Benthic Invertebrates

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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.

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

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).

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

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[1]	No. of organisms in subsamples	Total number of organisms in sample[2]
UAB002	38	350	921
UAB003	14	382	2729
UAB004	28	370	1321
UAB005	8	379	4738
UAB006	5	438	8760
UAB007	5	355	7100
Total		**2274**	**25569**

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
			% Sorting Efficiency	**99.9%**

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	Neoophora	Genus
	Neotaenioglossa	Genus
	Trombidiformes	Genus

Table 5. Excluded taxa

	Taxa
Aquatic	Copepoda, Ostracoda,
Non-aquatic	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	*Baetis*	130	124			
Ephemeroptera	Ameletidae	*Ameletus*	5	11		1	6 *Ameletus* misidentified as *Baetis*
Total						1	
Total organisms found in audit			382	382			
	Average % Identification Error Rate						**0.26%**

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

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

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

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

Average Identification Error Rate = 0.09% - Pass ($\leq$ 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
Order: Coleoptera							
Family: Elmidae							
Heterlimnius					660		660
Zaitzevia	8		4				11
Order: Diptera							
Family: Chironomidae							
Ablabesmyia	18		29		100	120	267
Brillia	3	7					10
Chironomus	16			25	80	1620	1741
Cricotopus	189	157	11	100	720	400	1577
Diamesa	3	7				40	50
Eukiefferiella	61	50	21	63	480	1060	1734
Neostempellina	3						3
Orthocladius	18	7	11		20	60	116
Pagastia		50			20	200	270
Parakiefferiella	32		4		40	120	195
Parametriocnemus	5	50			60	60	175
Polypedilum	68	86	14	63	140	20	391
Potthastia	3		4			240	246
Procladius	3						3
Zavrelimyia					180		180
Family: Empididae							
Oreogeton	11	7				100	118
Family: Psychodidae							
Pericoma	21	7	11		460	80	579
Family: Tipulidae							
Antocha					40		40
Order: Ephemeroptera							
Family: Ameletidae							
Ameletus	16	114	14	100	240		484
Family: Baetidae							
Baetis	142		86	100	2600	240	3168
Family: Ephemerellidae	5	43	4	100	220	100	472
Drunella	5		93	113	40	20	271
Ephemerella					40		40
Serratella	21	100	11	88	240	60	519
Family: Heptageniidae	16	529	39				584
Cinygmula	5				260		265
Epeorus	16	7	4				27
Rhithrogena	8	86	146	400	20		660
Family: Family: Leptophlebiidae	5	71		1088	100	380	1644
Leptophlebia	24	686	404	1350	380	1020	3863

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

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

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