CHAPTER 3:
USING LEAF PACKS AS A LEARNING TOOL

The methods in the Leaf Pack Stream Ecology Kit are very flexible and open to the type of design you would like to encourage in your students’ experiments. The kit can be used to establish baseline conditions of a local waterway as discussed in Chapter 2 and then to continue to monitor it regularly as a citizen science or civic action experience. The supplemental material in Chapter 3 allows the learning experience to focus on experimental design and experimental methods.

ACTION PLAN
Developing an action plan can guide you in setting up the experiment and participating in the Leaf Pack Network. This activity takes anywhere from 2-6 weeks to complete. You may choose to have students take part in all of the experiences or just participate in certain phases. Use some or all of the steps in the general action plan below as a guide to planning your leaf pack project.

If you would like suggestions and guidance for completing your action plan 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.

General Information
1. Which classes will participate in the Leaf Pack project?
2. How many students will participate? Which grades?
3. What stream will be used?
4. What watershed or sub-watershed?
5. What logistical issues need to be addressed as you prepare to implement the Leaf Pack experiment? [Include things like administrative, transportation, property access, schedules, etc.]
6. How do you envision this project serving your curricular needs? Which topics will be of particular interest to you?
Design the Experiment

1. When do you plan to begin your project?

2. Describe the stream and location that the class will investigate. What conditions exist that will make this an interesting and effective location?

3. If experimental leaf packs will be used, what will be investigated in the project? [Different leaf species? Different stream locations such as riffles and pools? Different streams?]

Work Plan/Timeline

Outline a basic work plan or timeline. Consider the following tasks. If time is a concern, it is not necessary to choose all of the components.

- Class Introduction
- Site Investigation
- Preparing the Leaf Packs for the Stream
- Placing the Leaf Packs in the Stream
- Collecting the Leaf Packs From the Stream
- Processing the Leaf Packs
- Sorting and Identification
- Water Quality Calculations
- Sharing Data on the Leaf Pack Network Online Portal introduction

Leaf Pack Network

Contributing to the Leaf Pack Network Online Data Portal

If you would like suggestions and guidance for completing your action plan 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.
Consider the following questions:

1. How and when will you contribute your project data to the data portal?
2. How will your class use the web-based LPN resources?
3. How will you engage students in communication of LPN data through group discussion, presentations, and/or written assignments?
4. How else do you see sharing the information your class generates? (e.g. conferences, school board meetings, township meeting, local conservation groups, etc.)
5. What other networks/projects do you utilize?
6. How can Stroud Water Research Center assist you in this project?
7. What other training and resources do you desire?

THE LEAF PACK EXPERIMENT

1. Introduction

- Introduce students to the concepts in Chapter 1: What is a Leaf Pack? Linking Trees to Streams, Leaf Packs as Habitat, Life Cycles of Aquatic Insects, Water Quality Indicators, Functional Feeding Groups, Stream Size, Freshwater Macroinvertebrates, and A Continuum of Life as well as basic stream ecology concepts (watersheds, pollution, monitoring).
- Discuss why macroinvertebrates are used for monitoring and why students should care about healthy streams.
- Have students research the history and water quality information for the stream. Take advantage of online resources like [www.ModelMyWatershed.org](http://www.ModelMyWatershed.org), a free watershed-modeling web app, to learn more about your local watershed.

General Tips:

- If students cannot make it streamside, take digital photos or make a short video of the stream site where the leaf packs were placed. Label photos to show upstream, right/left bank, and the leaf pack locations.
- Use a topographic map of the site to further enhance your students’ ability to fill out the Habitat Data Sheet and determine land use.
- As an alternative to transporting stream water back to the classroom, use unchlorinated tap water or leave 3 buckets of chlorinated tap water in a cool (but not freezing) place three days prior to retrieving the leaf packs from the stream. This allows the chlorine to dissipate so that the water will be safe to use during the processing of the leaf packs and keep the macroinvertebrates alive so that they can be returned to the stream at the completion of the project.
• Local monitoring groups, like Trout Unlimited can be contacted for macroinvertebrate identification assistance.

2. Materials and Safety

See Chapter 2 for a list of materials that are needed and tips for safely carrying out the experiment.

3. Defining the Experiment

Choosing an Experimental Test Factor

Consider the characteristics of the waterway to determine what the experiment will test. This is the focus of the experiment or the *experimental variable*. Leaf packs can be used to explore any one of the following possible experimental factors:

• food preference
• habitat preference
• seasonal differences/fluctuations
• habitat/water quality differences within and among streams

The focus of the experiment can be chosen from observations of a local stream, from classroom studies that have explored stream communities, or from the desire to learn more about a particular topic or aspect of stream ecology. Have students develop a hypothesis about the outcome of the experiment.
Choosing an Experimental Variable

A leaf pack experiment testing a **habitat preference** might test the following **experimental variables** (remember to keep everything similar except the experimental variable):

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>location in stream</td>
<td>a riffle area vs. a pool or slow-moving area of the same stream</td>
</tr>
<tr>
<td>location in stream</td>
<td>an area with a vegetated bank vs. an area with a steep, muddy, eroded bank</td>
</tr>
<tr>
<td>stream type</td>
<td>a forested stream vs. an unshaded meadow stream, or urban stream</td>
</tr>
</tbody>
</table>

A leaf pack experiment testing for a **food preference** might test the following **experimental variables** (remember to keep everything similar except the experimental variable):

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>leaf type</td>
<td>a mix or variety of leaves vs. one leaf type (e.g., maple) or native vs. non-native leaves</td>
</tr>
<tr>
<td>leaf type</td>
<td>grasses vs. tree leaves</td>
</tr>
<tr>
<td>leaf age</td>
<td>a mix of fresh green leaves vs. the same mix of dried brown leaves</td>
</tr>
</tbody>
</table>

A leaf pack experiment testing **seasonal differences** might test the following:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>time</td>
<td>autumn season vs. spring season</td>
</tr>
</tbody>
</table>

This would be the same experiment repeated once during autumn and then again in the spring (or vice versa). For instance, the same type of leaves would be placed in the same stream, in the same area, and at the same time of day.

**Two Examples of Possible Experiments**

**Experiment Example One**

A class has visited a local stream and discovered areas of heavy sediment runoff. The experimental variable could be habitat quality: “Do certain macroinvertebrates survive better in this stream or in a stream with no signs of sediment runoff?”

**Experiment Example Two**

The investigators are curious as to what types of leaves are more desirable to a variety of macroinvertebrates. The experimental variable could be food preference: “Do the macroinvertebrates prefer oak, maple, or willow leaves?”
Deciding How the Experimental Variable Will Be Tested
This is the fun part and is limited only to the creativity of the investigators.

Using the same examples:

**Experiment Setup – Example One**

The class has chosen to test how habitat quality affects the abundance of certain macroinvertebrates by comparing leaf pack colonization in a stream with sediment runoff vs. leaf pack colonization in a suspected “cleaner” stream. Stream habitat quality is the experimental variable. This is the one thing that will be different between the leaf pack experiments. The variable, polluted stream vs. healthy stream, is compared.

The leaf packs will be placed in similar types of habitats, or areas, of the two streams (e.g., all leaf packs are placed in a riffle area), on the same day, generally the same time, and each leaf pack is prepared using the same weight of the same leaf type. The one thing that will be different is the quality of the stream – one stream has a lot of sediment the other one does not. The variety of influences that impact the colonization of each leaf pack will be similar, so conclusions from the results can be based upon the difference in habitat quality.

**Experiment Setup – Example Two**

The investigators have chosen to test the food preferences of the macroinvertebrates by comparing leaf pack colonization of oak leaves vs. willow leaves vs. maple leaves. Leaf type is the experimental variable. This is the one thing that is different between leaf packs. The effect of the variable, oak leaves vs. willow leaves vs. maple leaves, is compared.

Leaf packs will be prepared with oak leaves, willow leaves, or maple leaves. All of the leaf packs will be placed in the same stream at the same location (e.g., all leaf packs are placed in the same general riffle area) on the same day at generally the same time, and each leaf pack is prepared using the same weight of leaves. The one thing that will be different is the type of leaves. The variety of influences that impact the colonization of each leaf pack will be similar so conclusions from the results can be based upon the difference in leaf type.
Deciding How Many Leaf Packs Will Be Needed

Because the abundance and diversity of freshwater macroinvertebrates can vary immensely even within the same riffle area, **replicate** leaf packs should be prepared for each experimental variable. Replication will improve accuracy and reduce errors, as well as provide an extra leaf pack so that the experiment can be completed should a leaf pack wash away during a flood event. The Leaf Pack Stream Ecology Kit includes sufficient materials to prepare and analyze a total of six leaf packs.

Using the same examples mentioned previously:

**Leaf Pack Preparation - Example One**

Prepare six identical leaf packs. Place three leaf packs in an area of the stream that has a lot of sediment. Place the other three leaf packs in an area of the stream that is “cleaner”.

**Experiment Setup – Example Two**

Prepare two replicate leaf packs representing each of the three leaf types into the stream [two oak leaf packs, two willow leaf packs and two maple leaf packs] for a total of six leaf packs.

Is A Control Leaf Pack Necessary?

Decide whether **control leaf packs** will be used. Control leaf packs contain leaves from the three most common trees around the stream and are placed in a riffle. The “control leaf pack” helps lead to conclusions about relationships between habitat, land use, and macroinvertebrate diversity and density, creating a clearer assessment of water quality because of the decision of what leaves will make up the contents of the leaf packs. These act as a point of comparison to ‘check’ on your experimental packs, but are not always necessary. For example, if you are simply comparing the health of two stream sites, in which your packs would contain the same weight and type of leaf species, control leaf packs would not be appropriate.

4. **Completing the Field Data Sheet and Site Map**

Complete the Field Data Sheet and Site Map.

5. **Collecting Leaves.**

Follow the procedures in Chapter 2.

6. **Preparing the Leaf Packs for the Stream.**

Follow the procedures in Chapter 2.

Before students engage in any of the processes, review the equipment and tools that are needed and
demonstrate each step. For example, weigh leaves as a demonstration so that students will understand how to use the scale and how many leaves are required.

7. Placing the Leaf Packs in the Stream.
Follow the procedures in Chapter 2.

8. Collecting the Leaf Packs from the Stream.
Follow the procedures in Chapter 2.

9. Processing the Leaf Packs
Follow the procedures in Chapter 2. If the experimental variable is the leaf type, and leaf pack contents are not the same, use a separate bucket to process each type of leaf pack.

10. Sorting and Identification.
Follow the procedures in Chapter 2. If leaf pack contents have been processed into more than one bucket, be sure to keep contents separate and labeled properly. Below are three examples of how the classroom activity of sorting and identifying the macroinvertebrates can be accomplished. Each example represents a unique leaf pack experiment.

Setting Up a Lab Area for Sorting

Example A
Experiment consists of two variables with three replicates for each variable (e.g., three for a polluted stream, three for a clean stream) for a total of six leaf packs. These are divided between six work stations: three for one variable (polluted stream), three for the second variable (clean stream).

Example A
2 Variables • 3 Replicates
**Example B**

Experiment consists of three variables with two replicates for each variable (e.g., two packs with oak leaves, two packs with willow leaves, and two packs with maple leaves) for a total of six leaf packs. These are divided between six work stations: two for the first variable (oak leaves), two for the second variable (willow leaves), and two for the third variable (maple leaves).

**Example C**

Experiment consists of two variables with one replicate for each variable (e.g., one pack for green leaves, one pack for dried leaves) for a total of two leaf packs. Each leaf pack is divided between three trays. These are then divided into six work stations: three for one variable (green leaves), three for the second variable (dried leaves).

*The step-by-step instructions throughout the rest of these procedures are based upon Example A."

An option for all experiments is to sort one leaf pack at a time while storing the remaining leaf packs for a later time (perhaps for a different group of students or the same group of students the next day). Leaf packs can be stored in the zipper-top bags (each containing a small amount of stream water) in a refrigerator or cooler overnight. It is not recommended that the macroinvertebrates be kept out of the stream for longer than 24 hours.
Work Stations
Set up six work stations (4-5 students recommended per station), one area for each leaf pack, according to Example A. The following materials are recommended for each station to explore the leaf packs and process macroinvertebrate data:

- Biotic Index Data Sheet
- 1 Freshwater Macroinvertebrate Sorting Sheet
- 10 Petri dishes
- Sorting Trays
- Paintbrushes [1 per student]
- Plastic spoons [1 per student]
- Hand lenses [1 per student] or MacroLens [1 per station]

If the experiment included control leaf packs and experimental leaf packs complete the Experiment Summary Data Sheet to compare the results.

11. Discussion Questions

1. What was the variable in your leaf pack experiment? Why was it better to use more than one leaf pack for each experimental variable?
2. Did the experimental variable affect the macroinvertebrates that were collected?
3. How many different kinds of freshwater macroinvertebrates did your leaf pack contain? What was the total number of macroinvertebrates?
4. How would you graph the results of your leaf pack experiment? How does visually modeling your data help you identify patterns in population density and biodiversity?
5. Define what is meant by biodiversity and bioindicators. How do these terms apply to your leaf pack experiments?
6. If there were no trees along the stream, would you expect the number of macroinvertebrates to change? How would this affect the stream community?
7. What was most surprising to you throughout this experiment?
8. Prior to human settlement, how do you think the land around your study site looked?
9. Were there potential sources of bias or error in your experiment that might have influenced your results?
ADDITIONAL ACTIVITIES

Activity 1 - CREATING A TREE LEAF IDENTIFICATION KEY

Students will create an identification key based on the characteristics of eight different leaves.

Materials

- Leaves, 8 per team
- Labels, 8 per team
- Tree Identification Worksheet, 8 per team

Procedure

Divide the students into teams. Each team should:

1. Mark the labels A through H.
2. Collect a leaf from a plant or tree. Place label A on the back of this leaf.
3. Complete question 1 and question 2 on a Tree Leaf Identification Worksheet for Tree A.
4. Repeat Step 2 and Step 3 for Tree B through H and Leaf B through H.

The rest of the activity can be completed off site.

5. Complete questions 3 – 6 for Leaf A through Leaf H.
6. Look at the Tree Leaf Identification Worksheet for Leaf A. Choose one characteristic of Leaf A. Write down the characteristic.
7. Sort the leaves into two piles based on the characteristic that was chosen for Leaf A. One pile will have Leaf A and all of the other leaves that share that characteristic with leaf A. The other pile will have all of the leaves that do not have that characteristic.
8. Continue to sort each pile into smaller piles based another characteristic until each pile only has a single leaf. Write down the characteristic that is chosen at each step.
9. Dividing items based on their characteristics creates a dichotomous key. Use the characteristic choices that you had chosen for sorting the leaves to draw a dichotomous key.

There are many possible dichotomous keys that can be created. Figure 9 is an example.
Identification Key

8 leaves

3 leaves - toothed edges

2 leaves - veins netted

Leaf A - light green

Leaf B - not netted

Leaf C - dark green

5 leaves - without toothed edges

3 leaves - veins netted

Leaf D - waxy

Leaf E - not waxy

2 leaves - not netted

2 leaves top rounded

Leaf F pointed tip

Leaf G wider than long

Leaf H

Figure 9. Example Identification Key. An identification key is created based on the characteristics of eight different leaves.

10. Place your leaves in a random arrangement with the label side facing down. Pick one leaf from the pile. Remove the label.

11. Trade your leaf and your dichotomous key with another team.

12. Use the dichotomous key to identify the mystery leaf.

Discussion Questions

1. Why might color and size not be good characteristics for a dichotomous key? Color and size are variable (e.g. leaves change color in the fall; size varies according to sun exposure; disease or insect damage could alter appearance).

2. Why are leaves better characteristics than flowers on a dichotomous key for trees? Flowers are more consistent in form than leaves on shrubs and herbaceous plants and are used for identification. However, flowers on trees are often hard to see and don’t stay on the trees as long as leaves.
Draw the Leaf

Answer the following questions, circle the description that best matches the tree and leaf.

1. The tree is: **evergreen** or **deciduous** (loses leaves seasonally).
2. Arrangement of leaves on branch: **opposite** or **alternate**.
3. Type of leaf: **simple** or **compound**.
4. Type of veins on leaf/leaflet: **single main vein with small side veins** or **several main veins from one point**.
5. Edge of leaf: **smooth** or **sinuate** (wavy) or **dentate** (toothed)
6. Is the base of the leaf symmetrical? **Yes** or **No**

**Identification of Tree**

Common Name

Latin Name
Activity 2 - DETERMINING STREAM DISCHARGE

The amount of water moving past a point of a stream can be calculated if four sources of data are known – width, depth, roughness coefficient and velocity.

$$\text{width (m)} \times \text{depth (m)} \times \text{roughness coefficient} \times \text{velocity (m/s)} = \text{discharge (m}^3/\text{s)}$$

When placing leaf packs, select a straight stretch of stream [no bends] which will have unobstructed riffles and runs, if possible. Complete the measurements before the leaf packs are placed in the stream.

**Materials**

- tape measure
- boots
- meter stick
- stop watch
- clipboard
- pencil
- floating object (orange, ball, walnut)

**Procedure**

Complete the measurements before the leaf packs are placed in the stream.

**Calculation of stream width:**

1. In the proximity of where the leaf packs will be placed, stretch a tape measure tightly across the stream.
2. Measure the distance at the water’s edge from one bank to the other.

$$\text{Stream width} = \underline{\text{________________________}} \text{ m}$$
Calculation of stream depth:
1. Use a meter stick to measure and record three equidistant depth measurements along the width transect. [See points 1, 2 and 3 in the diagram above]. The short edges of the meter stick should be facing upstream.

\[
\text{Average depth} = \frac{\text{m} + \text{m} + \text{m}}{3}
\]

2. Add the three measurements and divide by three to calculate the average depth.

Calculation of stream velocity:
1. Measure a 10 meter distance parallel to the stream where the leaf packs will be placed. Ideally, the majority of the 10 meter distance should be upstream from where the leaf packs will be placed. The time that it take for the floating object to travel 10 meters will be measured.

2. Place an observer at the start and at the end of the 10 meter course. You will also need an object dropper, a recorder and a timekeeper.

3. Drop the floating object about 30 cm above the starting point.

4. When the floating object passes the starting point the observer at the start should yell “Start” and the timer should start the stop watch. When floating object passes the ending point the observer at the end of the 10 meter course should yell “Stop” and the timer should stop the stop watch. The recorder should record the time.
5. Repeat the procedure three times. Add the three measurements and divide the total by three to calculate the average.

____________________ sec  ____________________ sec  ____________________ sec

Average time to travel 10 m: ____________________ sec

6. Calculate the stream velocity:

\[
\text{Velocity} = \frac{\text{distance}}{\text{time}} = \frac{10 \text{ m}}{\text{average time for 10 meters [in seconds]}}
\]

Calculation of Stream Discharge

The Roughness Coefficient is a value given for the type of stream bottom. Check the box that best describes the stream bottom:

- [ ] 0.9 – the stream bottom is smooth with silt, sand or bedrock
- [ ] 0.8 – the stream bottom is rough with rubble, stones or gravel

\[
\text{width} \times \text{average depth} \times \text{roughness coefficient} \times \text{velocity} = \text{discharge [m}^3/\text{s}]
\]

\[
\text{________ [m] \times _______ [m] \times _______ \times _______ [m/s] = _______ [m}^3/\text{s}]
\]

\[
\text{Stream Discharge} = \text{___________ m}^3/\text{s}
\]

Conversion factor: To convert cubic meters per second [m}^3/\text{s}] to cubic feet/sec [cfs] multiply by 35.31.

Note: Discard any trials where the floating object gets hung up by roots, cobbles, debris, etc.
Activity 3 - MACROINVERTEBRATE ID CARD GAME

Materials

- Freshwater Aquatic Macroinvertebrates: Identification Flash Cards
- Dichotomous Key
- Clothes pins, lanyard [optional]

Preparation:

- Brainstorm the names of familiar insects.
- Draw a generic insect body with the help of the participants.
- Show caterpillar photo asking if it is an insect.
- Discuss life stages and common larval stage found in aquatic systems.
- Generate a list of words regarding insect anatomy that are unfamiliar to the participants.
- Define the unfamiliar words and show examples on images of aquatic macroinvertebrates.
- Introduce the Dichotomous Key and the Freshwater Aquatic Macroinvertebrates: Identification Flash Cards.
- Play the card game activity.

Instructions

This activity is done in teams of two people.

1. One person selects a macroinvertebrate card and hands it to their partner, who doesn’t look at the card, and places it behind their back, photo side out. [A clothespin or lanyard can be used to fasten or hold the card in back of their partner.

2. The person with the card on their back holds the Dichotomous Key in front of them and tries to figure out what macroinvertebrate is on the flash card by going through the key and strictly asking questions that have a “Yes” or “No” answer.

3. The person answering the questions bases their answers only on the macroinvertebrate photo on the flash card.

4. Once the person with the key is certain of the macroinvertebrate fastened to their back, they can name the macroinvertebrate and discover whether they are correct.

5. Now reverse roles choosing a different macroinvertebrate card.
LEAF PACK STREAM ECOLOGY KIT GOALS

• To actively engage students in investigative and place-based watershed education in environmental science, technology, engineering, and math (E-STEM) content
• To promote student inquiry by using scientific methods involving observational and explanatory activities
• To raise awareness of the importance of streamside forests to the ecology of rivers and streams and to promote their stewardship
• To develop a diverse and dynamic network of groups that digitally share information about their backyard streams
• To use the Leaf Pack Stream Ecology Kit and other teaching resources to improve hands-on E-STEM education and teacher professional development

LEAF PACK STREAM ECOLOGY KIT OBJECTIVES

At the conclusion of the project, students will have:

• used the LPN as a tool to apply and understand scientific principles in a real-world situation
• conducted a research-oriented investigation of their local stream(s)
• engaged in inquiry-based, hands-on data gathering and monitoring
• observed and described aquatic food webs and resource availability among communities, populations, and organisms in a freshwater ecosystem
• identified local tree species and their function as food and habitat in freshwater ecosystems
• identified local macroinvertebrates
• explored macroinvertebrates’ roles as valuable indicators of stream health
• identified their local watershed using a map
• measured physical characteristics of a stream
• analyzed macroinvertebrate data by using indices to assess water quality
• formulated research questions related to leaf packs and E-STEM disciplines
• drawn conclusions based on empirical evidence about the relationships among habitat, land use, and macroinvertebrate diversity and density
• identified a way to improve water quality and minimize human impact in a local stream
• collected real data in real places to improve capacity for real-world decision-making surrounding water-quality impacts and watershed stewardship
ALIGNMENT WITH THE NEXT GENERATION SCIENCE STANDARDS (GRADES 5-12)

Leaf Pack Stream Ecology Kit

<table>
<thead>
<tr>
<th>Performance Expectations:</th>
<th>Disciplinary Core Ideas:</th>
<th>Cross Cutting Concepts:</th>
<th>Engineering Practices:</th>
</tr>
</thead>
</table>

DATA SHEETS, RESOURCES & REFERENCES

DATA SHEETS

- Field Data Sheet/Site Map
- Habitat Data Sheet
- Biotic Index Data Sheet
- Experiment Summary Data Sheet