Laboratory Methods

The following lab protocol, derived from Hilsenhoff 1987, was followed:

Equipment

Grided sorting tray, storage jars, vials/caps, random number set, forceps, labeling materials, 70% EtOH, illuminated magnifier

Protocol

After returning to the laboratory, sample jars completely filled with debris should be drained of ethanol by pouring through a screen or net with a 1.0 mm or smaller mesh. The ethanol should then be replaced with 70% ethanol. An alternative is to refrigerate the sample to allow ample time for diffusion of the ethanol throughout the debris and into the arthropods.

About 15 minutes before picking and sorting a sample in the laboratory, strain the ethanol from the sample and replace it with water. No arthropods should remain floating on the surface of the water.

Place the contents of the jar in a large, flat pan marked with a grid, add two or three additional jars full of water, and spread the contents evenly over the bottom of the pan.

If the jar is completely filled with fine debris, especially filamentous algae, only half of the sample should be initially placed in the pan for sorting, with care being taken to assure that each half of the sample contains the same amount and kinds of debris.

A 30 by 45-cm pan with a 5-cm grid is satisfactory. Select a starting square for each sample by picking a number from a box of corresponding numbers or from a table of random numbers.

Remove all arthropods from the starting square and then remove arthropods from each successively higher numbered square. An arthropod on a line is considered to be in the square that contains its head, or in the square closest to its head. After the highest numbered square has been sampled, return to square 1. Remove and preserve at least 100 arthropods. Remove all arthropods from the last square to be picked.

Not collected: Hemiptera, Coleoptera other than Dryopoidea (Dryopidae, Elmidae), arthropods less than 3 mm long (because most cannot be identified) . . . except for adult Elmidae and fifth instar Hydroptilidae larvae (which have expanded abdomens and are usually in cases)

An illuminated 5X magnifier on a long, movable arm (Luxo®) will facilitate finding and removing arthropods from the pan.

Preserve all arthropods in 70% ethanol for identification to genus or species. Isopropyl alcohol may also be used.

Sort and identify all arthropods to genus, except Chironomidae, which should be placed in a separate vial. When all samples have been identified to genus, species identification should be made whenever necessary and possible. This is best accomplished by working on one genus at a time and identifying species in that genus from all samples before identifying species in another genus.

Chironomidae are sorted to genus by placing those that look alike together. Head color, head size and shape, markings on the head, antennal length and structure, number and location of eye spots, general shape and pigmentation of the mentum, length and color of preanal papillae and setae, length of prolegs and color of their claws, and general coloration are among the characters that can be used to separate genera. Mount the two most dissimilar larvae from each group in Hoyer's medium under separate cover slips on the same slide. If both are found to be the same genus, the remainder may be assumed to be also the same and need not be mounted. If they are different, further sorting and slide mounting is needed or all must be mounted on slides. An alternative is to clear all larvae in 10% KOH and make temporary mounts in glycerine for identification.

Record the number of each species on a data sheet and multiply the number by the tolerance value for that species. Sum the products and divide by the total number of arthropods in the sample to obtain the biotic index for the stream. Table 1 is a general guide to the water quality of streams. Replicate samples, or both spring and fall samples, will add to the confidence of the evaluation.

Lab processing through step 4 above was carried out in 2004 by C. Garry; the same steps in 2005 were carried out by UW-River Falls graduate student Z. McCallister. Identification of specimens and HBI determinations were carried out by J. Dimick, Aquatic Entomology Laboratory, UW-Stevens Point.