

Protocol for Monitoring Springs at Ozark National Scenic Riverways, Missouri. Heartland I&M Network

SOP 7: Laboratory Processing and Identification of Invertebrates

Version 1.1 (01/29/2020)

Revision History Log:

Previous Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #
1.0	1/29/2020 and 2/26/2021	D.E. Bowles and H.R. Dodd	References updated and added to SOP; QA/QC procedures and certification clarified; sample processing and identification methods clarified	Practicality of having references associated with the SOP; Increase data integrity of sample processing	1.1

This SOP explains procedures for processing and storing samples after field collection as well as identification of specimens. Procedures for storing reference specimens are also described.

I. Preparing the Sample for Processing

Processing procedures apply to all benthic samples. This is an important and time consuming step. Particular care should be taken to ensure that samples are being processed thoroughly and efficiently. The purpose of sorting is to remove invertebrates from other material in the sample.

Procedure:

- A. Sample processing begins by pouring the original field sample into a USGS standard sieve (500- μ m) placed in a catch pan. The preservative that is drained from the sample should be placed back in the original sample container for eventual rehydration of remaining sample debris that is not sorted during the subsample procedure described below.
- B. Rinse the sample contents in the sieve with tap water to flush the residual preservative. Large debris material (>2 cm; i.e. leaves, sticks, rocks) should be removed by hand and rinsed into the sieve. Each piece of large debris removed from the bulk sample should be carefully inspected under a hand lens to ensure that all attached organisms are removed. The large rinsed debris material should then be placed back in the original sample container that contains the drained preservative. For samples that contain heavy amounts of aquatic vegetation (particularily algae or moss where invertebrates can be entangled or

attached), the vegetation should remain in the sieve for the subsampling procedure and processing under a microscope, as described below.

- C. A general rule on when to elutriate the sample: If the sample has little aquatic vegetation or large debris (covers <25% of the sieve) but fine sediment (sand/gravel) covers more than 50% of the sieve, elutriation should be used to separate the invertebrates and smaller organic debris from the fine sediment. To conduct elutriation, place the contents of the sieve in a 1 gallon bucket and add water to the sample such that at least 2 inches of water covers the sample. Swirl the contents of the bucket rapidly enough to allow organic material (invertebrates and small debris) to float on top of the water, then rapidly and carefully decant the water into the sieve without stirring up the fine sediment. This may take several washes to separate the organic material from the fine sediment. Carefully examine the inorganic content (fine sediment) using a magnifying lens for the presence of remaining invertebrates (especially mollusk shells and Trichoptera cases). Add these specimens to the organic debris portion that is in the sieve. Return the inorganic portion to the original sample container.
- D. The sieve containing the organic sample portion of the sample should be placed in a shallow pan of water. Enough water should be used such that the contents are floated until they are evenly distributed on the pan bottom. The sieve should then be carefully lifted from the water so that contents are not redistributed. The sieve is marked with eight (8) equal portions (Fig. 1) in order to select sections of the sieve for subsampling and sorting as described below.

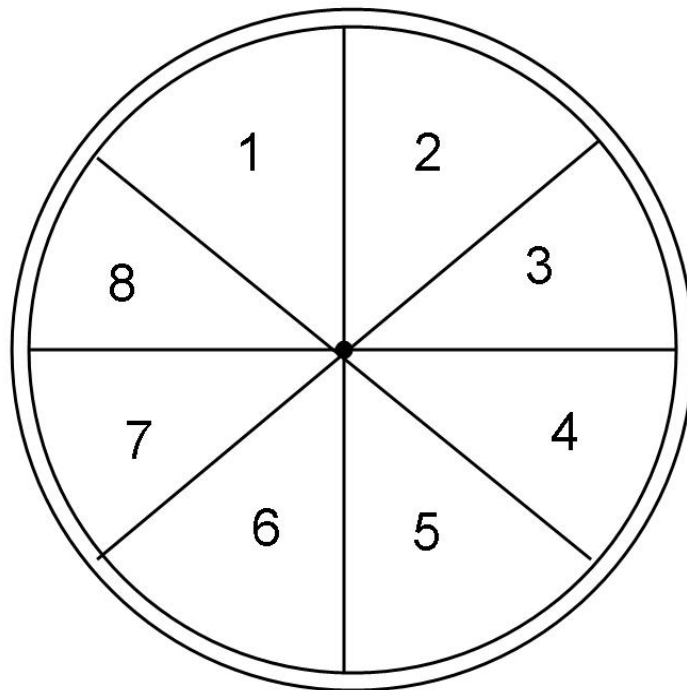


Figure 1. Diagram of a 500-µm USGS sieve marked into eight equal fractions.

II. Subsampling and Sorting the sample

In order to ensure that the subsample adequately represents the contents of the whole sample, a minimum of 200 organisms, if present, will be removed from the sample.

Procedure:

- A. Using the random table below (Fig. 2), the sorter will randomly pick one of the one-eighth fractions of the sample in the sieve to represent a minimum 12.5% subsample. To select the initial number of the fraction to sort, the sorter will look away from the random number table and then place their finger on a number. If additional fractions must be sorted, the sorter will use the next number down within the same column to select the next fraction.

5	7	2	5	3	2	1	2	2	1	1	7
4	6	7	2	8	3	5	8	5	5	7	8
6	3	3	7	7	8	6	1	7	2	4	3
1	8	4	8	5	5	4	3	1	7	3	1
2	2	8	1	1	6	3	5	6	8	8	5
7	1	6	4	4	4	2	7	3	4	2	4
8	4	5	6	6	1	8	6	8	6	6	2
3	5	1	3	2	7	7	4	4	3	5	6

Figure 2. Randomly generated integer sequences between 1 and 8. Sequences are oriented from top to bottom within a column.

- B. Using a putty knife or similar tool, the selected fraction contents are carefully scraped from the sieve. If the entire fraction is small, then place subsampled fraction into a petri dish with either 70% ethyl alcohol or tap water. If the entire fraction is too large to fit in a petri dish, then place the fraction into a small white sorting pan containing water until all of the fraction can be sorted under a microscope. The bottom of the sieve in the area where the subsample was removed should then be carefully inspected using a hand lens to ensure that no invertebrates remain.
- C. All samples will be sorted using a large petri dish under a dissecting microscope with a minimum 10X magnification.
- D. As invertebrates are removed from the fraction and placed in a labeled vial with ethanol, they should be counted with a hand-held enumerator. The target number of organisms to obtain in a subsample for accurate representation of the whole sample is 200 organisms. When the fraction has been completely sorted and at least 195 (at least 97.5% of the 200 organism target) have been removed, no additional sorting is necessary. If less than 195 organisms were removed, the sorter should remove another randomly selected one eighth fraction from the sieve, sort it entirely, and continue to count the organisms using the

enumerator. This process is repeated until at least 195 organisms have been removed or the entire sample has been sorted.

- E. Always completely sort the removed sample fractions regardless of how many organisms are present (*e.g.*, the first fraction removed possibly could contain 300 or more organisms).
- F. Counting damaged organisms: If a fragment includes the head, and, in the case of arthropods, the thorax, count the organism and place it in the vial. For oligochaetes, there may be several fragments. Place these fragments into the vial, but only count those that have a rounded end (*i.e.* anterior or posterior end). Organisms that have developed wings are typically not counted, except aquatic beetles (Elmidae, Dytiscidae, Hydrophilidae) and aquatic true bugs (Gerridae, Nepidae, Notonectidae). If the sorter is unsure, the organism should be placed in the vial but not counted on the enumerator.
- G. To assist with correctly sorting invertebrates from the sample, the sorter will review photos of typically collected taxa. These photos will be located in the HTLN aquatics laboratory at Missouri State University, Springfield, MO.
- H. Any invertebrates present in the subsampled fraction will be stored in a separate storage vial, preserved in 70% ethyl alcohol, and properly labeled (Fig 3).
 - 1. The sorter should clearly indicate on the vial label the percent of sample sorted: 12.5, 25, 37.5, 50, 62.5, 75, 87.5, 100%.
 - 2. **This information is transferred to the Aquatic Invertebrate Identification and Enumeration sheet by the person identifying the sample and is critical for estimating final benthic densities.** For example, to estimate density for the entire sample if only one eighth section (12.5%) is sorted, the number of specimens in this fraction must be multiplied by a factor of 8. Other fractions and their multiplication factors are: 2=4, 3= 2.67, 4=2, 5=1.6, 6=1.33, 7=1.14, and 8=1.
- I. On a spreadsheet provided by the project manager, the sorter will record the percent subsampled and number of organisms picked for each sample. The sorter should bring up any difficulties with sample sorting to the project manager who will note them on the Aquatic Invertebrate Identification & Enumeration Sheet (an example is located at the end of this SOP).
- J. Large and/or Rare: Additionally, a “large and/or rare” taxa component is included in the sample sorting process. These specimens will be used for reflecting accurate sample diversity estimates.
 - 1. A large and/or rare additional taxa search will be completed on the remaining unsorted bulk sample following the subsample routine. Any organisms that were **clearly not** in the sorted subsampled portion(s) should be placed in a separate large and/or rare vial with a label (Fig 3).

2. NOTE: Just because a specimen is large does not mean it should be removed during this process. It must fit the criterion that it was not present in the subsample.
 3. There may be several or no specimens for the large and/or rare vial depending on the sample.
 4. Examples of possible large and/or rare organisms are: *Corydalis cornutus*, *Pteronarcys pickettii*, tabanids, tipulids, dragonfly larvae, crayfish, gordian worms, large beetles, other unusual species, etc.
- K. Once the subsampled fraction has been sorted and large and rare has been conducted on the remaining unsorted fraction, the sorter places the unsorted sample back into the original container with the original labels (both inside and outside label) and 70% ethyl alcohol. An “X” is placed on the outside label of the container to note the sample has been sorted.

III. Sample Preservation and Labeling

- A. In summary, there are two or three containers at the end of the sorting process:
 1. original bulk jar with any materials not sorted
 2. sorted invertebrates from the subsampled fraction(s) in a vial
 3. large and/or rare vial (if applicable)
- B. All containers with sorted invertebrates or remaining bulk sample will be stored in 70-80% ethyl alcohol.
- C. Do not crowd the collected specimens excessively as it inhibits long term preservation. If there are a large number of specimens in the sorted subsampled fraction, then a second vial will be used. In this case, the sorter will create a second label with all the information in Fig 3 and add “1 of 2” and “2 of 2” on the labels for the sorted subsample vials.
- D. Labels:
 1. Written only on rag bond paper in permanent water proof ink.
 2. Label data should include (see Fig 3):
 - Park Name
 - Site Name
 - Transect with sample location (2, 4, 6, 8, 10; Left, Middle, Right),
 - Sampling Date
 - Collector Initials.
 3. Vial with subsampled fraction(s) must also include the percent subsampled
 4. Large and rare vial should be specifically labeled as such

<u>Label Type</u>	<u>Sample Information</u>
Original Field Sample:	Park Name: OZAR Site Name: Blue Spring Transect: 2 Left Collection Date: 22 July 2007 Collector initials: DEB, HRD
Bulk sample not sorted: (placed back in original jar)	Park Name: OZAR Site Name: Blue Spring Transect: 2 Left Collection Date: 22 July 2007 Collector initials: DEB, HRD
Vial with sorted subsample:	Subsample: 25% Park Name: OZAR Site Name: Blue Spring Transect: 2 Left Collection Date: 22 July 2007 Collector initials: DEB, HRD
Vial with sorted Large and Rare:	LARGE & RARE Park Name: OZAR Site Name: Blue Spring Transect: 2 Left Collection Date: 22 July 2007 Collector initials: DEB, HRD

Figure 3. Example specimen labels for each container used during the sorting process.

IV. Identification of Invertebrates

To the extent possible, all invertebrates should be identified to genus exclusive of the groups and selected conditions indicated below.

Procedure:

- A. Organisms should be placed in a large petri dish (with either 70% ethyl alcohol or tap water) under a dissecting microscope with at least 10X magnification.
- B. Taxonomic identification level: Most specimens will be identified to genus, when possible.

1. For some taxa only higher taxonomic levels can be obtained. For consistency among those identifying the samples, those taxa that will be left at higher taxonomic levels are listed in Table 1.
2. In most cases, much of the sample can be identified to the required level of genus or to the level in Table 1 for those specific taxa. However, samples may contain early instars or damaged specimens. In such cases, the specimen should be identified to the lowest level possible, which is typically family.

Table 1. Expected identification levels for taxa that are not required to be taken to genus level.

Phylum	Class	Order	Family
Nematoda			
Nematomorpha			
Annelida	Hirudinea	Branchiobdellida	
		Arhynchobdellida	Erpobdellidae, Hirudinidae
		Rhynchobdellida	Glossiphoniidae, Piscicolidae
Annelida	Oligochaeta		
Arthropoda	Arachnoidea	Trombiiformes	"Hydracarina"
Arthropoda	Crustacea	Ostracoda	
Arthropoda	Insecta	Diptera	Chironomidae

C. If **damaged organisms** can be identified, they are counted ONLY if:

1. The fragment includes the head, and, in the case of arthropods, the thorax. For Oligochaetes, the person identifying the sample should pull all fragments in the sample to one area of the petri dish to match the anterior and posterior portions in order to count the number of oligochaetes.
2. The mollusk shell (bivalve or gastropod) or caddisfly case is occupied by a specimen.
3. Damaged specimens may require identification to a higher taxonomic level, typically family.

D. **Early instar or juvenile** specimens may require identification at a higher taxonomic level than mature specimens, typically family.

E. Taxonomic keys and nomenclature: The primary keys will be Merritt *et al.* (2019) for identification of insects and Smith (2002) and Thorp and Covich (2016) for identification of non-insect invertebrates. Additional taxonomic references for specific groups *include* Moulton and Stewart (1996), Poulton and Stewart (1991) and Wiggins (1995). A list of taxa known or likely to occur at OZAR is included in SOP

#12 (Data Analysis). Accuracy of scientific names will be checked using the most recent keys for taxonomic identification (listed above) and relevant literature from experts in those taxa.

- F. Using enumerators or using tallies on the Aquatic Invertebrate Identification & Enumeration Sheet (Fig. 4), the total of each taxon for the sample is recorded. Final counts of each taxon should be entered on the sheet when a sample is complete (i.e., Tot Cnt column on enumeration sheet). All information from the sample label should be recorded on the top of the enumeration sheet along with the initials of the person identifying the specimens (i.e., determiner) and the date they were determined. The organisms from that sample are returned to their vial that contains the label and 70% ethyl alcohol. A “dot” is placed on the lid of the vial to denote the sample has been identified.
- G. Large and/or Rare samples are identified, and taxa counts are recorded on an enumeration sheet specifically labeled with “Large and Rare” (Fig 5). All information from the sample label is record on the top of the enumeration sheet along with the determiner’s initial and date the sample was identified. The organisms from the Large and Rare sample are returned to their vial that contains the label and 70% ethyl alcohol. A “dot” is placed on the lid of the vial to denote the sample has been identified.

V. Reference Collection

Procedure:

- A. A reference collection is maintained at the HTLN aquatics lab stationed at Missouri State University, Springfield, MO. Identified specimens for the reference collection are stored in vials with 70% ethyl alcohol and labeled with:
 - Park Name
 - Site Name
 - Transect location
 - Collection date
 - Taxon Name
 - Name of Determiner
- B. Regional or other taxonomist specialists should review the identifications of reference specimens for accuracy.

VI. QA/QC and Certification

Procedures:

Sample processing and sorting

The following QA/QC procedure (adapted from USEPA, 2004b) will be used on sorted invertebrate samples.

- A. Initially, a QA officer (Aquatic Program Leader, or experienced staff member certified in invertebrate sorting and identification) will use a microscope at 10x magnification to check **all sorted fractions from the first five samples processed by a sorter**. This applies to inexperienced sorters who have never sorted aquatic macroinvertebrate samples and to experienced sorters who are new to processing HTLN samples and has not completed the QA/certification process.
- B. **Experienced staff:** Certification for experienced sorters will occur when sorters are consistent in achieving >90% sorting efficiency after at least five consecutive samples have been checked. Once certified, these experienced sorters no longer need to be checked and can now check sorting efficiency of others. In the event that an individual fails to achieve >90% sorting efficiency, the next five consecutive samples sorted will undergo QC for certification of the sorter.
- C. **Inexperienced staff:** For inexperienced sorters who reach 90% efficiency in the first five samples, additional samples will undergo QC when the sorter starts processing another set of samples from a different spring (at least one sample from the spring; 1 out of 5). If an inexperienced sorter maintains >90% efficiency for multiple springs, they become certified and no longer need checked for sorting efficiency of spring samples.
- D. The QA officer will calculate percent sorting efficiency (PSE) for each sample as follows:

$$\text{PSE} = \text{A}/(\text{A}+\text{B}) (100)$$

where A = number of organisms found by the primary sorter, and B = number of recoveries (organisms missed by the primary sort and found by the QC check).

Sorting efficiency should not be calculated for samples processed by more than one individual.

- E. QA of Subsample: As the sorter is processing the subsampled fraction, each petri dish used to sort the subsample will be checked by the QA officer. If the amount of organics in the subsample is large, the sorter may use several petri dishes in order to sort the specimens from the smaller organic debris or vegetation. The QA officer will keep a running record of the number of specimens missed by the sorter for the entire subsampled fraction to calculate sorting efficiency. Once the QA officer has checked the petri dish, the contents can be discarded.

- F. QA of Large and Rare: The QA officer will use a hand lens to scan the remaining unsorted sample. This is completed to check the sorter's Large and Rare sample vial for any missed organisms. In order to complete this QA, the sorted subsample vial and the Large and Rare vial (if applicable) that was completed by the sorter will need to be examined by the QA officer to ensure that any specimens remaining in the unsorted sample are not present in these vials.

Specimen Identification

- A. A QA officer (i.e., Program Leader or staff member certified in identification) will QC samples of both inexperienced and experienced individuals conducting identifications of spring samples. Experienced individuals are those who have taxonomic identification skills but new to HTLN springs samples and have not completed identification certification process described below.
- B. Percent similarity between the identification of the individual and the QA officer must exceed 90% for 10 consecutive spring samples in order for the individual to be certified on identifications
1. For experienced individuals, once they reach this certification criteria in 10 samples, they no longer need to be checked and can check inexperienced individuals.
 2. For inexperienced individuals, identifications will be checked from at least 1 sample (i.e. 1 of 5) from each new spring they process until multiple springs are processed by this individual.
- C. If an individual fails to maintain a >90% identification efficiency during the 10 consecutive samples, then QC will be performed on an additional five consecutive samples until certification is reached.
- D. If any specimens are incorrectly identified, all specimens assigned to that taxon will be reexamined.
- E. If a taxon is especially difficult to identify or has not been collected from the park previously, the specimen will be identified by multiple certified determiners. If all determiners agree, the specimen is recorded as such on the identification sheet. If there is disagreement among determiners, then the specimen will be sent to a person with taxonomic expertise in invertebrates of the region. It is also important that the taxonomist maintains contact with other taxonomists through professional societies and other interactions, and stays current with the pertinent published literature.

VIII. References

- Merritt, R. W., K. W. Cummins, and M. B. Berg (editors). 2019. An introduction to the aquatic insects of North America. 5th edition. Kendall/Hunt Publishing Company, Dubuque, IA.
- Moulton, S. R., III, and K. W. Stewart. 1996. Caddisflies (Trichoptera) of the Interior Highlands of North America. Memoirs of the American Entomological Institute. The American Entomological Institute, Gainesville, FL.
- Pennak, R.W. 1989. Freshwater invertebrates of the United States, 3rd edition. J. Wiley and Sons, Inc. New York, NY.
- Poulton, B. C., and K. W. Stewart. 1991. The Stoneflies (Plecoptera) of the Ozark and Ouachita Mountains. Memoirs of the American Entomological Society 38:1-116.
- Smith, G. 2002. Pennak's Freshwater Invertebrates of the United States: Porifera to Crustacea, 4th Edition. Wiley Publishers, New York.
- Thorp and Covich. 2016. Volume II: Keys to the Nearctic Fauna, 4th Edition. Elsevier, Inc. London, England.
- Wiggins, G.B. 1995. Larvae of North American caddisfly genera (Trichoptera), 2nd edition. University of Toronto Press, Toronto, Canada.

Aquatic Invertebrate Identification & Enumeration Sheet

Page ____ of ____

Spring Name: _____
 Transect Number: 2 4 6 8 10 State: _____
 Sample date: _____ Date of Det: _____
 Subsampled portion: 12.5 25 37.5 50 65.5 75 87.5 100 Determiner(s): _____

ORDER (or higher)	FAMILY	GENUS	SPECIES	TALLEY/COUNT	Tot Cnt	RC
Diptera						
Coleoptera						
Ephemeroptera						
Plecoptera						
Trichoptera						
Amphipoda						
Other						

Figure 4. Aquatic Invertebrate Identification & Enumeration Sheet for subsample.

Aquatic Invertebrate Identification & Enumeration Sheet

Spring Name: _____
 Transect Number: 2 4 6 8 10 State: _____
 Sample date: _____ Date of Det: _____
Subsampled portion: 12.5 25 37.5 50 65.5 75 87.5 100 Determiner(s): _____

ORDER (or higher)	FAMILY	GENUS	SPECIES	TALLEY/COUNT	Tot Cnt	RC
Diptera						
Coleoptera						
Ephemeroptera						
Plecoptera						
Trichoptera						
Amphipoda						
Other						

Figure 5. Aquatic Invertebrate Identification & Enumeration Sheet for Large and Rare.

Table 2. Invertebrate taxa list with functional feeding groups and tolerance values for the seven large springs (Alley, Big, Blue, Phillips, Pulltite, Round, and Welch) at OZAR. Functional Feeding Groups: C = Collector, F = Filterer, He = Herbivore, Pa = Parsite, Pr = Predator, Sc = Scraper, Sh = Shredder.

Phylum	Class	Order	Family	Genus	Tolerance Value	Functional Feeding Group
Annelida	Hirudinea				10.0	C
Annelida	Hirudinea	Branchiobdellida			6.0	C
Annelida	Hirudinea	Rhynchobdellida	Glossiphoniidae		7.0	Pr
Annelida	Oligochaeta				8.0	
Arthropoda	Arachnida	Trombidiformes	"Hydracarina"		5.7	Pa,Pr
Arthropoda	Crustacea	Amphipoda			4.0	
Arthropoda	Crustacea	Amphipoda	Crangonyctidae	Crangonyx	8.0	C
Arthropoda	Crustacea	Amphipoda	Crangonyctidae	Stygobromus		C
Arthropoda	Crustacea	Amphipoda	Gammaridae	Gammarus	6.9	C
Arthropoda	Crustacea	Amphipoda	Hyalellidae	Hyalella	7.9	C
Arthropoda	Crustacea	Copepoda			8.0	
Arthropoda	Crustacea	Decapoda	Cambaridae		6.0	
Arthropoda	Crustacea	Decapoda	Cambaridae	Orconectes	2.7	C
Arthropoda	Crustacea	Isopoda			8.0	
Arthropoda	Crustacea	Isopoda	Asellidae	Caecidotea	8.0	C
Arthropoda	Crustacea	Isopoda	Asellidae	Lirceus	7.7	C
Arthropoda	Crustacea	Ostracoda			8.0	
Arthropoda	Insecta	Coleoptera				
Arthropoda	Insecta	Coleoptera	Dytiscidae		5.0	
Arthropoda	Insecta	Coleoptera	Elmidae	Dubiraphia	6.4	C
Arthropoda	Insecta	Coleoptera	Elmidae	Optioservus	2.7	C,Sh
Arthropoda	Insecta	Coleoptera	Elmidae	Stenelmis	5.4	C,Sc
Arthropoda	Insecta	Coleoptera	Hydrophilidae		5.0	
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Berosus	8.6	He,C
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Enochrus	8.0	C
Arthropoda	Insecta	Coleoptera	Hydrophilidae	Hydrobius	5.0	C,Pr
Arthropoda	Insecta	Coleoptera	Scirtidae	Prionocyphon		
Arthropoda	Insecta	Collembola				

Arthropoda	Insecta	Collembola	Sminthuridae			C
Arthropoda	Insecta	Diptera	Athericidae	Atherix	2.1	Pr
Arthropoda	Insecta	Diptera	Ceratopogonidae		6.0	C,Pr,Sc
Arthropoda	Insecta	Diptera	Ceratopogonidae	Atrichopogon	6.0	C
Arthropoda	Insecta	Diptera	Ceratopogonidae	Bezzia (Palpomyia)	6.0	Pr
Arthropoda	Insecta	Diptera	Ceratopogonidae	Dasyhelea	6.0	C, Sc
Arthropoda	Insecta	Diptera	Chironomidae		6.0	
Arthropoda	Insecta	Diptera	Dixidae	Dixa	2.8	C,Pr
Arthropoda	Insecta	Diptera	Empididae		6.0	
Arthropoda	Insecta	Diptera	Empididae	Chelifera	6.0	Pr
Arthropoda	Insecta	Diptera	Empididae	Clinocera	6.0	Pr
Arthropoda	Insecta	Diptera	Empididae	Hemerodromia	6.0	C,Pr
Arthropoda	Insecta	Diptera	Empididae	Neoplasta	6.0	
Arthropoda	Insecta	Diptera	Empididae	Trichoclinocera	6.0	Pr
Arthropoda	Insecta	Diptera	Empididae	Trichoclinocera	6.0	Pr
Arthropoda	Insecta	Diptera	Ephydriidae		5.5	C,Sh
Arthropoda	Insecta	Diptera	Ephydriidae	Scatella	6.0	
Arthropoda	Insecta	Diptera	Muscidae	Limnophora	7.0	Pr
Arthropoda	Insecta	Diptera	Psychodidae	Pericoma	4.0	C
Arthropoda	Insecta	Diptera	Psychodidae	Psychoda	9.9	C
Arthropoda	Insecta	Diptera	Simuliidae		4.0	
Arthropoda	Insecta	Diptera	Simuliidae	Prosimulium	2.6	F
Arthropoda	Insecta	Diptera	Simuliidae	Simulium	4.4	F
Arthropoda	Insecta	Diptera	Tabanidae	Tabanus	9.7	Pr
Arthropoda	Insecta	Diptera	Tipulidae		3.0	C
Arthropoda	Insecta	Diptera	Tipulidae	Antocha	4.6	C
Arthropoda	Insecta	Diptera	Tipulidae	Hexatoma	4.7	Pr
Arthropoda	Insecta	Diptera	Tipulidae	Pseudolimnophila	7.3	C
Arthropoda	Insecta	Diptera	Tipulidae	Tipula	7.7	C,Sh
Arthropoda	Insecta	Ephemeroptera	Baetidae		4.0	C
Arthropoda	Insecta	Ephemeroptera	Baetidae	Acentrella	3.6	C
Arthropoda	Insecta	Ephemeroptera	Baetidae	Acerpenna	3.7	C
Arthropoda	Insecta	Ephemeroptera	Baetidae	Baetis	6.0	C
Arthropoda	Insecta	Ephemeroptera	Baetidae	Dipheter	5.0	
Arthropoda	Insecta	Ephemeroptera	Baetidae	Procloeon	6.3	C

Arthropoda	Insecta	Ephemeroptera	Baetidae	Pseudocloeon	4.4	C
Arthropoda	Insecta	Ephemeroptera	Caenidae	Caenis	7.6	C,Sc
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae		1.0	C,Sc
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Attenella	1.0	C
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Ephemerella	1.7	C,Sc
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Eurylophella	3.0	C
Arthropoda	Insecta	Ephemeroptera	Ephemerellidae	Serratella	1.9	C
Arthropoda	Insecta	Ephemeroptera	Heptageniidae		4.0	F
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Stenacron	7.1	C
Arthropoda	Insecta	Ephemeroptera	Heptageniidae	Stenonema	3.4	C,Sc
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae		2.0	
Arthropoda	Insecta	Ephemeroptera	Leptophlebiidae	Paraleptophlebia	1.2	C,Sh
Arthropoda	Insecta	Ephemeroptera	Potamanthidae	Anthopotamus	4.0	C
Arthropoda	Insecta	Ephemeroptera	Tricorythidae	Tricorythodes	5.4	C
Arthropoda	Insecta	Hemiptera				Pr
Arthropoda	Insecta	Hemiptera	Corixidae		5.0	He, Pr
Arthropoda	Insecta	Hemiptera	Corixidae	Palmacorixa	5.0	Pr
Arthropoda	Insecta	Hemiptera	Corixidae	Trichocorixa	5.0	Pr
Arthropoda	Insecta	Hemiptera	Hebridae	Lipogomphus		Pr
Arthropoda	Insecta	Hemiptera	Veliidae		7.0	Pr
Arthropoda	Insecta	Hemiptera	Veliidae	Microvelia	6.4	Pr
Arthropoda	Insecta	Lepidoptera				
Arthropoda	Insecta	Lepidoptera	Crambidae	Petrophila	5.0	Sh, He
Arthropoda	Insecta	Lepidoptera	Noctuidae			Sh
Arthropoda	Insecta	Megaloptera				Pr
Arthropoda	Insecta	Megaloptera	Corydalidae	Corydalus	5.6	Pr
Arthropoda	Insecta	Megaloptera	Corydalidae	Nigronia	5.8	Pr
Arthropoda	Insecta	Megaloptera	Sialidae	Sialis	7.5	Pr
Arthropoda	Insecta	Neuroptera	Sisyridae	Climacia	6.5	Pr
Arthropoda	Insecta	Odonata	Aeshnidae	Boyeria	6.3	Pr
Arthropoda	Insecta	Odonata	Coenagrionidae		9.0	Pr
Arthropoda	Insecta	Odonata	Coenagrionidae	Argia	8.7	Pr
Arthropoda	Insecta	Odonata	Gomphidae	Hagenius	4.0	Pr
Arthropoda	Insecta	Plecoptera			2.0	
Arthropoda	Insecta	Plecoptera	Capniidae		2.0	

Arthropoda	Insecta	Plecoptera	Capniidae	Allocapnia	2.8	Sh
Arthropoda	Insecta	Plecoptera	Chloroperlidae		1.0	C,Pr
Arthropoda	Insecta	Plecoptera	Leuctridae		0.0	Sh
Arthropoda	Insecta	Plecoptera	Leuctridae	Leuctra	0.7	Sh
Arthropoda	Insecta	Plecoptera	Leuctridae	Zealeuctra	0.0	Sh
Arthropoda	Insecta	Plecoptera	Perlidae		1.0	C,Pr
Arthropoda	Insecta	Plecoptera	Perlidae	Agnetina	1.4	Pr
Arthropoda	Insecta	Plecoptera	Perlidae	Neoperla	1.6	Pr
Arthropoda	Insecta	Plecoptera	Perlodidae	Clioperla	4.8	Pr
Arthropoda	Insecta	Plecoptera	Perlodidae	Isoperla	3.2	C,Pr
Arthropoda	Insecta	Plecoptera	Taeniopterygidae	Strophopteryx	2.5	Sh
Arthropoda	Insecta	Plecoptera	Taeniopterygidae	Taeniopteryx	2.8	C,Sh
Arthropoda	Insecta	Trichoptera				
Arthropoda	Insecta	Trichoptera	Brachycentridae	Brachycentrus	1.0	F
Arthropoda	Insecta	Trichoptera	Glossosomatidae		0.0	
Arthropoda	Insecta	Trichoptera	Glossosomatidae	Glossosoma	1.5	C,SC
Arthropoda	Insecta	Trichoptera	Glossosomatidae	Protoptila	2.8	Sc
Arthropoda	Insecta	Trichoptera	Helicopsychidae	Helicopsyche	0.0	SC
Arthropoda	Insecta	Trichoptera	Hydropsychidae		4.0	
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Ceratopsyche	1.4	F
Arthropoda	Insecta	Trichoptera	Hydropsychidae	Cheumatopsyche	6.6	F
Arthropoda	Insecta	Trichoptera	Hydroptilidae		4.0	
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Hydroptila	6.2	He,SC
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Ochrotrichia	6.8	C, He
Arthropoda	Insecta	Trichoptera	Hydroptilidae	Oxyethira	3.0	C,He
Arthropoda	Insecta	Trichoptera	Lepidostomatidae	Lepidostoma	1.0	Sh
Arthropoda	Insecta	Trichoptera	Limnephilidae	Pycnopsyche	2.3	C,Sh
Arthropoda	Insecta	Trichoptera	Polycentropodidae	Polycentropus	3.5	C,Pr
Arthropoda	Insecta	Trichoptera	Psychomyiidae	Lype	4.3	C
Arthropoda	Insecta	Trichoptera	Psychomyiidae	Paduniella	0.0	C,Sc
Arthropoda	Insecta	Trichoptera	Rhyacophilidae	Rhyacophila	0.8	Pr
Mollusca	Bivalvia					F
Mollusca	Bivalvia	Veneroida	Pisidiidae			F
Mollusca	Bivalvia	Veneroida	Pisidiidae	Pisidium	6.8	F
Mollusca	Bivalvia	Veneroida	Pisidiidae	Sphaerium	7.7	F

Mollusca	Gastropoda				7.0	
Mollusca	Gastropoda	Basommatophora	Ancylidae	Ferrissia	6.9	Sc
Mollusca	Gastropoda	Basommatophora	Lymnaeidae	Fossaria	6.0	Sc
Mollusca	Gastropoda	Basommatophora	Lymnaeidae	Pseudosuccinea	7.2	Sc
Mollusca	Gastropoda	Basommatophora	Physidae		8.0	
Mollusca	Gastropoda	Basommatophora	Physidae	Physella	9.1	Sc
Mollusca	Gastropoda	Neotaenioglossa	Hydrobiidae		7.0	Sc
Mollusca	Gastropoda	Neotaenioglossa	Hydrobiidae	Amnicola	4.8	Sc
Mollusca	Gastropoda	Neotaenioglossa	Hydrobiidae	Fontigens		Sc
Mollusca	Gastropoda	Neotaenioglossa	Pleuroceridae	Elimia	2.5	Sc
Nematoda					5.0	
Nematomorpha					5.0	
Platyhelminthes	Turbellaria	Tricladida	Planariidae	Dugesia	7.5	C,Pr
Platyhelminthes	Turbellaria	Tricladida	Planariidae	Phagocata		Pr