Analysis of a biological sample:
Technical summary of methods and quality assurance procedures
Prepared for Washington County Conservation District
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by
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METHODS

Sample processing

One macroinvertebrate sample collected from Zavoral's Creek was delivered to Rhithron's laboratory facility in Missoula, Montana on September 20, 2013. The sample arrived in good condition. The sample was decanted prior to shipment; preservative was replenished immediately upon arrival at the laboratory. A chain of custody document containing sample identification information was provided by the Washington County Conservation District (WCD) Project Manager. Upon arrival, the sample was unpacked and examined, and checked against the WCD chain of custody. An inventory spreadsheet including project code and internal laboratory identification numbers was uploaded into the Rhithron database prior to sample processing.

A subsample of a minimum of 300 organisms was achieved using methods consistent with Minnesota Pollution Control Agency protocols (MPCA 2004). A Caton sub-sampling device (Caton 1991), divided into 30 grids, each approximately 6 cm by 6 cm was used. The sample was thoroughly mixed in its jar, poured out and evenly spread into the Caton tray, and individual grids were randomly selected. The contents of each grid were examined under stereoscopic microscopes using 10x-30x magnification. Grid selection, examination, and sorting continued until at least 300 organisms were sorted. All aquatic invertebrates from each selected grid were sorted from the substrate, and placed in 80% ethanol for subsequent identification.

After the target number of organisms was obtained in the subsample, a large/rare search was performed: the Caton tray was scanned for additional organisms that were not collected in the subsample. These organisms were placed in a separate vial and labeled as "Large/Rare Organisms". Unsorted sample fractions were retained and stored at the Rhithron laboratory.

Organisms were individually examined by certified taxonomists, using 10x – 80x stereoscopic dissecting scopes (Leica S8E) and identified to target taxonomic levels consistent with MPCA stream protocols (MPCA 2004), using appropriate published taxonomic references and keys.

Identification, counts, life stages, and information about the condition of specimens were recorded on electronic bench sheets. Organisms that could not be identified to the taxonomic targets because of immaturity, poor condition, or lack of complete current regionally-applicable published keys were left at appropriate taxonomic levels that were coarser than those specified. To obtain accuracy in richness measures, these organisms were designated as "not unique" if other specimens from the same group could be taken to target levels. Organisms designated as "unique" were those that could be definitively distinguished from other organisms in the sample. Taxonomists examined organisms in the Large/Rare vial and determined there were not any unique taxa collected. Identified organisms were preserved in 80% ethanol in labeled vials, and archived at the Rhithron laboratory.

Chironomids were carefully morphotyped using 10x – 80x stereoscopic dissecting microscopes (Leica S8E) and representative specimens were slide mounted and examined at 200x – 1000x magnification using an Olympus BX 51 or Leica DM 1000 compound microscope. Slide mounted organisms were archived at the Rhithron laboratory.

Quality control procedures

Quality control procedures for initial sample processing and subsampling involved checking sorting efficiency. An independent observer microscopically re-examined 25% of sorted substrate from the sample. Quality control procedures proceeded as follows:

The quality control technician poured the sorted substrate from the processed sample out into a Caton tray, redistributing the substrate so that 25% of it could be accurately lifted out by removing entire grids in a random fashion. Grids were selected, and re-examined until 25% of the substrate was re-sorted. All organisms that were missed were counted and this number was added to the total number obtained in the original sort. Sorting efficiency was evaluated by applying the following calculation:

$$SE = \frac{n_1}{n_1 + n_2} \times 100$$

where: SE is the sorting efficiency, expressed as a percentage, n_1 is the total number of specimens in the first sort, and n_2 is the total number of specimens expected in the second sort, based on the results of the re-sorted 25%.

Quality control procedures for taxonomic determinations of invertebrates involved checking accuracy, precision and enumeration. All organisms were re-identified and counted by an independent taxonomist. Taxa lists and enumerations were compared by calculating a Bray-Curtis similarity statistic (Bray and Curtis 1957), Percent Taxonomic Disagreement (PTD) and Percent Difference in Enumeration (PDE). Routinely, discrepancies between the original identifications and the QC identifications are discussed among the taxonomists, and necessary rectifications to the data are made. Discrepancies that cannot be rectified by discussions are routinely sent out to taxonomic specialists for identification. However, taxonomic certainty for identifications in this project was high and no external verifications were necessary.

Data analysis

Taxonomic data and counts were entered into the Rhithron laboratory database, and an uploadable spreadsheet file, consistent with MPCA stream data requirements, including taxonomy, counts, life stages, sample metadata, and other information was generated.

Taxa lists and counts were constructed and standard metric calculations for aquatic invertebrate assemblages were made using Rhithron's customized database software. A sites-by-taxa matrix in spreadsheet format was also created.

RESULTS

Quality Control Procedures

Sorting efficiency was 95.40%, taxonomic precision for identification and enumeration was 98.01% (Bray Curtis), 0.93% PTD and 1.07% PDE, and data entry efficiency was 100%. These similarity statistics fall within acceptable industry criteria (Stribling et al. 2003).

Data analysis

Taxa list and metric summary pages are given in the Appendix. Electronic spreadsheets were provided to the WCD Project Manager via email.

REFERENCES

Bray, J. R. and J. T. Curtis. 1957. An ordination of upland forest communities of southern Wisconsin. Ecological Monographs 27: 325-349.

Caton, L. W. 1991. Improving subsampling methods for the EPA's "Rapid Bioassessment" benthic protocols. Bulletin of the North American Benthological Society. 8(3): 317-319.

MPCA, 2004. Laboratory Analysis – Invertebrate Sample Processing and Identification. State of Minnesota Standard Operating Procedure.

Stribling, J.B., S.R Moulton II and G.T. Lester. 2003. Determining the quality of taxonomic data. J.N. Am. Benthol. Soc. 22(4): 621-631.

APPENDIX

Taxa list and metric summary

Washington County Conservation District, MN

Zavoral's Creek
September 2013

Taxa Listing

Project ID: WCD13ZC2
RAI No.: WCD13ZC2001

RAI No.: WCD13ZC2001 **Sta. Name:** Zavoral's Creek
Client ID:
Date Coll.: 9/17/2013 **No. Jars:** 1 **STORET ID:**

Taxonomic Name	Count	PRA	Unique	Stage	Qualifier	BI	Function
Other Non-Insect							
Acari	11	3.38%	Yes	Unknown		5	PR
Nemata	1	0.31%	Yes	Unknown		5	UN
Turbellaria	2	0.62%	Yes	Unknown		4	PR
Asellidae							
Caecidotea sp.	3	0.92%	Yes	Unknown		8	CG
Gammaridae							
Gammarus sp.	69	21.23%	Yes	Unknown		4	SH
Physidae							
Physa sp.	22	6.77%	Yes	Unknown		8	SC
Oligochaeta							
Oligochaeta	1	0.31%	Yes	Unknown		10	CG
Ephemeroptera							
Baetidae							
Baetis sp.	41	12.62%	Yes	Larva		5	CG
Leptophlebiidae							
Paraleptophlebia sp.	2	0.62%	Yes	Larva		1	CG
Plecoptera							
Capniidae							
Capniidae	16	4.92%	Yes	Larva	Early Instar	1	SH
Nemouridae							
Amphinemura sp.	1	0.31%	Yes	Larva		2	SH
Trichoptera							
Glossosomatidae							
Glossosoma sp.	1	0.31%	Yes	Larva		0	SC
Hydropsychidae							
Diplectrona modesta	2	0.62%	Yes	Larva		0	CF
Hydropsychidae	6	1.85%	No	Larva	Early Instar	4	CF
Lepidostomatidae							
Lepidostoma sp.	2	0.62%	Yes	Larva		1	SH
Limnephilidae							
Hesperophylax designatus	2	0.62%	Yes	Larva		5	SH
Limnephilus sp.	3	0.92%	Yes	Larva		3	SH
Psychomyiidae							
Lype diversa	4	1.23%	Yes	Larva		2	SC
Rhyacophilidae							
Rhyacophila sp.	9	2.77%	Yes	Larva		1	PR

Taxa Listing

Project ID: WCD13ZC2
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RAI No.: WCD13ZC2001 **Sta. Name:** Zavoral's Creek
Client ID:
Date Coll.: 9/17/2013 **No. Jars:** 1 **STORET ID:**

Taxonomic Name	Count	PRA	Unique	Stage	Qualifier	BI	Function
Diptera							
Ceratopogonidae							
Ceratopogoninae	1	0.31%	Yes	Larva	Early Instar	6	PR
Dixidae							
Dixa sp.	8	2.46%	Yes	Larva		1	CG
Empididae							
Empididae	1	0.31%	No	Larva	Early Instar	6	PR
Neoplasta sp.	1	0.31%	Yes	Larva		5	PR
Ptychopteridae							
Ptychoptera sp.	1	0.31%	Yes	Larva		7	CG
Simuliidae							
Simulium sp.	17	5.23%	Yes	Larva		6	CF
Chironomidae							
Chironomidae							
Corynoneura sp.	2	0.62%	Yes	Larva		7	CG
Eukiefferiella sp.	20	6.15%	Yes	Larva		8	CG
Heterotrissocladius sp.	1	0.31%	No	Pupa		0	CG
Heterotrissocladius sp.	1	0.31%	Yes	Larva		0	CG
Micropsectra sp.	4	1.23%	Yes	Larva		4	CG
Odontomesa sp.	3	0.92%	Yes	Larva		4	CG
Orthocladiinae	2	0.62%	No	Pupa	Damaged	6	CG
Parametriocnemus sp.	1	0.31%	No	Pupa		5	CG
Parametriocnemus sp.	16	4.92%	Yes	Larva		5	CG
Paratendipes sp.	1	0.31%	Yes	Larva		10	CG
Polypedilum sp.	2	0.62%	Yes	Larva		6	SH
Prodiamesa sp.	3	0.92%	Yes	Larva		3	CG
Synorthocladius sp.	2	0.62%	Yes	Larva		2	CG
Tanytarsus sp.	1	0.31%	No	Pupa		6	CF
Tanytarsus sp.	1	0.31%	Yes	Larva		6	CF
Thienemanniella sp.	2	0.62%	Yes	Larva		6	CG
Thienemannimyia Gr.	1	0.31%	Yes	Larva		5	PR
Tvetenia sp.	34	10.46%	Yes	Larva		5	CG
Zavrelimyia sp.	1	0.31%	Yes	Larva		8	PR
Sample Count	**325**						

Metrics Report

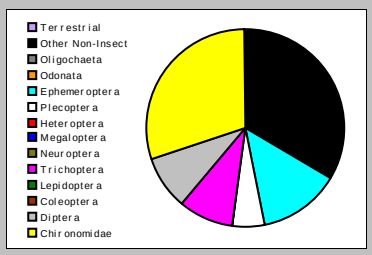
Project ID: WCD13ZC2
RAI No.: WCD13ZC2001
Sta. Name: Zavoral's Creek
Client ID:
STORET ID
Coll. Date: 9/17/2013
Latitude: **Longitude:**

Abundance Measures

Sample Count: 325
Sample Abundance: 1,950.00 16.67% of sample used
Coll. Procedure:
Sample Notes:

Taxonomic Composition

Category	R	A	PRA
Terrestrial			
Other Non-Insect	6	108	33.23%
Oligochaeta	1	1	0.31%
Odonata			
Ephemeroptera	2	43	13.23%
Plecoptera	2	17	5.23%
Heteroptera			
Megaloptera			
Neuroptera			
Trichoptera	7	29	8.92%
Lepidoptera			
Coleoptera			
Diptera	5	29	8.92%
Chironomidae	15	98	30.15%

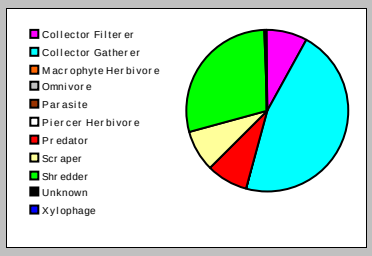


Dominant Taxa

Category	A	PRA
Gammarus	69	21.23%
Baetis	41	12.62%
Tvetenia	34	10.46%
Physa	22	6.77%
Eukiefferiella	20	6.15%
Simulium	17	5.23%
Parametriocnemus	17	5.23%
Capniidae	16	4.92%
Acari	11	3.38%
Rhyacophila	9	2.77%
Dixa	8	2.46%
Hydropsychidae	6	1.85%
Micropsectra	4	1.23%
Lype diversa	4	1.23%
Caecidotea	3	0.92%

Functional Composition

Category	R	A	PRA
Predator	7	27	8.31%
Parasite			
Collector Gatherer	17	148	45.54%
Collector Filterer	3	27	8.31%
Macrophyte Herbivore			
Piercer Herbivore			
Xylophage			
Scraper	3	27	8.31%
Shredder	7	95	29.23%
Omnivore			
Unknown	1	1	0.31%

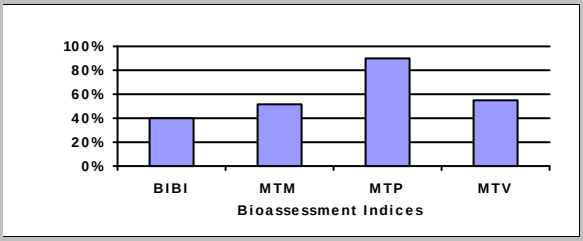


Metric Values and Scores

Metric	Value
Composition	
Taxa Richness	38
E Richness	2
P Richness	2
T Richness	7
EPT Richness	11
EPT Percent	27.38%
All Non-Insect Abundance	109
All Non-Insect Richness	7
All Non-Insect Percent	33.54%
Oligochaeta+Hirudinea Percent	0.31%
Baetidae/Ephemeroptera	0.953
Hydropsychidae/Trichoptera	0.276
Dominance	
Dominant Taxon Percent	21.23%
Dominant Taxa (2) Percent	33.85%
Dominant Taxa (3) Percent	44.31%
Dominant Taxa (10) Percent	78.77%
Diversity	
Shannon H (loge)	2.779
Shannon H (log2)	4.010
Margalef D	6.439
Simpson D	0.096
Evenness	0.054
Function	
Predator Richness	7
Predator Percent	8.31%
Filterer Richness	3
Filterer Percent	8.31%
Collector Percent	53.85%
Scraper+Shredder Percent	37.54%
Scraper/Filterer	1.000
Scraper/Scraper+Filterer	0.500
Habit	
Burrower Richness	3
Burrower Percent	0.92%
Swimmer Richness	3
Swimmer Percent	15.69%
Clinger Richness	6
Clinger Percent	16.00%
Characteristics	
Cold Stenotherm Richness	0
Cold Stenotherm Percent	0.00%
Hemoglobin Bearer Richness	2
Hemoglobin Bearer Percent	0.92%
Air Breather Richness	1
Air Breather Percent	0.31%
Voltinism	
Univoltine Richness	16
Semivoltine Richness	0
Multivoltine Percent	47.08%
Tolerance	
Sediment Tolerant Richness	1
Sediment Tolerant Percent	0.31%
Sediment Sensitive Richness	1
Sediment Sensitive Percent	0.31%
Metals Tolerance Index	3.316
Pollution Sensitive Richness	1
Pollution Tolerant Percent	30.46%
Hilsenhoff Biotic Index	4.665
Intolerant Percent	15.08%
Supertolerant Percent	14.77%
CTQa	91.147

Bioassessment Indices

BioIndex	Description	Score	Pct	Rating
BIBI	B-IBI (Karr et al.)	20	40.00%	
MTP	Montana DEQ Plains (Bukantis 1998)	27	90.00%	None
MTV	Montana Revised Valleys/Foothills (Bollman 1998)	10	55.56%	Slight
MTM	Montana DEQ Mountains (Bukantis 1998)	11	52.38%	Moderate



Friday, December 13, 2013