDNA | eDNA | | | |
| **BIVALVES** | | | | | | | | |
| **Order: Veneroida** | | | | | | | | |
| **Sphaeriidae** | | | | | | | | |
| *Musculium* | eDNA | eDNA | eDNA | | | | | |
| **Pisidiidae** | | Morph | | | | | | |
| *Pisidium* | | Morph | eDNA | | | | | |
| *Pisidium casertanum* | | eDNA | | | | | | |
| **GASTROPODS** | | Morph | | | | | | |
| **Order: Basommatophora** | | | | | | | | |
| **Lymnaeidae** | Morph | Morph | | | | Morph | | |
| *Fossaria* | | Morph | | | | | | |
| **Order: Littorinimorpha** | | | | | | | | |
| **Amnicolidae** | | | | | | | | |
| *Amnicola* | eDNA | | | eDNA | eDNA | eDNA | eDNA | eDNA |
| *Amnicola dalli* | | | eDNA | | | | | |
| **RIBBON WORMS** | | | | | | | | |
| **Order: Monostilifera** | | | | | | | | |
| **Tetrastemmatidae** | | | | | | | | |
| *Prostoma graecense* | eDNA | | eDNA | | | | | |
| **WATER BEARS** | | | | | | | | |
| **Order: Apochela** | | | | | | | | |
| **Milnesiidae** | | | | | | | | |
| *Milnesium* | | | eDNA | | | | | |
| **OLIGOCHAETE WORMS** | | | | | | | | |
| **Order: Lumbriculida** | | | | | | | | |
| **Lumbriculidae** | | | | | | | | |
| *Rhynchelmis* | | | Morph | | | | | |
| **Order: Tubificida** | | | | | | | | |
| **Enchytraeidae** | Morph | | | | | | Morph | |
| *Enchytraeus buchholzi* | eDNA | | | | | | | eDNA |
| **Lumbricidae** | Morph | Morph | | | Morph | | Morph | |
| *Eiseniella tetraedra* | eDNA | eDNA | | | eDNA | eDNA | eDNA | eDNA |
| **Naididae** | | | | | | | | |
| *Allonais* | eDNA | | | | eDNA | | | |
| Tubificinae | | Morph | | Morph | | | | |
| *Tubifex tubifex* | | | | eDNA | | | | |
| **NEMATODES (Nemata)** | | | Morph | Morph | Morph | Morph | | Morph |
| **FLAT WORMS (Turbellaria)** | | | | | Morph | | | |