



# Sample Collection, Sorting, Taxonomic Identification, and Analysis of Benthic Macroinvertebrate Communities Standard Operating Procedure

**March 15, 2012**

Approvals:

---

Dave Feldman, Biocriteria Specialist, Water Quality Standards Date

---

Robert Bukantis, WQ Standards Supervisor, Water Quality Planning Bureau Date

---

Mindy McCarthy, QA Officer, Water Quality Planning Bureau Date

---

Darrin Kron, Monitoring and Assessment Supervisor, Water Quality Planning Bureau Date

## REVISION HISTORY

Revision No.	Date	Modified By	Sections Modified	Description of Changes
1	10/21/2004	DF, MB, BB	4,5,7, Appendices, Attachment 1	Quality Assurance procedures were added. The taxa list (Appendix 3) was updated The site visit field forms (Attachment 1) were updated.
2	09/08/2006	DF, MB, MS, BB	1,2,5,6,7, Appendices	The Montana Observed/Expected (a.k.a. RIVPACS) Model and Multimetric Indices (MMIs) were added. The Appendices were relabeled by letter instead of number. The taxa list (Appendix A) was updated. The Glossary of metrics was updated for the MMIs. Appendix D was added to the SOP.
3	03/15/2012	DF, MM, KM, BB	All	The scope of the SOP was refined to include which river segments were unwadeable and beyond the scope of the sampling protocols. The justification for the index sampling period was slightly modified. The coldwater O/E model and Bray Curtis (BC) Index were added. The MMIs were removed. The rarefaction instructions to prepare the data for the MMIs in Appendix C were removed and replaced with example outputs from both O/E models. The code to calculate the coldwater O/E model and instructions on how to use it was added as Appendix D. The laboratory subsampling procedures were updated to require that the labs submit their SOPs, and use unbiased subsampling procedures.

## TABLE OF CONTENTS

Acronyms .....	iii
1.1 Scope and Applicability .....	1
1.1 General Considerations.....	1
1.1.1 Index Period .....	1
1.1.2 Site Selection.....	2
1.1.3 Sample Collection Methods .....	2
1.1.4 Checklist of Recommended Field Supplies .....	2
2.0 Sample Collection Procedures .....	3
2.1 Precautions .....	3
2.2 Sample Labeling .....	3
2.2.1 Sample Containers and Preservation.....	3
2.2.2 Sampling Quality Control .....	4
2.2.2.1 Field Replicates .....	4
2.2.3 Benthic macroinvertebrate sampling methods and protocols .....	4
2.2.3.1 Environmental Monitoring and Assessment Protocol (EMAP) .....	4
2.3 Supporting Field Data.....	6
3.0 Subsampling & Sorting.....	6
3.1 Pre-subsampling methods .....	7
3.1.1 Picking detritus.....	7
3.1.1.1 Elutriation.....	7
3.2 Sub-sampling and Sorting .....	7
3.2.1 Sorting Quality Control (QC) .....	8
3.2.1.1 Relative Percent Difference in Enumeration (RPDE) .....	8
4.0 Taxonomy.....	8
4.1 Taxonomic Identification .....	8
4.2 Quality Control for Taxonomy .....	9
4.2.1 Accuracy.....	9
4.2.2 Precision.....	9
4.2.2.1 Percent Taxonomic Disagreement (PTD) .....	10
4.2.3 Corrections to database.....	11
5.0 Data Analysis and Interpretation .....	11
5.1 Transfer sample data .....	11
5.2 Reduce sample data.....	11

5.2.1 Operational Taxonomic Units (OTUs) .....	11
5.2.2 Excluded Taxa.....	12
5.2.3 Re-Sampling .....	12
5.3 Identify site classes .....	12
5.3.1 Predictor Variables.....	12
5.4 Calculate Macroinvertebrate Indicator Results .....	13
5.4.1 Calculate the Montana-specific Hilsenhoff Biotic Index (HBI) .....	13
5.4.2 Calculate Observed/Expected.....	13
5.5 Validating Model Outputs.....	14
5.6 Interpret the Observed/Expected Index.....	14
5.6.1 Bray-Curtis Index.....	14
5.6.1.1 How the BC Index Can Be Helpful .....	15
5.7 Reporting Results .....	16
6.0 Literature Cited .....	16
Appendix A - Montana Macroinvertebrate Taxa List.....	A-1
Appendix B - An Example Taxa List Template .....	B-1
Appendix C - Example Output for the Observed/Expected Models .....	C-1
Appendix D - Montana Department of Environmental Quality Benthic Macroinvertebrate Observed/Expected Manual .....	D-1
Attachment 1 – Site Visit Form .....	Att. 1-1
Attachment 2 – Photograph Locations and Descriptions of Reach and/Or Sites .....	Att. 2-1
Attachment 3 – Site Sketch Form .....	Att. 3-1

## LIST OF TABLES

Table 1-1. Non-wadeable river segments within the state of Montana.....	1
Table 5-1. Predictive variables associated with each sampling station.....	13
Table 5-4. Biological test thresholds based on O/E results. ....	14
Table 5-6. A hypothetical example that demonstrates the O/E calculation, the absolute difference for the hypothetical observed (O) and expected (P) taxa, and sum of O and P for 5 taxa (adopted from Van Sickle 2008). ....	15
Table C-1. EDAS output table example. ....	C-2

## LIST OF FIGURES

Figure 2-1. Sampling locations for the reachwide benthic macroinvertebrate sample within the sampling reach (adopted from Peck et al. 2006). ....	6
--	---

## ACRONYMS

<b>Acronym</b>	<b>Definition</b>
CW	Cold Water (fisheries)
DEQ	Department of Environmental Quality (Montana)
EDD	Electronic Data Deliverable
EMAP	Environmental Monitoring and Assessment Protocol
EPA	Environmental Protection Agency (US)
ETOH	95% Ethanol
FFP	Frost-Free Period Point
GIS	Geographic Information System
GPS	Global Positioning System
HBI	Hilsenhoff Biotic Index
ITIS	Integrated Taxonomic Information System
MQO	Measurement Quality Objective
NRCS	National Resources Conservation Service
O/E	Observed/Expected
OTU	Operational Taxonomic Units
PTD	Percent Taxonomic Disagreement
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
RPD	Relative Percent Difference
RPDE	Relative Percent Difference in Enumeration
SAP	Sampling and Analysis Plan
SOP	Standard Operating Procedure
TMDL	Total Maximum Daily Load
USGS	United States Geological Survey
USU	Utah State University
WQX	EPA's Water Quality Exchange System



## 1.1 SCOPE AND APPLICABILITY

The methods described herein are used for collecting and analyzing macroinvertebrate data from wadeable streams (1st order or higher) by or for the Montana Department of Environmental Quality (DEQ). Benthic macroinvertebrate data collected on large rivers is beyond the scope of this method.

**Table 1-1** lists those large river segments.

**Table 1-1. Non-wadeable river segments within the state of Montana**

River Name	Segment Description
Big Horn River	Yellowtail Dam to mouth
Clark Fork River	Bitterroot River to state-line
Flathead River	Origin to mouth
Kootenai River	Libby Dam to state-line
Madison River	Ennis Lake to mouth
Missouri River	Origin to state-line
South Fork Flathead River	Hungry Horse Dam to mouth
Yellowstone River	State-line to state-line

### 1.1 GENERAL CONSIDERATIONS

Prior to conducting a benthic macroinvertebrate survey, determine the specific data quality objectives (DQOs) and the information sought. DQOs can be qualitative or quantitative statements developed to specify the quality of data needed to support specific decisions and conclusions about the information sought. The DQOs should be documented in a Sampling and Analysis Plan or Quality Assurance Project Plan.

#### 1.1.1 Index Period

The index period is the period of time that samples should be collected to minimize seasonal variation.

- The index period for Montana is generally the summer (June 21 to October 1), following runoff loeb (Richards D.C., 1996; Loeb and Spacie, 1994). Eastern Montana streams may be sampled May 1 to October 1.
- Sampling may occur outside of the index period, however this may reduce certainty that the data are comparable to historical data, reference conditions or indices (see discussion below).

The index period noted above was the temporal basis for reference conditions used in the development of the Observed/Expected (O/E) model. However, even within this index period, extreme runoff or summer drought conditions could affect these periods; professional judgment must be used when considering sampling schedules. Sampling macroinvertebrates during high flows, besides being potentially unsafe, is likely to increase sampling variance due to the difficulties associated with sampling these waters using the methods described herein.

When monitoring for trends at a particular site, minimize seasonal variation by sampling as close as possible to the same date each year.

### 1.1.2 Site Selection

Sampling requirements including the total number of sampling sites their location in the study area, and the spatial and temporal independence are outlined in each DEQ pollutant-specific assessment method. Some of the assessment methods do not require any biological samples to be collected for assessment decisions. Refer to the assessment method when determining the sampling design in order to achieve an appropriate assessment of the stream. Always consider potential constraints to collecting representative samples such as site access when selecting sites. Document any necessary changes due to site access restrictions.

The study design should specify requisite site specifications for the study. For example, many study designs use the riffle environment as the specific point of comparison between streams. A *rationale* described in the Sampling and Analysis Plan (SAP) for determining representative riffles for the anticipated stream types would be a *site selection specification*.

Although riffle areas with cobble substrates are generally the most diverse and productive habitat type, these may not be representative of the predominant type of habitat in the stream. If so, the study design should describe the variety of habitats, the rationale for representative sampling, and the various appropriate sampling techniques for the study.

There is ample evidence that the presence of lakes, impoundments, bridges, road crossings, and bedrock or large-boulder dominated substrates affect benthic invertebrate community composition; therefore, sampling sites should be located as far from these as is practical, *unless the objective of the study is to measure their localized influence*.

Always sample sufficiently upstream from the confluence of major tributaries or receiving waterbodies to minimize their influence on study sites. Otherwise, consider the mixing zone of the two waterbodies and select sites downstream of the confluence that are well mixed, and represent both the biological community and water quality conditions.

### 1.1.3 Sample Collection Methods

DEQ employs a semi-quantitative Environmental Monitoring and Assessment Protocol (EMAP) reach wide sampling technique to collect samples in a known area allowing the estimation of population density, in addition to diversity and abundance.

### 1.1.4 Checklist of Recommended Field Supplies

- Field Meter (pH, Spec. Conductance, DO)
- Macroinvertebrate sample bottles (1 liter wide mouth poly)
- Lab markers, external labels, clear tape, Parafilm-M
- Internal labels (paper)
- Sampler (D-frame net)
- Field notebook
- Field data sheets (Site Visit Form, Photo Documentation Form, Site Map Form)
- Camera
- GPS unit
- 95% Ethanol preservative
- 500 um sieve for washing and sorting out large objects
- Pencils



- ❑ Clipboard
- ❑ De-Ionized Water
- ❑ Hip boots or waders
- ❑ Maps
- ❑ Dishwashing brush
- ❑ Hard or soft-sided Coolers to store samples
- ❑ Small soft-sided coolers to transport samples

## 2.0 SAMPLE COLLECTION PROCEDURES

The following section discusses sample collection procedures used to sample macroinvertebrates in streams and wadeable rivers not listed in **Table 1-1**.

### 2.1 PRECAUTIONS

Care must be taken when looking for suitable sampling sites not to disturb the substrate or habitat in areas where samples might be collected. A sample collected where the substrate has been disturbed by walking may not be representative of the community.

Be sure to transfer the entire sample to the bottles. Only fill each bottle one-half to three-quarters full with sample to leave room for preservative. Capture all bugs by thoroughly cleaning off all large rocks and sticks. Excluding large rocks prevents grinding damage to bugs during transport. Discard cleaned rocks and sticks.

Pack and store collection bottles carefully and securely in order to protect the sample jars and prevent the loss of contents during transport or shipment.

### 2.2 SAMPLE LABELING

Accurate labeling of sample bottles is important to preserve critical information. Attach a preprinted label to the jar clearly identifying the sample (location, date, number of bottles, method of collection and sample number). Use pre-printed, fill-in-the-blank labels to help ensure that the sample information is complete.

#### 2.2.1 Sample Containers and Preservation

Collection jars: 1-liter wide-mouth polyethylene bottle. (Note: It may be necessary to use more than one 1-liter polyethylene bottle.)

Use 95% ethanol (ETOH) to preserve the macroinvertebrate samples. . Please remember that ETOH is flammable! The drawback of using ethanol is that the fluid composition of the sample needs to be dominated by ethanol to properly preserve the sample. Because of this, try to keep as much water out of the sample jar as possible, and fill the sample bottle only one half with sample and the remainder with ETOH to fill the bottle completely. Split the sample using an additional bottle(s) if necessary, and be sure to correctly label the bottles using a numeric sequence (i.e. 1 of 3, 2 of 3, 3 of 3).

## 2.2.2 Sampling Quality Control

### 2.2.2.1 Field Replicates

Field replicates are collected to measure total method error and should always be included in the sampling design at a predetermined frequency (usually 10%).

Field replicates are two or more samples, collected side-by-side or consecutively, at the sampling site. Replicate samples should be taken at places that are very similar in terms of depth, substrate, composition, and slope. **Always collect samples from downstream to upstream to avoid sample contamination.** Replicates are processed in the laboratory as discrete samples. They are compared directly to the original samples. Any difference represents total method error (e.g., heterogeneity of site, reproducibility of sampling technique, sorting error, and identification error).

Relative Percent Difference (RPD) is used to evaluate results differences between two replicate samples.

$$RPD = \frac{result1 - result2}{\frac{result1 + result2}{2}} \times 100$$

The results of replicated samples (i.e. O/E model results) should be < 20% RPD, however, each project must state its required replicate precision criteria based on project DQO's.

## 2.2.3 Benthic macroinvertebrate sampling methods and protocols

A variety of other sampling methods are available from EPA, USGS, and other organizations. It is beyond the scope of this SOP to discuss all available sampling methods, or the applicability of sampling design for determining the health of benthic macroinvertebrate communities using the Data Analysis and Interpretation tools presented in Section 5 of this SOP.

When sampling methods other than those listed in this SOP are used for water quality assessments by or for DEQ, the SOP(s) for the alternate methods must be attached or cited in the project's Quality Assurance Project Plan (QAPP) or Sampling and Analysis Plan (SAP). Any known (documented) or assumed comparability to the methods in this SOP should be cited or documented in these Quality Assurance (QA) documents.

### 2.2.3.1 Environmental Monitoring and Assessment Protocol (EMAP)

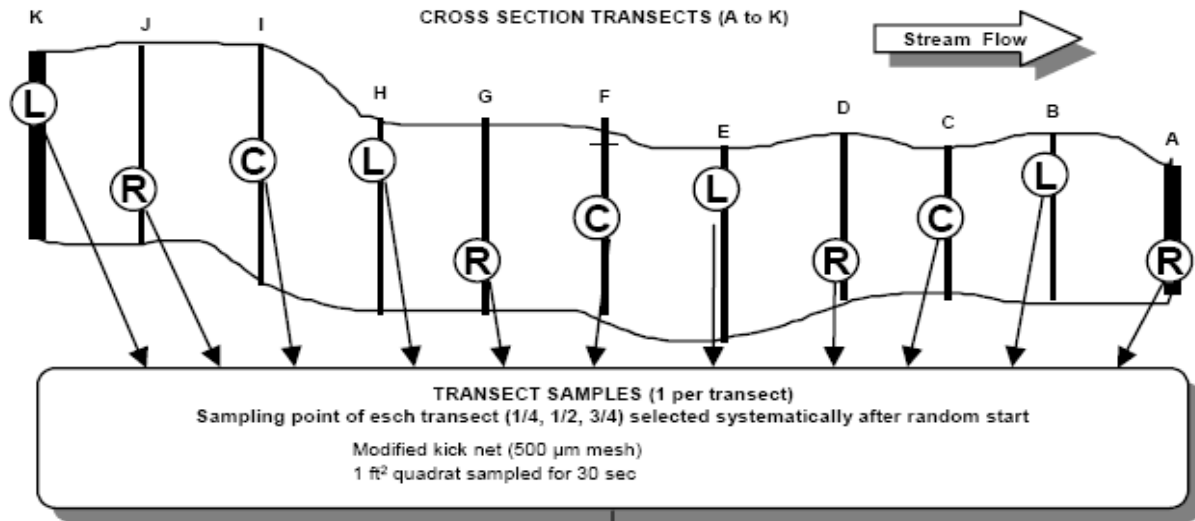
Currently, the DEQ Reference Project uses EMAP sampling protocols to identify and assess reference sites (Peck, 2006). These data were included in the compiled reference data collection used for the O/E models. Therefore, EMAP methods are considered valid sampling techniques for use with the tools presented in Section 5 of this SOP.

#### 2.2.3.1.1 Reachwide Sample

The EMAP sampling protocol requires that a kick sample be collected at each of 11 transects (A through K), sampling from downstream to upstream along the reach; these kick samples are composited to obtain a sample that is representative of the entire reach. Collect each kick sample at each transect (A through K) at the assigned sampling point (left, center, or Right). Position a 500um D-frame kick net within a representative portion of the designated channel location and hold the net vertically upright with the base of the frame in contact with the substrate and the open portion of the net facing into the flow. See **Figure 2-1** for a conceptual diagram of the EMAP reach, and where to collect each macroinvertebrate

kick sample. Note: if a sampling location that is unsafe to wade to is encountered, try to find a safer location either up or downstream approximately 1 meter of that location. Switch to another position (i.e., R,L,C) on the same transect if moving up or downstream to collect the sample isn't possible. Return to the initial sampling pattern at the next transect after the kick is collected. Here are some important points to consider when collecting macroinvertebrate samples:

- *Thoroughly* inspect and clean (with stream water) the net after each sampling event to ensure that all organisms have been removed to prevent contamination between sites. Consider the following procedures to ensure that all organisms are removed from the net and placed in the jars:
  - Rinse large rocks, sticks, and other debris into the net and thoroughly inspect them prior to discarding.
  - Use a small plastic spoon to scoop the sample into the sample jar.
  - Inspect the entire inner surface of the net and use clean forceps to carefully remove any organisms clinging to the net and place them into the sample jar.
  - Dump the contents of the net into a 500 um sieve, rinse stream water through the sieve to clean the sample of fine sediment and transfer the sample into the jar.
  - Elutriate each kick sample to separate the organic and inorganic portions before you transfer it into sample jars; this should help protect the soft-bodied macroinvertebrates from damage caused by inorganic material in the sample jar during sample transport and storage. Elutriate by submerging portions of the sample in a 5 gallon bucket containing some water, vigorously swirl the sample in the water to separate the organic and inorganic portions of the sample, then decant the floating material into the 500 micron sieve and transfer from the sieve into the sample jar. Repeat this process several times until almost the entire organic portion of the sample has been removed. Then transfer the inorganic portion to different sample jars and submit these along with the organic portions to the analytical laboratory.
  - When sampling a pool with little to no flow, follow the same procedure except use your hands or feet to push the sample into the net. If encountering a slackwater area choked with vegetation, sweep the net through the vegetation for 30 seconds over the 0.09m<sup>2</sup> sampling location.
  - Add enough ETOH to submerge the debris in each sample jar as soon as the material from each kick is added to the jar to prevent damage or loss of some taxa by opportunistic predators.
  - Spray organisms clinging to the net with a dilute (10%) ammonia or (95%) EtOH solution to detach them from the net. Partially immerse the net in the stream to concentrate the detached organisms at the base of the net and/or use forceps to transfer organisms directly into the jar.
  - To collect replicate samples using the reachwide approach, simply collect replicate kicks at each transect at another location on the same transect. For example, if the regular sample is collected following the Right, Left, Center pattern, the replicate sample could either be collected following the Left, Center, Right or Center, Right, Left pattern.
  - Always check all of the sampling gear between sampling events to make certain all organisms were removed. This prevents sample contamination between sites and prevents biased biological assessments



**Figure 2-1. Sampling locations for the reachwide benthic macroinvertebrate sample within the sampling reach (adopted from Peck et al. 2006).**

## 2.3 SUPPORTING FIELD DATA

Macroinvertebrate sample sites are described by metadata recorded at the time of sampling as well as any field observations taken at the time of sampling. Use the Site Visit Form (**Attachment 1**) to record metadata (GPS lat/long in decimal degrees, NAD 83) for the sampling site and record sample identifiers.

Digital photographs should be taken of the sampling site (upstream, downstream, and the site itself) and of the substrate sampled. Record metadata for the photos on the Photo Point Form (**Attachment 2**). As a back-up to digital photos, a sketch of the sampling site is recommended, along with an indication where the sample was taken at the site. A Site Sketch Form is included in this SOP (**Attachment 3**).

## 3.0 SUBSAMPLING & SORTING

Subsampling is using a representative portion of the field-collected sample for analysis. Sorting is the physical separation, counting, and grouping of organisms from the debris collected in the sample. All macroinvertebrate samples collected by and for DEQ should follow an unbiased subsampling procedure to achieve a target subsample count of  $500 \pm 10\%$  organisms.

It is important to understand the objectives of the project when selecting subsampling techniques. If the intention of the study is to measure population density (i.e., quantitative sampling), sort the entire sample. Full sample sorts can be used for samples collected by semi-quantitative collection methods if the study requires that all organisms are identified. However, full sample sorts are more time consuming for the taxonomist, adding additional expense to each sample.

## **3.1 PRE-SUBSAMPLING METHODS**

To get a relatively even distribution of organisms prior to subsampling or sorting, remove large debris from the sample. There are two approaches to this sample clean up. The debris can be removed from the sample (picking) or the sample can be removed from the debris (elutriation).

### **3.1.1 Picking detritus**

Picking the debris from the sample involves rinsing out fine material, removing large rocks, woody debris and setting them aside. Picking and rinsing should be performed in a 500 um sieve with a gentle stream of water to prevent splattering. Large rocks and sticks must be thoroughly scraped, rinsed and examined prior to setting them aside. The remaining sample is placed in an appropriate sized Caton tray for subsampling and sorting.

#### **3.1.1.1 Elutriation**

Elutriation is basically an extraction of the organisms from the substrate remaining in the sample. This technique works best when the substrate is inorganic material such as sand, pebbles, and rocks. Samples with a large amount of organic material are not amenable to this extraction. Field elutriations can be performed to reduce the number of jars a field crew transports to the macroinvertebrate laboratory or to separate soft-bodied macroinvertebrates from the coarse material in a sample.

Dump the sample into a 5-gallon bucket and fill the tray with water. Swish the sample around in the tray to liberate organic material and bugs from the substrate. The organic material and organisms should float in the water. Pour the water and all floating material and organisms into a 500um sieve. Add more water and repeat the process until no more organic material remains in the tray. Note: A 10% magnesium sulfate solution can be used instead of just water to increase the efficiency of the elutriation.

Once the sample is separated, use a magnifying lamp to check the inorganic portion of the sample for snails, clams, stone cased organisms, or any other organism too heavy to have floated. Have a fellow technician take a second look to verify that all organisms were removed. Any organisms found in the inorganic fraction must be added to the organisms in the sieve so they may be included in the sort. If a large number of organisms are found remaining in the inorganic fraction, the elutriation technique is not appropriate and the sample fraction in the sieve must be recombined with the inorganic fraction and sorted as a whole.

## **3.2 SUB-SAMPLING AND SORTING**

The techniques for subsampling and sorting vary slightly between the contractors used by DEQ. Rather than describing (in detail) each contractor's specific method, DEQ requires that each contractor documents their procedure in a SOP, which includes the following components:

- Magnifying glass or microscope used for subsample and sort. (Specify power)
- Standardized sorting bench sheets
- Random selection of cells
- Last cell chosen
- Quality Controls to determine sorting efficiency or precision

SOPs must be submitted to DEQ as part of the contractor qualification process.

### 3.2.1 Sorting Quality Control (QC)

#### 3.2.1.1 Relative Percent Difference in Enumeration (RPDE)

DEQ evaluates the precision of sample counts taken for the subsamples by calculating the relative percent difference of enumeration (RPDE). The RPDE is the standard formula used to evaluate how well a laboratory performs by comparing a sample result versus the result generated by a second laboratory evaluating the same sample (American Public Health Association, et al., 1998). Calculate the RPDE by comparing results from two independent laboratories or taxonomists using the formula:

$$RPDE = \frac{| \text{Sample Result}_1 - \text{Sample Result}_2 |}{(\text{Sample Result}_1 + \text{Sample Result}_2)/2}$$

where *sample result*<sub>1</sub> is the number of organisms in a sample counted by the first laboratory, and *duplicate result*<sub>2</sub> is the recount by the second taxonomist or laboratory (adapted from Standard Methods 20<sup>th</sup> Ed. - 1020 B (American Public Health Association, et al., 1998)). The purpose of this calculation is to highlight those samples where counts differ substantially and to focus attention on obtaining better consistency.

The measurement quality objective (MQO) for RPDE is 10%. Values for individual sample comparisons exceeding the MQO (> 10%) require an examination to determine causes of the difference. Upon completion of this investigation, DEQ will communicate when and if the RPDE exceeds 10% to the taxonomic contractor and QC taxonomist(s). This will occur as a conference call and/or e-mail correspondence with the contractor(s).

## 4.0 TAXONOMY

### 4.1 TAXONOMIC IDENTIFICATION

Consistency in macroinvertebrate taxonomy is critical for final site assessments and interpretation. It is the responsibility of the taxonomist to communicate the composition of samples to those personnel performing data entry, and to be able to defend taxonomic results to the ultimate data analysts. DEQ encourages taxonomists to identify specimens to the *lowest practical taxonomic level*. However, DEQ also understands that specimen condition (damage, early instar, poor slide mounts) may force the taxonomist into an unavoidable situation of having to leave identification at more coarse levels (e. g., family instead of genus). With the limitation of organism condition in mind, DEQ will judge consistency of data based on historical hierarchical levels that have been achieved, resulting in the Montana Master Taxa list (**Appendix A**).

*Count vs. non-count specimens.* In general, all specimens in the subsample should be identified and counted by the taxonomist, including pupae. However, there are several exceptions known as non-counts, which include:

- Empty mollusk shells (lacking soft tissue)
- Worm fragments lacking the anterior (head) end
- Body parts NOT INCLUDING at least the head and thorax
- Surface dwellers such as Collembola, Gerridae, and Veliidae

- Incidentally-collected terrestrial specimens

Some hierarchical inconsistency in taxonomic data is dealt with during coding and analysis (see **Section 5.2**). During development of the Observed/Expected (O/E) models (**Section 5.5**), operational taxonomic units (OTUs) are specified to reduce the influence of ambiguous taxonomy. The complete Montana taxa list with OTUs and other attributes is available from DEQ as a table within the DEQ macroinvertebrate database and as **Appendix A**. A template for tracking identification and enumerations is provided as **Appendix B**.

## 4.2 QUALITY CONTROL FOR TAXONOMY

All QC activities, including specification and response to corrective actions, should be completed *prior* to initiation of data reduction (metric calculation and indicator analysis). The aspects relevant to taxonomic data quality are: accuracy and precision (Stribling, et al., 2003).

### 4.2.1 Accuracy

Accuracy is defined as the nearness of a measurement to a specified analytical truth. For taxonomy, there are several options that can be used as the analytical truth, including:

- Museum-based type material (holotype, paratype, or other; the original specimen or series of specimens on which the original description of a species is based),
- The most current and accepted taxonomic literature (dichotomous keys with illustrated, diagnostic morphological characters), or
- A reference collection, verified by an independent taxonomic specialist.

Type material is usually found in museums, and direct interaction with museum curators is required to use it for comparisons. Thus, it is often not feasible, or even necessary, for routine monitoring and assessment programs or projects (Stribling et al. 2003). Contractors must provide at least one reference collection per year that can be verified by a qualified, independent macroinvertebrate taxonomist. Depending on the magnitude of an individual project, a project-specific reference collection may be requested by DEQ; what the reference collection is intended to represent will be determined by DEQ. Whether the reference collection is annual or project-based, specimens included in it are intended to represent the original taxonomist's concept of the morphological basis of each taxon. Verification of the reference collection, by DEQ staff or independent laboratories or taxonomists, documents the morphological and nomenclatural understanding of the original taxonomist. This will provide DEQ with some assurance of the contract taxonomist's use of up-to-date nomenclature (with the Integrated Taxonomic Information System [ITIS] as the standard), as well as verifying their understanding of the morphological bases of taxonomic groupings.

### 4.2.2 Precision

Precision is defined as nearness of different measures of the same property. Simply stated, it is a measure of consistency and repeatability; for production taxonomy (Stribling et al. 2003), quantification of taxonomic precision provides the ability to document and communicate the quality of taxonomic data within a project, or within a laboratory over time. Taxonomic precision is evaluated by direct comparison of the results (list of taxa and number of individuals of each) of a randomly-selected sample that is processed by 2 taxonomists or laboratories. Once a sample is identified by the primary taxonomist (T1), it is sent to an outside, independent taxonomist (T2), who re-identifies the entire (sub)sample. The sample should be sent to T2 blind, that is, without any identification labels associated

with the material. Each taxonomist will be provided with a hierarchical target list (standard level of effort for each taxon) and “counting rules” (need to be included as attachments). The total set of randomly-selected subsamples is a subset of the total number of samples collected for:

- A single project,
- Multiple projects within a sampling year, or
- >1 projects over several sampling years.

If multiple taxonomists within a project or laboratory have each identified enough samples, the subset can be stratified among the taxonomists so that specific checks can be made on individual taxonomists. Generally, and as a rule-of-thumb, the subset of samples can be approximately 10% of the total sample lot. For example, if a project has a total of 30 samples, there should be 3 samples randomly selected that will be re-identified by an outside/independent taxonomist. It is recommended that there be at least 3 samples selected and re-identified for sample lots of  $\leq 30$ . For large programs or projects, say  $> 500$  samples, it may be unnecessary to have 50 samples re-identified, but that decision will be made by DEQ.

Using the final results by the taxonomists, precision is quantified for both specimen enumeration and taxonomic identifications for each of the QC or re-id samples.

#### **4.2.2.1 Percent Taxonomic Disagreement (PTD)**

Use the formula below for determining Percent Taxonomic Disagreement (PTD):

$$PTD = \left[ 1 - \left( \frac{comp_{pos}}{N} \right) \right] \times 100$$

Where:  $comp_{pos}$  is the number of agreements, and N is the total number of organisms in the larger of the two counts (Stribling et al. 2003).

Three types of taxonomic errors are illuminated by this comparison process: 1) straight disagreements, 2) hierarchical differences, and 3) missing specimens. Effort will be made during the side by side comparison of individual samples to specify the types of differences that are observed, and which types predominate in each sample. The lower the PTD value, the greater is the overall taxonomic precision, indicating relative consistency in sample treatment. For the 2006 field season and beyond, an MQO for  $PTD \leq 15\%$  is established. Values for individual sample comparisons exceeding the MQO ( $> 15\%$ ) will prompt a more detailed examination of those individual samples to determine the cause or causes of the difference in counts. PTD results as well as parsing of the difference types will be used by DEQ to develop corrective actions. Corrective actions related to taxonomic disagreement will be forwarded in writing to the taxonomy laboratory responsible for the primary identifications. The corrective actions will require that the contractor(s) correct the entire sample lot, or only the 10% subset that were the QC samples.

##### **4.2.2.1.1 Bias**

Bias is defined as statistical or method error caused by systematically favoring some outcomes over others and can be characterized as the degree of departure from a true value. Taxonomic bias exists if there are consistent misinterpretations of dichotomous keys or morphological features, poor processing of samples (e.g., poor slide-mounting techniques), or inadequate optical equipment. There is no specific



MQO established for bias but it may be a factor in the review of accuracy, which is described as low bias (directional error) and high precision.

### **4.2.3 Corrections to database**

Any corrections to DEQ databases will be handled by the DEQ QA Officer, who will work directly with the DEQ MT-eWQX Coordinator and the DEQ macroinvertebrate specialist, who are responsible for the MT-eWQX and EDAS databases, respectively.

## **5.0 DATA ANALYSIS AND INTERPRETATION**

Data analysis and assessment of biological integrity should be performed following the six steps listed here and described in **Sections 5.1 – 5.6**.

1. Transfer sample data from laboratory output to a reliable database
2. Reduce sample data
  - a) condense to proper OTUs
  - b) exclude taxa marked
  - c) artificially reduce large samples
3. Compile site information (predictor variables) and identify site classes
4. Calculate the Montana-specific version of the Hilsenhoff Biotic Index (HBI)
5. Calculate a Observed/Expected(O/E) Model Result
6. Assess site biological integrity through interpretation of index results

Most of these steps are considerably simplified by using EDAS (Tetra Tech and Montana Department of Environmental Quality, 2006), which can be obtained from DEQ along with its User's Manual (Tetra Tech, 2006). Compiling site information (step 3), calculating the O/E model (step 4), and the interpreting the results (step 5) are accomplished using other technologies and procedures described below.

### **5.1 TRANSFER SAMPLE DATA**

Benthic macroinvertebrate taxa lists with individual counts should be transferred into EDAS (Tetra Tech 2006a) or another database in which taxa can be associated with the taxonomic attributes used in calculating metrics and predictive models. EDAS includes a function that automates import of taxonomic data after the data are properly formatted in a spreadsheet. With any data transfer procedure, the accuracy of the transfer should be checked to assure 100% accuracy.

### **5.2 REDUCE SAMPLE DATA**

#### **5.2.1 Operational Taxonomic Units (OTUs)**

For each taxon in a sample taxa list, the OTU must be determined. OTUs are identified in the Benthic Master Taxa table of EDAS. This is the level of identification at which taxonomic counts are condensed for calculations, for O/E (predictive model) calculations<sup>1</sup>. For instance, midges (Diptera: Chironomidae) have OTUs at the subfamily level because this is the level at which all agencies in Montana reliably

---

<sup>1</sup> Note: OTUs are not considered taxonomic target levels. Future DEQ macroinvertebrate tools may require different taxonomic requirements. Therefore, macroinvertebrate taxonomy should include identifications at the lowest practical taxonomic level, as stated in **Section 4-1**.

identify them. Numbers of individuals in multiple taxa identified at a more detailed level are summed within a sample if the OTU is at a more coarse level. OTUs for metric calculations include family and order level identifications. OTUs for predictive models exclude family and more coarse identifications for some taxa groups.

### 5.2.2 Excluded Taxa

In calculations of metrics that are counts of taxa (richness metrics), only those taxa that are unique within each sample should be counted. Ambiguous taxa are excluded from taxa counts. Such taxa include family level identifications within a sample that also includes genus level identifications from the same family. Likewise, order or coarser identifications may be ambiguously unique and should be excluded. Such exclusions should be made after converting taxa and individual counts at the appropriate OTU level. In EDAS, 'Excluded Taxa' are automatically marked as they are imported. Excluded taxa can also be marked manually. Within each sample, every OTU taxon that defines a group that is also represented by another OTU taxon identified at a more detailed taxonomic level should be marked for exclusion from metrics that count unique taxa.

### 5.2.3 Re-Sampling

Random re-sampling is the process by which samples larger than the target subsample size are reduced to the exact target subsample size. Random re-sampling generates a list of taxa and their abundances that would occur if the original sample had identified only the target number of individuals. Such a list is necessary for the predictive model, in which presence and absence of taxa for a standard sample size is required. It contrasts from rarefaction, which gives a probability for every taxon, but does not reduce the list of individuals or taxa.

In EDAS, random re-sampling is accomplished by assigning a random number to each individual in an oversized sample, ordering the list by the random numbers, and selecting the top 300 individuals to represent the new sample. By this method, some individuals and perhaps some uncommon taxa are eliminated from the list. Because it uses random number generators in determining the final list of taxa, random re-sampling can result in different taxa lists every time it is executed.

## 5.3 IDENTIFY SITE CLASSES

### 5.3.1 Predictor Variables

Site classes for the predictive model (O/E) are not distinct, but are calculated as probabilities within the model. Both indices require predictor variables for site classification (**Table 5-1**). These variables can be derived by associating geographically referenced data with the coordinates and delineated watersheds of each site. While it is possible to derive predictor values through map analysis, watershed delineation and spatial analyses are best performed with a Geographic Information System (GIS), such as ArcGIS 9.x or 10.x. The GIS analysis is outlined in Chinnayakanahalli et al. (2006). The basic GIS analytical procedures include the *association* of Station IDs with waterbody names with *geographic* coordinates, loading the spatially referenced stations into the GIS program, verifying station locations, delineating catchments using Digital Elevation Models (DEMs), and intersecting station points and catchments with the appropriate predictor data layers.

**Table 5-1. Predictive variables associated with each sampling station.**

Variable (EDAS name)	Description	Model	Data Source
Latitude (GIS_LAT)	Latitude (decimal), confirmed during delineation	O/E 2005; 2011	GIS or map
Longitude(GIS_LONG)	Longitude (decimal), confirmed during delineation	O/E 2005	GIS or map
Area (SQ_KM)	Catchment area (km <sup>2</sup> )	O/E 2005; 2011	GIS or map
Basin (Columbia River Basin)	Within the Columbia River Basin (1 = yes, 0 = no)	O/E 2005	GIS or map
Air Temperature (TMAX_PT)	Annual mean of daily maximum temperatures at the site between 1971-2000 (C x 10, e.g., 14.4 degrees C = 144)	O/E 2005	PRISM database <sup>a</sup>
Standard Deviation of the elevation within the watershed (ELEVsd_WS)	The standard deviation of the digital elevation model data within the watershed	O/E 2011	GIS
Air Temperature (TMAX_PT_2011)	Annual mean of daily maximum temperatures at the site between 1961-1990 (C x 10, e.g., 14.4 degrees C = 144)	O/E 2011	PRISM database <sup>a</sup>
Julian Day (DAY)	Numeric Day of the year (1-365)	O/E 2011	Calendar
Mountains (Mtn1)	Classified as a coldwater stream (1 = yes, 0 = no)	O/E 2011	ARM <sup>b</sup>

<sup>a</sup>PRISM: Parameter-elevation Regressions on Independent Slopes Model (Daly, et al., 2004), URL: <http://www.ocs.orst.edu/prism/>

<sup>b</sup>Administrative Rules of Montana

## 5.4 CALCULATE MACROINVERTEBRATE INDICATOR RESULTS

### 5.4.1 Calculate the Montana-specific Hilsenhoff Biotic Index (HBI)

The HBI metric represents the relative sensitivity of the sample to nutrient perturbation. It is calculated as the average tolerance value of all individuals in the sample (excluding those without tolerance values). The range of the Biotic Index values is 0-10, 0 being sensitive and 10 being pollution tolerant. The biotic index values are listed in the "TolVal" column in the Master Taxa List in **Appendix A**. The HBI results are calculated using a weighted average:

$$HBI = \sum (ni \times ai) / N$$

Where:

"n" is the number of individuals in a taxon, "i";

"a" is the index value assigned to that taxon;

N is the number of individuals in the sample with a Biotic Index value.

### 5.4.2 Calculate Observed/Expected

The O/E models are calculated as the ratio of the number of taxa observed (O) in the collected sample to the number expected (E) in that site type (Feldman and Jessup, 2012). The O/E ratio is calculated from sample taxa lists and predictive variables that are either uploaded to a website that runs the model or via the coldwater O/E model built using the R statistical language. The website is maintained by the Utah

State University's (USU) Western Center for Monitoring and Assessment of Freshwater Ecosystems (<http://www.cnr.usu.edu/wmc/>). The R code for the coldwater O/E model is detailed in **Appendix D**. Details for generating uploadable files from EDAS and running either of the models are available in the EDAS User's Manual (Tetra Tech 2010) and on the website. An explanation of the theory and processes of the model is included in Feldman and Jessup (2012).

## 5.5 VALIDATING MODEL OUTPUTS

Output from either O/E model provides information to determine the validity of the values (see **Appendix C**). Validity is dependent on having a minimum sample size after data reduction and, for the O/E, on model recognition of the combination of predictor variables. Sample sizes for the O/E may be different for the same sample because the O/E discounts some coarse taxa identifications (see **Section 5.2**). To determine whether the O/E could recognize the combination of predictor variables, refer to the website results labeled 'outlier'. An outlier O/E test result indicates a site that is outside of the experience of the model and the O/E value may be unreliable.

## 5.6 INTERPRET THE OBSERVED/EXPECTED INDEX

The interpretation of benthic macroinvertebrate data is a component within the broader Water Quality Assessment Method (Montana Department of Environmental Quality, 2011). Depending on the availability and rigor of other biological data, benthic macroinvertebrate data *may not*, be used exclusively for aquatic life and fisheries beneficial use support determinations. With this in mind, this section of the SOP uses the terms: "pass", or "fail" in relation to the biological model test in the context of the biological component within an established water quality assessment method (**Appendix D**).

The O/E model is used with established assessment methods to determine the integrity of the macroinvertebrate community. The O/E models are used to test how far a given macroinvertebrate community deviates from those sampled at reference sites.

O/E values in **Table 5-4** indicate when a macroinvertebrate sample either passes or fails the biological model test. Note: some DEQ stressor-specific assessment methods apply different O/E thresholds in a different manner than those presented in **Table 5-4** (see Suplee and Sada de Suplee, 2011). Follow the guidelines in those assessment methods when using the biological indicators for stressor-specific assessments. Otherwise use the thresholds in **Table 5-4**.

**Table 5-4. Biological test thresholds based on O/E results.**

O/E Model	Region (stream setting)	Pass	Fail
2005	Eastern MT (plains)	≥ 0.80	< 0.80
2011	Western MT (mountain/transitional)	≥ 0.90	< 0.90

### 5.6.1 Bray-Curtis Index

A supplemental measure to the O/E model is the Bray-Curtis Index (BC) (Van Sickle, 2008). The BC index is derived from some of the same information used to calculate the O/E score. It can be a useful tool for discerning counterintuitive O/E results. The BC Index is most helpful whenever a high (i.e. good) O/E score results from a sample collected from a known stressed stream. Occasionally this occurs when certain stressors (e.g. excess nutrients) cause some taxa to be **replaced** by other taxa that are more tolerant of the stressors, resulting in a high O/E score in a stream with high stressors. The BC Index

shows when new taxa have replaced others taxa above and beyond what the O/E model expected to measure in a given stream.

The formula for the BC Index is the sum of the absolute differences in the number of presence/absence (1,0) of observed taxa (O) minus their associated probabilities of capture (P) divided by the total sum of the O + P values:

$$BC = \frac{\sum |O - P|}{\sum (O + P)}$$

Note that the O in the formula above is not the raw count of individuals from a taxa group (e.g., 15 *Tricorythodes sp.* out of 100 total individual insects counted in a sample). Rather, it is a dummy variable indicating whether or not even a single individual of the taxa in question was observed in the sample (0 no, 1 yes). The BC Index is scaled in the opposite direction than the O/E model. The BC scores will range between 0 and 1; a score of 0 means that no taxa replacement has occurred, and 1 means that all of the taxa have been replaced with other taxa. Use the BC Index **only** to help guide your assessment decisions when you believe you are getting an incorrect O/E score. It is not meant to trump or replace the O/E score, just to clarify it. Van Sickle (2008) reported that the BC Index was more sensitive to several stressor gradients based upon nationwide datasets. You can use it to show when a community is shifting before the O/E model will react to the stressor.

#### 5.6.1.1 How the BC Index Can Be Helpful

Table 5-6 shows a hypothetical macroinvertebrate sample collected from a stream with a high measured stressor (e.g., a fine sediment source) upstream of the sampling location. For demonstration purposes, this is an extremely small sample (five taxa). Remember that the “E” in the O/E formula is the sum of the probability of capture values for all of the taxa in a sample.

**Table 5-6. A hypothetical example that demonstrates the O/E calculation, the absolute difference for the hypothetical observed (O) and expected (P) taxa, and sum of O and P for 5 taxa (adopted from Van Sickle 2008).**

Taxon	O	P	O - P	(O + P)
1	1	0.1	0.9	1.1
2	1	0.3	0.7	1.3
3	1	0.8	0.2	1.8
4	0	0.9	0.9	0.9
5	0	0.9	0.9	0.9
	O = sum = 3	E = sum = 3	Mean = 0.72; sum = 3.6	sum = 6

In this scenario, taxa 4 and 5 were expected by the model to be in the sample (i.e., probability of capture = 0.9; however, they were not collected, therefore O = 0 for both taxa). Taxa 1 and 2 were very unlikely to be collected, yet they were collected (O = 1 for each taxon). The probability of capture rates for taxa 1 and 2 was 0.1 and 0.3, respectively. The O/E score in this scenario = (3/3) = 1, and at first blush, this could be interpreted as a reference quality stream supporting a healthy macroinvertebrate community. In the example, however, recent habitat observations of high fine sediment deposition suggested an impairment really is occurring at the stream but it was not detected by O/E. The BC Index = 0.6 (3.6/6.0). This means that 40% of the taxa in this sample were replaced by rare, potentially tolerant, taxa. With

this combination of O/E and BC scores, you can say that the macroinvertebrate community was in the process of adjusting to the instream changes in stressor levels.

## 5.7 REPORTING RESULTS

Reports written for the DEQ following the methods described herein require:

- The EDAS output and a concise one paragraph ecological interpretation. The ecological interpretation includes a description of the invertebrates found in the sample and what their presence represents in terms of water quality.
- Taxonomic lists of macroinvertebrates, including tolerance values and functional feeding group designations used for each taxon. Taxa lists should include class, order, family, genus, and species designations where possible. Taxa lists should follow the taxonomic classification scheme shown in **Appendix A** to facilitate DEQ quality assurance activities.
- Copies of taxonomic bench sheets should be included (Taxa List Template example provided in **Appendix B**.)
- Number pages on all reports, including appendices.
- A clear and concise summary of findings and conclusions.
- Clearly document the dates samples were collected in any written report.
- If an internal reference is developed for the analysis, describe the internal reference used.
- Clear documentation of the proportion of sample subsampled.
- Submit data both in hard copy and in electronic form (EQUIS/WQX Compatible).
- If any further analysis is needed in which the O/E models cannot be used, contact David Feldman; Water Quality Standards Section; Montana Department of Environmental Quality, PO Box 200901, Helena MT 59620-0901 (406)-444-6764, dfeldman@mt.gov or the current biocriteria specialist for the state of Montana.

## 6.0 LITERATURE CITED

American Public Health Association, American Water Works Association, Control Federation Water Pollution, and Environment Federation Water. 1998. Standard Methods for the Examination of Water and Wastewater, 20 ed., American Public Health Association.

Chinnayakanahalli, K., C. Kroeber, and R. Hill. 2006. The Multiwatershed Delineation Tool: GIS Software in Support of Regional Watershed Analyses. *Utah State University, Logan*.

Daly, C., W. P. Gibson, M. Doggett, J. Smith, and G. Taylor. 2004. Up-to-Date Monthly Climate Maps for the Conterminous United States. In: Proceedings of 14th AMS Conference on Applied Climatology, 84th AMS Annual Meeting Combined Preprints. 13-16.

Feldman, D. and B. K. Jessup. 2012. The Proper Use and Interpretation of the Montana Observed/Expected (O/E) Models. **WQPBWQSTR-003**.

Loeb, S. L. and A. Spacie. 1994. Biological Monitoring of Aquatic Systems: CRC.

- Montana Department of Environmental Quality, Water Quality Planning Bureau. 2011. Water Quality Assessment Method. Helena, Montana: State of Montana.
- Peck, D. V. A. T. Herlihy B. H. Hill R. M. Hughes P. R. Kaufman D. J. Klemm J. M. Lazorchak F. H. McCormick S. A. Peterson P. L. Ringold T. Magee and M. Cappaert. 2006. Environmental Monitoring and Assessment Program-Surface Waters Western Pilot Study: Field Operations Manual for Wadeable Streams. Operations Manual for Wadeable Streams. EPA/620/R-06/003. Washington, D.C.: United States Environmental Protection Agency.
- Richards D.C. 1996. The Use of Aquatic Macroinvertebrates As Water Quality Indicators in Mountain Streams of Montana. Master of Science. Bozeman, Montana, USA: Montana State University, Bozeman.
- Stribling, J. B., S. R. Moulton, and G. T. Lester. 2003. Determining the Quality of Taxonomic Data. *Journal of the North American Benthological Society*. 22(4): 621-631.
- Suplee, M. W. and R. Sada de Suplee. 2011. 2011 Assessment Methodology for Determining Wadeable Stream Impairment Due to Excess Nitrogen and Phosphorus Levels. Helena, Montana: State of Montana. Report WQPBMASSTR-01.
- Tetra Tech. 2006. EDAS User's Manual for Montana. Report 1.
- EDAS for Montana. Microsoft Access Application. Ver. v3.3.2k . 2006.
- Van Sickle, J. 2008. An Index of Compositional Dissimilarity Between Observed and Expected Assemblages. *Journal of the North American Benthological Society*. 27(2): 227-235.





## **APPENDIX A - MONTANA MACROINVERTEBRATE TAXA LIST**

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Diptera	Chironomidae	Ablabesmyia	8	Tanypodinae	Ablabesmyia
Arthropoda	Insecta	Diptera	Chironomidae	Acalcarella	8	Chironominae	NA
Arthropoda	Arachnida	Acarina	Acarina	Acarina	5	Acarina	Acari
Arthropoda	Insecta	Ephemeroptera	Baetidae	Acentrella	4	Acentrella	Acentrella
Arthropoda	Insecta	Ephemeroptera	Baetidae	Acentrella insignificans	4	Acentrella	Acentrella
Arthropoda	Insecta	Ephemeroptera	Baetidae	Acentrella turbida	4	Acentrella	Acentrella
Arthropoda	Insecta	Lepidoptera	Pyralidae	Acentria		Lepidoptera	NA
Arthropoda	Insecta	Ephemeroptera	Baetidae	Acerpenna		Acerpenna	NA
Arthropoda	Insecta	Ephemeroptera	Baetidae	Acerpenna pygmaea		Acerpenna	NA
Arthropoda	Insecta	Diptera	Chironomidae	Acricotopus	6	Orthoclaadiinae	NA
Arthropoda	Insecta	Plecoptera	Perlidae	Acroneuria	0	Acroneuria	Perlidae
Arthropoda	Insecta	Plecoptera	Perlidae	Acroneuria abnormis	2	Acroneuria	Perlidae
Arthropoda	Insecta	Diptera	Culicidae	Aedes	7	Culicidae	NA
Arthropoda	Insecta	Odonata	Aeshnidae	Aeshna		Aeshna	NA
Arthropoda	Insecta	Odonata	Aeshnidae	Aeshnidae	5	Aeshnidae	NA
Arthropoda	Insecta	Coleoptera	Dytiscidae	Agabinus		Agabinus	Dytiscidae
Arthropoda	Insecta	Coleoptera	Dytiscidae	Agabus	5	Agabus	Dytiscidae
Arthropoda	Insecta	Trichoptera	Glossosomatidae	Agapetus	0	Agapetus	Glossosomatidae
Arthropoda	Insecta	Diptera	Blephariceridae	Agathon	0	Blephariceridae	NA
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Agraylea	8	Agraylea	Hydroptilidae
Arthropoda	Insecta	Hemiptera	Naucoridae	Ambrysus	3	Naucoridae	Other_Hemiptera
Arthropoda	Insecta	Hemiptera	Naucoridae	Ambrysus mormon	3	Naucoridae	Other_Hemiptera
Arthropoda	Insecta	Ephemeroptera	Ameletidae	Ameletus	0	Ameletus	Ameletidae
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Ametor		Ametor	Hydrophilidae
Arthropoda	Insecta	Ephemeroptera	Ametropodidae	Ametropus		Ametropus	NA
Arthropoda	Insecta	Trichoptera	Brachycentridae	Amiocentrus	3	Amiocentrus	Amiocentrus
Arthropoda	Insecta	Trichoptera	Brachycentridae	Amiocentrus aspilus	3	Amiocentrus	Amiocentrus
Mollusca	Gastropoda	Neotaenioglossa	Hydrobiidae	Amnicola		Hydrobiidae	NA
Arthropoda	Insecta	Odonata	Coenagrionidae	Amphiagrion	7	Amphiagrion	Zygoptera
Arthropoda	Insecta	Trichoptera	Limnephilidae	Amphicosmoecus canax		Amphicosmoecus	Limnephilidae
Arthropoda	Insecta	Plecoptera	Nemouridae	Amphinemura	2	Amphinemura	NA
Arthropoda	Insecta	Plecoptera	Nemouridae	Amphinemura banksi	2	Amphinemura	NA
Crustacea	Malacostraca	Amphipoda	.	Amphipoda		Amphipoda	Amphipoda
Arthropoda	Insecta	Coleoptera	Amphizoidae	Amphizoa		Amphizoa	NA
Arthropoda	Insecta	Trichoptera	Limnephilidae	Anabolia		Anabolia	Limnephilidae

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Anacaena	5	ANACAENA	Hydrophilidae
Arthropoda	Insecta	Trichoptera	Glossosomatidae	Anagapetus	0	Anagapetus	Glossosomatidae
Arthropoda	Insecta	Ephemeroptera	Acanthametropodidae	Analetris eximia		Analetris	NA
Arthropoda	Insecta	Odonata	Aeshnidae	Anax	5	Anax	NA
Arthropoda	Insecta	Odonata	Aeshnidae	Anax junius		Anax	NA
Annelida	Annelida	Annelida	Annelida	Annelida		Annelida	NA
Arthropoda	Insecta	Diptera	Culicidae	Anopheles	10	Culicidae	NA
Arthropoda	Insecta	Diptera	Tipulidae	Antocha	3	Antocha	Antocha
Arthropoda	Insecta	Trichoptera	Apataniidae	Apatania	3	Apatania	Apataniidae
Arthropoda	Insecta	Diptera	Chironomidae	Apedilum		Chironominae	NA
Mollusca	Gastropoda	Basommatophora	Physidae	Aplexa		Aplexa	Physidae
Arthropoda	Insecta	Diptera	Chironomidae	Apsectrotanypus	8	Tanypodinae	Macropelopiini
Arthropoda	Insecta	Odonata	Lestidae	Archilestes	9	Archilestes	Zygoptera
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Arctopsyche	2	Arctopsyche	Arctopsyche
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Arctopsyche grandis	2	Arctopsyche	Arctopsyche
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Arctopsychinae		Arctopsychinae	NA
Arthropoda	Insecta	Odonata	Coenagrionidae	Argia	7	Argia	Zygoptera
Arthropoda	Insecta	Ephemeroptera	Leptohyphidae	Asioplax		Asioplax	NA
Arthropoda	Insecta	Ephemeroptera	Leptohyphidae	Asioplax edmundsi		Asioplax	NA
Arthropoda	Insecta	Diptera	Athericidae	Athericidae	2	Atherix	NA
Arthropoda	Insecta	Diptera	Athericidae	Atherix	5	Atherix	NA
Arthropoda	Insecta	Diptera	Athericidae	Atherix pachypus	4	Atherix	NA
Arthropoda	Insecta	Diptera	Ceratopogonidae	Atrichopogon		Forcipomyiinae	Ceratopogonidae
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Attenella	3	Attenella	NA
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Attenella delantala		Attenella	NA
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Attenella margarita	3	Attenella	NA
Annelida	Oligochaeta	Haplotaxida	Tubificidae	Aulodrilus	10	Oligochaeta	Tubificidae
Arthropoda	Insecta	Diptera	Chironomidae	Axarus		Chironominae	NA
Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetidae	4	Baetidae	NA
Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetis	5	Baetis	NA
Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetis alius	1	Baetis	NA
Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetis bicaudatus	2	Baetis	Baetis_bicaudatus
Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetis flavistriga	5	Baetis	Baetis_flavistriga
Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetis intercalaris		Baetis	NA
Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetis propinquus	6	Baetis	NA

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetis punctiventris		Baetis	NA
Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetis tricaudatus	4	Baetis	Baetis_tricaudatus
Arthropoda	Insecta	Ephemeroptera	Baetiscidae	Baetisca		Baetisca	NA
Arthropoda	Insecta	Hemiptera	Belostomatidae	Belostomatidae	10	Belostomatidae	Other_Hemiptera
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Berosus	5	Berosus	Hydrophilidae
Arthropoda	Insecta	Diptera	Ceratopogonidae	Bezzia		Ceratopogoninae	Ceratopogonidae
Arthropoda	Insecta	Diptera	Blephariceridae	Bibliocephala	0	Blephariceridae	NA
Arthropoda	Insecta	Diptera	Blephariceridae	Bibliocephala grandis	0	Blephariceridae	NA
Arthropoda	Insecta	Diptera	Blephariceridae	Blephariceridae	0	Blephariceridae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Boreochlus	1	Podonominae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Boreoheptagyia		Diamesinae	NA
Arthropoda	Insecta	Trichoptera	Brachycentridae	Brachycentridae	1	Brachycentridae	NA
Arthropoda	Insecta	Trichoptera	Brachycentridae	Brachycentrus	1	Brachycentrus	Brachycentrus
Arthropoda	Insecta	Trichoptera	Brachycentridae	Brachycentrus americanus	1	Brachycentrus	Brachycentrus
Arthropoda	Insecta	Trichoptera	Brachycentridae	Brachycentrus occidentalis	2	Brachycentrus	Brachycentrus
Arthropoda	Insecta	Ephemeroptera	Caenidae	Brachycercus	3	Brachycercus	Caenidae
Annelida	Hirudinea	Branchiobdellida	.	Branchiobdellida		Branchiobdellida	NA
Arthropoda	Insecta	Diptera	Chironomidae	Brillia	4	Orthocladiinae	Brillia
Arthropoda	Insecta	Diptera	Chironomidae	Brundiniella	3	Tanypodinae	Macropelopiini
Arthropoda	Insecta	Coleoptera	Haliplidae	Brychius	5	Brychius	Haliplidae
Bryozoa	Tubellaria	.	.	Bryozoa		Bryozoa	NA
Arthropoda	Insecta	Hemiptera	Notonectidae	Buenoa		Notonectidae	Other_Hemiptera
Crustacea	Malacostraca	Isopoda	Asellidae	Caecidotea	8	Asellidae	NA
Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenidae	8	Caenidae	Caenidae
Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	8	Caenis	Caenidae
Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis latipennis	7	Caenis	Caenidae
Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis youngi	8	Caenis	Caenidae
Arthropoda	Copepoda	Calanoida	.	Calanoida		Calanoida	NA
Arthropoda	Insecta	Plecoptera	Perlidae	Calineuria californica	2	Calineuria	Perlidae
Arthropoda	Insecta	Ephemeroptera	Baetidae	Callibaetis	9	Callibaetis	Callibaetis
Arthropoda	Insecta	Hemiptera	Corixidae	Callicorixa		Corixidae	Corixidae
Arthropoda	Insecta	Diptera	Stratiomyidae	Caloparyphus	7	CALOPARYPHUS/EUPARYPHUS	Stratiomyidae
Crustacea	Malacostraca	Decapoda	Cambaridae	Cambaridae		Cambaridae	NA
Arthropoda	Insecta	Ephemeroptera	Baetidae	Camelobaetidium	4	Camelobaetidium	NA
Arthropoda	Insecta	Diptera	Chironomidae	Camptocladius	6	Orthocladiinae	NA

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Plecoptera	Capniidae	Capniidae	1	Capniidae	Capniidae
Arthropoda	Insecta	Diptera	Chironomidae	Cardiocladius	5	Orthoclaadiinae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Cardiocladius albiplumus	5	Orthoclaadiinae	NA
Arthropoda	Insecta	Plecoptera	Perlodidae	Cascadopera trictura	2	Cascadopera	Perlodidae
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Caudatella	0	Caudatella	Caudatella
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Caudatella cascadia		Caudatella	Caudatella
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Caudatella edmundsi	0	Caudatella	Caudatella
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Caudatella heterocaudata	0	Caudatella	Caudatella
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Caudatella hystrix	0	Caudatella	Caudatella
Arthropoda	Insecta	Hemiptera	Corixidae	Cenocorixa		Corixidae	Corixidae
Arthropoda	Insecta	Ephemeroptera	Baetidae	Centroptilum	2	Centroptilum	NA
Arthropoda	Insecta	Trichoptera	Leptoceridae	Ceraclea	3	Ceraclea	Leptoceridae
Arthropoda	Insecta	Diptera	Ceratopogonidae	Ceratopogonidae	6	Ceratopogonidae	Ceratopogonidae
Arthropoda	Insecta	Diptera	Ceratopogonidae	Ceratopogoninae	6	Ceratopogoninae	Ceratopogonidae
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Ceratopsyche	5	Hydropsyche_Ceratopsyche	NA
Arthropoda	Insecta	Ephemeroptera	Caenidae	Cercobrachys		Cercobrachys	Caenidae
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Cercyon		Cercyon	Hydrophilidae
Arthropoda	Insecta	Diptera	Chironomidae	Chaetocladius	6	Orthoclaadiinae	Chaetocladius
Annelida	Oligochaeta	Haplotaxida	Naididae	Chaetogaster		Oligochaeta	Naididae
Annelida	Oligochaeta	Haplotaxida	Naididae	Chaetogaster diaphanus	6	Oligochaeta	Naididae
Annelida	Oligochaeta	Haplotaxida	Naididae	Chaetogaster limnaei	6	Oligochaeta	Naididae
Arthropoda	Insecta	Diptera	Chaoboridae	Chaoboridae	8	Chaoboridae	Chaoboridae
Arthropoda	Insecta	Diptera	Chaoboridae	Chaoborus		Chaoborus	Chaoboridae
Arthropoda	Insecta	Diptera	Empididae	Chelifera	5	Chelifera_Metachela_Neoplasta	Empididae
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Cheumatopsyche	5	Cheumatopsyche	Cheumatopsyche
Arthropoda	Insecta	Trichoptera	Philopotamidae	Chimarra utahensis	4	Chimarra	Philopotamidae
Arthropoda	Insecta	Diptera	Chironomidae	Chironomidae	10	Chironomidae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Chironominae	7	Chironominae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Chironomini	6	Chironominae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Chironomus	10	Chironominae	Chironomus
Arthropoda	Insecta	Plecoptera	Chloroperlidae	Chloroperlidae	1	Chloroperlidae	NA
Arthropoda	Insecta	Plecoptera	Chloroperlidae	Chloroperlinae		Chloroperlidae	NA
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Choroterpes	2	Choroterpes	Leptophlebiidae
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Choroterpes albiannulata	2	Choroterpes	Leptophlebiidae
Arthropoda	Insecta	Diptera	Tabanidae	Chrysops	10	Tabanidae	Tabanidae

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Trichoptera	Limnephilidae	Chyrandra	2	Chyrandra	Limnephilidae
Arthropoda	Insecta	Trichoptera	Limnephilidae	Chyrandra centralis	2	Chyrandra	Limnephilidae
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Cinygma	0	Cinygma	NA
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Cinygmula	0	Cinygmula	Cinygmula
Arthropoda	Insecta	Plecoptera	Perlidae	Claassenia	2	Claassenia sabulosa	Perlidae
Arthropoda	Insecta	Plecoptera	Perlidae	Claassenia sabulosa	3	Claassenia sabulosa	Perlidae
Arthropoda	Branchiopoda	Diplostraca	.	Cladocera		Cladocera	Cladocera
Arthropoda	Insecta	Diptera	Chironomidae	Cladopelma		Chironominae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Cladotanytarsus	7	Chironominae	Cladotanytarsus
Arthropoda	Insecta	Coleoptera	Elmidae	Cleptelmis	4	Cleptelmis	Cleptelmis
Arthropoda	Insecta	Coleoptera	Elmidae	Cleptelmis addenda		Cleptelmis	Cleptelmis
Arthropoda	Insecta	Coleoptera	Elmidae	Cleptelmis ornata	4	Cleptelmis	Cleptelmis
Arthropoda	Insecta	Diptera	Empididae	Clinocera	5	Clinocera	Empididae
Arthropoda	Insecta	Odonata	Coenagrionidae	Coenagrion		Coenagrionidae	Zygoptera
Arthropoda	Insecta	Odonata	Coenagrionidae	Coenagrionidae	7	Coenagrionidae	Zygoptera
Arthropoda	Insecta	Coleoptera	Dytiscidae	Colymbetes		Colymbetes	Dytiscidae
Arthropoda	Insecta	Diptera	Chironomidae	Conchapelopia	6	Tanypodinae	Other_Tanypodinae
Arthropoda	Insecta	Diptera	Chironomidae	Constempellina	8	Chironominae	NA
Arthropoda	Copepoda	Copepoda	Copepoda	Copepoda		Copepoda	Copepoda
Arthropoda	Insecta	Coleoptera	Dytiscidae	Coptotomus	5	Coptotomus	Dytiscidae
Mollusca	Bivalvia	Corbiculacea	Corbiculidae	Corbicula		Corbicula	NA
Arthropoda	Insecta	Odonata	Cordulegastridae	Cordulegaster		Cordulegaster	NA
Arthropoda	Insecta	Odonata	Cordulegastridae	Cordulegastridae		Cordulegastridae	NA
Arthropoda	Insecta	Odonata	Corduliidae	Corduliidae		Corduliidae	NA
Arthropoda	Insecta	Hemiptera	Corixidae	Corisella		Corixidae	Corixidae
Arthropoda	Insecta	Hemiptera	Corixidae	Corisella tarsalis		Corixidae	Corixidae
Arthropoda	Insecta	Hemiptera	Corixidae	Corixidae	9	Corixidae	Corixidae
Arthropoda	Insecta	Diptera	Chironomidae	Corynoneura	7	Orthocladiinae	Corynoneura
Crustacea	Malacostraca	Amphipoda	Crangonyctidae	Crangonyx		Crangonyx	Amphipoda
Arthropoda	Insecta	Diptera	Chironomidae	Cricotopus	8	Orthocladiinae	Cricotopus
Arthropoda	Insecta	Diptera	Chironomidae	Cricotopus (Cricotopus)	8	Orthocladiinae	Cricotopus
Arthropoda	Insecta	Diptera	Chironomidae	Cricotopus (Isocladius)	8	Orthocladiinae	Cricotopus
Arthropoda	Insecta	Diptera	Chironomidae	Cricotopus (Nostococladius)	8	Orthocladiinae	Cricotopus
Arthropoda	Insecta	Diptera	Chironomidae	Cricotopus bicinctus	9	Orthocladiinae	Cricotopus
Arthropoda	Insecta	Diptera	Chironomidae	Cricotopus tremulus	8	Orthocladiinae	Cricotopus

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Diptera	Chironomidae	Cricotopus trifascia	8	Orthocladiinae	Cricotopus
Arthropoda	Insecta	Diptera	Chironomidae	Cricotopus trifasciatus	8	Orthocladiinae	Cricotopus
Arthropoda	Insecta	Trichoptera	Limnephilidae	Cryptochia	3	Cryptochia	Limnephilidae
Arthropoda	Insecta	Diptera	Chironomidae	Cryptochironomus	8	Chironominae	Cryptochironomus
Arthropoda	Insecta	Diptera	Tipulidae	Cryptolabis		Cryptolabis	Other_Tipulidae
Arthropoda	Insecta	Diptera	Chironomidae	Cryptotendipes	6	Chironominae	Cryptotendipes
Arthropoda	Insecta	Diptera	Culicidae	Culex	10	Culicidae	NA
Arthropoda	Insecta	Diptera	Culicidae	Culicidae	10	Culicidae	NA
Arthropoda	Insecta	Diptera	Ceratopogonidae	Culicoides	10	Ceratopogoninae	Ceratopogonidae
Arthropoda	Insecta	Trichoptera	Glossosomatidae	Culoptila	2	Culoptila	Glossosomatidae
Arthropoda	Insecta	Plecoptera	Perlodidae	Cultus	2	Cultus	Perlodidae
Arthropoda	Insecta	Coleoptera	Curculionidae	Curculionidae		Curculionidae	NA
Arthropoda	Copepoda	Cyclopoida	.	Cyclopoida	8	COPEPODA	NA
Arthropoda	Branchiopoda	Diplostraca	Daphniidae	Daphniidae		Cladocera	Cladocera
Crustacea	Malacostraca	Decapoda	.	Decapoda	6	Decapoda	NA
Arthropoda	Insecta	Diptera	Chironomidae	Demicryptochironomus	8	Chironominae	NA
Annelida	Oligochaeta	Haplotaxida	Naididae	Dero digitata		Oligochaeta	Naididae
Arthropoda	Insecta	Coleoptera	Dytiscidae	Deronectes	5	Deronectes	Dytiscidae
Arthropoda	Insecta	Coleoptera	Dytiscidae	Desmopachria		Desmopachria	Dytiscidae
Arthropoda	Insecta	Plecoptera	Leuctridae	Despaxia	2	Leuctridae	Leuctridae
Arthropoda	Insecta	Plecoptera	Leuctridae	Despaxia augusta	2	Leuctridae	Leuctridae
Arthropoda	Insecta	Diptera	Deuterophlebiidae	Deuterophlebia	0	Deuterophlebia	NA
Arthropoda	Insecta	Diptera	Chironomidae	Diamesa	5	Diamesinae	Diamesa
Arthropoda	Insecta	Diptera	Chironomidae	Diamesinae	4	Diamesinae	NA
Arthropoda	Insecta	Trichoptera	Limnephilidae	Dicosmoecus	2	Dicosmoecus	Limnephilidae
Arthropoda	Insecta	Trichoptera	Limnephilidae	Dicosmoecus atripes	2	Dicosmoecus	Limnephilidae
Arthropoda	Insecta	Trichoptera	Limnephilidae	Dicosmoecus gilvipes		Dicosmoecus	Limnephilidae
Arthropoda	Insecta	Diptera	Tipulidae	Dicranota	0	Dicranota	Dicranota
Arthropoda	Insecta	Diptera	Chironomidae	Dicrotendipes	8	Chironominae	Dicrotendipes
Arthropoda	Insecta	Ephemeroptera	Baetidae	Dipheter hageni	5	Dipheter	Dipheter
Arthropoda	Insecta	Diptera	Chironomidae	Diplocladius	5	Orthocladiinae	NA
Arthropoda	Insecta	Diptera	.	Diptera		Diptera	NA
Arthropoda	Insecta	Plecoptera	Perlodidae	Diura	2	Diura	Perlodidae
Arthropoda	Insecta	Plecoptera	Perlodidae	Diura knowltoni	2	Diura	Perlodidae
Arthropoda	Insecta	Diptera	Dixidae	Dixa	4	Dixa	Dixidae

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Diptera	Dixidae	Dixella		Dixella	Dixidae
Arthropoda	Insecta	Diptera	Dixidae	Dixidae	4	Dixidae	Dixidae
Arthropoda	Insecta	Diptera	Dolichopodidae	Dolichopodidae	4	Dolichopodidae	NA
Arthropoda	Insecta	Trichoptera	Philopotamidae	Dolophilodes	0	Dolophilodes	Philopotamidae
Arthropoda	Insecta	Plecoptera	Perlidae	Doroneuria	0	Doroneuria	Perlidae
Arthropoda	Insecta	Plecoptera	Perlidae	Doroneuria theodora	0	Doroneuria	Perlidae
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Drunella	1	Drunella	NA
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Drunella coloradensis	0	Drunella coloradensis/flavilinea	Drunella_coloradensis/ flavilinea
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Drunella doddsi	1	Drunella doddsi	Drunella_doddsi
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Drunella flavilinea	2	Drunella coloradensis/flavilinea	Drunella_coloradensis/ flavilinea
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Drunella grandis	2	Drunella grandis	Drunella_grandis
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Drunella spinifera	0	Drunella spinifera	Drunella_spinifera
Arthropoda	Insecta	Coleoptera	Dryopidae	Dryopidae		Dryopidae	NA
Arthropoda	Insecta	Coleoptera	Elmidae	Dubiraphia	6	Dubiraphia	Dubiraphia
Platyhelminthes	Tubellaria	Tricladida	Planariidae	Dugesia		Turbellaria	Turbellaria
Arthropoda	Insecta	Coleoptera	Dytiscidae	Dytiscidae	5	Dytiscidae	Dytiscidae
Arthropoda	Insecta	Coleoptera	Dytiscidae	Dytiscus	5	Dytiscus	Dytiscidae
Arthropoda	Insecta	Trichoptera	Limnephilidae	Ecclisocosmoecus		Ecclisocosmoecus	Limnephilidae
Arthropoda	Insecta	Trichoptera	Limnephilidae	Ecclisomyia	4	Ecclisomyia	Limnephilidae
Annelida	Oligochaeta	Lumbriculida	Lumbriculidae	Eclipidrilus	4	Oligochaeta	Eclipidrilus
Arthropoda	Insecta	Diptera	Simuliidae	Ectemnia		Simuliidae	Simuliidae
Arthropoda	Insecta	Diptera	Chironomidae	Einfeldia	8	Chironominae	NA
Arthropoda	Insecta	Coleoptera	Elmidae	Elmidae	4	Elmidae	NA
Arthropoda	Insecta	Diptera	Empididae	Empididae	6	Empididae	Empididae
Arthropoda	Insecta	Odonata	Coenagrionidae	Enallagma	7	Coenagrionidae	Zygoptera
Annelida	Oligochaeta	Haplotaxida	Enchytraeidae	Enchytraeidae	4	Oligochaeta	NA
Arthropoda	Insecta	Diptera	Chironomidae	Endochironomus	10	Chironominae	Endochironomus
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Enochrus	5	Enochrus	Hydrophilidae
Arthropoda	Insecta	Trichoptera	Limnephilidae	Eocosmoecus		Eocosmoecus	Limnephilidae
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Epeorus	2	Epeorus	Epeorus
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Epeorus albertae	2	Epeorus	Epeorus
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Epeorus deceptivus	0	Epeorus	Epeorus
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Epeorus grandis	0	Epeorus	Epeorus
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Epeorus longimanus	1	Epeorus	Epeorus
Arthropoda	Insecta	Ephemeroptera	Ephemeridae	Ephemera	2	Ephemera	NA



Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Ephemeroptera	Ephemeridae	Ephemera simulans	1	Ephemera	NA
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Ephemerella	2	Ephemerella	Ephemerella
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Ephemerella aurivillii	0	Ephemerella aurivillii	Ephemerella
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Ephemerella inermis	4	Ephemerella	Ephemerella
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Ephemerella infrequens	2	Ephemerella	Ephemerella
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Ephemerellidae	1	Ephemerellidae	NA
Arthropoda	Insecta	Ephemeroptera	Ephemeridae	Ephemeridae	4	Ephemeridae	NA
Arthropoda	Insecta	Ephemeroptera	.	Ephemeroptera		Ephemeroptera	NA
Arthropoda	Insecta	Ephemeroptera	Polymitarcyidae	Ephoron	2	Ephoron	NA
Arthropoda	Insecta	Ephemeroptera	Polymitarcyidae	Ephoron album	2	Ephoron	NA
Arthropoda	Insecta	Diptera	Ephydriidae	Ephydriidae	6	Ephydriidae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Epoicocladus		Orthoclaadiinae	NA
Arthropoda	Insecta	Diptera	Tipulidae	Erioptera	7	Erioptera	Other_Tipulidae
Arthropoda	Insecta	Odonata	Gomphidae	Erpetogomphus	3	Gomphidae	Gomphidae
Annelida	Hirudinea	Arhynchobdellida	Erpobdellidae	Erpobdella		Erpobdellidae	Hirudinea
Annelida	Hirudinea	Arhynchobdellida	Erpobdellidae	Erpobdellidae	8	Erpobdellidae	Hirudinea
Arthropoda	Insecta	Diptera	Chironomidae	Eukiefferiella	4	Orthoclaadiinae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Eukiefferiella brehmi	2	Orthoclaadiinae	Eukiefferiella_Brehmi_Gr
Arthropoda	Insecta	Diptera	Chironomidae	Eukiefferiella claripennis	5	Orthoclaadiinae	Eukiefferiella_Claripennis_Gr
Arthropoda	Insecta	Diptera	Chironomidae	Eukiefferiella coerulescens	4	Orthoclaadiinae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Eukiefferiella devonica	4	Orthoclaadiinae	Eukiefferiella_Devonica_Gr
Arthropoda	Insecta	Diptera	Chironomidae	Eukiefferiella gracei	4	Orthoclaadiinae	Eukiefferiella_Gracei_Gr
Arthropoda	Insecta	Diptera	Chironomidae	Eukiefferiella pseudomontana		Orthoclaadiinae	Eukiefferiella_Pseudomontana_Gr
Arthropoda	Insecta	Diptera	Chironomidae	Eukiefferiella similis		Orthoclaadiinae	NA
Arthropoda	Insecta	Diptera	Stratiomyidae	Euparyphus	7	CALOPARYPHUS/EUPARYPHUS	Stratiomyidae
Arthropoda	Insecta	Ephemeroptera	Baetidae	Fallceon quilleri	5	Fallceon	NA
Mollusca	Gastropoda	Basommatophora	Ancylidae	Ferrissia	6	Ferrissia	NA
Mollusca	Gastropoda	Basommatophora	Ancylidae	Ferrissia parallelus	6	Ferrissia	NA
Mollusca	Gastropoda	Neotaenioglossa	Hydrobiidae	Fluminicola		Hydrobiidae	NA
Arthropoda	Insecta	Diptera	Ceratopogonidae	Forcipomyiinae	6	Forcipomyiinae	Ceratopogonidae
Mollusca	Gastropoda	Basommatophora	Lymnaeidae	Fossaria	6	Lymnaeidae	Lymnaeidae
Crustacea	Malacostraca	Amphipoda	Gammaridae	Gammaridae	4	Gammaridae	Amphipoda
Crustacea	Malacostraca	Amphipoda	Gammaridae	Gammarus	4	Gammarus	Amphipoda
Annelida	Hirudinea	Rhynchobdellida	Glossiphoniidae	Glossiphonia		Glossiphoniidae	Hirudinea
Annelida	Hirudinea	Rhynchobdellida	Glossiphoniidae	Glossiphonia complanata	9	Glossiphoniidae	Hirudinea

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Annelida	Hirudinea	Rhynchobdellida	Glossiphoniidae	Glossiphoniidae	9	Glossiphoniidae	Hirudinea
Arthropoda	Insecta	Trichoptera	Glossosomatidae	Glossosoma	0	Glossosoma	Glossosomatidae
Arthropoda	Insecta	Trichoptera	Glossosomatidae	Glossosoma traviatum	0	Glossosoma	Glossosomatidae
Arthropoda	Insecta	Trichoptera	Glossosomatidae	Glossosoma velona	0	Glossosoma	Glossosomatidae
Arthropoda	Insecta	Trichoptera	Glossosomatidae	Glossosomatidae	0	Glossosomatidae	Glossosomatidae
Arthropoda	Insecta	Diptera	Pelecorhynchidae	Glutops	1	Pelecorhynchidae	Glutops
Arthropoda	Insecta	Trichoptera	Limnephilidae	Glyphopsyche	1	Glyphopsyche	Limnephilidae
Arthropoda	Insecta	Diptera	Chironomidae	Glyptotendipes	10	Chironominae	NA
Arthropoda	Insecta	Trichoptera	Goeridae	Goeracea		Goeridae	NA
Arthropoda	Insecta	Odonata	Gomphidae	Gomphidae	2	Gomphidae	Gomphidae
Arthropoda	Insecta	Odonata	Gomphidae	Gomphus		Gomphidae	Gomphidae
Arthropoda	Insecta	Coleoptera	Hydraenidae	Gymnochthebius		Gymnochthebius	NA
Mollusca	Gastropoda	Basommatophora	Planorbidae	Gyraulus	8	Planorbidae	Planorbidae
Arthropoda	Insecta	Coleoptera	Gyrinidae	Gyrinus	5	GYRINUS	NA
Arthropoda	Insecta	Coleoptera	Haliplidae	Haliplidae	8	Haliplidae	Haliplidae
Arthropoda	Insecta	Coleoptera	Haliplidae	Haliplus	8	Haliplus	Haliplidae
Annelida	Oligochaeta	Haplotaxida	Haplotaxidae	Haplotaxidae		Oligochaeta	NA
Annelida	Oligochaeta	Haplotaxida	Haplotaxidae	Haplotaxis		Oligochaeta	NA
Arthropoda	Insecta	Diptera	Chironomidae	Harnischia		Chironominae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Heleniella	6	Orthocladinae	Heleniella
Arthropoda	Insecta	Coleoptera	Dryopidae	Helichus	5	Helichus	NA
Arthropoda	Insecta	Coleoptera	Dryopidae	Helichus striatus	5	Helichus	NA
Arthropoda	Insecta	Trichoptera	Helicopsychidae	Helicopsyche	3	Helicopsyche	NA
Arthropoda	Insecta	Trichoptera	Helicopsychidae	Helicopsyche borealis	3	Helicopsyche	NA
Arthropoda	Insecta	Trichoptera	Helicopsychidae	Helicopsychidae	3	Helicopsychidae	NA
Mollusca	Gastropoda	Basommatophora	Planorbidae	Helisoma	6	Planorbidae	Planorbidae
Mollusca	Gastropoda	Basommatophora	Planorbidae	Helisoma anceps	7	Planorbidae	Planorbidae
Annelida	Hirudinea	Rhynchobdellida	Glossiphoniidae	Helobdella	10	Glossiphoniidae	Hirudinea
Annelida	Hirudinea	Rhynchobdellida	Glossiphoniidae	Helobdella stagnalis	10	Glossiphoniidae	Hirudinea
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Helophorus	5	Helophorus	NA
Arthropoda	Insecta	Diptera	Empididae	Hemerodromia	6	Hemerodromia	Empididae
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Heptagenia	4	Heptagenia	NA
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Heptageniidae	2	Heptageniidae	NA
Arthropoda	Insecta	Diptera	Tipulidae	Hesperoconopa	1	Hesperoconopa	Other_Tipulidae
Arthropoda	Insecta	Hemiptera	Corixidae	Hesperocorixa		Corixidae	Corixidae

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Hemiptera	Corixidae	Hesperocorixa laevigata	9	Corixidae	Corixidae
Arthropoda	Insecta	Plecoptera	Perlidae	Hesperoperla pacifica	1	Hesperoperla	Perlidae
Arthropoda	Insecta	Trichoptera	Limnephilidae	Hesperophylax	3	Hesperophylax	Limnephilidae
Arthropoda	Insecta	Odonata	Calopterygidae	Hetaerina americana		Calopterygidae	Zygoptera
Arthropoda	Insecta	Coleoptera	Elmidae	Heterlimnius	3	Heterlimnius	Heterlimnius
Arthropoda	Insecta	Coleoptera	Elmidae	Heterlimnius corpulentus	3	Heterlimnius	Heterlimnius
Arthropoda	Insecta	Coleoptera	Heteroceridae	Heteroceridae		Heteroceridae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Heterotrissocladius	0	Orthoclaadiinae	Heterotrissocladius
Arthropoda	Insecta	Diptera	Chironomidae	Heterotrissocladius marcidus		Orthoclaadiinae	Heterotrissocladius
Arthropoda	Insecta	Ephemeroptera	Ephemeridae	Hexagenia	6	Hexagenia	NA
Arthropoda	Insecta	Ephemeroptera	Ephemeridae	Hexagenia limbata	6	Hexagenia	NA
Arthropoda	Insecta	Diptera	Tipulidae	Hexatoma	2	Hexatoma	Hexatoma
Annelida	Hirudinea	.	.	Hirudinea	9	Hirudinea	Hirudinea
Arthropoda	Insecta	Trichoptera	Limnephilidae	Homophylax	2	Homophylax	Limnephilidae
Crustacea	Malacostraca	Amphipoda	Talitridae	Hyaella	8	Hyaella	Amphipoda
Crustacea	Malacostraca	Amphipoda	Hyalellidae	Hyaella azteca	8	Hyaella	Amphipoda
Crustacea	Malacostraca	Amphipoda	Hyalellidae	Hyalellidae	8	Hyalellidae	Amphipoda
Coelenterata	Hydrozoa	Hydroida	Hydridae	Hydra	5	Hydridae	NA
Arthropoda	Insecta	Coleoptera	Hydraenidae	Hydraena		Hydraena	NA
Arthropoda	Insecta	Diptera	Chironomidae	Hydrobaenus	8	Orthoclaadiinae	Hydrobaenus
Mollusca	Gastropoda	Neotaenioglossa	Hydrobiidae	Hydrobiidae		Hydrobiidae	NA
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Hydrobius		Hydrobius	Hydrophilidae
Arthropoda	Insecta	Coleoptera	Hydrochidae	Hydrochus	7	Hydrochus	NA
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Hydrophilidae	5	Hydrophilidae	Hydrophilidae
Arthropoda	Insecta	Coleoptera	Dytiscidae	Hydroporus		Hydroporus	Dytiscidae
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Hydropsyche	5	Hydropsyche_Ceratopsyche	Hydropsyche
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Hydropsyche bronta	5	Hydropsyche_Ceratopsyche	Hydropsyche
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Hydropsyche cockerelli	4	Hydropsyche_Ceratopsyche	Hydropsyche
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Hydropsyche morosa	6	Hydropsyche_Ceratopsyche	Hydropsyche
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Hydropsyche occidentalis	5	Hydropsyche_Ceratopsyche	Hydropsyche
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Hydropsyche oslari	4	Hydropsyche_Ceratopsyche	Hydropsyche
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Hydropsyche simulans		Hydropsyche_Ceratopsyche	Hydropsyche
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Hydropsyche slossonae	4	Hydropsyche_Ceratopsyche	Hydropsyche
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Hydropsychidae	4	Hydropsychidae	NA
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Hydropsychinae		HYDROPSYCHINAE	NA

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Hydroptila	6	Hydroptila	Hydroptilidae
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Hydroptilidae	4	Hydroptilidae	Hydroptilidae
Coelenterata	Hydrozoa	.	.	Hydrozoa	5	Hydrozoa	NA
Arthropoda	Insecta	Coleoptera	Dytiscidae	Hygrotus		Hygrotus	Dytiscidae
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Ironodes	0	Ironodes	NA
Arthropoda	Insecta	Odonata	Coenagrionidae	Ischnura	7	Coenagrionidae	Zygoptera
Arthropoda	Insecta	Plecoptera	Perlodidae	Isogenoides	3	Isogenoides	Perlodidae
Arthropoda	Insecta	Plecoptera	Perlodidae	Isogenoides elongatus	3	Isogenoides	Perlodidae
Arthropoda	Insecta	Ephemeroptera	Isonychiidae	Isonychia	2	Isonychia	NA
Arthropoda	Insecta	Plecoptera	Perlodidae	Isoperla	2	Isoperla	Perlodidae
Arthropoda	Insecta	Plecoptera	Perlodidae	Isoperla fulva	3	Isoperla	Perlodidae
Arthropoda	Insecta	Plecoptera	Perlodidae	Isoperla sobria	2	Isoperla	Perlodidae
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Ithytrichia	4	Ithytrichia	Hydroptilidae
Arthropoda	Insecta	Plecoptera	Chloroperlidae	Kathroperla	1	Kathroperla	NA
Arthropoda	Insecta	Plecoptera	Chloroperlidae	Kathroperla perdita	1	Kathroperla	NA
Arthropoda	Insecta	Plecoptera	Perlodidae	Kogotus	1	Kogotus	Perlodidae
Arthropoda	Insecta	Diptera	Chironomidae	Krenosmittia	1	Orthocladinae	Krenosmittia
Arthropoda	Insecta	Ephemeroptera	Baetidae	Labiobaetis		Labiobaetis	NA
Arthropoda	Insecta	Diptera	Chironomidae	Labrundinia	7	Tanypodinae	Labrundinia
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Laccobius		Laccobius	Hydrophilidae
Arthropoda	Insecta	Coleoptera	Dytiscidae	Laccophilus	5	Laccophilus	Dytiscidae
Arthropoda	Insecta	Ephemeroptera	Oligoneuriidae	Lachlania		Oligoneuriidae	NA
Arthropoda	Insecta	Coleoptera	Lampyridae	Lampyridae		Lampyridae	NA
Arthropoda	Insecta	Coleoptera	Elmidae	Lara	1	Lara	Lara
Arthropoda	Insecta	Coleoptera	Elmidae	Lara avara	1	Lara	Lara
Arthropoda	Insecta	Diptera	Chironomidae	Larsia	6	Tanypodinae	Larsia
Arthropoda	Insecta	Trichoptera	Lepidostomatidae	Lepidostoma	1	Lepidostoma	Lepidostomatidae
Arthropoda	Insecta	Trichoptera	Lepidostomatidae	Lepidostoma cascadense		Lepidostoma	Lepidostomatidae
Arthropoda	Insecta	Trichoptera	Lepidostomatidae	Lepidostoma pluviale		Lepidostoma	Lepidostomatidae
Arthropoda	Insecta	Trichoptera	Lepidostomatidae	Lepidostomatidae	1	Lepidostomatidae	Lepidostomatidae
Arthropoda	Insecta	Trichoptera	Leptoceridae	Leptoceridae	4	Leptoceridae	Leptoceridae
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Leptophlebia	3	Leptophlebia	Leptophlebiidae
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Leptophlebiidae	2	Leptophlebiidae	Leptophlebiidae
Arthropoda	Insecta	Odonata	Lestidae	Lestes	9	Lestes	Zygoptera
Arthropoda	Insecta	Hemiptera	Belostomatidae	Lethocerus	10	Belostomatidae	Other_Hemiptera

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Odonata	Libellulidae	Leucorrhinia		Libellulidae	Libellulidae
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Leucotrichia	2	Leucotrichia	Hydroptilidae
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Leucotrichia pictipes	2	Leucotrichia	Hydroptilidae
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Leucrocuta	1	Leucrocuta	NA
Arthropoda	Insecta	Plecoptera	Leuctridae	Leuctridae	1	Leuctridae	Leuctridae
Arthropoda	Insecta	Odonata	Libellulidae	Libellula	9	Libellulidae	Libellulidae
Arthropoda	Insecta	Odonata	Libellulidae	Libellulidae	9	Libellulidae	Libellulidae
Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilidae	3	Limnephilidae	Limnephilidae
Arthropoda	Insecta	Trichoptera	Limnephilidae	Limnephilus	3	Limnephilus	Limnephilidae
Annelida	Oligochaeta	Haplotaxida	Tubificidae	Limnodrilus	10	Oligochaeta	Tubificidae
Annelida	Oligochaeta	Haplotaxida	Tubificidae	Limnodrilus hoffmeisteri	10	Oligochaeta	Tubificidae
Arthropoda	Insecta	Diptera	Tipulidae	Limnophila	3	Limnophila	Limnophila
Arthropoda	Insecta	Diptera	Muscidae	Limnophora	7	Muscidae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Limnophyes	6	Orthocladiinae	Limnophyes
Arthropoda	Insecta	Diptera	Tipulidae	Limonia		Limonia	Other_Tipulidae
Arthropoda	Insecta	Coleoptera	Dytiscidae	Liodessus		Liodessus	Dytiscidae
Arthropoda	Insecta	Diptera	Chironomidae	Lopescladius	2	Orthocladiinae	NA
Annelida	Oligochaeta	Haplotaxida	Lumbricidae	Lumbricidae	4	Oligochaeta	NA
Annelida	Oligochaeta	Haplotaxida	Lumbricidae	Lumbricina	4	Oligochaeta	NA
Annelida	Oligochaeta	Lumbriculida	Lumbriculidae	Lumbriculidae	4	Oligochaeta	NA
Annelida	Oligochaeta	Lumbriculida	Lumbriculidae	Lumbriculus		Oligochaeta	NA
Mollusca	Gastropoda	Basommatophora	Lymnaeidae	Lymnaeidae	6	Lymnaeidae	Lymnaeidae
Arthropoda	Insecta	Diptera	Chironomidae	Macropelopia	6	Tanypodinae	Macropelopiini
Arthropoda	Insecta	Plecoptera	Nemouridae	Malenka	1	Malenka	Malenka
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Mayatrichia	1	Mayatrichia	Hydroptilidae
Arthropoda	Insecta	Plecoptera	Perlodidae	Megarcys	1	Megarcys	Perlodidae
Mollusca	Gastropoda	Basommatophora	Planorbidae	Menetus		Planorbidae	Planorbidae
Arthropoda	Insecta	Diptera	Dixidae	Meringodixa		Meringodixa	Dixidae
Nematoda	Polychaeta	Mermithida	Mermithidae	Mermithidae	5	Nematoda	NA
Arthropoda	Insecta	Diptera	Chironomidae	Mesosmittia		Orthocladiinae	NA
Arthropoda	Insecta	Hemiptera	Mesoveliidae	Mesovelia			Other_Hemiptera
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Metrichia		Metrichia	Hydroptilidae
Arthropoda	Insecta	Diptera	Chironomidae	Metriocnemus		Orthocladiinae	NA
Arthropoda	Insecta	Trichoptera	Brachycentridae	Micrasema	1	Micrasema	Micrasema
Arthropoda	Insecta	Trichoptera	Brachycentridae	Micrasema bactro	1	Micrasema	Micrasema

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Coleoptera	Elmidae	Microcylloepus	5	Microcylloepus	NA
Arthropoda	Insecta	Coleoptera	Elmidae	Microcylloepus pusillus	5	Microcylloepus	NA
Arthropoda	Insecta	Diptera	Chironomidae	Micropsectra	4	Chironominae	Micropsectra
Arthropoda	Insecta	Diptera	Chironomidae	Microtendipes	6	Chironominae	Microtendipes
Arthropoda	Insecta	Trichoptera	Molannidae	Molanna	6	Molanna	NA
Mollusca	Ostracoda	.	.	Mollusca		Mollusca	NA
Arthropoda	Insecta	Diptera	Tipulidae	Molophilus		Molophilus	Other_Tipulidae
Arthropoda	Insecta	Diptera	Chironomidae	Monodiamesa	7	Prodiamesinae	NA
Annelida	Hirudinea	Arhynchobdellida	Erpobdellidae	Mooreobdella	10	Erpobdellidae	Hirudinea
Annelida	Hirudinea	Arhynchobdellida	Erpobdellidae	Mooreobdella melanostoma		Erpobdellidae	Hirudinea
Arthropoda	Insecta	Plecoptera	Leuctridae	Moselia infuscata		Leuctridae	Leuctridae
Arthropoda	Insecta	Diptera	Muscidae	Muscidae	9	Muscidae	NA
Arthropoda	Insecta	Trichoptera	Leptoceridae	Mystacides	4	Mystacides	Leptoceridae
Annelida	Oligochaeta	Haplotaxida	Naididae	Naididae	8	Oligochaeta	Naididae
Annelida	Oligochaeta	Haplotaxida	Naididae	Nais	9	Oligochaeta	Naididae
Annelida	Oligochaeta	Haplotaxida	Naididae	Nais behningi	8	Oligochaeta	Naididae
Annelida	Oligochaeta	Haplotaxida	Naididae	Nais bretscheri		Oligochaeta	Naididae
Annelida	Oligochaeta	Haplotaxida	Naididae	Nais simplex		Oligochaeta	Naididae
Annelida	Oligochaeta	Haplotaxida	Naididae	Nais variabilis	10	Oligochaeta	Naididae
Arthropoda	Insecta	Diptera	Chironomidae	Nanocladius	3	Orthoclaadiinae	Nanocladius
Arthropoda	Insecta	Coleoptera	Elmidae	Narpus	2	Narpus	Narpus
Arthropoda	Insecta	Coleoptera	Elmidae	Narpus concolor	2	Narpus	Narpus
Arthropoda	Insecta	Diptera	Chironomidae	Natarsia		Tanypodinae	Other_Tanypodinae
Arthropoda	Insecta	Plecoptera	Chloroperlidae	Neaviperla	1	Chloroperlidae	NA
Arthropoda	Insecta	Trichoptera	Leptoceridae	Nectopsyche	2	Nectopsyche	Leptoceridae
Nematoda	Polychaeta	.	.	Nematoda	5	Nematoda	Nematoda
Nematomorpha	Polychaeta	.	.	Nematomorpha		Nematomorpha	NA
Arthropoda	Insecta	Trichoptera	Limnephilidae	Nemotaulius	3	Nemotaulius	Limnephilidae
Arthropoda	Insecta	Diptera	Stratiomyidae	Nemotelus	7	Nemotelus	Stratiomyidae
Arthropoda	Insecta	Plecoptera	Nemouridae	Nemouridae	2	Nemouridae	NA
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Neochoroterpes	2	Neochoroterpes	Leptophlebiidae
Arthropoda	Insecta	Coleoptera	Dytiscidae	Neoclypeodytes		Neoclypeodytes	Dytiscidae
Arthropoda	Insecta	Trichoptera	Uenoidae	Neophylax	3	Neophylax	Uenoidae
Arthropoda	Insecta	Trichoptera	Uenoidae	Neophylax occidentis		Neophylax	Uenoidae
Arthropoda	Insecta	Trichoptera	Uenoidae	Neophylax rickeri	3	Neophylax	Uenoidae

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Trichoptera	Uenoidae	Neophylax splendens		Neophylax	Uenoidae
Arthropoda	Insecta	Diptera	Empididae	Neoplasta	6	Chelifera_Metachela_Neoplasta	Empididae
Arthropoda	Insecta	Hemiptera	Pleidae	Neoplea		Pleidae	Other_Hemiptera
Arthropoda	Insecta	Trichoptera	Uenoidae	Neothremma	1	Neothremma	Uenoidae
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Neotrichia	2	Neotrichia	Hydroptilidae
Annelida	Hirudinea	Arhynchobdellida	Erpobdellidae	Nephelopsis		Erpobdellidae	Hirudinea
Arthropoda	Insecta	Trichoptera	Polycentropodidae	Neureclipsis	6	Polycentropodidae	NA
Arthropoda	Insecta	Odonata	Corduliidae	Neurocordulia		Corduliidae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Nilotanypus	7	Tanypodinae	Other_Tanypodinae
Arthropoda	Insecta	Hemiptera	Notonectidae	Notonecta	5	Notonectidae	Other_Hemiptera
Arthropoda	Insecta	Hemiptera	Notonectidae	Notonectidae	7	Notonectidae	Other_Hemiptera
Arthropoda	Insecta	Trichoptera	Polycentropodidae	Nyctiophylax	5	Polycentropodidae	NA
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Ochrotrichia	4	Ochrotrichia	Hydroptilidae
Arthropoda	Insecta	Coleoptera	Hydraenidae	Ochthebius		Ochthebius	NA
Arthropoda	Insecta	Odonata	Gomphidae	Octogomphus		Gomphidae	Gomphidae
Arthropoda	Insecta	Diptera	Chironomidae	Odontomesa	4	Prodiamesinae	Odontomesa
Arthropoda	Insecta	Diptera	Stratiomyidae	Odontomyia		Hedriodiscus/Odontomyia	Stratiomyidae
Arthropoda	Insecta	Trichoptera	Leptoceridae	Oecetis	8	Oecetis	Leptoceridae
Annelida	Oligochaeta	.	.	Oligochaeta	8	Oligochaeta	NA
Arthropoda	Insecta	Trichoptera	Uenoidae	Oligophlebodes	3	Oligophlebodes	Uenoidae
Arthropoda	Insecta	Trichoptera	Limnephilidae	Onocosmoecus	3	Onocosmoecus	Limnephilidae
Arthropoda	Insecta	Trichoptera	Limnephilidae	Onocosmoecus unicolor	3	Onocosmoecus	Limnephilidae
Annelida	Oligochaeta	Haplotaxida	Naididae	Ophidonais	6	Oligochaeta	Naididae
Annelida	Oligochaeta	Haplotaxida	Naididae	Ophidonais serpentina	6	Oligochaeta	Naididae
Arthropoda	Insecta	Odonata	Gomphidae	Ophiogomphus	5	Gomphidae	Gomphidae
Arthropoda	Insecta	Coleoptera	Elmidae	Optioservus	5	Optioservus	Optioservus
Arthropoda	Insecta	Coleoptera	Elmidae	Optioservus seriatus	4	Optioservus	Optioservus
Crustacea	Malacostraca	Decapoda	Cambaridae	Orconectes		Cambaridae	NA
Arthropoda	Insecta	Coleoptera	Elmidae	Ordobrevia	5	Ordobrevia	NA
Arthropoda	Insecta	Coleoptera	Elmidae	Ordobrevia nubifera	5	Ordobrevia	NA
Arthropoda	Insecta	Coleoptera	Dytiscidae	Oreodytes	5	Oreodytes	Dytiscidae
Arthropoda	Insecta	Diptera	Empididae	Oreogeton	4	Oreogeton	Empididae
Arthropoda	Insecta	Diptera	Tipulidae	Ormosia	6	Ormosia	Other_Tipulidae
Arthropoda	Insecta	Diptera	Chironomidae	Orthocladiinae		Orthocladiinae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Orthocladius	7	Orthocladiinae	Orthocladius

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Diptera	Chironomidae	Orthocladius annectens		Orthocladiinae	Orthocladius
Arthropoda	Insecta	Diptera	Chironomidae	Orthocladius rivulorum		Orthocladiinae	Orthocladius
Crustacea	Ostracoda	.	.	Ostracoda		Ostracoda	Ostracoda
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Oxyethira	3	Oxyethira	Hydroptilidae
Crustacea	Malacostraca	Decapoda	Astacidae	Pacifastacus	6	Astacidae	NA
Crustacea	Malacostraca	Decapoda	Astacidae	Pacifastacus leniusculus		Astacidae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Pagastia	1	Diamesinae	Pagastia
Arthropoda	Insecta	Hemiptera	Corixidae	Palmacorixa	9	Corixidae	Corixidae
Arthropoda	Insecta	Hemiptera	Corixidae	Palmacorixa buenoi	9	Corixidae	Corixidae
Arthropoda	Insecta	Hemiptera	Corixidae	Palmacorixa gillettei	9	Corixidae	Corixidae
Arthropoda	Insecta	Diptera	Chironomidae	Parachaetocladus		Orthocladiinae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Parachironomus	10	Chironominae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Paracladius	8	Orthocladiinae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Paracladopelma	7	Chironominae	Paracladopelma
Arthropoda	Insecta	Ephemeroptera	Baetidae	Paracloeodes		Paracloeodes	NA
Arthropoda	Insecta	Diptera	Chironomidae	Paracricotopus		Orthocladiinae	NA
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Paracymus		Paracymus	Hydrophilidae
Arthropoda	Insecta	Diptera	Chironomidae	Parakiefferiella	6	Orthocladiinae	Parakiefferiella
Arthropoda	Insecta	Diptera	Chironomidae	Paralauterborniella	8	Chironominae	Paralauterborniella
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Paraleptophlebia	1	Paraleptophlebia	Leptophlebiidae
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Paraleptophlebia bicornuta	2	Paraleptophlebia	Leptophlebiidae
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Paraleptophlebia debilis	1	Paraleptophlebia	Leptophlebiidae
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Paraleptophlebia heteronea	1	Paraleptophlebia	Leptophlebiidae
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Paraleptophlebia temporalis	1	Paraleptophlebia	Leptophlebiidae
Arthropoda	Insecta	Plecoptera	Leuctridae	Paraleuctra	2	Leuctridae	Leuctridae
Arthropoda	Insecta	Diptera	Chironomidae	Paramerina		Tanypodinae	Paramerina/Zavreliomyia
Arthropoda	Insecta	Diptera	Chironomidae	Parametricnemus	5	Orthocladiinae	Parametricnemus
Arthropoda	Insecta	Plecoptera	Chloroperlidae	Paraperla	1	Paraperla	NA
Arthropoda	Insecta	Diptera	Chironomidae	Paraphaenocladus	4	Orthocladiinae	NA
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Parapsyche	0	Parapsyche	Parapsyche
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Parapsyche almota		Parapsyche	Parapsyche
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Parapsyche elsis	1	Parapsyche	Parapsyche
Arthropoda	Insecta	Diptera	Chironomidae	Paratanytarsus	6	Chironominae	Paratanytarsus
Arthropoda	Insecta	Diptera	Chironomidae	Paratendipes	10	Chironominae	Paratendipes
Arthropoda	Insecta	Diptera	Chironomidae	Parochlus	1	Podonominae	NA



Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Diptera	Chironomidae	Parorthocladius	6	Orthocladiinae	Parorthocladius
Arthropoda	Insecta	Diptera	Tipulidae	Pedicia	6	Pedicia	Other_Tipulidae
Arthropoda	Insecta	Coleoptera	Haliplidae	Peltodytes	8	Peltodytes	Haliplidae
Arthropoda	Insecta	Diptera	Chironomidae	Pentaneura	6	Tanypodinae	Other_Tanypodinae
Arthropoda	Insecta	Diptera	Chironomidae	Pentaneurini	6	Tanypodinae	Other_Tanypodinae
Arthropoda	Insecta	Diptera	Psychodidae	Pericoma	4	Pericoma/Telmatoscopus	Psychodidae
Arthropoda	Insecta	Odonata	Libellulidae	Perithemis		Libellulidae	Libellulidae
Arthropoda	Insecta	Plecoptera	Perlidae	Perlidae	2	Perlidae	Perlidae
Arthropoda	Insecta	Plecoptera	Perlodidae	Perlinodes	1	Perlinodes	Perlodidae
Arthropoda	Insecta	Plecoptera	Perlodidae	Perlinodes aurea	1	Perlinodes	Perlodidae
Arthropoda	Insecta	Plecoptera	Perlodidae	Perlodidae	2	Perlodidae	Perlodidae
Arthropoda	Insecta	Plecoptera	Leuctridae	Perlomyia		Leuctridae	Leuctridae
Arthropoda	Insecta	Lepidoptera	Pyralidae	Petrophila	5	Lepidoptera	NA
Arthropoda	Insecta	Diptera	Chironomidae	Phaenopsectra	7	Chironominae	Phaenopsectra
Arthropoda	Insecta	Trichoptera	Limnephilidae	Philarctus quaeris		Philarctus	Limnephilidae
Arthropoda	Insecta	Trichoptera	Philopotamidae	Philopotamidae	3	Philopotamidae	Philopotamidae
Arthropoda	Insecta	Diptera	Blephariceridae	Philorus		Blephariceridae	NA
Arthropoda	Insecta	Trichoptera	Phryganeidae	Phryganea		Phryganeidae	NA
Arthropoda	Insecta	Trichoptera	Phryganeidae	Phryganeidae	4	Phryganeidae	NA
Mollusca	Gastropoda	Basommatophora	Physidae	Physa		Physa_Physella	Physidae
Mollusca	Gastropoda	Basommatophora	Physidae	Physella	8	Physa_Physella	Physidae
Mollusca	Gastropoda	Basommatophora	Physidae	Physidae	8	Physidae	Physidae
Arthropoda	Insecta	Diptera	Tipulidae	Pilaria	7	Pilaria	Other_Tipulidae
Annelida	Hirudinea	Rhynchobdellida	Piscicolidae	Piscicolidae		Piscicolidae	Hirudinea
Mollusca	Bivalvia	Veneroida	Pisidiidae	Pisidiidae	8	Pisidiidae	Pisidiidae
Mollusca	Bivalvia	Veneroida	Pisidiidae	Pisidium	8	Pisidiidae	Pisidiidae
Mollusca	Bivalvia	Veneroida	Pisidiidae	Pisidium milium	8	Pisidiidae	Pisidiidae
Annelida	Hirudinea	Rhynchobdellida	Glossiphoniidae	Placobdella		Glossiphoniidae	Hirudinea
Platyhelminthes	Tubellaria	Tricladida	Planariidae	Planariidae		Turbellaria	Turbellaria
Mollusca	Gastropoda	Basommatophora	Planorbidae	Planorbella		Planorbidae	Planorbidae
Mollusca	Gastropoda	Basommatophora	Planorbidae	Planorbidae	6	Planorbidae	Planorbidae
Mollusca	Gastropoda	Basommatophora	Planorbidae	Planorbula		Planorbidae	Planorbidae
Arthropoda	Insecta	Ephemeroptera	Baetidae	Plauditus	5	Plauditus	NA
Arthropoda	Insecta	Ephemeroptera	Baetidae	Plauditus punctiventris	5	Plauditus	NA
Arthropoda	Insecta	Plecoptera	Nemouridae	Podmosta	2	Podmosta	NA

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Platyhelminthes	Tubellaria	.	.	Polycelis coronata	4	Turbellaria	Turbellaria
Arthropoda	Insecta	Trichoptera	Polycentropodidae	Polycentropodidae	6	Polycentropodidae	NA
Arthropoda	Insecta	Trichoptera	Polycentropodidae	Polycentropus	6	Polycentropus	NA
Arthropoda	Insecta	Diptera	Chironomidae	Polypedilum	6	Chironominae	Polypedilum
Porifera	Hydrozoa	.	.	Porifera		Porifera	NA
Mollusca	Gastropoda	Neotaenioglossa	Hydrobiidae	Potamopyrgus antipodarum		Potamopyrgus	NA
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Potamyia	4	Potamyia	NA
Arthropoda	Insecta	Diptera	Chironomidae	Potthastia	2	Diamesinae	Potthastia
Arthropoda	Insecta	Diptera	Chironomidae	Potthastia gaedii	3	Diamesinae	Potthastia
Arthropoda	Insecta	Diptera	Chironomidae	Potthastia longimana	4	Diamesinae	Potthastia
Annelida	Oligochaeta	Haplotaxida	Naididae	Pristina	10	Oligochaeta	Naididae
Arthropoda	Insecta	Diptera	Chironomidae	Procladius	9	Tanypodinae	Procladius
Arthropoda	Insecta	Ephemeroptera	Baetidae	Procloeon	7	Procloeon	NA
Arthropoda	Insecta	Diptera	Chironomidae	Prodiamesa	3	Prodiamesinae	Prodiamesa
Arthropoda	Insecta	Diptera	Simuliidae	Prosimulium	3	Simuliidae	Simuliidae
Arthropoda	Insecta	Plecoptera	Nemouridae	Prostoia besametsa	3	Prostoia	NA
Nemertea	Enopla	Hoploneuridae	Tetrastemmatidae	Prostoma		Nemertea	NA
Arthropoda	Insecta	Diptera	Tanyderidae	Protanyderus	5	Tanyderidae	NA
Arthropoda	Insecta	Trichoptera	Glossosomatidae	Protoptila	1	Protoptila	Glossosomatidae
Arthropoda	Insecta	Diptera	Chironomidae	Psectrocladius	8	Orthocladiinae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Psectrotanypus	10	Tanypodinae	Macropelopiini
Arthropoda	Insecta	Diptera	Chironomidae	Pseudochironomus	5	Chironominae	Pseudochironomus
Arthropoda	Insecta	Ephemeroptera	Baetidae	Pseudocloeon	4	Pseudocloeon	NA
Arthropoda	Insecta	Diptera	Chironomidae	Pseudodiamesa	2	Diamesinae	Pseudodiamesa
Arthropoda	Insecta	Diptera	Chironomidae	Pseudorthocladius	0	Orthocladiinae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Pseudosmittia	6	Orthocladiinae	NA
Mollusca	Gastropoda	Basommatophora	Lymnaeidae	Pseudosuccinea		Lymnaeidae	Lymnaeidae
Arthropoda	Insecta	Diptera	Psychodidae	Psychoda	4	Psychoda	Psychodidae
Arthropoda	Insecta	Diptera	Psychodidae	Psychodidae	4	Psychodidae	Psychodidae
Arthropoda	Insecta	Trichoptera	Limnephilidae	Psychoglypha	0	Psychoglypha	Limnephilidae
Arthropoda	Insecta	Trichoptera	Psychomyiidae	Psychomyia	2	Psychomyia	NA
Arthropoda	Insecta	Trichoptera	Psychomyiidae	Psychomyia flavida	2	Psychomyia	NA
Arthropoda	Insecta	Plecoptera	Pteronarcyidae	Pteronarcella	4	Pteronarcella	NA
Arthropoda	Insecta	Plecoptera	Pteronarcyidae	Pteronarcella badia	3	Pteronarcella	NA
Arthropoda	Insecta	Plecoptera	Pteronarcyidae	Pteronarcyidae	2	Pteronarcyidae	NA

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Plecoptera	Pteronarcyidae	Pteronarcys	2	Pteronarcys	NA
Arthropoda	Insecta	Plecoptera	Pteronarcyidae	Pteronarcys californica	2	Pteronarcys	NA
Arthropoda	Insecta	Plecoptera	Pteronarcyidae	Pteronarcys dorsata	2	Pteronarcys	NA
Arthropoda	Insecta	Trichoptera	Phryganeidae	Ptilostomis		Phryganeidae	NA
Arthropoda	Insecta	Diptera	Ptychopteridae	Ptychoptera	7	Ptychopteridae	Ptychopteridae
Arthropoda	Insecta	Trichoptera	Limnephilidae	Pycnopsyche		Pycnopsyche	Limnephilidae
Mollusca	Gastropoda	Neotaenioglossa	Hydrobiidae	Pyrgulopsis		Hydrobiidae	NA
Annelida	Oligochaeta	Haplotaxida	Tubificidae	Quistradrilus multisetosus		Oligochaeta	Tubificidae
Mollusca	Gastropoda	Basommatophora	Lymnaeidae	Radix auricularia		Radix auricularia	Lymnaeidae
Arthropoda	Insecta	Hemiptera	Nepidae	Ranatra	7	Ranatra	Other_Hemiptera
Arthropoda	Insecta	Diptera	Tipulidae	Rhabdomastix	1	Rhabdomastix	Rhabdomastix
Arthropoda	Insecta	Coleoptera	Dytiscidae	Rhantus		Rhantus	Dytiscidae
Arthropoda	Insecta	Diptera	Chironomidae	Rheocricotopus	4	Orthoclaadiinae	Rheocricotopus
Arthropoda	Insecta	Diptera	Chironomidae	Rheosmittia		Orthoclaadiinae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Rheotanytarsus	6	Chironominae	Rheotanytarsus
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Rhithrogena	0	Rhithrogena	Rhithrogena
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Rhithrogena robusta	0	Rhithrogena	Rhithrogena
Annelida	Oligochaeta	Haplotaxida	Tubificidae	Rhyacodrilus		Oligochaeta	Tubificidae
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila	1	Rhyacophila	NA
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila alberta	0	Rhyacophila alberta gr.	Rhyacophila_alberta_gr
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila angelita	0	Rhyacophila angelita gr.	Rhyacophila_Angelita_Gr
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila arnaudi		Rhyacophila arnaudi	NA
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila betteni	1	Rhyacophila betteni gr.	Rhyacophila_Betteni_Gr
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila blarina		Rhyacophila sibirica gr.	NA
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila brunnea	0	Rhyacophila brunnea/vemna Gr.s	Rhyacophila_Brunnea_Gr
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila coloradensis	0	Rhyacophila coloradensis gr.	NA
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila hyalinata	1	Rhyacophila hyalinata gr.	Rhyacophila_Hyalinata_Gr
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila iranda		Rhyacophila vofixa gr.	NA
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila narvae	0	Rhyacophila sibirica gr.	Rhyacophila_narvae
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila nevadensis		Rhyacophila nevadensis gr.	NA
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila pellisa	0	Rhyacophila sibirica gr.	Rhyacophila_pellisa/valuma
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila rotunda		Rhyacophila rotunda gr.	NA
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila sibirica		Rhyacophila sibirica gr.	NA
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila vagrita	0	Rhyacophila vagrita gr.	NA
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila verrula	0	Rhyacophila verrula gr.	Rhyacophila_Verrula_Gr

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila vofixa	0	Rhyacophila vofixa gr.	Rhyacophila_vofixa_Gr
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophilidae	0	Rhyacophilidae	NA
Annelida	Oligochaeta	Lumbriculida	Lumbriculidae	Rhynchelmis		Oligochaeta	Rhynchelmis
Arthropoda	Insecta	Diptera	Chironomidae	Robackia	4	Chironominae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Saetheria	8	Chironominae	NA
Arthropoda	Insecta	Hemiptera	Saldidae	Saldula		Saldula	Other_Hemiptera
Arthropoda	Insecta	Diptera	Sciomyzidae	Sciomyzidae		Sciomyzidae	NA
Arthropoda	Insecta	Trichoptera	Uenoidae	Sericostriata		Sericostriata	Uenoidae
Arthropoda	Insecta	Trichoptera	Uenoidae	Sericostriata surdickae		Sericostriata	Uenoidae
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Serratella	2	Serratella	Serratella
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Serratella micheneri	0	Serratella	Serratella
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Serratella tibialis	2	Serratella	Serratella
Arthropoda	Insecta	Plecoptera	Perlodidae	Setvena	0	Setvena	Perlodidae
Arthropoda	Insecta	Plecoptera	Perlodidae	Setvena bradleyi	0	Setvena	Perlodidae
Arthropoda	Insecta	Hemiptera	Sialidae	Sialis	4	Sialis	Sialis
Arthropoda	Branchiopoda	Diplostraca	Sididae	Sididae		Cladocera	Cladocera
Arthropoda	Insecta	Hemiptera	Corixidae	Sigara	9	Corixidae	Corixidae
Arthropoda	Insecta	Hemiptera	Corixidae	Sigara alternata		Corixidae	Corixidae
Arthropoda	Insecta	Hemiptera	Corixidae	Sigara decorata		Corixidae	Corixidae
Arthropoda	Insecta	Hemiptera	Corixidae	Sigara decoratella		Corixidae	Corixidae
Arthropoda	Insecta	Hemiptera	Corixidae	Sigara grossolineata		Corixidae	Corixidae
Arthropoda	Insecta	Hemiptera	Corixidae	Sigara lineata		Corixidae	Corixidae
Arthropoda	Insecta	Diptera	Simuliidae	Simuliidae	6	Simuliidae	Simuliidae
Arthropoda	Insecta	Diptera	Simuliidae	Simulium	5	Simuliidae	Simuliidae
Arthropoda	Insecta	Ephemeroptera	Siphonuridae	Siphonurus	2	Siphonuridae	NA
Arthropoda	Insecta	Plecoptera	Perlodidae	Skwala	3	Skwala	Perlodidae
Mollusca	Bivalvia	Veneroida	Sphaeriidae	Sphaeriidae	8	Pisidiidae	Pisidiidae
Mollusca	Bivalvia	Veneroida	Pisidiidae	Sphaerium	8	Pisidiidae	Pisidiidae
Mollusca	Gastropoda	Basommatophora	Lymnaeidae	Stagnicola	6	Lymnaeidae	Lymnaeidae
Arthropoda	Insecta	Diptera	Chironomidae	Stempellina	2	Chironominae	Stempellina
Arthropoda	Insecta	Diptera	Chironomidae	Stempellinella	4	Chironominae	Stempellinella
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Stenacron	4	Stenacron	NA
Arthropoda	Insecta	Coleoptera	Elmidae	Stenelmis	5	Stenelmis	NA
Arthropoda	Insecta	Coleoptera	Elmidae	Stenelmis occidentalis		Stenelmis	NA
Arthropoda	Insecta	Diptera	Chironomidae	Stenochironomus	5	Chironominae	NA

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Diptera	Chironomidae	Stictochironomus	5	Chironominae	Stictochironomus
Arthropoda	Insecta	Coleoptera	Dytiscidae	Stictotarsus		Stictotarsus	Dytiscidae
Arthropoda	Insecta	Diptera	Chironomidae	Stilocladius	6	Orthoclaadiinae	Stilocladius
Arthropoda	Insecta	Diptera	Stratiomyidae	Stratiomyidae	7	Stratiomyidae	Stratiomyidae
Arthropoda	Insecta	Diptera	Stratiomyidae	Stratiomys		Stratiomys	Stratiomyidae
Arthropoda	Insecta	Odonata	Gomphidae	Stylurus		Gomphidae	Gomphidae
Arthropoda	Insecta	Diptera	Chironomidae	Sublettea	2	Chironominae	NA
Arthropoda	Insecta	Plecoptera	Chloroperlidae	Suwallia	1	Chloroperlidae	Suwallia
Arthropoda	Insecta	Plecoptera	Chloroperlidae	Sweltsa	0	Chloroperlidae	Sweltsa
Arthropoda	Insecta	Odonata	Libellulidae	Sympetrum	9	Libellulidae	Libellulidae
Arthropoda	Insecta	Diptera	Chironomidae	Symposiocladius	6	Orthoclaadiinae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Sympotthastia	2	Diamesinae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Synendotendipes		Orthoclaadiinae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Synorthoclaadius	2	Orthoclaadiinae	NA
Arthropoda	Insecta	Diptera	Tabanidae	Tabanidae	10	Tabanidae	Tabanidae
Arthropoda	Insecta	Diptera	Tabanidae	Tabanus	10	Tabanidae	Tabanidae
Arthropoda	Insecta	Plecoptera	Taeniopterygidae	Taenionema	2	Taeniopterygidae	Taeniopterygidae
Arthropoda	Insecta	Plecoptera	Taeniopterygidae	Taeniopterygidae	2	Taeniopterygidae	Taeniopterygidae
Arthropoda	Insecta	Diptera	Chironomidae	Tanypodinae		Tanypodinae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Tanypus	10	Tanypodinae	Tanypus
Arthropoda	Insecta	Diptera	Chironomidae	Tanytarsini	6	Chironominae	NA
Arthropoda	Insecta	Diptera	Chironomidae	Tanytarsus	6	Chironominae	Tanytarsus
Arthropoda	Insecta	Diptera	Thaumaleidae	Thaumalea		Thaumaleidae	NA
Arthropoda	Insecta	Diptera	Thaumaleidae	Thaumaleidae		Thaumaleidae	NA
Annelida	Hirudinea	Rhynchobdellida	Glossiphoniidae	Theromyzon		Glossiphoniidae	Hirudinea
Arthropoda	Insecta	Diptera	Chironomidae	Thienemanniella	6	Orthoclaadiinae	Thienemanniella
Arthropoda	Insecta	Diptera	Chironomidae	Thienemannimyia		Tanypodinae	Thienemannimyia_Gr
Arthropoda	Insecta	Diptera	Chironomidae	Thienemanniola		Chironominae	NA
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Timpanoga	2	Timpanoga	NA
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Timpanoga hecuba	2	Timpanoga	NA
Arthropoda	Insecta	Diptera	Tipulidae	Tipula	4	Tipula	Tipula
Arthropoda	Insecta	Diptera	Tipulidae	Tipulidae	3	Tipulidae	Other_Tipulidae
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Traverella	2	Traverella	Leptophlebiidae
Arthropoda	Insecta	Trichoptera	Leptoceridae	Triaenodes	6	Triaenodes	Leptoceridae
Arthropoda	Insecta	Diptera	Chironomidae	Tribelos		Chironominae	NA

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Diptera	Empididae	Trichoclinocera		Trichoclinocera	Empididae
Arthropoda	Insecta	Hemiptera	Corixidae	Trichocorixa	9	Corixidae	Corixidae
Arthropoda	Insecta	Hemiptera	Corixidae	Trichocorixa borealis	9	Corixidae	Corixidae
Arthropoda	Insecta	Trichoptera	.	Trichoptera		Trichoptera	NA
Platyhelminthes	Tubellaria	Tricladida	.	Tricladida	4	Tubellaria	Tubellaria
Arthropoda	Insecta	Ephemeroptera	Tricorythidae	Tricorythidae	4	Tricorythidae	NA
Arthropoda	Insecta	Ephemeroptera	Leptohyphidae	Tricorythodes	4	Tricorythodes	Tricorythodes
Arthropoda	Insecta	Ephemeroptera	Leptohyphidae	Tricorythodes minutus	4	Tricorythodes	Tricorythodes
Arthropoda	Insecta	Plecoptera	Chloroperlidae	Triznaka		Chloroperlidae	NA
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Tropisternus		Tropisternus	Hydrophilidae
Annelida	Oligochaeta	Haplotaxida	Tubificidae	Tubifex		Oligochaeta	Tubificidae
Annelida	Oligochaeta	Haplotaxida	Tubificidae	Tubifex tubifex	10	Oligochaeta	Tubificidae
Annelida	Oligochaeta	Haplotaxida	Tubificidae	Tubificidae	10	Oligochaeta	Tubificidae
Platyhelminthes	Tubellaria	.	.	Tubellaria	4	Tubellaria	Tubellaria
Arthropoda	Insecta	Diptera	Chironomidae	Tvetenia	5	Orthoclaadiinae	Tvetenia
Arthropoda	Insecta	Diptera	Chironomidae	Tvetenia bavarica		Orthoclaadiinae	Tvetenia
Arthropoda	Insecta	Diptera	Chironomidae	Tvetenia discoloripes		Orthoclaadiinae	Tvetenia
Arthropoda	Insecta	Diptera	Chironomidae	Tvetenia vitracies		Orthoclaadiinae	Tvetenia
Arthropoda	Insecta	Diptera	Simuliidae	Twinnia	7	Simuliidae	Simuliidae
Arthropoda	Insecta	Trichoptera	Uenoidae	Uenoidae	2	Uenoidae	Uenoidae
Mollusca	Bivalvia	Unionida	Unionidae	Unionidae		Unionidae	NA
Mollusca	Gastropoda	Heterostropha	Valvatidae	Valvata	3	Valvata	NA
Mollusca	Gastropoda	Heterostropha	Valvatidae	Valvatidae	3	Valvatidae	NA
Arthropoda	Insecta	Plecoptera	Nemouridae	Visoka cataractae	0	Visoka	Visoka
Arthropoda	Insecta	Diptera	Empididae	Wiedemannia		Wiedemannia	Empididae
Arthropoda	Insecta	Trichoptera	Philopotamidae	Wormaldia	0	Wormaldia	Philopotamidae
Arthropoda	Insecta	Diptera	Chironomidae	Xenochironomus	4	Chironominae	NA
Arthropoda	Insecta	Trichoptera	Leptoceridae	Ylodes		Ylodes	Leptoceridae
Arthropoda	Insecta	Plecoptera	Peltoperlidae	Yoraperla	0	Yoraperla	Peltoperlidae
Arthropoda	Insecta	Plecoptera	Peltoperlidae	Yoraperla brevis		Yoraperla	Peltoperlidae
Arthropoda	Insecta	Coleoptera	Elmidae	Zaitzevia	5	Zaitzevia	Zaitzevia
Arthropoda	Insecta	Coleoptera	Elmidae	Zaitzevia parvulus		Zaitzevia	Zaitzevia
Arthropoda	Insecta	Plecoptera	Nemouridae	Zapada	2	Zapada	NA
Arthropoda	Insecta	Plecoptera	Nemouridae	Zapada cinctipes	3	Zapada	Zapada_cinctipes
Arthropoda	Insecta	Plecoptera	Nemouridae	Zapada columbiana	2	Zapada	Zapada_columbiana

Phyla	Class	Order	Family	FinalID	TolVal	OTU_2005	OTU_2011
Arthropoda	Insecta	Plecoptera	Nemouridae	Zapada frigida	1	Zapada	NA
Arthropoda	Insecta	Plecoptera	Nemouridae	Zapada Oregonensis		Zapada	Zapada_oregonensis_gr
Arthropoda	Insecta	Diptera	Chironomidae	Zavreliomyia	8	Tanypodinae	Paramerina/Zavreliomyia
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Zumatrichia	3	Zumatrichia	Hydroptilidae
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Zumatrichia notosa	3	Zumatrichia	Hydroptilidae







## **APPENDIX C - EXAMPLE OUTPUT FOR THE OBSERVED/EXPECTED MODELS**

The following page shows an example output table from EDAS (Tetra Tech 2006a). These are used to illustrate index results for samples. The EDAS output result comes as a direct output saved as an MS Excel (2003) spreadsheet (**Table C-1**). The spreadsheet contains all of the relevant sampling location information and O/E values. EDAS can also generate Multimetric Index values if a project requires them (not shown in the example).

**Table C-1. EDAS output table example.**

Station ID	Waterbody Name	Lat_Dec	Long_Dec	Cld Wrm Type	SiteClass	Four Code HUC	TMDL Planning Area	Reference	ActivityID	CollDate	CollMeth	Ben Samp ID	Rep Num	OE model test	Total OE Bugs	O/E_2005	BC_2005	CW_OE_2011	CW_outlier 05_2011	CW_BC_pc>half_2011
C04FIREC01	Fire Creek	46.8884031	-114.804544	Cold	Mountains	17010204	MIDDLE CLARK FORK	Reference	2523-MAC-R	13-Jun-06	MAC-R-500	17087	0	P	300	0.80	0.62	0.64	1	0.43
C04FIREC01	Fire Creek	46.8884031	-114.804544	Cold	Mountains	17010204	MIDDLE CLARK FORK	Reference	2705-MAC-R	08-Sep-06	MAC-R-500	17089	0	P	300	0.80	0.14	0.94	0	0.25

## **APPENDIX D - MONTANA DEPARTMENT OF ENVIRONMENTAL QUALITY BENTHIC MACROINVERTEBRATE OBSERVED/EXPECTED MANUAL**

### **D1.0 Introduction**

#### **D1.1 Background**

This manual is intended to guide users in creating and applying predictive models of observed and expected (O/E) benthic macroinvertebrate taxa in samples from streams in Montana. O/E models have been developed in 2005 and 2011 by consultants to the Montana Department of Environmental Quality (DEQ). With this guidance and associated software, development of new models and application of existing models should be possible for DEQ staff.

The O/E model is an assessment tool that can be used to determine the degree to which a sample contains the number and type of taxa expected after considering relevant characteristics of each site's environmental setting. Literature on the O/E predictive models is abundant, and the theories behind the responsiveness of the models to stress can be reviewed in those articles (Clarke et al. 1996, Clarke et al. 2002, Clarke et al. 2003, Hawkins and Carlisle 2001, Hawkins et al. 2000, Hawkins and Norris 2000, Hawkins et al. 2009, Hubler 2008, Van Sickle et al. 2005, Van Sickle et al. 2006, and Van Sickle et al. 2007). The Bray-Curtis (BC) index is used as a secondary indicator and its development and application are also presented in this document. It is developed using theories similar to the O/E index (Van Sickle 2008).

#### **D1.2 Purpose and Goals**

This document is intended as a guide for developing, interpreting, and applying O/E and BC indices. It is especially tailored to the needs of DEQ in assessing benthic macroinvertebrate conditions in streams of Montana. Following the steps of this manual should allow DEQ staff to calibrate new predictive models, interpret the model output, and apply developed models for assessment purposes.

#### **D1.3 Data organization and software**

Data for analyses must conform to DEQ standards for sample collection for taxa and other data standards for reference designations and predictor data. The necessary information for development and application of the O/E and BC indices include two types: One of taxonomic composition in samples and another of site and sample characteristics (predictors) for the sampling locations. DEQ uses the Ecological Data Analysis System (EDAS) for generating macroinvertebrate indicator of water quality results. EDAS is also used to generate taxonomic and predictor data in formats that can be used for model development and application. Some formats require further manipulation after export from EDAS. In this manual, we assume that the analyst is familiar with EDAS and its functions. Specific format for analyses will be described in following sections.

The instructions include references to existing software. While some details on the use of the software are provided, analysts are expected to have basic knowledge of the software and can investigate software application issues through other manuals specific to those programs. The software referenced most often includes Excel (Microsoft Corporation), PC-ORD (McCune and Mefford 2006), R (<http://www.r-project.org>), Statistica (StatSoft, Inc.), and "SubSample.exe" (a DOS program). Alternative software could be used for some steps of the analyses.

## D2.0 Applying an Observed/Expected model

Once the O/E and BC models are built on calibration data and saved, they can be applied to test data. Test data are samples from any site that was not used as a reference sample in the model calibration. This could include samples from reference-quality sites sampled in years following the calibration effort or from sites that have some know degree of disturbance.

### D2.1 Required files and scripts

The files required to calculate O/E scores from an established model include 2 data files and multiple R scripts. The taxa and predictor matrix should have the same format described for model building. Taxa identifiers should be the 8-character OTUs, predictors must include all predictors used in the models, and records for the taxa and predictor files must have the same sample identifiers in the same order. The same predictor file used for model building can be used for model application, where the 'RefCalVal' field includes 'test' designations. Only the sample identifiers and the model predictors are required in the test predictor file, but the other fields can be included as well.

The model can be applied either at the end of the 'model.build.V4.1.R' script or from the 'model.apply.V4.1.R' script. During its execution, the script calls on other files, including 'model.predict.v4.1.r', 'MyModel.Version1.Rdata', and 'assess.one.sample.4.1.r'. If new data are prepared for calculation of O/E and BC scores, first create the taxon and predictor files as described above. Then run the 'model.apply.V4.1.R' script as described below.

The required fields in the predictor files are as follow. All spellings and cases must be exact.

SampID	Also called BenSamp-Rep (rename to SampID)
RefCalVal	With allowed values 'Calib', 'Valid', or 'test' (case sensitive) (optional field)
ELVsd_WS	Standard deviation of Elevation in the watershed
GIS_LAT	Latitude
JulianDay	Sequential day of the year (cold only)
log_sqkm	logarithm of watershed square kilometers (also called 'log_km2' [rename])
Mtns1	Designation of the mountains ecoregions (1), or not (0)
Tmax_PT	Maximum monthly average temperature at the point (also TMAX_PT_2011)
FFP_PT	Frost-Free Period Point (For Warm-Water model only)
WDmin_PT	Minimum number of wet days (For Warm-Water model only)

There are alternate names for two variables because these were also used in the 2005 O/E model. In the case of Tmax\_PT, the value is calculated differently among models, so that in EDAS the value is labeled Tmax\_PT\_2011, but it is recognized in the model as 'Tmax\_PT'.

### D2.2 Running model application R code

The model application R code is named 'model.apply.V4.1.R'. The code is run in sections so that errors can be corrected sequentially (and to avoid wasting time running subsequent erred sections). In the next report section, the code for the cold-water model is contained in text boxes. It is recommended that the code be run in the blocks between bullets as shown below. All model development files should be kept

in one file folder (or “directory”). Retaining distinct runs in separate directories is a good idea when experimenting and interpreting.

- Introduction and set-up.

```
# R code modified to allow application only (cut out much of the building steps)

# Ben Jessup and Dave Feldman modified original code by John Van Sickle, US Environmental
Protection Agency;
#Version 3: For application of an existing model (established through the model.build.v4.1 script)

#####;

## First clear the workspace
rm(list=ls())

#load required packages;
library(gtools); library(MASS);
library(cluster); library(Hmisc);
require(scatterplot3d)
require(maps)

# change directory based on where have script and data files (EWL)
# but to keep code the same substitute with "getwd"
# change the working directory to the correct one using "setdir". Notice that the filepath uses "/"
setdir ("C:/working directory")
myDir <- getwd()
```

- Load the predictor file and display example data, which should be in the same directory as the script.

```
# STEP 1 -- INITIAL SETUP -- Input and organize the bug and predictor data;
# The exact code for this step is very particular for each data set, ;
# but similar operations will need to be done on every data set;
# Data setup below is an example which contains both reference and nonreference sites;

#####;
# Input data are predictor data and a (site x taxa) matrix of abundance for all bugs;
# Predictor data file must include a column to identify the calibration, validation and test sites;

# Step 1a - Read and organize predictor data;
# Input the predictor data, tab delimited. Use the sample/site ID as the row name;

# Import Data
myFile = "data.cold.preds.tst.tab"
predall=read.delim(myFile,row.names=1)

head(predall); #look at 1st 5 rows, all columns;
dim(predall); # number of rows and columns;
```

- Load the bug file and display dimensions of the file (# rows and # columns)

```
## Step 1b - Input the assemblage data (bug data), as a site-by-taxa matrix;
# The bug matrix is the result of fixed-count subsampling and matlify or cross-tab functions;

bugall<-read.table(paste(myDir,"data.cold.bugs.tst.tab",sep="/"),row.names="SampID",
                  header=T,sep="\t");
dim(bugall)
```

- Make sure that the predictor and bug files have records in the same order, with the same identifiers.
- If records are aligned, “true” will appear for every record.

```
## Step 1c - Align bug and predictor data, by site/sample;
#check sample(row) alignment of bug and predictor data;
row.names(bugall)==row.names(predall);
# If samples are not aligned, fix by aligning bugs data to predictor data;
bugall<-bugall[row.names(predall),];
#check alignment again -- alignment OK;
row.names(bugall)==row.names(predall);
```

- Change taxa abundance to presence/absence.

```
#Create a Presence/absence (1/0) matrix (site by taxa) for the bugs;
bugall.pa<-bugall;
bugall.pa[bugall.pa>0]<-1;
```

- The following lines are mostly informative.
- The source and load lines call on scripts and files that should be in the same directory as the script.
- The last line displays taxa names.

```

# Step 7 (continuation of steps in model.build.v4.1) - Making predictions for new (test) data.
# first, source the prediction script and also load the desired model;
source("model.predict.v4.1.r");
load('MyModel.Version1.Rdata');

# User must supply a sample-by-taxa matrix of taxa abundance or else presence/absence
#(coded as 1 or 0), for all new samples;
# User must also supply a corresponding file of predictor data for those same samples;
# These 2 files should have similar formats as the original taxa and predictor data sets;
# Notes on file formats --
# A) The sample ID column in both files should be read into R as a row name (see Step 1 examples).
# B) Predictor data set -- Must include columns with the same names, units, etc.,
# as the model's predictor variables. All other columns will be ignored;
# Column order does not matter;
# Predictions and calculations of O/E will be made only for those samples that have;
# complete data for all model predictors.;
# C) Sample-by-taxa matrix. Can contain abundance or presence/absence (1 or 0).
# Missing or empty cells now allowed;
# Sample ID's (row names) must match those of predictor data.
# Any names for new taxa (column names) are acceptable, in any order;
# HOWEVER - Only those new-data taxa names that match the names in the
# calibration data can be used to calculate observed richness;
# All other taxa (columns) in the new-data bug matrix are ignored;
# To see a list of the calibration-taxa names, do:
names(bugcal.pa)[colSums(bugcal.pa)>0];

```

- Define subset of data if desired
- Limit analysis to sites with complete data

```

# Example predictions: non-reference sites are labeled "test" (see Step 1);

pred.test<-predall; #predictor data - ALL sites;
bug.test.pa<-bugall.pa; #Bug presence/absence matrix, ALL sites;

#To limit to test sites only, use this code;
#pred.test<-predall[as.character(predall[, 'RefCalVal'])=='test',];
#bug.test.pa<-bugall.pa[as.character(predall[, 'RefCalVal'])=='test',];

#Drop all samples/sites that do not have complete data for the model predictors;
pred.test<-pred.test[complete.cases(pred.test[,preds.final]),];
bug.test.pa<-bug.test.pa[row.names(pred.test),];

```

- Calculate and display O/E, BC, and outlier status
- Results are not automatically saved and should be copied from the R console



- Looking at capture probabilities and predicted group occurrences is optional

```
#makes predictions for test data; *** Check PC at end of line, modify if needed
  #(may want to run BC at PC=1.e-14) **
OE.assess.test<-model.predict.v4.1(bugcal.pa,grps.final,preds.final,
  grpmns,covpinv,prednew=pred.test,bugnew=bug.test.pa,Pc=0.5);

# Look at O/E and BC scores of test-data samples;
OE.assess.test$OE.scores;
##### COPY these from the R console to a spreadsheet

# Look at predicted capture probabilities (1st 5 rows) for all calibration taxa, for test data;
Head (OE.assess.test$Capture.Probs);
# Look at predicted group occurrence probabilities, for all test samples;
OE.assess.test$Group.Occurrence.Probs;
```

- Calculate increaser/decreaser statistics
- Results are not automatically saved and should be copied from the R console
- For each of taxon, we list the 'Sensitivity Index'. The index is calculated from the average predicted probability of detection (assuming sites were under reference condition), the number of test sites at which taxa were predicted to occur, the number of test sites at which taxa were observed, and the ratio of observed sites to expected sites for each taxon (the 'Sensitivity Index'). We interpret it as a measure of sensitivity of a taxon to whatever stressors are influencing a taxon within the set of test sites submitted for assessment. A ratio > 1 indicates the taxon was found at more sites than expected and was thus an 'increaser' or tolerant taxon. A ratio < 1 indicates the taxon was found at fewer sites than expected and was thus a 'decreaser' or intolerant taxon.

```
# Increaser/Decreaser taxa
## use the output from this (in test sites) to find increasers and decreasers
## also called the sensitivity index
## Increaser values will be >1 (or >2 for certain increasers)
## Decreaser values will be <1 (or <0.5 for certain decreasers)

# limit above to test sites only
taxa.pc<-OE.assess.test$Capture.Probs[as.character(predall[, 'RefCalVal'])=='test',];
bug.test.pa<-bugall.pa[as.character(predall[, 'RefCalVal'])=='test',]; #Bug presence/absence matrix,
test sites;
# calculate observed occurrences in all test sites over predicted occurrences in all test sites
Inc.dec.taxa<-apply(bug.test.pa,2,sum) / apply(taxa.pc,2,sum);
Inc.dec.taxa
```

- Calculating O/E for an individual sample
- This gives more detailed results than the routine above, especially for individual taxa
- First identify the script and load the data
- Other lines are informative

```

## Assessing an individual sample or site;
  source("assess.one.sample.4.1.r")
  bug.test.pa<-bugall.pa
#This function assesses a single site or sample from a new (test) data set to which
# model.predict.v4.1() has already been applied.
# assess.one.sample() compares observed occurrences with the model-predicted
# probabilities of occurrence for all taxa;

# Input parameters are:
# case -- A selected sample ID, for which a prediction has already been made ;
  # The selected case must be among those assessed above;
# result.prd -- Output from O/E calculation above.
# bugnew -- Sample-by-taxa matrix of new samples.
# Pc -- Cutoff for capture probabilities for inclusion of taxa in O/E;

```

- Enter the name of the sample between quotes after *case=*

```

# The function produces a data frame with one row per taxon, and the following columns:
# observed presence(1) or absence(0);
# predicted capture probability;
# Big.diff = "Yes", if there is a big difference (>=0.5) between observed and predicted;
# In.OtoE = "Yes" if the taxon would be included in the O/E calculation for this sample,
  # given the stated value of Pc;

#By default, the function displays the results with its rows (taxa) sorted by
  # the magnitude of (observed-predicted),
  # as suggested in Van Sickle, J. (2008), JNABS 27:227-235;

site1.result<-assess.one.sample.4.1(case="11591_0",result.prd=OE.assess.test, bugnew=bug.test.pa,
Pc=0.5);

# See below for other sorting possibilities;
# Alternative display is to sort the taxa by their predicted occurrence probabilities;
site1.result[order(site1.result$predicted,decreasing=TRUE),];
# Another alternative is to sort alphabetically by taxon name;
site1.result[order(row.names(site1.result)),];

```

- End of script

## References Cited

- Clarke, R.T., M.T. Furse, J.F. Wright, and D. Moss. 1996. Derivation of a biological quality index for river sites: comparison of the observed with the expected fauna. *Journal of Applied Statistics* 23:311-332.
- Clarke, R.T., M.T. Furse, R.J.M. Gunn, J.M. Winder, and J.F. Wright. 2002. Sampling variation in macroinvertebrate data and implications for river quality indices. *Freshwater Biology* 47:1735-1751.
- Clarke, R.T., J.F. Wright, and M.T. Furse. 2003. RIVPACS models for predicting the expected macroinvertebrate fauna and assessing the ecological quality of rivers. *Ecological Modeling* 160:219-233.
- Hawkins, C.P., R.H. Norris, J.N. Hogue, and J.W. Feminella. 2000. Development and evaluation of predictive models for measuring the biological integrity of streams. *Ecological Applications* 10:1456-1477.
- Hawkins, C.P. and R.H. Norris. 2000. Effects of taxonomic resolution and use of subsets of the fauna on the performance of RIVPACS-type models. Pages 217-228 in J.F. Wight, D.W. Sutcliffe, and M.T. Furse, editors. *Assessing the biological quality of fresh waters: RIVPACS and other techniques*. Freshwater Biological Association, Ambleside, Cumbria, UK.
- Hawkins, C.P. and D.M. Carlisle. 2001. Use of predictive models for assessing the biological integrity of wetlands and other aquatic habitats. *Bioassessment and management of North American Wetlands* Pages 59-83 in R.B. Rader, D.P. Batzer. John Wiley & Son, New York.
- Hawkins, Charles P. 2009. Revised Invertebrate RIVPACS Model and O/E Index for Assessing the Biological Condition of Colorado Streams. *Prepared for*: Colorado Department of Public Health and Environment, Water Quality Control Division – Monitoring Unit, Denver, Colorado. *Prepared by*: Western Center for Monitoring and Assessment of Freshwater Ecosystems, Department of Watershed Sciences, Utah State University, Logan, Utah.
- Hubler, S. 2008. PREDATOR: Development and use of RIVPACS-type macroinvertebrate models to assess the biotic condition of wadeable Oregon streams Oregon Department of Environmental Quality. DEQ08-LAB-0048-TR.
- McCune, B. and M. J. Mefford. 2006. PC-ORD. Multivariate Analysis of Ecological Data. Version 5.18. MjM Software, Gleneden Beach, Oregon, U.S.A.
- Suplee, M., R. Sada de Suplee, D. Feldman, and T. Laidlaw. 2005. Identification and Assessment of Montana Reference Streams: A Follow-up and Expansion of the 1992 Benchmark Biology Study (DRAFT 2.5). Montana Department of Environmental Quality, Planning, Prevention and Assistance Division, Water Quality Planning Bureau, Water Quality Standards Section, 1520 E. 6th Ave, Helena, MT.
- Van Sickle, J., C.P. Hawkins, D.P. Larsen, and A.H. Herlihy. 2005. A null model for the macroinvertebrate assemblage expected in unimpaired streams. *Journal of the North American Benthological Society* 24:178-191.

Van Sickle, J., D.D. Huff, C.P. Hawkins. 2006. Selecting discriminant function models for predicting the expected richness of aquatic macroinvertebrates. *Freshwater Biology* 51:359–372.

Van Sickle, J., D.P. Larsen, C.P. Hawkins. 2007. Exclusion of rare taxa affects performance of the O/E index in bioassessments. *Journal of the North American Benthological Society* 26(2):319-331. 2007.

Van Sickle, J. 2008. An index of compositional dissimilarity between observed and expected assemblages. *Journal of the North American Benthological Society* 27(2):227–235.



## **ATTACHMENT 1 – SITE VISIT FORM**

Place Site Visit Label Here

## Site Visit Form

(One Station per page)

Project ID: \_\_\_\_\_

Date: \_\_\_\_\_ Time: \_\_\_\_\_ Personnel: \_\_\_\_\_

Waterbody: \_\_\_\_\_ Location: \_\_\_\_\_

Station ID: \_\_\_\_\_ Visit #: \_\_\_\_\_ HUC: \_\_\_\_\_ County: \_\_\_\_\_

Latitude: \_\_\_\_\_ Longitude \_\_\_\_\_ Lat/Long Verified?  By: \_\_\_\_\_

Elevation: \_\_\_\_\_ ft m Geo Method: **GPS** Other: \_\_\_\_\_ Datum: NAD27 **NAD83** WGS84

Samples Collected:	Sample ID:	Sample Collection Information/Preservation:
<b>Water</b> <input type="checkbox"/>		GRAB EW1
Analysis:		Preserved: HNO <sub>3</sub> H <sub>2</sub> SO <sub>4</sub> H <sub>3</sub> PO <sub>4</sub> HCL Ice Frozen None
Analysis:		Preserved: HNO <sub>3</sub> H <sub>2</sub> SO <sub>4</sub> H <sub>3</sub> PO <sub>4</sub> HCL Ice Frozen None
Analysis:		Preserved: HNO <sub>3</sub> H <sub>2</sub> SO <sub>4</sub> H <sub>3</sub> PO <sub>4</sub> HCL Ice Frozen None
Analysis:		Preserved: HNO <sub>3</sub> H <sub>2</sub> SO <sub>4</sub> H <sub>3</sub> PO <sub>4</sub> HCL Ice Frozen None
Analysis:		Preserved: HNO <sub>3</sub> H <sub>2</sub> SO <sub>4</sub> H <sub>3</sub> PO <sub>4</sub> HCL Ice Frozen None
Analysis:		Preserved: HNO <sub>3</sub> H <sub>2</sub> SO <sub>4</sub> H <sub>3</sub> PO <sub>4</sub> HCL Ice Frozen None
Analysis:		Preserved: HNO <sub>3</sub> H <sub>2</sub> SO <sub>4</sub> H <sub>3</sub> PO <sub>4</sub> HCL Ice Frozen None
Analysis:		Preserved: HNO <sub>3</sub> H <sub>2</sub> SO <sub>4</sub> H <sub>3</sub> PO <sub>4</sub> HCL Ice Frozen None
<b>Sediment</b> <input type="checkbox"/>		SED-1
Analysis:		Preserved: None Other:
<b>Benthic Chl-a</b> <input type="checkbox"/>		Sample Method: C=Core H=Hoop T=Template N=None
Composite at Lab <input type="checkbox"/> Ash-Free Dry Weight <input type="checkbox"/>		Sample Location: R=Right C=Center L=Left
Transect: A - B - C - D - E - F - G - H - I - J - K -		
<b>Phytoplankton Chl-a</b> <input type="checkbox"/>		D1 Filtered: _____ mL D2 Filtered: _____ mL
<b>Phytoplankton CNP</b> <input type="checkbox"/>		D1 Filtered: _____ mL D2 Filtered: _____ mL
<b>Algae</b> <input type="checkbox"/>		PERI-1-MOD PERI-1 OTHER:
<b>Macroinvertebrates</b> <input type="checkbox"/>		MAC-R-500 HESS OTHER:
Collection Reach Length (m):	# of Jars:	Mesh Size: 500 OTHER:

Field Measurements:	Time:	Field Assessments:
Water Temp: _____ °C °F	am pm	Field Forms: Fish Cover Form <input type="checkbox"/> Photographs <input type="checkbox"/>
Bar. Pressure: _____ mm/Hg	Air Temp: _____ °C °F	Aquatic Plant Tracking Form <input type="checkbox"/> Rosgen Form <input type="checkbox"/>
pH: _____ DO: _____ mg/L	SC: _____ umho/cm	NRCS Form <input type="checkbox"/> MAP Forms <input type="checkbox"/> Summary Form <input type="checkbox"/>
Flow: _____ cfs	Flow Comments: Dry Bed <input type="checkbox"/> No Measurable Flow <input type="checkbox"/>	Channel Cross-Section <input type="checkbox"/> Other: _____
Flow Method: Meter <input type="checkbox"/> Float <input type="checkbox"/> Gage <input type="checkbox"/> Visual Est. <input type="checkbox"/>		Data Loggers: Temperature <input type="checkbox"/> YSI <input type="checkbox"/> TruTrack <input type="checkbox"/>
Turbidity: Clear <input type="checkbox"/> Slight <input type="checkbox"/> Turbid <input type="checkbox"/> Opaque <input type="checkbox"/>		AquaRods <input type="checkbox"/> Weather Station <input type="checkbox"/> Surveyor <input type="checkbox"/>

<b>Comments</b>	Only Transect F <input type="checkbox"/> Total Site Length _____ m Average Wetted Width _____ m Transect Length _____ m

Chemistry Lab Information:		
Lab Samples Submitted to:	Account #:	Term Contract Number:
Contact Name & Phone:	EDD <input checked="" type="checkbox"/> Format: MT-eWQX Compatible	
1) Relinquished By & Date/Time:	1) Shipped By: Hand <input type="checkbox"/> FedEx/UPS <input type="checkbox"/> USPS <input type="checkbox"/>	1) Received By & Date/Time:
2) Relinquished By & Date/Time:	2) Shipped By: Hand <input type="checkbox"/> FedEx/UPS <input type="checkbox"/> USPS <input type="checkbox"/>	2) Received By & Date/Time:

Lab Use Only - Delivery Temperature: Wet Ice \_\_\_\_\_ °C Dry Ice \_\_\_\_\_ °C

Rev. 3/18/2011

### Site Visit Form Instructions

Place a Site Visit Code label in the upper left corner (ONLY 1 SITE VISIT CODE PER FORM).

Place a Trip Label in the upper right corner. (Covering Project ID and Trip ID with label is alright.)

1. **Project ID:** If you do not have a Trip Label, enter the Project ID assigned by Data Management. If Project ID is not assigned, leave blank for Water Quality Database Manager.
2. **Trip ID:** If you do not have a Trip Label, enter the Trip ID assigned by Data Management. If Trip ID is not assigned, leave blank for Water Quality Database Manager.
3. **Date/Time:** Enter the date and time of the station visit.
4. **Personnel:** Enter the first and last name(s) of the personnel conducting field activities.
5. **Waterbody:** Enter the name of the waterbody such as “Missouri River”.
6. **Location:** Description of sample location such as “upstream from bridge on Forest Service road 100”. For confidentiality please DO NOT use proper names of people in the location field.
7. **Station ID:** If you have a Trip Label, enter the established ID. If there is no ID on the Trip Label, leave the field blank and Data Management will generate a Station ID when the Site Visit Form is submitted.
8. **Visit #:** Enter “1” if this is a new station. Leave blank if visit number is unknown.
9. **HUC:** If you do not have a Trip Label, enter the fourth code (8 digit) HUC the station falls within.
10. **County:** If you do not have a Trip Label, enter the county in which the station falls within.
11. **Lat/Long:** Latitude and Longitudes should be obtained in decimal degrees using a GPS unit reading **NAD83** whenever possible. If a lat/long is obtained by another method, the datum and method must be recorded in the Site Visit Comments.
12. **Lat/Long Verified:** Latitudes and Longitudes should be verified immediately upon return from the field. Verify by plotting on a paper map or using a mapping website. Once the lat/long has been verified check the Verified box and enter initials after “By”. Do not make minor adjustments to measured values during verification; they are assumed to be correct within the limitations of the measurement system. Gross errors should be corrected as follows: 1) Draw a single line through the erroneous value(s) and initial. Do not erase the original reading. 2) Write the corrected value in the comment field along with the method and datum used to derive the corrected value.
13. **Elevation:** Record elevation collected by GPS and circle the GPS datum used. If elevation is obtained by another method, the datum and method must be recorded in the Site Visit Comments.
14. **Samples Collected:** Check the box next to each activity that is collected during the station visit.
15. **Sample ID:** Write the Sample ID (Site Visit Code-sample identifier) for all of the samples collected.
16. **Sample Collection Procedure:** Circle the appropriate Sample Collection Procedure ID.  
For each Chlorophyll-*a* transect, record the sample collection method in the first space provided and the sample location in the second space provided (example: A: T - R).  
For Phytoplankton, record the volume filtered for each sample collected.
17. **Analysis Requested:** Record the requested laboratory analysis for each chemistry sample and circle the preservative used.
18. **Field Measurements:** Record your field measurements in the spaces provided.
19. **Field Assessments:** Check the boxes next to each type of field assessment completed.
20. **Site Visit Comments:** Record general comments about the station visit, samples, and field measurements.
21. **Chemistry Lab Information:** If chemistry lab samples were taken, complete this section.  
Lab Samples Submitted to: Enter name of laboratory where samples will be sent.  
Account #: Enter account number at laboratory where samples will be sent.  
Date Submitted: Record date the samples were received by the laboratory.  
Sign and date the form each time the samples change possession.





## **ATTACHMENT 2 – PHOTOGRAPH LOCATIONS AND DESCRIPTIONS OF REACH AND/OR SITES**

**PHOTOGRAPH LOCATIONS AND DESCRIPTIONS  
OF REACH AND/OR SITES**

<b>Date:</b>		<b>Site Visit Code(s):</b>	
--------------	--	----------------------------	--

<b>Photo No:</b> _____	<b>Lat</b> _____	<b>Long</b> _____
Description:		

<b>Photo No:</b> _____	<b>Lat</b> _____	<b>Long</b> _____
Description:		

<b>Photo No:</b> _____	<b>Lat</b> _____	<b>Long</b> _____
Description:		

<b>Photo No:</b> _____	<b>Lat</b> _____	<b>Long</b> _____
Description:		

<b>Photo No:</b> ____	<b>Lat</b> _____	<b>Long</b> ____
Description:		

**PHOTOGRAPH LOCATIONS AND DESCRIPTIONS-CONTINUED**

<b>Photo No:</b> _____	<b>Lat</b> _____	<b>Long</b> _____	
Description: _____			
<b>Photo No:</b> _____	<b>Lat</b> _____	<b>Long</b> _____	
Description: _____			
<b>Photo No:</b> _____	<b>Lat</b> _____	<b>Long</b> _____	
Description: _____			
<b>Photo No:</b> _____	<b>Lat</b> _____	<b>Long</b> _____	
Description: _____			
<b>Photo No:</b>		<b>Lat</b>	
Description:			



## ATTACHMENT 3 – SITE SKETCH FORM

Date: \_\_\_\_\_ Site Visit Code: \_\_\_\_\_

Waterbody: \_\_\_\_\_

Personnel: \_\_\_\_\_

