



**Benthic Invertebrates**

**Report February 2023**

**Taxonomy, Methods and Quality Control**

**for**

**Athabasca Watershed Council**

**Athabasca, AB**

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## Sample Reception

Six sample jars containing benthic invertebrates were received by ABI Environmental Services (3911 Varsity Dr. NW, Calgary, Alberta) on November 3, 2022. Samples were received, counted, inspected, and compared to the packing list. The shipping box arrived after an extended period and damaged with two (UAB003 & UAB006) of the sample containers being cracked and leaking into the shipping box. The contents of the damaged containers were deemed salvageable because the containers had been placed in Ziplock bags and only one container per bag had ruptured. The contents from the broken containers were inspected and transferred to new containers. The preservative was replaced with 70% ethanol in each of the containers. (Table 1).

Label information was:

- UAB002: 2022-10-02: Upper McLeod-Gregg River
- UAB003: 2022-10-03: Upper McLeod – Whitehorse Creek
- UAB004: 2022-10-03: Upper McLeod – McLeod River

UAB005: 2022-10-03: Upper McLeod – McLeod River  
UAB006: 2022-10-04: Upper McLeod – Embarras River  
UAB007: 2022-10-02: Upper McLeod – Gregg River

We recommend that each container be placed in a separate ziplock bag in case of breakage.

## Sample Processing

Large plant material like twigs and leaves were rinsed and then removed from the samples and discarded. The smaller macrophytes and the silt/mud, gravel and sand were reduced in the samples by washing and sieving to separate the invertebrates from this debris. Samples were emptied on to a series of stacked sieves, in order from the top: 13.3 mm, 1000 um and 400 um, and gently washed with water. A pan or basin was placed under the bottom sieve. The vegetation and substrate resting on the 13.3 mm and 1000 um sieves was gently washed with water, inspected for invertebrates, and then discarded. Any large invertebrates captured on these coarse sieves were transferred to a labelled wide mouth jar. The smaller contents resting on the 1000 and 400 um sieves were inverted into separate basins and gently washed off these sieves to remove as much of the plant debris and substrate as was practical. These fractions were then transferred in water to the Marchant box. The fraction that passed through the 400-um sieve was inspected for invertebrates and none to a few were found. This procedure was repeated for all 6 samples.

## Subsampling and Sample Sorting

Initially samples were poured into a white pan and roughly counted to determine if subsampling was necessary. Invertebrates were found to be abundant in all six of the samples (Table 2). The method of subsampling was accomplished following the CABIN procedure (McDermott 2014). Briefly, the samples were transferred to a Marchant box, mixed with water, inverted, swirled, and righted. Using a random number generator in Excel, the first five cells were selected, and the contents removed to watch glasses using a transfer pipette. If at least 300 organisms of the taxa of interest (listed in Table 4) were not reached from these five cells, additional cells were randomly chosen until this criterion was reached. If the count was met partway through the cell the entire cell contents was counted as per the CABIN protocol. During sorting the invertebrates were rough sorted into major taxa groups. These invertebrates were placed in labeled glass vials with 70% ethanol. Excluded taxa were identified and noted (Table 5). After counting and sorting, these residues were bulked together, transferred to a new jar, labeled as “sorted” and retained for auditing. The unsorted cells were poured out of the Marchant box, returned to their original container, labelled as “unsorted” and retained.

## Sorting Audit Protocol

Three of the six samples (50%) were randomly chosen for resorting by another team member. Sorting precision was calculated as percent sorting efficiency (% SE) using the CABIN method.

$$\%SE = \left(1 - \frac{\# \text{ of Organisms Missed}}{\text{Total \# of Organisms Found}}\right) * 100$$

The sorting efficiency is in Table 3 and exceeded the CABIN protocol of 95% with an average sorting efficiency of 99.9% (Table 3).

## Identification and Taxonomy

The rough sorted samples were further examined to identify organisms to the family and genus level. Taxa were entered on paper data sheets and then transferred to an excel spreadsheet and the counts summarized using a Pivot Table. The CABIN Protocols for effort and identification level of respective taxa were followed as closely as possible. In the case of Chironomidae, temporary glycerine slide mounts of dissected specimens were made to confirm identifications to the genus level. Where there were disarticulated specimens only those with heads were counted to avoid double counting specimens. There was also exuviae from larval moults in some samples that were not counted as this may have been double counting specimens present or counting specimens that were not in the portion of the stream bed as exuviae tend to float downstream after a moult. This was especially true for Ephemeroptera. Where possible pupal keys were used to get fly pupae to family/genus.

All samples contained a high number of invertebrates which enabled subsampling. The number of organisms (included taxa) identified in this study was 2274. To facilitate comparisons among the samples, the subsampled collections were scaled up to a full sample. The total number of organisms would then be 25569 (Tables 2 and 7). These organisms were distributed among 31 families and 60 genera (Table 7). Eighty-nine (89%) of the organisms could be identified to the genus level. The remaining were either too immature or damaged or identification keys didn't exist to be confidently identified lower than family. The CABIN analysis protocol will provide further information on site indices and statistics.

## Auditing Protocol

The auditing protocol was performed on the same sample as the sorting efficiency. We followed the CABIN protocol for determining the Identification Error Rate and tabulated the incorrect identifications and missed organisms (Table 6).

$$\% \text{ Identification Error} = \frac{\# \text{ Incorrect Identifications}}{\text{Total Organisms Found in Audit}} * 100$$

The average Identification Error Rate for the QAQC samples was 0.09% (Table 6). This error rate is well within the tolerances for CABIN (5% or less).

## Taxonomic Keys and References

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### **Equipment List**

- Tyler stainless steel and brass sieves: 13.3 mm, 4000 um, 1000 um and 400 um
- Catchment pan and basin.
- Marchant box for subsampling
- Tools: Transfer pipettes, forceps, slides, cover slips, watch glasses, glass vials with screw tops, acid free paper, squeeze bottles
- Jars: 125, 250, 500 and 1000 ml
- Glycerine for temporary slides
- 70% ethanol
- Dissecting microscopes: Leica MZ6 and Leica MS5 (6.3 – 80X)
- Compound microscope Olympus CX41 (40-1000X)
- Light sources: MI-150 Fiber-lights

## Tables

**Table 1. Number, size and condition of plastic collection jars**

	Site ID	Sample name	Number of jars	Jar size	Collection Date	Condition
1	UAB002	UAB-2022-002	1	500 ml	2022-10-02	Free from damage
2	UAB003	UAB-2022-003	1	500 ml	2022-10-03	Extensive damage to the sampling jar. Jar content was recovered.
3	UAB004	UAB-2022-004	1	500 ml	2022-10-03	Free from damage
4	UAB005	UAB-2022-005	1	500 ml	2022-10-03	Free from damage
5	UAB006	UAB-2022-006	1	500 ml	2022-10-04	Extensive damage to the sampling jar. Jar content was recovered.
6	UAB007	UAB-2022-007	1	500 ml	2022-10-02	Free from damage

**Table 2. Subsampling effort - measured and calculated number of invertebrates per sample**

Sample name	Number of cells sampled from Marchant box <sup>1</sup>	No. of organisms in subsamples	Total number of organisms in sample <sup>2</sup>
UAB002	38	350	921
UAB003	14	382	2729
UAB004	28	370	1321
UAB005	8	379	4738
UAB006	5	438	8760
UAB007	5	355	7100
<b>Total</b>		<b>2274</b>	<b>25569</b>

1. Marchant box has 100 cells

2. Scaled up to a full sample

**Table 3. QA-QC Sorting efficiency for three randomly selected sample.**

Sample	Original Count	QA Audit Count	Comments	% SE
UAB003	381	382	Missed 1. Organisms: 1 Chironomid	99.7%
UAB005	379	379	Missed 0.	100
UAB006	438	438	Missed 0.	100
<b>% Sorting Efficiency</b>				<b>99.9%</b>

Percent Sorting Efficiency 99.9% = Pass ( ≥95%)

**Table 4. Standard taxonomic effort for practical Identification**

Group	Taxa	Attained Level of Identification
Insects	Coleoptera	Family/Genus
	Diptera	Family/Genus
	Ephemeroptera	Genus
	Plecoptera	Family/Genus
	Trichoptera	Family/Genus
Non-insects	Neophora	Genus
	Neotaenioglossa	Genus
	Trombidiformes	Genus

**Table 5. Excluded taxa**

	Taxa
<b>Aquatic</b>	Copepoda, Ostracoda,
<b>Non-aquatic</b>	Hemiptera, Hymenoptera (Formicidae)

**Table 6A. QA-QC Identification error rate for sample UAB003.**

Order	Family	Genus	Raw Count	Audit Count	Audit Flag	IE Error	Comments
Ephemeroptera	Baetidae	<i>Baetis</i>	130	124			
Ephemeroptera	Ameletidae	<i>Ameletus</i>	5	11		1	6 <i>Ameletus</i> misidentified as <i>Baetis</i>
<b>Total</b>						1	
<b>Total organisms found in audit</b>			382	382			
<b>Average % Identification Error Rate</b>							<b>0.26%</b>

**Table 6B. QA-QC Identification error rate for sample UAB005.**

Order	Family	Genus	Raw Count	Audit Count	Audit Flag	IE Error	Comments
<b>Total</b>						0	
<b>Total organisms found in audit</b>			379	379			
<b>Average % Identification Error Rate</b>							<b>0%</b>

**Table 6C. QA-QC Identification error rate for sample UAB006.**

Order	Family	Genus	Raw Count	Audit Count	Audit Flag	IE Error	Comments
<b>Total</b>						0	
<b>Total organisms found in audit</b>			438	438			
<b>Average % Identification Error Rate</b>							<b>0%</b>

**Average Identification Error Rate = 0.09% - Pass ( ≤ 5%)**

**Table 7. Total count of benthic macroinvertebrates per field site, Upper McLeod River - Athabasca, October 2022.**

Taxonomic Group	UAB002	UAB003	UAB004	UAB005	UAB006	UAB007	Total
<b>Order: Coleoptera</b>							
<b>Family: Elmidae</b>							
<i>Heterlimnius</i>					660		660
<i>Zaitzevia</i>	8		4				11
<b>Order: Diptera</b>							
<b>Family: Chironomidae</b>							
<i>Ablabesmyia</i>	18		29		100	120	267
<i>Brillia</i>	3	7					10
<i>Chironomus</i>	16			25	80	1620	1741
<i>Cricotopus</i>	189	157	11	100	720	400	1577
<i>Diamesa</i>	3	7				40	50
<i>Eukiefferiella</i>	61	50	21	63	480	1060	1734
<i>Neostempellina</i>	3						3
<i>Orthocladius</i>	18	7	11		20	60	116
<i>Pagastia</i>		50			20	200	270
<i>Parakiefferiella</i>	32		4		40	120	195
<i>Parametriocnemus</i>	5	50			60	60	175
<i>Polypedilum</i>	68	86	14	63	140	20	391
<i>Potthastia</i>	3		4			240	246
<i>Procladius</i>	3						3
<i>Zavrelimyia</i>					180		180
<b>Family: Empididae</b>							
<i>Oreogeton</i>	11	7				100	118
<b>Family: Psychodidae</b>							
<i>Pericoma</i>	21	7	11		460	80	579
<b>Family: Tipulidae</b>							
<i>Antocha</i>					40		40
<b>Order: Ephemeroptera</b>							
<b>Family: Ameletidae</b>							
<i>Ameletus</i>	16	114	14	100	240		484
<b>Family: Baetidae</b>							
<i>Baetis</i>	142		86	100	2600	240	3168
<b>Family: Ephemerellidae</b>							
<i>Drunella</i>	5	43	4	100	220	100	472
<i>Ephemerella</i>					40		40
<i>Serratella</i>	21	100	11	88	240	60	519
<b>Family: Heptageniidae</b>							
<i>Cinygmula</i>	5				260		265
<i>Epeorus</i>	16	7	4				27
<i>Rhithrogena</i>	8	86	146	400	20		660
<b>Family: Family: Leptophlebiidae</b>							
<i>Leptophlebia</i>	5	71		1088	100	380	1644
	24	686	404	1350	380	1020	3863

**Table 7. Total count of benthic macroinvertebrates per field sites Upper McLeod River – Athabasca, October 2022.**

<b>Taxonomic Group</b>	<b>UAB002</b>	<b>UAB003</b>	<b>UAB004</b>	<b>UAB005</b>	<b>UAB006</b>	<b>UAB007</b>	<b>Total</b>
<b>Order: Neoophora</b>							
<b>Family: Planariidae</b>							
<i>Polycelis</i>		50	7				57
<b>Order: Neotaenioglossa</b>							
<b>Family: Hydrobiidae</b>							
<i>Probythinella lacustris</i>	3						3
<b>Order: Plecoptera</b>							
<b>Family: Capniidae</b>							
<i>Capnia</i>	16	43	11	100	20	20	209
<b>Family: Chloroperlidae</b>							
<i>Plumiperla</i>		64	32	13			109
<i>Suwallia</i>	13	157	18				188
<i>Sweltsa</i>	3	7	86	25	20	40	180
<b>Family: Leuctridae</b>							
<i>Despaxia</i>		14			40		54
<i>Paraleuctra</i>		7					7
<i>Perlomyia</i>		7	21	25			54
<i>Pomoleuctra</i>			4				4
<b>Family: Nemouridae</b>							
<i>Amphinemura</i>	3						3
<i>Malenka</i>	61				120	220	401
<i>Zapada</i>		136	7	150	280	380	953
<b>Family: Perlidae</b>							
<i>Hesperoperla</i>	3				20		23
<b>Family: Perlodidae</b>							
<i>Diura</i>	11			25	80	60	176
<i>Isoperla</i>	34	7			60		101
<i>Megarcys</i>		7					7
<b>Family: Taeniopterygidae</b>							
<i>Oemopteryx</i>			4				4
<i>Taenionema</i>	3	57	118	575	420	100	1273

**Table 7. Total count of benthic macroinvertebrates per field sites Upper McLeod River – Athabasca, October 2022.**

<b>Taxonomic Group</b>	<b>UAB002</b>	<b>UAB003</b>	<b>UAB004</b>	<b>UAB005</b>	<b>UAB006</b>	<b>UAB007</b>	<b>Total</b>
<b>Order: Trichoptera</b>							
<b>Family: Brachycentridae</b>							
<i>Brachycentrus</i>	34		7	25	220	40	326
<i>Micrasema</i>					40		40
<b>Family: Glossosomatidae</b>							
<i>Glossosoma</i>						20	20
<b>Family: Hydropsychidae</b>							
<i>Arctopsyche</i>					40	20	60
<i>Parapsyche</i>	3	14	11	25		20	73
<b>Family: Lepidostomatidae</b>							
<i>Lepidostoma</i>						20	20
<b>Family: Limnephilidae</b>							
<i>Ecclisomyia</i>						20	20
<b>Family: Rhyacophilidae</b>							
<i>Rhyacophila</i>	3	14			40	60	117
<b>Order: Trombidiformes</b>							
<b>Family: Feltriidae</b>							
<i>Feltria</i>						40	40
<b>Family: Hydrachnidae</b>							
<i>Hydrachna</i>		7					7
<b>Family: Lebertiidae</b>							
<i>Lebertia</i>	8	14	36	38	40		135
<b>Family: Sperchontidae</b>							
<i>Sperchon</i>		43	50	100	60	40	293
<b>Family: Stygothrombiidae</b>							
<i>Stygothrombium</i>			4				4
<b>Family: Torrenticolidae</b>							
<i>Torrenticola</i>	3	14		50	120	60	247
<b>Total</b>	<b>921</b>	<b>2729</b>	<b>1321</b>	<b>4738</b>	<b>8760</b>	<b>7100</b>	<b>25569</b>