TIEE

Teaching Issues and Experiments in Ecology - Volume 7, August 2011

EXPERIMENTS

Using Stream Leaf Packs to Explore Community Assembly

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This image shows a stream environment in upstate New York where we have tested this experiment, a sieve with leaves from a leaf pack, and a macroinvertebrate. Photo by Cornelia Harris.

ABSTRACT
Students will explore functional and taxonomic diversity in a stream ecosystem, learn about food web relationships, and learn about the ways in which abiotic and biotic factors determine what organisms are present in a community. Students will make and install artificial leaf packs in a stream, wait for the leaf packs to be colonized by stream organisms, measure abiotic variables that could influence leaf pack colonization, retrieve the leaf packs and classify the organisms they find in both taxonomic and functional ways, and participate in a class discussion of how the leaf pack community is situated within a larger ecosystem.

KEYWORD DESCRIPTORS

- **Ecological Topic Keywords:** Abiotic factors, Aquatic Ecology, Biodiversity, Biotic Factors, Community Ecology, Competition, Dispersal, Food Web, Herbivores, Microorganisms, Mutualism, Predation, Stream Ecology

- **Science Methodological Skills Keywords:** classification, collecting and presenting data, data analysis, experimental design, field observational skills, field work, formulating hypotheses, graphing data, hypothesis generation and testing, identify biotic-abiotic interactions, quantitative data analysis, quantitative sampling, systematics, taxonomy, use of dichotomous keys, use of spreadsheets, use of graphing programs
- **Pedagogical Methods Keywords:** assessment, cooperative learning, concept mapping, inquiry, pairs check

CLASS TIME

1 session (30-45 minutes) to discuss the experiment and for students to formulate their research questions, 1 session (2-3 hours) to prepare and install leaf packs, 1 session (3-4 hours) to retrieve and identify organisms in leaf packs and begin data analysis. 1 optional session (20-30 minutes) for an instructor-led discussion of the conclusions students made based on their data.

OUTSIDE OF CLASS TIME

3-5 hours to finish data analysis and complete worksheets

STUDENT PRODUCTS

- Pre-Lab Worksheet
- Lab Worksheet
- Post-Lab Worksheet

SETTING

This experiment was developed for use in a variety of stream systems that receive allochthonous inputs and has been tested in Santa Barbara, Denver, Baltimore, upstate New York and rural Michigan. Students will need to wade into streams to install artificial leaf packs that need to remain in the stream for 2-4 weeks, so we recommend that this activity not be conducted in larger, swift-moving streams. Students will need to bring leaf packs back to a laboratory classroom where access to dissecting microscopes would be advantageous.

COURSE CONTEXT

This experiment has been used successfully in an introductory Ecology laboratory and in advanced high school Biology courses (8-24 students). This experiment would also be appropriate for introductory Biology laboratories.

INSTITUTION

Medium-sized public university, public high schools

TRANSFERABILITY

This experiment is transferable to non-majors courses and perhaps to upper division Ecology courses. A modified version of a longer, high school version (see acknowledgements) was used in a non-majors Environmental Issues class For example, analysis of data can range from displaying means (high school biology, non-majors undergraduate biology) to calculating richness indexes and conducting t-tests in Excel (majors biology, ecology). Experimental design support can range from little instructor support (ecology) to instructor's providing examples or generating the questions (high school, non-majors). This experiment can be used in a variety of geographical regions as long as students have access to a stream in which aquatic invertebrates are living.

ACKNOWLEDGEMENTS

A longer, high school version of this activity was developed as a teaching intervention to help students become more literate about biodiversity http://edr1.educ.msu.edu/EnvironmentalLit/publicsite/html/tm_be.html This research is supported in part by a grant from the National Science Foundation: Targeted Partnership: Culturally relevant ecology, learning progressions and environmental literacy (NSF-0832173). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. We acknowledge the researchers involved: Andy Anderson, Marcia Angle, Mitch Burke, Terry Grant, Michele Johnson, Shawna McMahon, John Moore, Liz Ratashak, Michael Schiebout, Jonathon Schramm, Scott Simon, Lori Spindler, and Brook Wilke.

SYNOPSIS OF THE EXPERIMENT

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SYNOPSIS OF THE EXPERIMENT

Principal Ecological Question Addressed

What factors influence the invertebrate community that colonizes leaf packs?

What Happens

During the first session, the instructor leads a discussion about community assembly, characteristics of streams, and the factors (dispersal, abiotic conditions/resources, and biotic interactions) that influence community assembly in general and community assembly of a stream environment specifically. Students work in groups to come up with a hypothesis and experimental design. Students collect leaves as homework between the first and second lab sessions. During the second lab session, students use their collected leaves to make artificial leaf packs and travel to a stream to install them. During the third session (2-4 weeks after the second session), students return to the stream to retrieve their leaf packs. They take the leaf packs back to their laboratory to sort through them and identify the organisms they contain. Students summarize and analyze their data. Students use their data to draw conclusions about their hypotheses. Instructors may choose to devote additional time to an instructor-led discussion of the patterns students found in their data and the explanations they came up with for those patterns in a fourth session.

Experiment Objectives

At the end of this lab exercise students will be able to:

1. Explain how dispersal, abiotic conditions and resources, and biotic interactions affect the community of invertebrates in a leaf pack.

2. Classify organisms based on similarities and differences in morphology, evolutionary relatedness, and biotic (e.g., type of prey or food available) and abiotic (e.g., the concentration dissolved oxygen, the amount of sunlight) requirements.

3. Use the scientific method appropriately to answer a question, including generating hypotheses, designing an experiment, and statistically analyzing data.

Equipment/ Logistics Required

- Plastic mesh bags (we used 24" onion/potato/seafood bags purchased from WebStaurant.com, price was $65 for 1000 bags)
- Leaves (students will collect these)
- Waterproof tags to label leaf bags (We have also used small stones with information written on them with a water-proof marker)
- String, bricks, or rocks to anchor litter bags in stream
- Flags or flagging tape to mark leaf bag sites, if needed
- Scale (0.0) to weigh leaves or 2 cup container or 500 ml beaker to measure volume
- Waders or appropriate shoes to place bags in stream
- First Aid Kit, throw rope, and any other safety equipment you think necessary
- Thermometer
- Dissolved oxygen sensor (e.g., Vernier DO-BTA) or test kit (e.g., CHEMetrics, Inc. K-7512)
- Test for turbidity (e.g., Secchi disk, Ben Meadows Company 224217)
- Stream velocity measurements: flow rate monitor OR orange/ping pong ball, meter tape, stopwatch (optional)
- Scissors
- Gallon size plastic storage bags for transporting leaf packs to and from the stream
- Macroinvertebrate identification guide(s). Each resource below has trade-offs in use so instructors should pick an identification guide that is appropriate for their students (e.g., the Save our Streams guide is a poster while the Stroud key is a multi-page packet).
- Online, printable resources include:
    - Save our Streams Monitor's Guide to Aquatic Macroinvertebrates, by Loren Larkin Kellogg for the Izaak Walton League of America, 1992. http://www.lccd.org/watershed/wav_riverkey.pdf
    - Stroud Water Research Center's dichotomous key: http://www.stroudcenter.org/lpn/LPNmanual/lpn_english/5_MacroKeyForWeb.pdf
- Online keys are available at:
    - http://people.virginia.edu/~sos-iwla/Stream-Study/Key/MacroKeyIntro.HTML
    - http://www.dec.ny.gov/animals/35772.html.

- Resources available for purchase:
    - Voshell, J.R. 2002. A Guide to Common Freshwater Invertebrates of North America. The McDonald & Woodward Publishing Company, Virginia
    - Aquatic Macroinvertebrate Insect Life Cycle & Habitat Flashcards (e.g.5946) or Aquatic Macroinvertebrate Insect Identification Flashcards (e.g., 5882-SA1) available from Connecticut Valley Biological Supply
- Leaf pack sorting sheets (optional; 1 per student group, purchased from Connecticut Valley Biological). This is a placemat that students can put at their desk or use in the field. It has spots to put petri dishes for sorting and next to each petri dish are pictures and descriptions of the most commonly found aquatic invertebrates.
- Petri dishes (9 per student group)
- Plastic spoons, forceps, transfer pipettes, turkey basters to pick out organisms
- White sorting trays (e.g., enamel baking dishes, plastic serving trays or Specimen sorting trays, Rose Entomology st210)
- Strainer or sieve and buckets for rinsing invertebrates from leaves, if desired
- Wash bottles
- Hand lenses or dissecting microscopes
- Stream Biology Briefs (provided in Appendices). This is a document that provides a short synopsis of the characteristics of the most commonly found aquatic invertebrates.

Summary of What is Due

- A pre-lab worksheet on which students explain their research question, hypotheses, and rationale for their hypotheses.
- A lab worksheet on which students answer questions about their experimental methods.
- A post-lab worksheet on which students record and interpret their data, answer questions about the difference between phylogenetic and functional diversity, and draw food webs based on the data they collected.

DETAILED DESCRIPTION OF THE EXPERIMENT

Introduction

A defining question in ecology is "what determines which types and how many organisms exist in a given location?" Before reading further, take a moment to write down your answer to this question.

Three components are useful in thinking about this question (Belyea and Lancaster 1999).

> **Dispersal - Can the organism get there?** Organisms can't live in a specific time and location if they can't get there; we call this "dispersal," the ability to travel to a new habitat (e.g., direct organism movement, water, wind). Is it likely that there are barriers to migration or dispersal that prevent the organisms of interest from being present at a given location? (e.g., dams). There may also be corridors between habitats that will facilitate movement.
>
> **Abiotic resources and conditions - Can the organism survive and reproduce given these abiotic resources and conditions?** Abiotic resources and conditions (e.g., light, water, oxygen, nitrogen, phosphorus, temperature, pesticide pollution, etc.) influence whether organisms are able to survive and reproduce in a specific time or location. In addition, organisms influence the abiotic environment around them.
>
> **Biotic interactions - Can the organism survive and reproduce given the range of biotic interactions?** Does the organism have food, does something eat it, what are the organism's competitors, mutualists, habitat-forming organisms, diseases, etc.?

Streams receive inputs of leaves from the riparian vegetation around them. These leaves build up in piles in the stream called *leaf packs*. One can consider these leaf packs "new habitats" and "new food sources" for stream organisms, and hence are excellent model systems for studying community assembly. You will be designing a leaf pack experiment in which you examine one or more of the components of community assembly listed above. You will essentially be answering the question "what determines which types and how many macroinvertebrates colonize leaf packs in a given location?"

Think about the physical, chemical and biological components of the stream habitat that you will be using for this activity.

What is the physical environment like? Attributes to consider are the texture of the sediment and how tightly it is packed, the width of the stream, the amount of meandering, the stream velocity, the volume of water that moves through the stream, the depth of the stream at the deepest points, how turbid the stream is, the temperature of the water, what material the stream bank is composed of, how close vegetation is to the stream and whether the vegetation shades the stream, and what the land use around your stream is like. If you have access to a study sites that are far apart (e.g upstream site in the mountains vs. downstream site flowing through town) you might choose to compare community assembly in those two sites. The stream/river should be wider, deeper, and slower as you move downstream (Colorado Division of Wildlife 2006).

What is the chemical environment like? Attributes to consider are the amounts of dissolved oxygen, dissolved organic matter, or nutrients (sulfate, chloride, fluoride, nitrogen (N, NH_4, NH_3^+, NO_3, NO_2), phosphorus, the pH and, presence of pharmaceuticals, petroleum products, or other wastes generated by human activity. Different parts of the stream may have different chemical environments. You may choose to place your leaf packs in paired sites (e.g., pools vs. riffles). The riffles of a stream are waters that move very rapidly (50 cm/second or faster), have a high oxygen concentration (at least 10mg/L) and a healthy pH value (above 7). Pools are much quieter than riffles. Water in pools moves more slowly, is cloudier, and has lower oxygen levels.

You may choose to compare an upstream and a downstream site. As a river moves downstream, it picks up dissolved nutrients along the way from both allochthonous (material like leaves falling in the stream) inputs and autochthonous inputs (material that comes from life that grows within the stream) (Vannote et al. 1980). As a river moves downstream, dissolved oxygen also decreases (Colorado Division of Wildlife 2006).

You may choose to compare community assembly in leaf packs containing one type of plant species to leaf packs containing another type of plant species. The types of leaves that fall into a stream can influence the biotic community that colonizes those leaves because leaves differ in chemical composition. Some leaves have high lignin content; lignin is a complex, structural molecule that is hard to break down. Some have high nitrogen content; nitrogen is a nutrient that is often in short supply. Some have tannins or toxins that negatively affect the palatability of leaves. For example, deciduous and coniferous leaves differ in their chemical composition, which affects what can and will eat them. Deciduous leaves are made of compounds that are easy to break down (e.g. cellulose) and have a relatively low C:N ratio (i.e. there is more N per unit C). Both of these characteristics make them easy for microorganisms to break down. Coniferous needles, on the other hand, contain more compounds that are difficult to break down (e.g., lignin and tannins) and have a higher C:N ratio (i.e. less N per unit C). As they break down, they also release organic acids, which lower the pH of the surrounding environment. Not all organisms are equally tolerant to acid, so the community that can live on coniferous needles could be different from deciduous leaves (Perry 1994). Deciduous leaves will breakdown faster the coniferous leaves, so you might also see differences in abundance of organisms living in the packs.

What is the biotic environment like? Organisms to consider are fish, tetrapods, macroinvertebrates, microorganisms, plankton, and aquatic vegetation. Also think about what stage the various organisms are in their life cycles. Think about whether vegetation is emergent or submergent. Think about whether the organisms live in the river during all stages of their life cycle or just some of them. Think about the relationships of the organisms to one another.

You can divide the stream macroinvertebrate community into general groups based on how they get their food: <u>scrapers</u>, <u>collectors</u>, <u>shredders</u>, and <u>predators</u>. Scrapers obtain food by scraping algae off of surfaces. Collectors (includes filter feeders) eat small particles of organic matter and small organisms floating in the water. Shredders eat bacteria and fungi on leaf surfaces. They tear up leaves into small pieces. Predators eat other organisms.

If you have access to a study sites that are far apart (e.g upstream site in the mountains vs. downstream site flowing through town) you might choose to compare community assembly in those two sites. In small rivers, that start at high altitudes, most of the food and energy sources come from allocthonous (outside) inputs. There is not very much primary production in these rivers because they are generally cold, shaded, and, if the stream is at a higher altitude the growing seasons will be short. Since most of the food in these rivers is course particulate organic matter, there are many shredders and collectors, and few grazers. In medium streams, there is more primary productivity because streams are wider, experiencing less shading from surrounding vegetation, and often times warmer, or at lower elevation. Because there is more algal primary productivity, you would expect to find more scrapers than you would in small rivers. There are also collectors and predators, but fewer shredders. In large streams, the community is dominated by collectors and predators. In large streams you would also find more aquatic plants and floating phytoplankton, and less attached algae (Vannote et al. 1980, Colorado Division of Wildlife 2006).

Once you install your leaf packs, they will begin to be colonized by organisms. From where will those organisms come? You might choose to place some of your leaf packs near and some of your leaf packs far from natural leaf packs already in the stream. These natural leaf packs could serve as sources of colonizing organisms for your artificial leaf packs.

Materials and Methods

This study can be conducted in any stream that supports aquatic invertebrates. Choose a stream and decide where in that stream you will be able to enter and install leaf packs. Streams should have an access point for you to safely enter the stream and streams should not be moving so fast that leaf packs will be washed away within two to four weeks. The sediment of the stream does not necessarily have to be soft. Leaf packs can either be anchored in sediment with pins or sunk by putting rocks in the bags or on the handles of the bags; you can also tie the bags to a tree or root at the side of the stream.

Overview of Data Collection and Analysis Methods

Pre-Lab Session: Participate in a class discussion about community assembly, characteristics of streams, and the factors (dispersal, abiotic conditions/resources, and biotic interactions) that influence community assembly in general and community assembly of a stream environment specifically. Assemble into groups of 4. As a group, go through the lab and answer the pre-lab questions on the Pre-Lab Worksheet. The questions are meant to help you develop a testable research question, hypotheses, and a plan to collect the leaves you will use to build your leaf packs.

Lab Session 1: This session includes both lab and field components.
Lab

1. Complete the Pre-Lab worksheet, which includes determining the question that you would like to answer through this experiment.

2. Decide how many leaf packs of each type you will need to make in order to carry out your experiment. On the Lab Worksheet, explain your decision.

3. Decide what abiotic data you will need to collect in order to carry out or interpret your experiment. Gather the equipment you will need. On the Lab Worksheet, explain your decisions about what abiotic data you will collect.

4. Construct your leaf packs, including labeling them by group and treatment in some way with a tag, token, or stone inside each bag. On the Lab Worksheet, explain how you standardized the amount of leaf material in your bags and how you labeled your bags.

Field

1. Travel with your class to the stream where you will install the leaf packs. You will need to bring the abiotic measurement tools, flagging, and equipment to anchor the bags (e.g. string, tent pegs, etc.).

2. If your instructor says to do so, complete the Stream Physical Characteristics Data Sheet.

3. Before entering the stream, describe to your instructor the safety measures you will take to ensure that your body, your possessions, and the lab equipment are safe.

4. Secure the leaf packs at the edge of a stream by anchoring them in the sediment or tying them to a tree or bush with the string and placing a rock or brick on top of the pack to keep the bag underwater. You can also tie the packs to several bricks or rocks. In a high flow stream, you can bury the bricks (with the packs attached on a longer string) in the ground along the edge of the stream, making sure the packs are securely tied to the brick prior to burial. Plan to leave the packs in the stream for 2-4 weeks.

5. Take notes on the Lab Worksheet that describe the location where they put each treatment and explain why they chose those locations. You may want to take a GPS reading or put a flag on the bank to help you relocate the bags in 2-4 weeks.

6. Take and record any necessary abiotic measurements.

7. Assist with cleaning and storing field equipment as directed by your instructor.

Lab Session 2: This session includes both lab and field components.

Field

1. Gather your notes from previous sessions and travel to the stream to retrieve your leaf packs. Make sure you have the equipment you need to gather necessary abiotic data. You will need to bring a gallon-sized plastic storage bag for each leaf pack. Scissors are helpful to cut knarled string and flagging, but you may need to remind students to collect all of their trash.

2. Review the safety procedures that you came up with on the first day.

3. Remove each leaf pack from the stream and place each pack in a separate gallon-sized plastic bag.

4. Take and record any abiotic measurements that your experiment requires. Take a minute to remind yourself of your rationale for collecting each type of abiotic data.

Lab

1. Take a moment to make a plan for not mixing up your samples once you take them out of their labeled bags. Good scientists anticipate possible mistakes and make plans to avoid making them.

2. First, before you empty your bags out, observe how the litter looks (is it green, brown, decaying, in clumps, etc.). Do you see any organisms? Are any of them together? What parts of the leaf pack are the organisms in or on? Record your observations on your data sheet.

3. Open the leaf pack, tease apart the leaves, and pick out the macroinvertebrates. Use the keys provided to identify each invertebrate in each of your packs. Separate the animals you find into major groups using the key, sorting mat and Petri dishes provided. Look carefully at the leaves themselves; many organisms will be attached to the leaves.

4. Now, count the numbers of invertebrates in each Petri dish on your sorting mat and record your data on your data sheet. Take notes about relative sizes and any other things you noticed about the invertebrates in your leaf pack. Repeat for each leaf pack.

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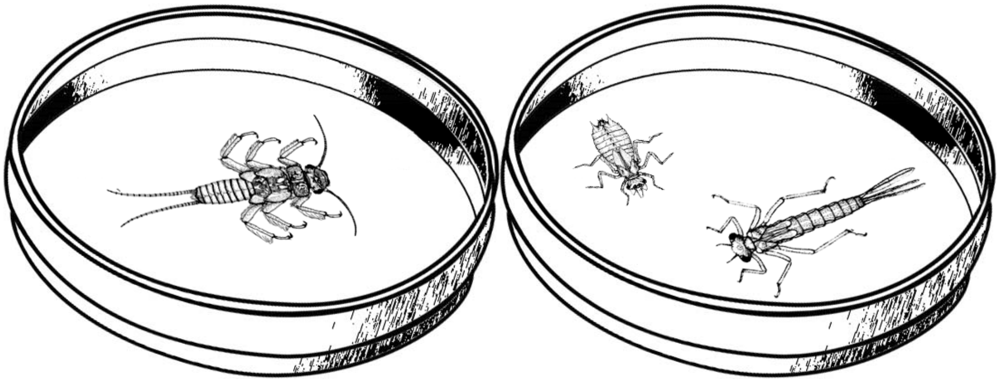
Example



Major Groups Common name (Order)	Number of individuals	Notes
Stoneflies (Order Plecoptera)	1	Seen eating a smaller invert
Dragonflies and Damselflies (Order Odonata)	2	Sizes: One big with skinny tail and one small with fat tail

Data Analysis and interpretation

1. Enter your data into Excel and make sure that everyone in your group has the spreadsheet. Begin analyzing your data and complete the Post-Lab Worksheet. You should finish data analysis and interpretation at home and be prepared to discuss your results in the next class.
2. To analyze your data and look for patterns you should calculate the average number of individuals for each group of organisms for each treatment. You should then graph the averages in a bar graph. Make sure to label the x and y axes of their sketched graph and write a figure legend. What patterns do you observe?
3. You may also want to calculate the following for your samples and inspect the results for patterns. Obtain a handout from your instructor to help you make these calculations.
    a. Richness of your samples. Richness is a count of the number of unique taxa (e.g., species, order, genus) in a given plot. For this activity, since we are not identifying to species, you will use “taxa” richness, usually meaning order in this case. It can be done in the same manner as species richness.

b. Relative Abundance: the proportion (p_i) of a taxa's abundance relative to the total abundance of all taxa for each individual taxa.
c. Shannon Diversity (H'): $H' = -\sum p_i \ln(p_i)$, where H' = the Shannon Diversity index, p_i = the proportion of the ith species/group, and ln = natural log. The summation is for all species/groups in a given grouping.
d. Shannon Evenness (J'): $J' = H'/\ln(S)$, where S = the number of unique species (i.e., richness)

4. Describe any patterns you observe in your data.

5. Make some conclusions about your research question and hypotheses based on the data you got from your leaf packs and the abiotic data you collected. Make sure to frame your conclusions in terms of community assembly.

6. Explain your conclusion. Why do you think the patterns you observed might exist?

7. Complete the rest of the Post-Lab Worksheet. This part of the worksheet helps you think about how the biota you found are related to each other phylogenetically and functionally. You will also think about feeding relationships among the organisms you found and the organisms in the surrounding ecosystem.

Questions for Further Thought and Discussion (some of these questions are also on the lab worksheets). Answer or discuss the questions that your instructor chooses for you.

1. Define dispersal and discuss the implications of dispersal on community assembly. Choose two organisms that you might find in a stream. Do you think organisms of that order or species can move from one stream to another? If so, how? If not, why not.
2. Give two examples of abiotic conditions and explain how each might be important to community assembly in a stream leaf pack. How do you think dissolved oxygen might affect survivorship in aquatic invertebrates? Can you think of some examples of human-induced changes to the abiotic environment of a stream?
3. Give two examples of biotic interactions and why they might be important to community assembly in a stream leaf pack. How is competition different and similar to predation?

4. If you took the same leaf packs and put them right next to each other in the same place, do you think they would be colonized by the same suite of organisms? Why or why not?
5. What kind of characteristics would be good to use when classifying organisms?
6. Define functional redundancy. Was there functional redundancy in your leaf packs? What evidence are you basing this off of?
7. What would happen to the abiotic and biotic environment if all collectors disappeared from the stream?
8. What would happen to the abiotic and biotic environment if there were three types of collectors and one of them disappeared from the stream?
9. You have just learned how an organism can change its abiotic environment in a way that also affects the other members of biological community. Can you think of any changes to a biological community in another ecosystem that would change the abiotic environment and therefore affect the biological community?
10. Imagine you have been investigating a stream where you find four different kinds of consumers in your leaf pack. Explain why there is more than one type of consumer rather than a "best adapted consumer".

References

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Protection Agency, Office of Water and Office of Research and Development, Washington, D.C., 106 pp.

Links

Invertebrates as Indicators
http://www.epa.gov/bioindicators/html/invertebrate.html

Benthic Macroinvertebrates in Wadeable Streams
http://cfpub.epa.gov/eroe/index.cfm?fuseaction=detail.viewInd&r=89189&lv=list.listByQues

Tools for Assessment of Student Learning Outcomes

Students can be assessed on three aspects of this project: 1) the pre-lab worksheet in which students come up with a research question, formulate hypotheses, and provide rationales for your hypotheses, 2) the lab worksheet in which students explain their methods and what they learned about aspects of doing work in the field, 3) the post-lab worksheet in which they will summarize and analyze their results, explain taxonomical relationships among organisms, explain functional relationships among organisms, and explain foraging relationships among organisms.

NOTES TO FACULTY

Challenges to anticipate and solve

Students will be working in groups. Working in groups is an important skill to learn, but often there are grandstanders and wallflowers in groups. If you think your students will have trouble working in groups, at the start of the lab or course, you can have students brainstorm a list of attributes that make a person a good group member. Once the students are in groups, have the group members tell each other things about themselves (e.g., where they are from, what the worst job they ever had was, and what their favorite hobbies are) and their strengths and weakness related to the lab (e.g., I am really good at tedious things, so I think I can count lots of insects. However, I can't swim, so I'd rather not be the one to anchor the bags in the stream). At the start of each lab session, ask the students to assess what tasks need to be completed that day and to equitably divide those tasks amongst each other.

Using EXCEL (Microscoft, Inc): Students seem to have varying experience with Excel. We ask students to tell us whether they are "very comfortable", "somewhat comfortable", or "not at all comfortable" with EXCEL. We then assign at least one person who is comfortable with Excel to every group so there can be some near-peer teaching. If you want your students to run a t-test on their results using EXCEL, here is a guide (click here for t-test instructions).

Because this is a field experiment and students are testing authentic hypotheses, it is difficult to anticipate the results. In situations where the leaf packs were colonized by more organisms than the students could reasonably count, we have suggested that students devise a way to subsample their leaf packs (e.g., sample each leaf pack for a set period of time such as 25 minutes). We recommend doing as many replicate leaf packs per treatment as possible. A good way to increase the sample size for any treatment is for multiple student groups to work together on one research question.

There may be situations where the leaf packs are colonized by very few or no organisms. In these situations, you may wish to have some data from the literature or past semesters for students to work with. You can also have them use the student data that we included or you can cut out pictures of organisms and have students identify and classify the organisms from the pictures. We have found the following comparisons to usually provide different communities: living vs. dead or senesced leaves, deciduous vs. coniferous leaves, and streams surrounded by different land uses.

Students may lose bags because of swift moving currents or vandalism. If you think that will be an issue, you may want to have students make 1-2 extra bags per treatment. If all of the bags are retrieved, you may tell the students to analyze only a subset of the bags.

This study can be conducted in any stream that supports aquatic invertebrates. Choose a stream and decide where in that stream you will be able to enter and install leaf packs. Streams should have an access point for students to safely enter the stream and streams should not be moving so fast that leaf packs will be washed away within two to four weeks. The sediment of the stream does not necessarily have to be soft. Leaf packs can either be anchored in sediment with pins or sunk by putting rocks in the bags or on the handles of the bags; you can also tie the bags to a tree or root at the side of the stream. If the stream you are using has large rocks, you can use those rocks to anchor the tops of the bags. We had some success with using 3 inch river rocks from a craft store as both labels and anchors in bags. Students wrote their names and treatment information on the rocks, put 3-4 rocks in each bag, and sealed the bag.

Experiment Description

Introducing the Experiment to Your Students

Pass out pictures of a stream with leaves in it or trees around it. If you can, use a picture from the stream you plan on visiting for the field trip. In pairs, students should brainstorm what they think lives in the stream. As a class, summarize student answers by creating a list of organisms on the board or a sheet of poster paper. Ask students to brainstorm how each of the organisms get food (i.e., how they get matter for growth and reproduction and energy for life's processes). Students may tend to focus on large animals; if so, remind them to think about smaller animals and organisms that are not animals.

Tell the students that leaves fall into the stream and make habitat for stream organisms. Talk about how leaves get into a stream when they fall from trees and build up in piles in the stream called *leaf packs*, and explain how leaf packs start out with few things living in them and slowly become colonized by many types of organisms (e.g., insects, algae).

In small groups or pairs ask students to brainstorm about what could affect the organisms that might colonize the leaf packs. The abiotic conditions (e.g. temperature, light, dissolved oxygen), the leaves themselves (i.e. shape, size, chemical composition for more advanced students), and what types of organisms are in the stream will influence what will live in the leaves. You may need to prompt students to focus their thinking. When thinking about what might live in a certain place students should ask themselves the following three questions, in this order:

Dispersal - Can the organism get there?

Abiotic resources and conditions - Can the organism survive and reproduce given these abiotic resources and conditions?

Biotic resources and interactions - Can the organism survive and reproduce given the range of biotic resources and interactions?

After the small group discussion, start a list on the board of the students' ideas. Group their ideas in three categories: things that might affect dispersal of organisms, abiotic factors, and biotic interactions.

Tell the students that they are going to see what colonizes leaf packs by making experimental leaf packs and placing them in a stream. Now is the time to distribute handouts and worksheets. Ask the student groups to come up with a research question and hypotheses. After 5-10 minutes, ask each group to share their question, their hypotheses, and the rationale for their hypotheses. Ask another group to provide a question or suggestion to the presenting group.

The most productive questions your students can ask are generally paired comparisons. If your students have trouble coming up with an ecologically interesting comparison you may want to suggest some possible treatments:

- Leaf packs with leaves of species A, leaf packs with leaves of species B
- Leaf packs with one type of leaf, leaf packs with 2 or more types of leaves
- Leaf packs with green leaves of species A, leaf packs with dried/senesced leaves of species A
- Leaf packs placed in a pool, leaf packs placed in a riffle
- Leaf packs placed in an area of the stream surrounded by farms, roads, or town, leaf packs placed in an area in a forest or other natural area
- Leaf packs placed by other leaves in the stream (e.g., a natural leaf pack) or in the middle of the stream away from a source population.

You can modify when the pre-lab worksheet is introduced to your students. For a lower level course, the worksheet could be given along with the Introduction, where most of the answers can be found. For an upper level course, the worksheet could be given after a short verbal introduction to the lab, but prior to giving students the written Introduction to the lab. For added interaction, you could have the students complete the pre-lab worksheet prior to reading the Introduction and then have the students use a different colored pen to modify their pre-lab worksheet based on what they learned from reading the Introduction.

Data Collection and Analysis Methods Used in the Experiment

If you have time and want students to learn how to characterize a habitat, we have included an optional extra data sheet called "Stream Physical Characteristics", which asks students to characterize the stream width, stream velocity, habitat, substrate size, cobble embeddedness, stream bank vegetation, and human impacts and land use.

Students will need one "Macroinvertebrate Data Collection" data sheet for each of their treatments. The data sheets have space for 4 replicates per treatment. You can do more or fewer depending on how large your groups will be and how long your class period is.

A good way for students to divide the labor of sorting through the leaf packs is for each person in the group to take one pack.

The Leaf Pack sorting sheets purchased from Connecticut Valley Biological are very helpful (and they are waterproof!). If you don't have the funds to purchase those sheets, you can make something similar. The sorting sheets have circles drawn where students should place individual petri dishes. Next to each circle is a description of the group of macroinvertebrates and some line drawings that show important characteristics. Students will pick organisms out of their leaf packs and place them in the appropriate petri dish for later counting.

If you think your students will take too long to sort the organisms from their whole pack, you can have students count a subsample of the pack or just sort for a set amount of time (e.g., 25 minutes). As they are sorting, prompt small groups to think about what they are doing: How many types of organisms are they finding? How can they tell when organisms are different kinds? How are the organisms different from one another? Do they think all of these organisms eat the same thing? Why or why not? Are all the organisms in a Petri dish the same? Prompt them to observe the organisms' mouthparts, particularly to identify what "counts" as a "mouthpart."

Students should calculate the average number of individuals for each group of organisms for each treatment using their data. They should then graph the averages in a bar graph as shown in the Excel template. Students often have trouble seeing the best ways to present visual data summaries. Students often make the mistake of putting raw data on graphs. To help students, you may ask them to sketch possible figures before they organize their EXCEL data sheets. They should label the x and y axes of their sketched graph and write a figure legend.

Depending on the level of student, you may want students to do more sophisticated calculations with their data. Here are some metrics we suggest and integrated into the investigation materials:

Taxon Richness: the count of all unique taxa in a given plot. For this activity, since we are not identifying to species, explain to students that we will use "taxon" richness, usually meaning order in this case. It can be done in the same manner as species richness, as long as it identifies the appropriate scale of classification.

Relative Abundance: the proportion (p_i) of a species/group abundance relative to the total abundance of all species/groups.

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Shannon Diversity (H'): $H' = -\sum p_i \ln(p_i)$, where H' = the Shannon Diversity index, p_i = the proportion of the ith species/group, and ln = natural log. The summation is for all species/groups in a given grouping

Shannon Evenness (J'): $J' = H'/\ln(S)$, where S = the number of unique species (i.e., richness)

We have provided a short handout that walks students through the different diversity measures and calculations as an appendix.

We suggest having students make bar graphs to represent a comparison of their two treatments based on:

- Total taxa richness
- Total taxa diversity
- Taxa richness by major functional groups
- Taxa richness by phylogenetic groups.

If you choose not to have students do more sophisticated analyses of their data, you will need to modify the directions on page 1 of the Post-Lab Worksheet.

Questions for Further Thought

1. Define dispersal and discuss the implications of dispersal on community assembly. Choose two organisms that you might find in a stream. Do you think organisms of that group can move from one stream to another? If so, how? If not, why not. How might dispersal limitations contribute to speciation?

   Dispersal is something we think students can easily grasp, yet very few students think about dispersal as a part of community assembly unless they are specifically prompted. This question asks students to think about how physical characteristics of organisms can facilitate or hinder their dispersal. The question also asks students to tie dispersal to larger and longer scale phenomena like speciation.

3. Give two examples of biotic interactions and why they might be important to community assembly in a stream leaf pack. How is competition different and similar to predation?

   Students might not at first understand what we mean by biotic interactions. Students should think about how biotic interactions affect relative resource acquisition by the species that are interacting.

4. If you took the same leaf packs and put them right next to each other in the same place, do you think they would be colonized by the same suite of organisms? Why or why not?

    Students should think about both stochasticity and how organisms modify their environments in ways that affect other organisms. This question requires systems thinking.

5. What kind of characteristics would be good to use when classifying organisms?

    The most useful type of characteristic to classify an organism is something that doesn't change over time, with different conditions, or different life stages. The most commonly used type of characteristic in history has been morphology – the way things look, especially traits that don't change. A better characteristic is DNA, because similar DNA sequences reflect a common and recent evolutionary ancestor and the DNA sequence of an individual doesn't change with time or environmental conditions. Students might also talk about the other ways to classify organisms including how they interact with other organisms (e.g. position in a food web) and behavioral characteristics.

6. Define functional redundancy. Was there functional redundancy in your leaf packs? What evidence are you basing this off of?

    A common misconception that we see is that students think that if one species were removed from a system, that the system would collapse. We would like them to have a more nuanced and complex understanding. To have that understanding they need to understand the functions of the species in the system, the relative abundance of the species, and whether there are other species that perform similar functions. A useful analogy might be to talk about a high school football team. If there were 5 guys who could all play quarterback well, the team wouldn't suffer if one of those guys transferred to a different school. But, if there was only 1 guy who could QB, then the team would have some losing games if that student moved away.

7. What would happen to the abiotic environment if collectors disappeared from the stream?

    The water would get cloudier and less sunlight would get into the water.

8. What would happen to the abiotic and biotic environment if there were three types of collectors and one of them disappeared from the stream?

    It depends on if the other two types of collectors can live all the places the one that disappeared lived. If they can live in the same habitats (e.g. if they can live in with the same oxygen amounts) then those two types would become more abundant and there may not be changes in *the abiotic environment. If they cannot live in the same habitats, then there might be an increase in the cloudiness in those habitats.*

9. You have just learned how an organism can change its abiotic environment in a way that also affects the other members of biological community. Can you think of any changes to a biological community in another ecosystem that would change the abiotic environment and therefore affect the biological community?

    Various answers that are parallel to the stream (e.g. decomposers do the same thing in the forest as the stream). Another example: Trees create shady (and cool) environments underneath them by absorbing the sunlight. If there are a lot of deer (eating saplings) or humans cutting down trees the sunlight reaching the ground might increase. This will let small plants (e.g. shrubs or grasses) that need a lot of sunlight grow more. It might also change the animal community by restricting those animals that need a temperature.

10. Imagine you have been investigating a stream where you find four different kinds of consumers in your leaf pack. Explain why there is more than one type of consumer rather than a "best adapted consumer".

    We would like students to think about how no two species have exactly the same niche. We also want them to understand that the environment is heterogeneous in space and time. This heterogeneity means that the competitive advantage goes to different species in different times and places.

Comments on the Assessment of Student Learning Outcomes:

Assessment for this lab is in the form of three worksheets. The pre-lab worksheet asks students to come up with a research question, hypotheses, and rationales for their hypotheses. You can collect these and provide comments to the groups before they leave the lab. However, a more beneficial way to evaluate these worksheets would be to have each group present their research question, hypotheses, and rationales to the class. The class can critique and offer advice. If the class misses something, you can verbally make comments too. This technique is more student-centered and is less onerous to the instructor.

The second worksheet presents students with a series of questions to help them pay attention to the design and implementation of their experiment. For example, the worksheet asks students how they will standardize the amount of leaves the put in their leaf packs, how they will replicate their experiment, and how they will install their leaf packs in the field.

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The third worksheet has students interpret their data. You can modify the worksheet if you want to increase the level of quantitative reasoning required. For example, you can ask students to calculate species diversity, species overlap, or other measures. You may also want students to perform t-tests to look at statistical significance of differences in their two treatments. This worksheet also asks students to create concept maps that group organisms phylogenetically and functionally. This is meant to help students understand what characteristics scientists look for when they group organisms in each of these two ways and help students realize that organisms in the same functional group may not be closely related phylogenetically.

Comments on Formative Evaluation of this Experiment:

Assessments used in this lab are outlined above. With regard to the pre-lab worksheet, students seem to have difficulty coming up with research questions that are testable, specific, and relevant. You may want to talk about these criteria with your students. With regard to the post-lab worksheet, students seem to have difficulty summarizing their data appropriately. They also struggle with explaining functional redundancy and making the less obvious connections between the biotic and abiotic conditions.

Comments on Translating the Activity to Other Institutional Scales or Locations:

The experiment can be conducted in any number of streams. We have used a river flowing through urban Denver, Colorado, streams in rural Michigan, and streams in upstate New York. This experiment may not be suitable for use with extremely large classes (e.g., 150 or more) because you may not be able to put enough leaf packs in the stream. We suggest that you consult with an aquatic biologist in your locale to determine the best time of year to conduct this activity, which should generally correspond with the timing of natural leaf fall in your area (e.g., October through February in Michigan, though you can find smaller organism throughout the summer).

STUDENT DATA COLLECTED IN THIS EXPERIMENT

We included actual student data sheets from a student group that compared community assembly in leaf packs filled with live cottonwood leaves to leaf packs filled with senesced cottonwood leaves. We have also provided an Excel worksheet with the data along with a sample graph. The data show a greater diversity of taxa in the bags filled with senesced cottonwood leaves (Student's t-test in Excel, t=3.26, p = 0.005). There is also a non-significant trend for more organisms in those packs.

GENERIC DISCLAIMER

Adult supervision is recommended when performing this lab activity. We also recommend that common sense and proper safety precautions be followed by all participants. No responsibility is implied or taken by the contributing author, the editors of this Volume, nor anyone associated with maintaining the TIEE web site, nor by their academic employers, nor by the Ecological Society of America for anyone who sustains injuries as a result of using the materials or ideas, or performing the procedures put forth at the TIEE web site, or in any printed materials that derive therefrom.