Analysis of biological samples:
Technical summary of methods and quality assurance procedures
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by
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METHODS

Sample processing: sorting and identification procedures

One hundred and thirty macroinvertebrate samples collected for the King County EPA Project were delivered to Rhithron's laboratory facility in Missoula, Montana on October 3, 2011. An inventory document containing sample identification information was provided by the King County (KC) Project Manager. Upon arrival, samples were unpacked and examined, and checked against the KC inventory. An inventory spreadsheet was created. This spreadsheet included project code and internal laboratory identification numbers and was uploaded into the Rhithron database prior to sample processing.

Standard sorting protocols (Plotnikoff and Wiseman 2001) were applied to achieve representative subsamples of a minimum of 500 organisms. Caton sub-sampling devices (Caton 1991), divided into 30 grids, each approximately 5 cm by 6 cm were used. Each individual sample was thoroughly mixed in its jar(s), 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. All aquatic invertebrates from each selected grid were sorted from the substrate, and placed in 95% ethanol for subsequent identification. Grid selection, examination, and sorting continued until at least 500 organisms were sorted. The final grid was completely sorted of all organisms. 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". When samples contained less than 500 organisms, the entire sample was sorted. All 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 S6E) and identified to target taxonomic levels consistent with Washington Department of Ecology requirements, using appropriate published taxonomic references and keys.

Midges and worms were carefully morphotyped using 10x – 80x stereoscopic dissecting microscopes (Leica S8E and S6E) and representative specimens were slide mounted and examined at 200x – 1000x magnification using an Olympus BX 51 compound microscope.

Identification, counts, life stages, and information about the condition of specimens were recorded on 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. Large/Rare organisms were identified, and these were recorded with a count of "1". Identified

organisms were preserved in 95% ethanol in labeled vials, and archived at the Rhithron laboratory.

Sample processing: sample recombinations and combinations

Many samples were collected in variable-area aliquots, which were subsampled and recombined in various ways. Forty-two samples ("Chico type") were collected as a 3 ft² (sampled area) aliquot and a 5 ft² aliquot. For these samples, the 3 ft² aliquot was subsampled and identified. Substrate from the 3 ft² aliquot was retained as the sorted fraction and the unsorted fraction.

After identification, organisms from the 3 ft² sample aliquot were recombined with the sorted and unsorted substrate from that aliquot. These aliquots were carefully reconstituted to insure complete dispersion of the identified organisms in the substrate. The reconstituted 3 ft² aliquot was then combined with the 5 ft² aliquot to achieve an 8 ft² sample. The 8 ft² sample was then subsampled, and organisms were identified.

Ten samples were collected as a 1 ft² aliquot, a 3 ft² aliquot, and a 5 ft² aliquot. For these samples, the "Chico type" procedure was followed, with additional treatment. Sorted and unsorted substrate from the 8 ft² aliquot was recombined with the identified organisms from that aliquot, and this reconstituted sample was then combined with the 1 ft² aliquot to achieve a 9 ft² sample. As before, this sample was subsampled, and organisms were identified.

Two samples (from Bellingham) were collected as 4 - 2ft² aliquots and a single 3ft² aliquot. For these samples, the aliquots were subsampled and identified separately, and no reconstitutions or combinations were performed.

One sample (from Pierce County) was collected as 3 – 1ft² aliquots and a single 5 ft² aliquot. For this sample, each 1 ft² aliquot was subsampled and identified separately. Then the identified organisms from each 1 ft² aliquot was recombined with their respective sorted and unsorted substrate fractions, and all of these were combined with the 5 ft² aliquot, to achieve an 8 ft² sample. This sample was then subsampled, and organisms were identified.

Two samples (from Redmond) were collected as 9 ft² samples, and these were subsampled separately and organisms were identified.

Quality control procedures

Quality control procedures for initial sample processing and subsampling involved checking sorting efficiency. These checks were conducted on 100% of the samples by independent observers who microscopically re-examined at least 20% of sorted substrate from each sample. Quality control procedures for each sample proceeded as follows:

The quality control technician poured the sorted substrate from a processed sample out into a Caton tray, redistributing the substrate so that 20% of it could be accurately lifted out by removing entire grids in a random fashion. Grids were selected, and re-examined until 20% 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 20%.

Quality control procedures for taxonomic determinations of invertebrates involved checking accuracy, precision and enumeration. Thirteen samples were randomly selected and all organisms 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) for each selected sample. 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.

Four taxa in these samples were not identifiable to target level, because they are not described in the taxonomic literature. Representatives of these specimens were sent to taxonomic specialists for identification. These taxa were assigned a provisional laboratory identifier, until definitive identifications could be made. These were: Empididae sp. (RAI Taxon # 0001), 7 total individuals in 3 different samples; Orthocladiinae sp. (RAI Taxon # 0001), 20 total individuals in 7 different samples; Orthocladiinae sp. (RAI Taxon # 0004), 23 total individuals in 9 different samples; and Orthocladiinae sp. (RAI Taxon # 0011), 199 total individuals in 33 different samples.

Data analysis

Taxa and counts for each sample were entered into Rhithron's customized database software. Rhithron's customized database application was used to produce species lists and counts in upload files for the King County Macroinvertebrate Data Management System.

RESULTS

Quality Control Procedures

Results of quality control procedures for subsampling and taxonomy are given in Table 1. Sorting efficiency averaged 98.68%, taxonomic precision for identification and enumeration averaged 96.00% for the randomly selected QA samples, and data entry efficiency was 100% for the project. These similarity statistics fall within acceptable industry criteria (Stribling et al. 2003).

Data analysis

Appropriate data files were uploaded to the Puget Sound Stream Benthos website.

Table 1. *Results of internal quality control procedures for subsampling and taxonomy.* King County EPA Project, 2011.

Rhithron ID	SampleID: Puget Sound Benthos	Collection Date	Sorting efficiency	Bray-Curtis similarity for taxonomy and enumeration
KC11EPA001	08CED4192_11_3sf	9/19/2011	99.18%	
KC11EPA002	08CED4192_11_8sf	9/19/2011	100.00%	
KC11EPA003	08CED5032_11_3sf	8/17/2011	100.00%	
KC11EPA004	08CED5032_11_8sf	8/17/2011	99.19%	
KC11EPA005	08EAS2272_11_3sf	9/12/2011	98.39%	
KC11EPA006	08EAS2272_11_8sf	9/12/2011	100.00%	
KC11EPA007	08ISS3877_11_3sf	8/30/2011	96.23%	
KC11EPA008	08ISS3877_11_8sf	8/30/2011	98.38%	97.11%
KC11EPA009	08ISS4724_11_3sf	9/8/2011	99.18%	
KC11EPA010	08ISS4724_11_8sf	9/8/2011	98.26%	
KC11EPA011	08ISS4748_11_3sf	8/30/2011	100.00%	
KC11EPA012	08ISS4748_11_8sf	8/30/2011	96.82%	
KC11EPA013	08LAK3879_11_3sf	8/30/2011	100.00%	
KC11EPA014	08LAK3879_11_8sf	8/30/2011	96.97%	
KC11EPA015	08LIT2585_11_3sf	9/12/2011	97.62%	
KC11EPA016	08LIT2585_11_8sf	9/12/2011	97.67%	97.24%
KC11EPA017	08SAM2862_11_3sf	9/12/2011	99.18%	
KC11EPA018	08SAM2862_11_8sf	9/12/2011	97.58%	
KC11EPA019	08WES0622_11_3sf	8/18/2011	100.00%	
KC11EPA020	08WES0622_11_8sf	8/18/2011	99.20%	
KC11EPA021	08WES0629_11_3sf	8/18/2011	98.40%	
KC11EPA022	08WES0629_11_8sf	8/18/2011	100.00%	
KC11EPA023	08WES0903_11_3sf	8/18/2011	96.86%	
KC11EPA024	08WES0903_11_8sf	8/18/2011	99.17%	
KC11EPA025	09COV1756_11_3sf	9/6/2011	98.38%	
KC11EPA026	09COV1756_11_8sf	9/6/2011	96.79%	
KC11EPA027	09DUW0225_11_3sf	9/1/2011	100.00%	
KC11EPA028	09DUW0225_11_8sf	9/1/2011	98.38%	
KC11EPA029	09JEN1357_11_3sf	9/8/2011	96.78%	
KC11EPA030	09JEN1357_11_8sf	9/8/2011	98.36%	
KC11EPA031	09LOW0751_11_3sf	9/15/2011	100.00%	
KC11EPA032	09LOW0751_11_8sf	9/15/2011	98.43%	
KC11EPA033	09MID1958_11_3sf	8/17/2011	97.57%	
KC11EPA034	09MID1958_11_8sf	8/17/2011	100.00%	
KC11EPA035	09MID2426_11_3sf	9/6/2011	98.41%	
KC11EPA036	09MID2426_11_8sf	9/6/2011	100.00%	97.03%
KC11EPA037	09NEW1657_11_3sf	8/17/2011	98.42%	
KC11EPA038	09NEW1657_11_8sf	8/17/2011	98.41%	
KC11EPA039	09SOO1022_11_3sf	8/3/2011	96.97%	
KC11EPA040	09SOO1022_11_8sf	8/3/2011	99.17%	
KC11EPA041	09SOO1130_11_3sf	9/15/2011	100.00%	
KC11EPA042	09SOO1130_11_8sf	9/15/2011	99.22%	96.13%
KC11EPA043	09SOO1283_11_3sf	8/3/2011	99.18%	
KC11EPA044	09SOO1283_11_8sf	8/3/2011	100.00%	
KC11EPA045	KCSSWM003_11_3sf	8/24/2011	97.56%	
KC11EPA046	KCSSWM003_11_8sf	8/24/2011	100.00%	96.34%
KC11EPA047	KCSSWM006_11_3sf	8/23/2011	98.35%	
KC11EPA048	KCSSWM006_11_8sf	8/23/2011	99.22%	
KC11EPA049	KCSSWM007_11_3sf	8/24/2011	99.20%	
KC11EPA050	KCSSWM007_11_8sf	8/24/2011	98.42%	

Table 1 (cont). *Results of internal quality control procedures for subsampling and taxonomy.* King County EPA Project, 2011.

Rhithron ID	SampleID: Puget Sound Benthos	Collection Date	Sorting efficiency	Bray-Curtis similarity for taxonomy and enumeration
KC11EPA051	KCSSWM009_11_3sf	8/23/2011	97.54%	
KC11EPA052	KCSSWM009_11_8sf	8/23/2011	99.22%	
KC11EPA053	KCSSWM011_11_3sf	8/29/2011	100.00%	
KC11EPA054	KCSSWM011_8sf	8/29/2011	98.35%	
KC11EPA055	KCSSWM030_11_3sf	8/29/2011	100.00%	
KC11EPA056	KCSSWM030_11_8sf	8/29/2011	98.42%	95.25%
KC11EPA057	KCSSWM034_11_3sf	8/25/2011	96.94%	
KC11EPA058	KCWSWM034_11_8sf	8/25/2011	100.00%	
KC11EPA059	KCSSWM038_11_3sf	8/25/2011	99.17%	
KC11EPA060	KCSSWM038_11_8sf	8/25/2011	100.00%	
KC11EPA061	KCSSWM040_11_3sf	8/29/2011	98.36%	
KC11EPA062	KCSSWM040_11_8sf	8/29/2011	100.00%	
KC11EPA063	McAleer_187_3sf	9/17/2011	100.00%	
KC11EPA064	McAleer_187_8sf	9/17/2011	96.77%	
KC11EPA065	LackeyCk_11_3sf	8/31/2011	100.00%	
KC11EPA066	LackeyCk_11_8sf	8/31/2011	97.56%	
KC11EPA067	PurdyCreek_11_3sf	8/26/2011	100.00%	
KC11EPA068	PurdyCreek_11_8sf	8/26/2011	97.56%	
KC11EPA069	Bagley07_11_3sf	9/28/2011	98.39%	
KC11EPA070	Bagley07_11_8sf	9/28/2011	96.87%	
KC11EPA071	Bagley07_11_9sf	9/28/2011	98.20%	
KC11EPA072	JCL_11_3sf	8/28/2011	98.42%	
KC11EPA073	JCL_11_8sf	8/28/2011	99.55%	
KC11EPA074	JCL_11_9sf	8/28/2011	98.22%	
KC11EPA075	Morse1pt7_11_3sf	9/22/2011	100.00%	
KC11EPA076	Morse1pt7_11_8sf	9/22/2011	98.51%	
KC11EPA077	Morse1pt7_11_9sf	9/22/2011	98.25%	95.03%
KC11EPA078	Siebert06_11_3sf	9/13/2011	97.58%	
KC11EPA079	Siebert06_11_8sf	9/13/2011	98.45%	
KC11EPA080	Siebert06_11_9sf	9/13/2011	100.00%	
KC11EPA081	Tumwater01a_11_3sf	9/8/2011	100.00%	
KC11EPA082	Tumwater01a_11_8sf	9/8/2011	100.00%	
KC11EPA083	Tumwater01a_11_9sf	9/8/2011	96.47%	
KC11EPA084	WTwin_11_3sf	9/19/2011	98.37%	
KC11EPA085	WTwin_11_8sf	9/19/2011	100.00%	
KC11EPA086	WTwin_11_9sf	9/19/2011	96.37%	97.27%
KC11EPA087	PIMA_11_3sf	8/22/2011	100.00%	
KC11EPA088	PIMA_11_8sf	8/22/2011	96.77%	
KC11EPA089	PIMA_11_9sf	8/22/2011	98.18%	
KC11EPA090	TNMA6462_11_3sf	8/22/2011	98.37%	
KC11EPA091	TNMA6462_11_8sf	8/22/2011	100.00%	
KC11EPA092	TNMA6462_11_9sf	8/22/2011	97.40%	
KC11EPA093	Chuckanut_Arroyo_R1_2sqft	9/28/2011	96.76%	
KC11EPA094	Chuckanut_Arroyo_R2_2sqft	9/28/2011	98.36%	
KC11EPA095	Chuckanut_Arroyo_R3_2sqft	9/28/2011	96.03%	
KC11EPA096	Chuckanut_Arroyo_R4_2sqft	9/28/2011	100.00%	95.71%
KC11EPA097	Chuckanut_Arroyo_3sqft	9/28/2011	99.20%	
KC11EPA098	Squalicum_IronGate_R1_2sqft	9/28/2011	99.19%	
KC11EPA099	Squalicum_IronGate_R2_2sqft	9/28/2011	99.19%	
KC11EPA100	Squalicum_IronGate_R3_2sqft	9/28/2011	99.16%	

Table 1 (cont). *Results of internal quality control procedures for subsampling and taxonomy.* King County EPA Project, 2011.

Rhithron ID	SampleID: Puget Sound Benthos	Collection Date	Sorting efficiency	Bray-Curtis similarity for taxonomy and enumeration
KC11EPA101	Squalicum_IronGate_R4_2sqft	9/28/2011	97.53%	
KC11EPA102	Squalicum_IronGate_3sqft	9/28/2011	99.22%	95.17%
KC11EPA103	SwanCk_11_R1_1sf	8/19/2011	100.00%	
KC11EPA104	SwanCk_11_R2_1sf	8/19/2011	100.00%	
KC11EPA105	SwanCk_11_R3_1sf	8/19/2011	100.00%	
KC11EPA106	SwanCk_11_8sf	8/19/2011	100.00%	
KC11EPA107	08BEA3650_11_3sf	9/7/2011	98.43%	
KC11EPA108	08BEA3650_11_8sf	9/7/2011	96.03%	
KC11EPA109	08BEA3650_11_9sf	9/7/2011	100.00%	95.07%
KC11EPA110	08BEA3650_11_9sf_900um	9/7/2011	98.22%	
KC11EPA111	08BEA3474_11_3sf	9/7/2011	96.05%	
KC11EPA112	08BEA3474_11_8sf	9/7/2011	98.38%	
KC11EPA113	08BEA3474_11_9sf	9/7/2011	100.00%	
KC11EPA114	08BEA3474_11_9sf_900um	9/7/2011	98.19%	
KC11EPA115	Benson_11_3sf	8/8/2011	96.83%	
KC11EPA116	Benson_11_8sf	8/8/2011	98.38%	
KC11EPA117	Boulder_11_3sf	8/15/2011	97.51%	
KC11EPA118	Boulder_11_8sf	8/15/2011	100.00%	95.04%
KC11EPA119	CCJensen_11_3sf	8/16/2011	99.18%	
KC11EPA120	CCJensen_11_8sf	8/16/2011	100.00%	
KC11EPA121	JimWhite_11_3sf	8/8/2011	99.20%	
KC11EPA122	JimWhite_11_8sf	8/8/2011	100.00%	
KC11EPA123	PILC_11_3sf	8/16/2011	98.18%	
KC11EPA124	PILC_11_8sf	8/16/2011	96.87%	
KC11EPA125	Squire_11_3sf	8/15/2011	100.00%	
KC11EPA126	Squire_11_8sf	8/15/2011	100.00%	97.14%
KC11EPA127	Tiger_11_3sf	8/8/2011	100.00%	
KC11EPA128	Tiger_11_8sf	8/8/2011	96.97%	
KC11EPA129	TR30_11_3sf	8/16/2011	97.57%	
KC11EPA130	TR30_11_8sf	8/16/2011	98.41%	

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.

Plotnikoff, R. and C. Wiseman. 2001. Benthic Macroinvertebrate Biological Monitoring Protocols for Rivers and Streams: 2001 Revision. Washington Department of Ecology. Olympia.

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.