

The Alliance for Aquatic Resource Monitoring

# Biological Monitoring Manual



April 2009



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# Table of Contents

Background on ALLARM..... 3

Background on Manual..... 3

What is a Macroinvertebrate? ..... 4

Method of Collection ..... 4

    Site Selection..... 4

    Rocky Bottom Sampling ..... 5

    Muddy Bottom Sampling ..... 7

Identification Categories..... 9

    Sensitive ..... 9

    Somewhat Sensitive ..... 11

    Tolerant..... 13

Quantifying Your Results..... 14

    EASI Tally Sheet (Example)..... 15

    Macroinvertebrate Survey (Example)..... 16

    EASI Tally Sheet..... 17

    Macroinvertebrate Survey ..... 18

Glossary..... 20

References ..... 21



## Background on ALLARM:

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The Alliance for Aquatic Resource Monitoring (ALLARM) is a project of the Environmental Studies Department at Dickinson College. Since its founding in 1986, ALLARM has become a nationally recognized technical and programmatic support center for community organizations interested in watershed assessment, protection, and restoration. ALLARM program goals are to:

- 1) Enhance local action for the protection and restoration of Pennsylvania watersheds by empowering communities with scientific knowledge and tools to implement watershed assessments;
- 2) Provide Dickinson College students with opportunities to participate in community-based participatory research thereby enhancing the quality of undergraduate science education; and
- 3) Be the leader in volunteer monitoring in Pennsylvania and a national model for college-community partnerships.



Through the work of student and professional staff, ALLARM offers comprehensive services to enable groups to use critical scientific tools to enhance environmental quality and fully participate in community decision-making. The program staff includes a Director, an Assistant Director, a faculty Science Director, and 12-14 undergraduate student staff.

For more information on please visit: [www.dickinson.edu/allarm](http://www.dickinson.edu/allarm) or email: [allarm@dickinson.edu](mailto:allarm@dickinson.edu)

## Background on Manual:

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The Environmental Alliance for Senior Involvement (EASI) developed a method for macroinvertebrate assessment that enables watershed groups to easily monitor the long-term biological condition of streams (Senior Environmental Corp). This is one of the few assessments that provide information regarding impacts that continuously influence aquatic life. In addition to biological monitoring, it is equally important to conduct chemical and visual assessments. It is critical to keep in mind that water chemistry may not be the only influential factor on biological life. Since macroinvertebrates spend almost their entire life in the stream, the condition of in-stream habitat is just as important as the chemical condition of the water.

ALLARM uses this protocol to train volunteers on biological monitoring. This manual is a culmination of tools and resources ALLARM has developed to complement training workshops using the EASI protocol.

Project funding provided by the Foundation for Pennsylvania Watersheds.



**Foundation for Pennsylvania Watersheds**

# What is a Macroinvertebrate?

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Most macroinvertebrates are crustaceans or insect larvae that have no vertebral column (invertebrate) and can be seen with the naked eye (macro). They spend the majority of their life living in streams or other aquatic environments.

Macroinvertebrates are most abundant in **riffle zones** of streams where oxygen is more plentiful or in areas around banks that provide more protection. Figure 1 shows examples of riffle zones and areas with protective bank coverage that are likely to support healthy populations of macroinvertebrates. The availability of food and oxygen along with chemical input will influence the type and abundance of macroinvertebrates in these areas.

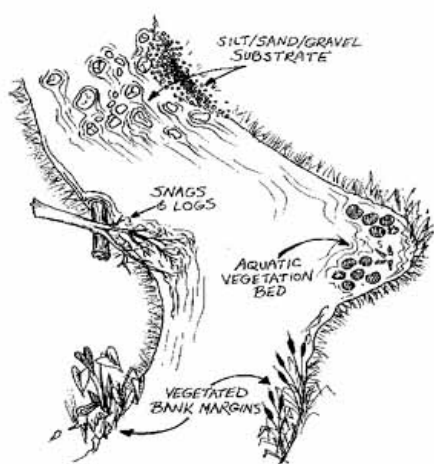


Figure 1. Section of stream illustrating natural habitats for macroinvertebrates

## Method of Collection:

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In general, macroinvertebrate assessments should be conducted twice a year, once in the spring (March-May) and once in the fall (August-October). Because of hatching and mating patterns, it is best for northern areas of the state to conduct assessments in the earlier parts of the seasons while southern areas should conduct them in the later parts of the seasons.

### Site Selection:

In order to have a good idea of the macroinvertebrate population that would normally reside in a stream, it is important to decide on a reference site that is the least impacted by pollution and has a variety of habitats that will provide the greatest abundance and diversity of macroinvertebrates in that area. For example, when investigating the impact of a discharge site, a site located upstream of the discharge is typically used as a **reference site** since it is essentially “unimpacted” by the discharge. *One* macroinvertebrate assessment consists of *three* sites within a 30-meter stretch of stream. Site #1 is the most downstream location, #2 is the midstream reach, and #3 is the site furthest upstream. For each location, you will thus have a total of three sites which will all be composited into one large sample. The



three sites should contain riffle zones; however, different methods will need to be used depending on the type of stream being sampled, for example rocky bottom versus muddy bottom.

### Rocky Bottom Sampling:

This method is used for streams that have riffles and **substrates** composed of gravel and/or cobble.

#### Necessary equipment:

- Measuring tape
- 1m X 1m, 500 $\mu$ m kick net or D-frame net
- Collection containers
- Forceps
- Magnifying glass/ hand lens
- Ethanol (to preserve macroinvertebrates if not identifying same day as collection)
- Waders
- Fishing license if collecting >50 organisms



#### Procedure:

1. Identify the sampling area and mark off the 30-meter stretch you will be analyzing. Choose three spots within that stretch. These three sites should be riffles areas and, if possible, should have different flow velocities and substrate types in order to get a better representation of the in-stream habitat diversity. It is often helpful to draw a sketch of the stream and label the three sampling spots (#1 is downstream, #2 is midstream, and #3 is upstream). If you are using a kicknet, proceed to step 2; if you are using a D-frame net, jump to step 6.
2. *Always* start at the downstream site first and also approach the location from downstream. This prevents you from disturbing the other sites and the site you are about to sample with sediment or other dislodged macroinvertebrates.

If you are using a kicknet, select your first 1 m X 1 m riffle area and have a team member position the net, facing upstream. The net should be held at a 45 degree angle to the water's surface. However, depending on the stream depth, this angle can be adjusted accordingly as long as water **DOES NOT** flow over the net. This may result in the loss of macroinvertebrates! Also ensure that the bottom of the net is fit to the bottom of the stream. This can be done by using rocks *from the sampling area* and anchoring the bottom of the net. Make sure to rub the rocks either before or after kicking to remove any macroinvertebrates that may be clinging to the rocks.

3. The second team member should stand in front of the kick net, facing downstream. First pick up any large rocks in the 1m X 1m area and rub them thoroughly in the water so that any macroinvertebrates clinging to the rock will flow into the net. Be sure to check for case-building caddisflies because they tend to



stick securely to the rocks. Place the “clean” rocks outside of kicking area. Starting at the upstream end of the 1m X 1m area, beginning kicking up the streambed to at least two or three inches in depth. Once you reach the net, remove the rocks that are anchoring the bottom of the net.

4. When removing the net, it is important to make sure water does not flow over the top because it will take away some of the organisms that you have just collected! The net holder should grasp the top of the handles while the kicker grabs the bottom handles and lift with a forward scooping motion. You can then either pick directly from the net or flush the contents into a bucket. Do not keep any fish, amphibians, or reptiles. If you collect crayfish, it may be a good idea to make note of them (because they are included in the assessment) and release them as well.



If you intend to identify the macroinvertebrates later, put them in containers with enough ethanol to cover all of the organisms. Make sure to label the containers according to which location they are from.

5. Repeat steps 2 through 4 for the other two sites and combine all of the organisms from all three sites. This will provide a better representation of the stream’s overall macroinvertebrate community. Now continue on to step 10.
6. To sample with a D-frame net, you will complete 20 jabs in the 30 m stretch you have previously selected. When using the jab method, samples should typically be taken from riffle areas, but you should also get samples of the overall habitat in the stream. The majority of the jabs should be taken from riffle areas (at least 10) and the remaining can be distributed among other stream habitats.
7. Be sure to check the undersides of rocks in the area you a jabbing for any macroinvertebrates that may be clinging to the rock, especially case-building caddisflies.
8. The contents from each jab can be dumped into a large bucket with stream water, which will be sorted later.
9. After completing 20 jabs, sort through all of the debris for anything that moves and transfer the organisms into the sorting trays.
10. When sorting, look for any movement and be sure to check on all sides of leaves or other debris for macroinvertebrates. The longer the net is out of the water, the less movement you will see so a magnifying glass may also be helpful



to make sure all of the organisms are collected. Ice cube trays work well to sort the macroinvertebrates into like-organisms.

11. Once you feel they are sufficiently sorted, use a hand lens and the following identification sheets to identify the organisms to the Order level. If you plan to identify the organisms at a later point, put them in a container with ethanol to help preserve them. Tally the number of individuals in each Order on the field data sheet (attached). You are now ready to begin calculating the stream water quality.

## Muddy Bottom Sampling:

This method is used for streams that lack riffles and have muddy, silty, or sandy bottoms.

### *Necessary Equipment:*

- D-frame net
- Collection containers
- Forceps
- Ethanol (to preserve macroinvertebrates if not identifying same day as collection)
- Fishing license if collecting >50 organisms

### *Procedure:*

1. There are typically four types of habitat found in muddy bottom streams: **vegetated bank margins, snags and logs, aquatic vegetation beds, and decaying organic matter**. For muddy bottom streams, 20 “jabs” with a D-frame net in different habitat areas are combined to get a representative sample of the macroinvertebrate population.
2. Note on your data sheet the types of habitats present and the number of jabs taken from each. Some suggestions for distributing the jabs are as follows:
  - a. If all four types are present, jab vegetated banks 10 times and divide the other 10 among remaining habitats.
  - b. If three types are present, jab the least productive habitat (silt/sand/gravel) 5 times and divide the remaining 15 between the more productive habitats.
  - c. If only two types are present, the silt/sand/gravel will likely be the most abundant, so jab it 5 times and the more productive habitat 15 times.
  - d. If some habitats are plentiful and others are sparse, sample what you can of the sparse habitats and take the remaining jabs of the most productive habitat available.
3. Begin sampling from the furthest downstream site and work upstream. The four habitat types have different ways to collect the macroinvertebrates:
  - a. Vegetated Bank Margins—keeping the net underwater, jab vigorously with an upward motion, brushing against vegetation and roots.



- b. Snags and Logs—holding the net under the submerged wood, use your other hand (which should be gloved) to rub about 1 ft<sup>2</sup> of the log. This equals one jab.
  - c. Aquatic Vegetation Beds—jab vigorously with an upward motion through the plant bed
  - d. Silt/sand/gravel—push the net forward about 1 foot upstream to dislodge the first few inches of substrate. To avoid a netful of mud, you can sweep the net through the water (as long as water does not run over the top of the net) to filter out some of the sediments.
4. The contents from each jab can be dumped into a large bucket with stream water, which will be sorted later.
5. After completing 20 jabs, sort through all of the debris for anything that moves and transfer the organisms into the sorting trays.
6. Once you feel that you have found everything, use a hand lens and the following identification sheets to identify the organisms to the Order level. If you plan to identify the organisms at a later point, put them in a container with ethanol to help preserve them. Tally the number of individuals in each Order on the field data sheet (attached). You are now ready to begin calculating the stream water quality.



# Identification Categories:

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## Sensitive:

### Water Penny Larvae—Order Coleoptera

- ✓ Wing pads absent
- ✓ Flattened disc-like body
- ✓ 6 legs under dorsal plate



Meyers, 2009

### Mayfly Nymph—Order Ephemeroptera

- ✓ 6 legs
- ✓ 3 tails
- ✓ Gills on abdomen
- ✓ Single claw on end of legs



Clapp, 2006

### Dobsonfly Larvae (Hellgrammite)—Order Megaloptera

- ✓ 6 legs
- ✓ 8 abdominal segments each with a filament
- ✓ 2 anal prolegs with hooks
- ✓ Well developed chewing parts
- ✓ Poorly developed eyes



Neuswanger, 2009

### Gilled Snail—Class Gastropoda

- ✓ Vary in size
- ✓ Presence of operculum is characteristic of gilled snail (seals the opening to the shell when the foot is retracted)
- ✓ Opening is on right hand side



Lake County, Ohio, 2009

### Riffle Beetle—Order Coleoptera

- ✓ Oval elongate body
- ✓ 6 legs



Peckarsky, 1990

### Non-Net-spinning Caddisfly Larvae—Order Trichoptera

- ✓ 6 legs
- ✓ 2 anal hooks
- ✓ Worm-like body
- ✓ Often build cases



Neuwinger, 2005  
University of Wisconsin  
Extension, 2007



### Stonefly Nymph—Order Plecoptera

- ✓ 6 legs
- ✓ Usually 2 tails
- ✓ Gills on thorax
- ✓ 2 claws on end of each leg



Meyers, 2009

## Somewhat Sensitive:

### Beetle Larvae—Order Coleoptera

- ✓ Abdomen composed of 8-10 segments
- ✓ Thoracic legs usually present
- ✓ No terminal prolegs



University of Minnesota, 2003

### Clams—Class Pelecypoda

- ✓ Two piece shell
- ✓ Commonly oval
- ✓ Approximately 2-250 mm in size



Clapp, 2006

### Crane Fly Larva-Order Diptera

- ✓ No wing pads
- ✓ Worm-like body
- ✓ 8-10 abdominal segments
- ✓ May have prolegs and terminal processes



Marietta College, 2009

Neuswanger, 2009

### Crayfish—Order Decapoda

- ✓ 2 large claws
- ✓ 8 legs
- ✓ 2 long antennae



Wisconsin Department of Natural Resources, 2008

### Damselfly/Dragonfly Nymph—Order Odonata

- ✓ Both have large labium (lower lip) and moderately developed eyes
- ✓ No gills along the body
- ✓ Dragonfly larvae have 3 wedge-shaped "tails" and the damselfly larvae have 3 feather-like "tails"



Dragonfly larva

Clapp, 2006



Damselfly larva

Neuswanger, 2009

**Scud**—Order Amphipoda

- ✓ Laterally flattened
- ✓ Swims sideways
- ✓ 7 pairs of legs
- ✓ Resembles a shrimp

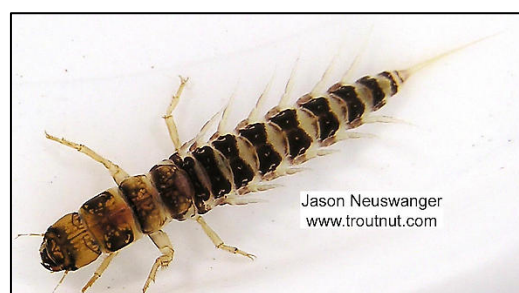


California Department of Fish and Game, 2009

North Carolina Museum of Natural Sciences, 2005

**Alderfly Larva**—Order Megaloptera

- ✓ Abdomen with 7 pairs of 4-5 segmented lateral filaments
- ✓ A single unbranched terminal filament



Neuswanger, 2009

**Sowbug**—Order Isopoda

- ✓ 7 pairs of legs and 2 antennae
- ✓ Dorsally flattened



Clapp, 2006

**Net-spinning Caddisfly Larva**—Order Trichoptera

- ✓ 1-3 sclerotized (hardened) thoracic segments
- ✓ 3 pairs of legs
- ✓ branched gills may be present on ventral side of abdomen
- ✓ anal hooks may also have tufts of longer hair

**Fishfly Larva**—Order Megaloptera

- ✓ No gill tufts underneath abdomen
- ✓ Resembles a small hellgrammite





## Tolerant:

### Aquatic Worm-- Class Oligochaeta

- ✓ Elongate cylindrical worms usually 1-30 mm in length
- ✓ Anywhere from 7-500 body segments



Texas Flyfishers, 2006

### Blackfly Larva—Order Diptera

- ✓ Cylindrical body with one end wider
- ✓ Head with fan-like mouth brushes



### Leech-- Order Hirudinea

- ✓ Dorsoventrally flattened
- ✓ Anterior and posterior ventral suckers



(AECOS, 2002)

### Midge Fly Larva—Order Diptera

- ✓ May have prolegs on thorax
- ✓ Terminal segment of abdomen may have processes on it
- ✓ Worm-like body
- ✓ No wing pads
- ✓ May resemble caddisfly



Neuswanger, 2009

### Other Snails--Class Gastropoda

- ✓ Non-gill breathing snails
- ✓ Do not have an operculum
- ✓ Opening is usually on left side



(Environmental Protection Agency, 2009)

## Quantifying Your Results:

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On your data sheet, assign one of the following codes to each group of organisms:

**R (rare)** = 1 to 9 organisms

**C (common)** = 10 to 99 organisms

**D (dominant)** = 100 or more organisms

The data sheets separate the macroinvertebrates into pollution tolerance groups where:


















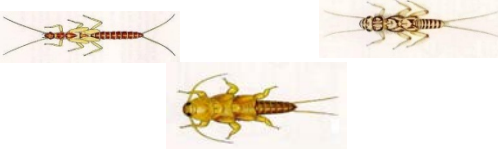




**Group I** represents organisms that are very sensitive to pollution and tend to live only in good-quality water

**Group II** represents organisms that are somewhat sensitive to pollution and are able to survive in water quality that is either good or fair.

**Group III** represents organisms that are tolerant of pollution and are able to survive in good, fair, and poor water quality

Once you have assigned the R, C, and D codes to the different groups, you are able to calculate the index value as described on the data sheet. An example of this scoring is provided for you.

*EASI Tally Sheet (Example):*

Group I Sensitive	Count	Group II Somewhat Sensitive	Count	Group III Tolerant	Count
Water Penny Larvae 	32	Beetle Larvae 	0	Aquatic Worms 	13
Hellgrammites 	1	Clams 	0	Blackfly Larvae 	0
Mayfly Nymphs 	46	Crane-fly Larvae 	0	Leeches 	0
		Crayfish 	2		
Gilled Snails 	0	Damselfly Nymphs 	1	Midge Larvae 	3
		Scuds 	0		
Riffle Beetles (adult) 	0	Sowbugs 	12	Snails 	5
Stonefly Nymphs 	7	Fishflies 	0	Site Designation:	
		Alderflies 	0		
Non Net-Spinning Caddisfly Larvae 	103	Net-Spinning Caddisfly Larvae 	41	Team Members:	

**Calculating the EASI Index Value to Rate the Water Quality of the Stream**

### Macroinvertebrate Survey (Example):

## MACROINVERTEBRATE SURVEY

Type of Sampling (check one)

Page 64

Rocky bottom  Muddy bottom

Muddy Bottom Sampling Only: Record the number of jabs taken in each habitat type.

Page 68

Vegetated bank margin

Snags and logs

Aquatic vegetation beds

Silt/sand/gravel substrate

## MACROINVERTEBRATE COUNT

Page 70

1. Identify the macroinvertebrates in your sample and assign them letter codes based on their abundance: R (rare) = 1-9 organisms; C (common) = 10-99 organisms; and D (dominant) = 100 plus organisms.

Group I Sensitive	Group II Somewhat-Sensitive	Group III Tolerant
<u>C</u> Water penny larvae	<u>N/a</u> Beetle larvae	<u>C</u> Aquatic worms
<u>R</u> Hellgrammites	<u>N/a</u> Clams	<u>N/a</u> Blackfly larvae
<u>C</u> Mayfly nymphs	<u>N/a</u> Crane fly larvae	<u>N/a</u> Leeches
<u>N/a</u> Gilled snails	<u>R</u> Crayfish	<u>R</u> Midge larvae
<u>N/a</u> Riffle beetle adult	<u>R</u> Damselfly nymphs	<u>R</u> Snails
<u>R</u> Stonefly nymphs	<u>N/a</u> Scuds	
<u>D</u> Non net-spinning caddisfly larvae	<u>C</u> Sowbugs	
	<u>N/a</u> Fishfly larvae	
	<u>R</u> Alderfly larvae	
	<u>C</u> Net-spinning caddisfly larvae	

## WATER QUALITY RATING

Page 70

2. To calculate the index value, add the number of letters found in the three Groups above and multiply by the indicated weighting factor.

Group I	Group II	Group III
<u>2</u> (# of R's) x 5.0 = <u>10</u>	<u>3</u> (# of R's) x 3.2 = <u>9.6</u>	<u>2</u> (# of R's) x 1.2 = <u>2.4</u>
<u>2</u> (# of C's) x 5.6 = <u>11.2</u>	<u>2</u> (# of C's) x 3.4 = <u>6.8</u>	<u>1</u> (# of C's) x 1.1 = <u>1.1</u>
<u>1</u> (# of D's) x 5.3 = <u>5.3</u>	<u>0</u> (# of D's) x 3.0 = <u>0</u>	<u>0</u> (# of D's) x 1.0 = <u>0</u>
Sum of the Index value for Group I = <u>25.5</u>	Sum of the Index value for Group II = <u>16.4</u>	Sum of the Index value for Group III = <u>3.5</u>

- To calculate the water quality score for the stream site, add together the index values for each group. The sum of these values equals the water quality score.

Water quality score = 45.4


















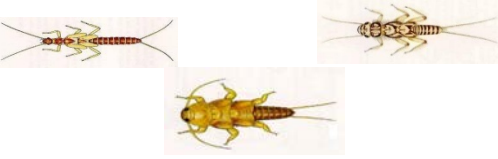




- Compare this score to the following number ranges to determine the quality of your stream site.

<u>0</u>	Good	>40
<u>0</u>	Fair	20 - 40
<u>0</u>	Poor	<20

NOTE: The tolerance groupings (Group I, II, and III) and the water quality rating categories were developed for streams in the Mid-Atlantic states. A trained biologist familiar with local stream fauna should help determine if these tolerance and water quality rating categories should be modified for your geographic region and program.



*EASI Tally Sheet:*

Group I Sensitive	Count	Group II Somewhat Sensitive	Count	Group III Tolerant	Count
Water Penny Larvae 		Beetle Larvae 		Aquatic Worms 	
Hellgrammites 		Clams 		Blackfly Larvae 	
Mayfly Nymphs 		Crane-fly Larvae 		Leeches 	
		Crayfish 			
Gilled Snails 		Damselfly Nymphs 		Midge Larvae 	
		Scuds 			
Riffle Beetles (adult) 		Sowbugs 		Snails 	
Stonefly Nymphs 		Fishflies 		Site Designation:	
		Alderflies 			
Non Net-Spinning Caddisfly Larvae 		Net-Spinning Caddisfly Larvae 		Team Members:	

*Macroinvertebrate Survey:*

## MACROINVERTEBRATE SURVEY

Type of Sampling (check one)

Page 64

Rocky bottom \_\_\_\_\_ Muddy bottom \_\_\_\_\_

Muddy Bottom Sampling Only: Record the number of jabs taken in each habitat type.

Page 68

Vegetated bank margin \_\_\_\_\_

Snags and logs \_\_\_\_\_

Aquatic vegetation beds \_\_\_\_\_

Silt/sand/gravel substrate \_\_\_\_\_

## MACROINVERTEBRATE COUNT

Page 70

1. Identify the macroinvertebrates in your sample and assign them letter codes based on their abundance: R (rare) = 1-9 organisms; C (common) = 10-99 organisms; and D (dominant) = 100 plus organisms.

Group I Sensitive	Group II Somewhat-Sensitive	Group III Tolerant
_____ Water penny larvae	_____ Beetle larvae	_____ Aquatic worms
_____ Hellgrammites	_____ Clams	_____ Blackfly larvae
_____ Mayfly nymphs	_____ Crane fly larvae	_____ Leeches
_____ Gilled snails	_____ Crayfish	_____ Midge larvae
_____ Riffle beetle adult	_____ Damselfly nymphs	_____ Snails
_____ Stonefly nymphs	_____ Scuds	
_____ Non net-spinning caddisfly larvae	_____ Sowbugs	
	_____ Fishfly larvae	
	_____ Alderfly larvae	
	_____ Net-spinning caddisfly larvae	

## WATER QUALITY RATING

Page 70

2. To calculate the index value, add the number of letters found in the three Groups above and multiply by the indicated weighting factor.

Group I	Group II	Group III
_____ (# of R's) x 5.0	_____ (# of R's) x 3.2	_____ (# of R's) x 1.2
= _____	= _____	= _____
_____ (# of C's) x 5.6	_____ (# of C's) x 3.4	_____ (# of C's) x 1.1
= _____	= _____	= _____
_____ (# of D's) x 5.3	_____ (# of D's) x 3.0	_____ (# of D's) x 1.0
= _____	= _____	= _____
Sum of the Index value for Group I = _____	Sum of the Index value for Group II = _____	Sum of the Index value for Group III = _____

To calculate the water quality score for the stream site, add together the index values for each group. The sum of these values equals the water quality score.

Water quality score = \_\_\_\_\_

Compare this score to the following number ranges to determine the quality of your stream site.

0	Good	>40
0	Fair	20 - 40
0	Poor	<20

**NOTE:** The tolerance groupings (Group I, II, and III) and the water quality rating categories were developed for streams in the Mid-Atlantic states. A trained biologist familiar with local stream fauna should help determine if these tolerance and water quality rating categories should be modified for your geographic region and program.

# Glossary:

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**Aquatic Vegetation Beds**—beds of submerged, green/leafy plants attached to stream bottom; typically productive

**Decaying Organic Matter**- typically leaves or once-living organisms that are in the process of decaying

**Reference site**- a site on the stream which is unimpaired or minimally impaired compared to the other sites being monitored

**Riffle zone**- an area of the stream that is shallower than the rest and has faster moving water, usually broken up by rocks

**Substrate**—a material or substance on which an organisms grows and lives, for example rocks or soil

**Snags and logs**—submerged wood, usually dead trees, logs, branches, roots, and leaf packs; very productive

**Vegetated Bank Margin**—consists of overhanging bank vegetation and submerged root mats attached to banks as well as submerged, decomposing leaf packs; highly productive and most abundant type of habitat.



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