

CHAPTER 2: MONITORING WITH LEAF PACKS

MATERIALS LIST

Leaf Pack Stream Ecology Kit Contents

Included Items

QTY	DESCRIPTION
6	Plastic mesh bags
6	Bag Tags
6	Large zipper-top bags
60	Petri dishes
30 ft	Nylon twine
1 set	Thermometers
1	Waterproof marker
1	Scale
12	Brushes
1	Sieve, 500 micron mesh
6	Sorting trays, white, plastic
1	MacroLens™
6	Hand lenses
12	Spoons, white, plastic
2	Rulers
1	Tree Identification Guide
1	Leaf Pack Stream Ecology Kit User's Manual
6	Freshwater Macroinvertebrate Sorting Sheets
1 Set	Freshwater Aquatic Macroinvertebrates: Identification Flash Cards
1	Freshwater Macroinvertebrate Dichotomous Key

Additional Items Needed

- Dried tree leaves, 180g [30g per leaf pack]
- Scissors
- Cooler and ice packs

Optional Items

- Rock hammer or sledge hammer
- Hollow cinder blocks or bricks
- Rebar [reinforcement bar], 1 meter section, or strong stakes.
- Dissecting microscope
- Buckets
- Freshwater Aquatic Macroinvertebrates: Life Cycle and Habitat Flash Cards [Code 5946]

The Leaf Pack Stream Ecology Kit provides materials for six leaf packs and six sorting stations. Procedures are written to be used with the manual and items that are included in the kit. Substitutions with equivalent items can be made.

SAFETY

Safety and health are important factors to consider when planning to monitor with leaf packs. Below are tips that will help you to ensure that the experience is enjoyable and safe.

- Follow all school or organization safety rules and guidelines regarding laboratory and outdoor activities.
- Follow state or country regulations for collecting macroinvertebrates. A fishing or collecting license may be required if macroinvertebrates are considered to be fish bait.
- Ensure that the sampling site is on public property or if stream access is on private property make sure that permission is obtained.
- The stream or river site should be wadeable. Do not enter a waterway where the water level is no higher than your knees. When working in streams, take special care to avoid slipping and falling into deep water.
- When working near deep or fast-moving water, wear a personal flotation device.
- On cold or windy days, it is especially important to provide dry clothes or blankets in case someone gets wet.
- Check weather reports and schedule field activities accordingly.
- If lightning is seen or thunder is heard, do not work in or near the water. Go indoors immediately.
- If the water quality is uncertain, wear protective gloves and boots when coming into contact with the water. Wash hands after deploying, collecting and processing the leaf packs. Never drink the water.
- Carry a first aid kit and cell phone.
- Tell someone where you are going and when you expect to return.
- Read the instructions for all procedures before beginning the project.



DEFINING A GOAL

Monitoring with leaf packs is a 3-4 week process and takes some time and planning. Before beginning, it is important to determine the question that will be answered and the focus of the project - the goal. Is monitoring being done to establish baseline conditions to understand the health of a stream or river, or will the results be used in a school setting to teach students about experimental design? For more information about using leaf packs as teaching tools see Chapter 3.

SELECTING A WATERWAY TO MONITOR

Another consideration is to determine which waterway will be monitored or studied and whether the stream has legal access. The ideal waterway would be a small stream where leaf packs can be placed in shallow riffle habitats or even runs, but not a stream with deep waters such as pools or larger rivers. A good rule of thumb is to place leaf packs in shallow water where the water level is no higher than your knees.

For help in making decisions about methods, goals, and how to get started, contact the Leaf Pack Network Administrator with Stroud Water Research Center at leafpacknetwork@stroudcenter.org. The Leaf Pack Network also offers in-person 1-2 day workshops.

COLLECTING LEAVES

Materials List

Additional Materials	Optional Materials
<ul style="list-style-type: none">Leaves, 3 types	<ul style="list-style-type: none">NewspaperPaper bags for storage of dried leaves

The artificial leaf packs will replicate a natural leaf pack in a stream. Natural leaf packs have layers of leaves, typically trapped against a rock, with stream water flowing rapidly over them, which keeps the leaves in place.



Procedure

Prior to monitoring, leaves that are strong enough to stay intact and attract macroinvertebrates as a food source and habitat will have to be collected and dried. If a stream is being monitored for baseline conditions, leaves from a species of tree that is native to the watershed should be chosen. It is recommended that leaves from three dominant species be included.



Tips for collecting leaves:

- Gather leaves that have already fallen from trees. If this is not possible, “green” or live leaves can be used but they will have to be dried.
- Gather dried leaves. If leaves are damp, lay them out on newspaper, indoors, until they are completely dry. If necessary, dry leaves can be stored in paper bags until they are needed.
- Gather enough leaves - approximately 15-30 g for each leaf pack.
- Choose leaves that are hand-sized or smaller.
- To select a leaf that will not break down in the stream too quickly, bend a dry leaf in half. If it easily breaks, it is a good leaf to use in a leaf pack. If it crumbles into lots of pieces, resembling crumbs, do not use it for a leaf pack.
- Leaves that have been stored for a long time most likely will be too old and crumble into tiny pieces. They will not function well in a leaf pack and should be discarded.

PREPARING LEAF PACKS FOR THE STREAM

Materials List

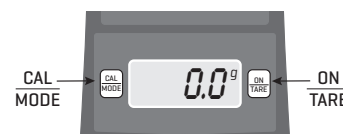
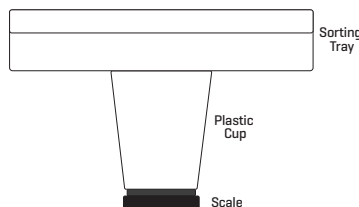
Included in Kit	Additional Materials	Optional Materials
<ul style="list-style-type: none">• Plastic mesh bags, 3• Bag tags, 3• Nylon twine• Waterproof marker• Scale• Thermometer• Field Data Sheet/Site Map• Tree Identification Guide	<ul style="list-style-type: none">• Leaves, 3 types• Scissors• Cup, lightweight plastic	<ul style="list-style-type: none">• Rebar or hollow cinder block• Mallet

* The larger the diameter of the plastic cup opening, the more stable the components of the weighing procedure will be. A diameter of 3.5" or greater is recommended. The height of the cup should be great enough to raise a sorting tray above the scale so that the display is visible.

Procedure

Each artificial leaf pack will consist of approximately 10 grams of each of three leaf types for a total weight of around 30 grams in a mesh bag.

1. Identify the three most common trees or shrubs near the stream. Choose three native species that are found in the watershed if monitoring for baseline conditions or if monitoring water quality.
2. Collect leaves and separate them by type. Dry the leaves as necessary.
3. Place the scale on a flat, level surface.
4. Place a lightweight plastic cup on the scale.
5. Place a sorting tray on top of the cup.
6. Press the ON/TARE button to turn the scale on and zero it. Make sure that the scale is in the gram [g] mode.

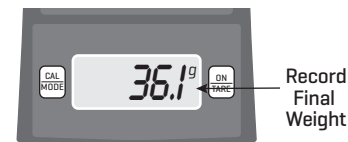
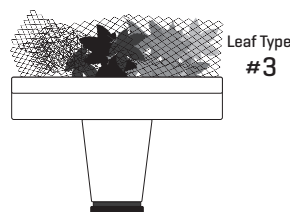
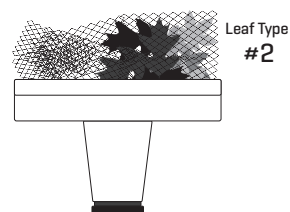
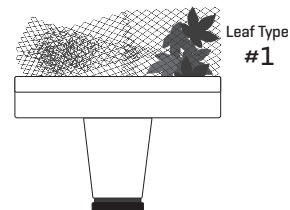
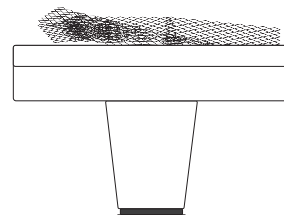



7. Place an empty plastic mesh bag in the tray. Record the weight of the mesh bag.
8. Remove the bag from the tray.
9. Open the bag all the way to the bottom. Tie a knot if the bag is not sealed.
10. Add approximately 10 grams of leaf type #1.
11. Press the ON/TARE button to turn the scale on and zero it.
12. Place the mesh bag with the leaves in the tray. Record the weight.
13. Remove the bag from the tray.
14. Add approximately 10 grams of leaf type #2.
15. Press the ON/TARE button to turn the scale on and zero it.
16. Place the mesh bag with the leaves in the tray. Record the weight.
17. Remove the bag from the tray.
18. Add approximately 10 grams of leaf type #3.
19. Press the ON/TARE button to turn the scale on and zero it.
20. Place the mesh bag with the leaves in the tray. Record the final weight.
21. Repeat steps 6 – 20 to create two more leaf packs.

Note: If the stream flow is minimal, it is acceptable to create smaller leaf packs so that the leaf packs can remain submerged [e.g. instead of three 30 g leaf packs, create six 15 g leaf packs].

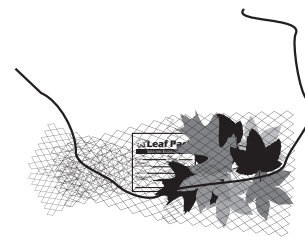
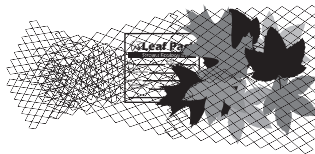
22. Complete a Bag Tag for each mesh bag using the waterproof marker. Record the information on the Field Data Sheet

- date
- leaf pack location
- leaf pack number
- leaf pack contents/weight
- organization/school



 Leaf Pack Stream Ecology Kit	
Date / Fecha	Number of packets of leaves
Organization / School Organización / Escuela	
Leaf Pack Location Ubicación del paquete de hojas	
Leaf Pack Contents/Weight Contenido del paquete de hojas	

23. Place a Bag Tag in each leaf pack bag.
24. Tie one knot in each leaf pack bag to close it.
25. Loop a long length of nylon twine through the mesh of each bag so that the leaf pack can be attached to a large rock or rebar.



For example

The empty mesh bag weighs 5.9 grams, there are 10.4 grams of Leaf Type #1, 9.8 grams of Leaf Type #2 and 10.0 grams of Leaf Type #3 for a total weight of 36.1 grams.

Empty Mesh Bag	5.9 g
Bag [5.9 g] + Leaf Type #1 [10.4 g]	16.3 g
Bag [5.9 g] + Leaf Type #1 [10.4 g] + Leaf Type #2 [9.8 g]	26.1 g
Bag [5.9 g] + Leaf Type #1 [10.4 g] + Leaf Type #2 [9.8 g] + Leaf Type #3 [10.0 g]	36.1 g
Final Weight of Pack	36.1 g

Note: The procedure is based on the 30 second auto off feature of the scale and the assumption that it will probably take greater than 30 seconds to place leaves in the bag.



PLACING LEAF PACKS IN THE STREAM

Materials List

Included in Kit	Additional Materials
<ul style="list-style-type: none">• Nylon twine• Thermometers• Ruler• Field Data Sheet/ Site Map• Habitat Data Sheet	<ul style="list-style-type: none">• Leaf packs, 3• Pencil

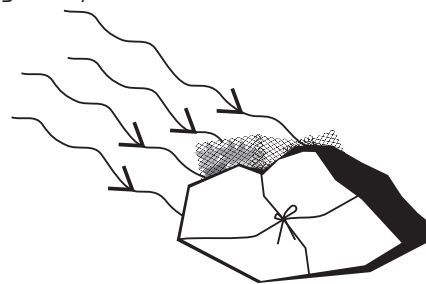


Procedure

Note: If Stream Discharge will be determined, do so before placing leaf packs in the stream. Follow the procedure found in Additional Activities in Chapter 3.

1. Find an area of a small stream where a clump of leaves would naturally form, such as the upstream side of a large rock or log. Make sure the water is deep enough for the packs to be totally submerged when placed in the stream. Place the leaf packs at least 1-2 feet from each other in the same riffle, on the upstream side of the rock or log so that as much surface area of the pack is facing the current as possible.
2. It is best to tie the leaf packs directly to existing rocks in the stream in a riffle habitat. If there are no rocks, use overhanging roots or use cinder blocks with reinforcing rods [rebar]. Use a sledgehammer to drive a piece of rebar through the hollow part of each cinder block to secure it to the streambed. Remember to remove the cinder blocks and rebar when the project has been completed.
3. Make sure that all leaf packs are submerged, securely tied, and not floating up and down in the water column. Leaf packs that flap with the current are not properly placed and will negatively influence the colonization of macroinvertebrates. If the leaf pack is not securely tied, the pack may dislodge and float downstream.
4. Record the information about the site and placement of the leaf packs on the Field Data Sheet/Site Map.

NOTE: Data fields such as Site Code and Site Name will be created in the Leaf Pack Network data portal.



USING THE THERMOMETERS

There are 2 thermometers included in the Leaf Pack kit. They have adhesive backs. Before going out into the stream, adhere each one to a ruler to make holding onto them a little easier.

The Low Range thermometer will show liquid crystal numbers when it is activated by low temperatures. The High Range thermometer has liquid crystal windows. The exact temperature is indicated by a GREEN display. The green display will usually be between a blue and a tan/red display.

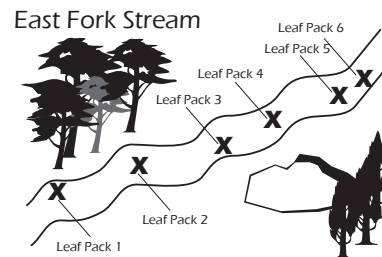
Low
Range °C



High
Range °C



5. Sketch the stream area on the Site Map. Show the position of each leaf pack in the stream and note any landmarks that may help in locating them [e.g. foot path, large Sycamore tree, rope swing, etc.]. Leaf packs may become covered with sediment and algae making them hard to locate weeks later. It may be useful to place markers along the bank of the stream to indicate the location of each leaf pack. However, they can also draw attention to the leaf packs which may lead to vandalism.



6. Complete the Habitat Data Sheet [optional].
7. Leave the leaf packs in the stream for 3-4 weeks. If possible, check the leaf packs periodically to make sure that they remain stable and submerged, especially after storms. Note any storms, flooding or drought on the Field Data Sheet.

If a storm is forecasted for after the leaf packs have been in the stream for at least 3 weeks, remove the leaf packs before the storm. If a storm occurs within the first or second week of being submerged, check each leaf pack for excess sediment. If a large amount of sediment is present, rinse each leaf pack with stream water and leave them in the stream for an additional week, or longer, than originally planned. Removing the leaf packs before 3 - 4 weeks will affect the results.



COLLECTING LEAF PACKS FROM THE STREAM

Materials List

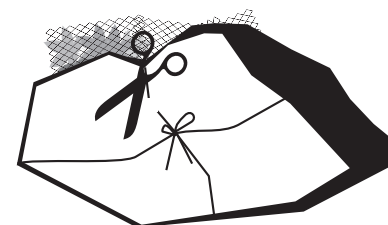
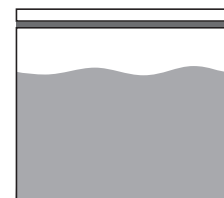
Included in Kit	Additional Materials	Optional Materials
<ul style="list-style-type: none">• Zipper-top bags• Sieve• Field Data Sheet with completed Site Map	<ul style="list-style-type: none">• Scissors• Collecting license or permit for aquatic macroinvertebrates, if necessary	<ul style="list-style-type: none">• Bucket, 3 – 5 gallon• Cooler and ice packs• Air stone

Procedure

Leaf packs should not be collected immediately after a storm because the macroinvertebrates may have been washed downstream by the current or burrowed in the stream substrate.

Note: All macroinvertebrates will be returned to the stream alive after collecting, sorting and counting.

1. Refer to the Site Map to identify the location of each leaf pack. The leaf packs will be collected starting with the pack farthest downstream and working upstream.
2. Complete the remaining information on the Field Data Sheet.
3. Collect additional stream water in a bucket so that it can be used during macroinvertebrate sorting. Keep the water cool. Tap water may be used. Chlorinated tap water must sit for three days before use to allow the chlorine to dissipate. Chlorine will kill the macroinvertebrates.
4. Put enough stream water in the bottom of a zipper-top bag to entirely cover the leaf packs. A bucket can also be used.
5. When retrieving leaf packs from the stream, gently hold onto the submerged leaf pack with one hand while holding the sieve in the water with the other hand just below the leaf pack as if to catch it. Have a second person cut the twine securing the leaf pack to the rock or block.
6. Quickly and gently pick up the leaf pack with the sieve. Place the leaf pack in the zipper-top bag. Rinse the sieve off into the bag. Seal the bag. Work quickly because some of the insects are very fast and will try to escape.
7. Repeat with remaining leaf packs.
8. Streamside processing is recommended or place the zipper-top bags in a cooler to bring the leaf packs indoors. Some invertebrates are very sensitive to changes in temperature. Therefore, try to keep them consistently cool and return them to the stream as soon as possible. If immediate processing is



not possible, most aquatic insects will survive in leaf packs that are kept in coolers with ice or refrigerated overnight. If macroinvertebrates are in a bucket, an aquarium aerator/bubbler/air stone is recommended.

To maintain the macroinvertebrates overnight:

- Place the zipper-top bags with the leaf packs in a cooler that is filled with stream water and leave them outdoors if the temperature is lower than 15 °C. Keep the cooler out of direct sunlight.
- Put freezer packs in the cooler for storage indoors.
- Store the zipper-top bags with the leaf packs bags in a refrigerator.
- If the insects are loose in a bucket of stream water, use an insulated bucket [bait bucket] with an air stone or aquarium bubbler.



PROCESSING THE LEAF PACKS

[streamside or indoors]

Materials List

Included in Kit	Additional Materials	Optional Materials
<ul style="list-style-type: none">• Sorting trays• Sieve	<ul style="list-style-type: none">• Buckets• Stream water• Cooler & ice packs/ refrigerator	<ul style="list-style-type: none">• Squirt bottles

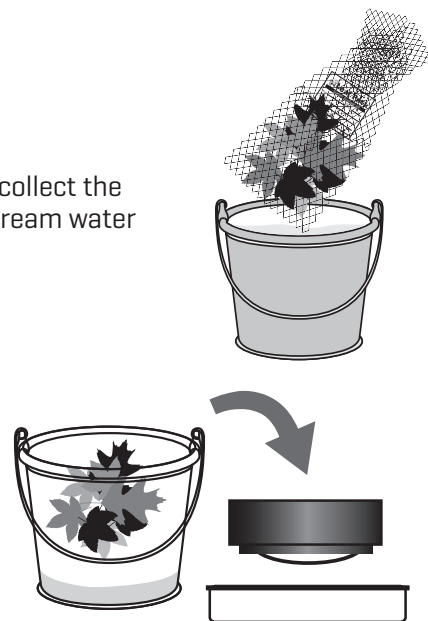
Procedure

Streamside processing is preferable. Keep the leaf packs in a cooler with ice packs or in a refrigerator until they are processed.

1. Fill a bucket three-fourths full with stream water.
2. Remove the twine from a leaf pack by untying it or cutting it.
3. Carefully untie the knot in the mesh bag.

4. Transfer the contents of the leaf pack to the bucket.
5. Rinse remaining contents of zipper-top bag into the bucket.
6. Repeat steps 2-4 with each leaf pack.
7. Pour the contents of the bucket through the sieve into another bucket to collect the macroinvertebrates in the sieve. If there is a lot of sediment pour some stream water through the sieve to rinse off the sediment.
8. Proceed to Preparation for Sorting.

If sorting and identification will take place in another location, transfer the macroinvertebrates in the sieve to a bucket that contains enough stream water to cover the contents. It may be helpful to use a squirt bottle to squirt stream water through the back of the sieve to dislodge the macroinvertebrates.



SORTING & IDENTIFICATION

Materials List

Included in Kit	Additional Materials	Optional Materials
<ul style="list-style-type: none"> • Sorting trays • Petri dishes • Spoons • Brushes • Hand lenses • MacroLens • Freshwater Macroinvertebrate Sorting Sheet 	<ul style="list-style-type: none"> • Freshwater Aquatic Macroinvertebrate ID Cards • Dichotomous Identification Key to Freshwater Macroinvertebrates • Biotic Index Data Sheet 	<ul style="list-style-type: none"> • Buckets • Stream water • Cooler & ice packs/ refrigerator • Aerator/Air stone/ bubbler • Cups • Bowls • Squirt bottles • Dissecting scope • Identification keys

Procedure - Preparation for Sorting

Six plastic trays and six sorting sheets are included in the Leaf Pack Stream Ecology Kit. The sorting procedure is written for dividing the contents from one leaf pack among several trays so that a group or team will sort the macroinvertebrates from one leaf pack.

1. Divide the pack contents among Sorting trays that contain a couple of inches of stream water. If time allows, check the reserved leaves in the zipper-top bag for the leaf pack being sorted for any macroinvertebrates that may have been overlooked. Add them to the trays. Proceed to Sorting and Identification.
2. Repeat for each leaf pack.



If the water in the trays begins to become too warm, place the trays on a plastic bag filled with ice to keep the macroinvertebrates cool.

Procedure - Sorting and Identification

Fill out a **Biotic Index Data Sheet** for each Leaf Pack.

1. Place a Petri dish on each circle on the Freshwater Macroinvertebrate Sorting Sheet.
2. Use a spoon to fill each Petri dish full of water.
3. Use a brush or spoon to transfer the macroinvertebrate from the trays to the Petri dish next to the drawing it resembles. Place all macroinvertebrates that look alike in the same Petri dish.

Use the hand lenses and the MacroLens to check for the special characteristics of each macroinvertebrate. Refer to the Freshwater Aquatic Macroinvertebrate ID Cards and Dichotomous Key for a list of macroinvertebrate characteristics. Keep the macroinvertebrates in water at all times.



Procedure - Calculating the Biotic Index

Through controlled experiments and field observations, scientists have learned that some aquatic organisms are particularly sensitive to specific types and levels of pollutants. Many freshwater invertebrates require a specific range of physical and chemical parameters to flourish. The presence or absence of these organisms, along with their abundance in a stream, can be used to reveal overall water quality.

Biotic indices are a widely used method of using a scale to indicate organic and nutrient pollution based on the abundance of organisms with known tolerances to environmental stress. There are many variations of biotic indices across the United States and around the world that have been developed to interpret regional differences in **taxa** sensitivities. The biotic indices in the Leaf Pack Stream Ecology Kit — The **Pollution Tolerance Index Score** and the **Pollution Tolerance Index Rating** — are created from a select subset of indices from across the United States.

Each organism is assigned a sensitivity group (1, 2 or 3) depending on the organism's ability to tolerate stressors such as pollution or lack of habitat within the stream. The organisms that are very sensitive are in Group 1 [sensitive], those that are somewhat sensitive are in Group 2, and those that can survive or tolerate poor water quality are in Group 3 [tolerant].

1. Count the number of individual macroinvertebrates in the Petri dishes for each taxa [e.g. mayflies, planarians, damselflies]. Record the quantity in the box to the left of the taxa name on the **Biotic Index** Data Sheet.
2. Determine the **Sum of All Individuals** by adding the numbers in the boxes next to all of the the taxa names. Record the total in the **Sum of All Individuals** box on the far right.
3. Count how many boxes in each sensitivity group column have a quantity entered. [Group 1 and Group 2: maximum 8, Group 3: maximum 7]. Enter the **Number of TAXA** in the box at the bottom of each column.
4. Multiply the **Number of TAXA** by the weighting factor (3, 2 or 1) at the bottom of the column to obtain the Index Value for each Sensitivity Group.
5. Add the **Index Values** for the three groups to determine the **Pollution Tolerance Index (PTI) Score**. Enter the **PTI Score** in the box.
6. Determine the **Pollution Tolerance Index Rating** from the PTI Score.



7. Repeat for each leaf pack. If the leaf packs are being used for monitoring the water quality of the stream, create a combined Biotic Index Data Sheet by averaging the data from all of the leaf packs to determine an overall Biotic Index.
8. Enter the values and the abundance levels into the data portal found on monitormywatershed.org
 NOTE: Only include counts of taxa groups found on the data sheet and those that are found alive (e.g. mussel shells or snail shells that are empty do not count). Make note of other taxa that may be found but do not include them in the index calculations.

Procedure - Calculating the EPT Index

The EPT index is a measure of the percentage of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) found in the total number of macroinvertebrates in a sample. Because these orders of macroinvertebrates tend to be sensitive to pollution, a high percentage of them per sample indicates good water quality.

$$\frac{E + P + T}{\text{Total Number of Macroinvertebrates}} = \boxed{}$$

Change to a percent by multiplying the answer by 100

$$\underline{} \times 100 = \boxed{} \% \text{EPT}$$

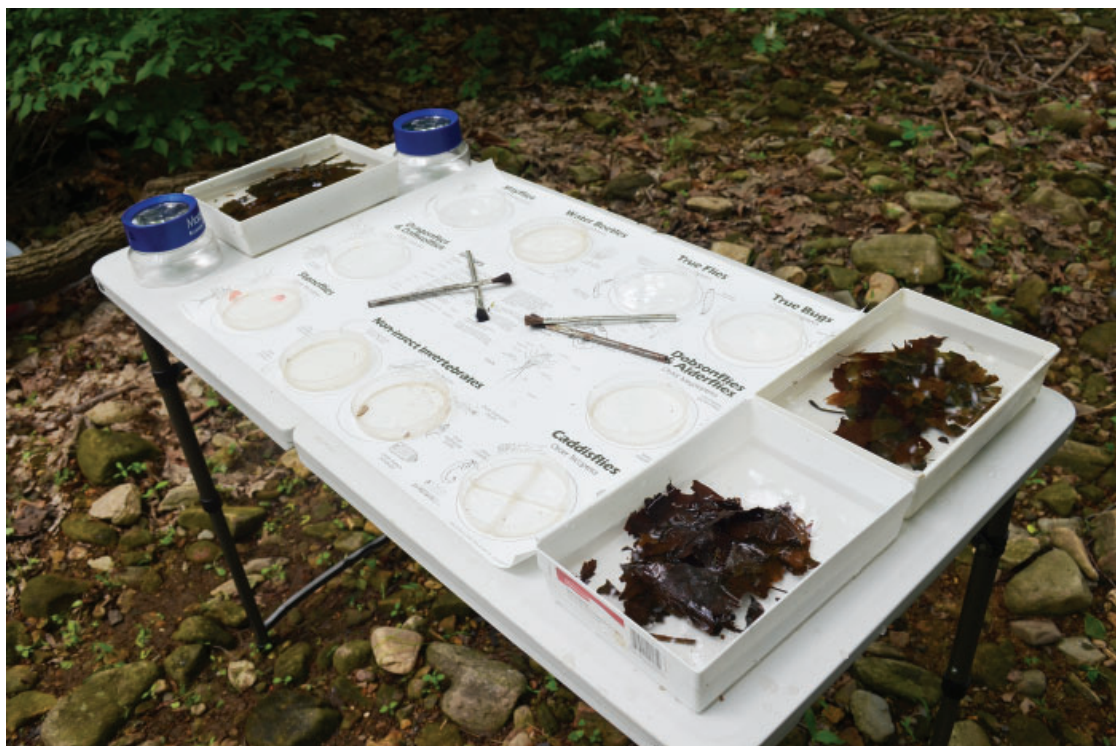
Calculating the EPT Index (as a Percentage)

1. Add together the number of Mayflies (E), Stoneflies (P), and other Caddisflies (T) found in the sample.
 Note: The common net-spinning caddisfly (Hydropsychidae) is not included in the EPT calculation because this one Family of caddisflies is not as sensitive to pollution.
2. Divide the EPT Total by the total number of all macroinvertebrates in the sample.
3. Multiply this number by 100 to convert it to percent. This is the EPT Index value. The higher the percentage, the better the water quality.

Procedure - Clean Up

Return the macroinvertebrates to the location from where they were collected as soon as possible.

- Macroinvertebrates that die can be preserved for a reference collection if placed in 70% ethyl alcohol.
- Wash and dry all equipment before storing.



CONCLUSION

What do the values and ratings mean?

The aquatic macroinvertebrate community is an indication of both the water quality and the available habitat. In a healthy stream a high diversity of all three sensitivity groups should be found, as well as a high abundance of the sensitive and somewhat sensitive groups. If high diversity but low abundances are found, the habitat survey should be referenced to see if there is a component of the survey that looks to be poor. If the results show low diversity but high numbers, there is a potential for pollution in the form of nutrients, sediment or excessive algal growth. For streams that appear healthy but show low diversity and low numbers, or little to no macroinvertebrates, it is time to examine the stream for high levels of pollution and again, revisit the habitat survey for any indicators of stressors. More serious pollution can come from sodium chloride pulses, acids, heavy metals, oil, soaps, insecticides, herbicides and more.

ENTERING DATA INTO THE LEAF PACK NETWORK ONLINE PORTAL

To contribute your data to the Leaf Pack Network go to www.wikiwatershed.org/monitor and follow the directions in the Leaf Pack Network section. Data fields within the Leaf Pack Network mirror the data fields in the online portal.

The basic steps to entering data [for the first time]:

1. Conduct a Leaf Pack project.
2. Create an account in the online portal.
3. Register your site.
4. Enter your data.
5. Explore your data and those contributed by others.

