December 20, 2021

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Dear Sarah,

Please find following our report on the benthic invertebrates from sites in the Athabasca watershed collected in 2021. In this report you will find a list of the benthic invertebrates and their occurrence per sample. We have followed the CABIN protocol as outlined in the CABIN Laboratory Methods (May 2014) for subsampling, sorting, taxa identification and the auditing protocols.

We have identified 98% of the organisms used in the CABIN analysis to the genus-level. In a few cases, invertebrates were identified to the family level because they were too immature, no keys were available to the generic level or were too damaged to proceed to the genus level. Tabulated counts per sample are provided in this report. A digital copy in Excel is also provided with the first tab containing the data in the format for uploading to CABIN and the second tab contains the count data with Phylum, Class, Order, Family and Genus, as well as the excluded taxa.

It has been our pleasure to work on the taxonomy portion of this project. We hope that these data meet your expectations, and we would be delighted to work with the Athabasca Watershed Council again. Photography will commence in the new year.

If you have any questions or require further information, please don't hesitate to contact us.

Yours sincerely,

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Benthic Invertebrates

Report December 2021
Taxonomy, Methods and Quality Control
for
Athabasca Watershed Council
Athabasca, AB

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Sample Reception

Five sample containers containing 4 samples were received by ABI Environmental Services (3911 Varsity Dr. NW, Calgary, Alberta) on October 8, 2021. Samples were received, counted, inspected, and compared to the packing list. All sample containers were free from damage and leaks. Samples were in one or two labelled containers (Table 1).

Label information was:

UAB001: 10/01/2021, Athabasca River – Solomon Creek
UAB002: 10/02/2021, Athabasca River-Gregg River
UAB003: 10/02/2021, Athabasca River – Whitehorse Creek
UAB004: 10/02/2021, Athabasca River – Mcleod River

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, 4000 um, 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 4000 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 glass mason jar. The contents resting on the 1000 and 400 um sieves were inverted into separate basins and gently washed through these sieves to remove as much of the plant debris and substrate as was practical and then transferred to the above mason jar with 70% ethanol. The fraction that passed through the 400 um sieve was inspected for invertebrates and none were found. This procedure was repeated for all 4 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 four 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 a watch glass using a transfer pipette. If at least 300 organisms of the taxa of interest (listed in Table 4) were not reached in the 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. 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). For each of the samples, the cells that were sorted (invertebrates removed) were bulked together labeled as “sorted” and retained for auditing. For each of the subsampled samples, the unsorted cells were labelled as “unsorted” and transferred to containers and retained.

Sorting Audit Protocol

One of the four samples (25%) was randomly chosen for resorting by another team member. Sorting precision was calculated as percent sorting efficiency (% SE) using the CABIN method.

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

The sorting efficiency is in Table 3, the sample exceeded the CABIN protocol of 95% with an average sorting efficiency of 98% (Table 3).

Identification and Taxonomy

The rough sorted samples were further examined to identify organisms to the 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 1332. Another nine organisms were identified but were in the CABIN excluded taxa of Hemerobiidae, Planariidae and Gordiidae. These organisms were excluded from the analysis. To facilitate comparisons among the samples, the subsampled collections were scaled up to a full sample. The total number of organisms would then be 12865 (Table 7). These organisms were distributed among 28 families and 56 genera (Table 7). We were pleased that 98% could be identified to the genus level. The remaining two percent 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).

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

The Identification Error Rate for sample UAB002 was 1.7%. This error rate is within the tolerances for CABIN. The persistent errors were the misidentification of small and gill-less *Drunella* with *Ephemerella* and small and gill-less *Leptophlebia* with *Rithrogena*. These errors were corrected and all the Ephemerellidae and Heptageniidae were re-examined in the non-audited samples.

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
- Mason 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 and size of plastic collection jars

Sample name	Number of jars	Jar size
UAB001	2	500 ml
UAB002	1	500 ml
UAB003	1	500 ml
UAB004	1	500 ml

Table 2. Sub sampling effort, and measured and calculated number of invertebrates per sample

Sample name	Number of cells sampled from Marchant box[1]	No. of organisms in subsamples	Total number of organisms in sample[2]
UAB001	10	326	3260
UAB002	12	362	3017
UAB003	7	326	4657
UAB004	16	309	1931
Total		**1323**	**12865**

1. Marchant box has 100 cells
2. Scaled up to a full sample

Table 3. Sorting efficiency for one randomly selected sample.

Sample	**Original Count**	**QA Audit Count**	**Comments**	**% SE**
UAB002			Missed 8. Organisms: 6 small chironomids, 1 mite and 1 Ephemeroptera.	98%
			% Sorting Efficiency	**98%**

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	Heterostropha	Genus
	Lumbriculida	Genus
	Neophora	Genus
	Trombidiformes	Family/Genus
	Tubificida	Genus

Table 5. Excluded taxa

	Taxa
Aquatic	Copepoda, Ostracoda, Grodioidea (Nematomorpha), Platyhelminthes
Non-aquatic	Neuroptera: Hemerobiidae

Table 6. Identification error rate for sample UAB002.

Order	Family	Genus	Raw Count	Audit Count	Audit Flag	IE Error	Comments
Diptera	Psychodidae	*Pericoma/ Telmatoscopus*	17	18			Enumeration (+1)
Ephemeroptera	Ephemerellidae	*Ephemerella*	52	49	52		Misidentification, 3 specimens
Ephemeroptera	Ephemerellidae	*Drunella*	13	16		3	3 Misidentified as Ephemerella, no front legs nor abdominal gill
Ephemeroptera	Heptageniidae	*Rithrogena*	19	16	19		Misidentification of 3 individuals
Ephemeroptera	Leptophlebiidae	*Leptophlebia*	0	3		3	Misidentification; identified as Rithrogena; small, no gills
Plecoptera	Chloroperlidae	*Sweltsa*	9	8			1 rolled up to family
Plecoptera	Chloroperlidae		0	1			
Total			110	111	71	6	
Total organisms found in audit			361	362			
	Average % Identification Error Rate						**1.7%**

Table 7. Total number of benthic macroinvertebrates per sample

Taxonomic Group	UAB001	UAB002	UAB003	UAB004	Total
Class: Clitellata					
Order: Lumbriculida					
Family: Lumbriculidae					
Lumbriculus	170				170
Order: Tubificida					
Family: Naididae					
Specaria	10				10
Class: Euchelicerata					
Order: Trombidiformes					
Family: Hydrachnidae					
Hydrachna		8			8
Family Lebertiidae					
Lebertia		58		81	140
Family: Sperchontidae					
Sperchon		25	86	75	186
Class: Gastropoda					
Heterostropha					
Family: Valvatidae					
Valvata	40				40
Class: Insecta					
Order: Coleoptera					
Family: Elmidae					
Narpus		42		13	54
Zaitzevia		25			25
Order: Diptera					
Family: Chironomidae					
Orthocladius	110	17	14	56	197
Diamesa			157		157
Cricotopus	250	50	86		386
Polypedilum	390	158	343	6	897
Brillia		17	29	25	70
Parametriocnemus			14		14
Pagastia	220	25	57		302
Potthastia		50	57		107
Eukiefferiella	40	8		75	123
Psectrocladius				25	25
Abiskomyia				19	19
Prodiamesa	10				10
Parakiefferiella	50	58			108
Cardiocaldius	40				40
Chironomus	10				10
Ablabesmyia		8			8

Table 7. Total number of benthic macroinvertebrates per sample

Taxonomic Group	UAB001	UAB002	UAB003	UAB004	Total
Family: Empididae					
Neoplasta	10	17		6	33
Oreogeton		17			17
Family: Psychodidae					
Pericoma/Telmatoscopus	10	150		6	166
Family: Simuliidae					
Prosimulium	90			6	96
Simulium	100				100
Family: Tipulidae					
Hexatoma				6	6
Dicranota	30		14		44
Order: Ephemeroptera					
Family: Ameletidae					
Ameletus	110	133	29		272
Family: Baetidae					
Baetis	640	975	557	100	2272
Family: Ephemerellidae					
Drunella		133	14	319	466
Ephemerella	150	408	14	94	666
Family: Heptageniidae	40				40
Cinygmula	10	83			93
Epeorus				6	6
Rhithrogena		133	257	119	509
Family: Leptophlebiidae					
Leptophlebia		25	2157	269	2451
(blank)	10				10
Order: Plecoptera					
Family: Capniidae					
Capnia			14		14
Family: Chloroperlidae		8			8
Plumiperla	20		29	6	55
Sweltsa		67	14	6	87
Family: Leuctridae					
Paraleuctra		17	14		31
Family: Nemouridae	20				20
Amphinemura				19	19
Malenka	200	75	14	31	321
Zapada	320	33	400	13	766

Table 7. Total number of benthic macroinvertebrates per sample

Taxonomic Group	UAB001	UAB002	UAB003	UAB004	Total
Family: Perlidae		8		6	15
Hesperoperla		42		6	48
Family: Perlodidae					
Cultus		17		6	23
Diura	40	50		13	103
Kogotus			14	31	46
Megarcys			29		29
Family: Taeniopterygidae					
Taenionema	40	17	214	356	627
Order: Trichoptera					
Family: Brachycentridae					
Brachycentrus		42		38	79
Family Glossosomatidae					
Glossosoma		17		6	23
Family: Hydropsychidae					
Arctopsyche	30		14		44
Parapsyche				13	13
Family: Rhyacophilidae					
Rhyacophila	50		14	75	139
Grand Total	**3260**	**3017**	**4657**	**1931**	**12865**