Abstract:
This two-part field lesson for mid- to upper-level undergraduates connects allochthonous inputs to the invertebrate consumers that depend on them. After preparing students with background information such as the stream continuum concept and ‘brown’ versus ‘green’ food web components, students will be ready to embark into the stream. During the first part, students will sample organic matter using a variety of equipment (learning methods), sort the organic matter into leaf packs, and deploy the leaf packs. During the second part, students will collect the leaf packs, inventory the inhabiting aquatic macroinvertebrates, and identify functional feeding groups. Students will reflect on their experience by drawing a diagram of a detritus-based food web.

Tags/Keywords:
Macroinvertebrates, Functional Feeding Groups, Allochthonous, Organic Matter, River Field Studies Network, leaf packs

Instructor Notes:
- **Target audience level** - Undergraduate students of any level, but the lesson may be most applicable for upper level courses. There are no prerequisites, but co-enrollment in an introductory level biology class is recommended.
- **Summary of the lesson** - Students are sampling detritus, building leaf packs, and exploring macroinvertebrates associated with detritus in a river or stream. The lesson can be completed in two short periods separated by time for leaf pack colonization (minimum of 24 hours between class periods). The aim is to engage students in thinking about food webs and terrestrial-aquatic linkages.
- **Student context factors** - Briefly describe how student context factors may influence student’s learning of the lesson and ability to participate in the lesson.
  - **Worldview** - Students’ socioeconomic status could prevent them from recreating at rivers, preventing them from understanding that rivers/streams are a system or from knowing water safety. In agricultural areas or in some cultures, insects may be viewed as pests, which is a bias the instructor will have to work through. Similarly, some students may view rivers and streams as ‘dirty’ and be wary of participating.
  - **Interests** - The most aligned interests with this lesson will be in ecology, biology, or environmental science. Students more focused on policy may engage in later stages where the content is applied to the bigger picture of a river system. Non-science majors will be engaged by human curiosity, sense of place, and connections of the material to their interests or majors.
- **Identity** - Students who have not seen themselves represented outdoors may struggle in a field environment. Indigenous students may view certain water bodies as culturally important, and their interactions with that body may be governed by that. Students who identify as having physical limitations may be unfamiliar with how to navigate terrain and lack functional field clothes. Lastly, students may feel uncomfortable or unsafe if the field area is in a space largely inhabited by people who do not share their identity.

- **Prior Knowledge and Skills** - Students who have taken previous biology classes may be able to make more connections and understand more vocabulary, but the lesson is not designed to be built on prior knowledge.

- **Motivation and Expectations** - Students will be motivated by team building and exploration. The anticipation of seeing the invertebrates in Part B can also be a motivator. Students can expect to get a taste of fieldwork and research, which they can decide to pursue further afterwards.

- **Prior Experiences** - Students should be familiar with how a field trip works and the dynamics within a class. Their prior experiences with rivers and streams or their worldview may influence their initial reaction to the activities, but no prior experience with fieldwork is necessary. We recommend that instructors use a geographically familiar stream/river to their campus to facilitate familiarity of surroundings for the students.

- **Personal Needs** - Instructors should help students acquire wading gear whenever possible and give students a list of things to bring (water bottle, sunscreen, etc.). We recommend introducing a code of conduct before leaving for the trip and hosting a Q&A about how the activity will work. Lastly, choose an accessible location and let students know that they can participate without contacting the water if desired. Students should wade in to collect leaf packs and insects, and handling the insects is expected, but for cases of extreme aversion, observing can be fine.

- **Approach to engagement** - Describe ideas for engaging students in the lesson and outcomes
  - 5E Approach:
    - **Engage** students by putting a spin on the food web: plants are always the foundation, but are they always living plants? Can food webs from two ecosystems connect?
    - Have students **explore** a shallow, slow section of a river or stream to sample detritus, and return later to **explore** aquatic invertebrates associated with that detritus. As they sample, give them different sampling equipment to test which items work better under certain conditions.
    - In the field, ask students to **explain** the process by which that detritus arrived in the stream, and why it’s important to the invertebrates. This can be a group discussion. What types of detritus were found and what are the origins? What types of invertebrates are there, and how might they
use detritus? Do they have any feeding strategies or natural history adaptations for detritus?

- Students should *elaborate* by following the food web further: where do the nutrients from the detritus go (i.e. to microbes, then invertebrates)? Where do the invertebrates take/send the nutrients (i.e. to birds, fish, spiders, mice…)?

- Finally, *evaluate* students through assigning a diagram of the food web. This will demonstrate students’ understanding of the importance of detritus and connections between terrestrial and aquatic ecosystems.

- Students might be interested in thinking about the role of detritivores in the ecosystem, both those invisible to the eye and large enough to see. They also might be interested in thinking about different ecosystems that have different types of detritus (i.e. deserts have limited deciduous leaf material, wetland macrophyte detritus, temperate autumn leaf pulse versus tropical steady leaf input, Mara River wildebeest carcasses (Subalusky et al., 2017), etc.).

**Logistics and materials needed**

- **Logistics**: the lesson has two parts (A & B), which should be taught on different days to allow invertebrate colonization of litter. The section of the river should be wadeable and have gentle velocity. An area without cutbanks is recommended for easy access, and perhaps a place with a paved ramp for accessibility needs. This lesson may work best in autumn when deciduous leaves have fallen, but can be done any time of year. Consider students’ access to river-appropriate clothing and footwear and how they will stay warm or cool as appropriate (for a 3-hour field period).

- **Materials**: the following should be provided
  - Core sampler (such as 5 gallon bucket with bottom cut off)
  - meter stick for measuring water depth in core
  - drift net + rebar + hammer for capturing drifting detritus
  - scale for weighing detritus
  - mesh bags (i.e. for produce) for building leaf packs
  - string for tying off leaf packs
  - trays/pans/bins for assembling and disassembling leaf packs
  - labels and writing utensils for leaf pack IDs
  - family-level invertebrate key (laminated)
  - hand lenses for inspecting invertebrates
  - forceps / tweezers for handling invertebrates
  - ice cube trays for sorting invertebrates
  - clicker counters for enumerating invertebrates
  - plastic zipper bags (quarts and/or gallons) for holding leaf packs and contents upon removal (or transport with trays)
  - kick net for additional invertebrate exploration
  - *worksheets* for student diagrams and notes
  - *data sheets* for students to record on

**Further reading for the instructor**
Learning Outcomes & Objectives:
At the conclusion of this lesson, students will have gained experience in field skills and data collection by learning how to use different types of equipment for sampling macroinvertebrates and discussion on when to use different collection methods. By collecting and identifying macroinvertebrates in the field, students will increase their field observation skills and identification skills. They will also learn the best practices for doing field identifications. From a big picture perspective, students will have increased scientific understanding of the ‘less charismatic’ members of the food webs in their local streams. As well as a stronger sense of place by increasing their knowledge of local species and who the students share their community with.

- Define the terms detritus, invertebrate, benthic, and ...
- Identify the type of equipment needed to sample benthic versus water column macroinvertebrates or detritus for qualitative and quantitative analyses (A & B)
- Compare detritus quantities and types between benthic and drift (A)
- Describe traits of various invertebrate orders (i.e. FFG, strategies for feeding) (B)
- Classify the different invertebrates collected in the leaf packs (e.g. using an order and/or family-level guide) by type of litter (A & B)
- Diagram the path and the role of detritus in the food web ('brown side' vs 'green side') (A & B)

Assessment of Learning Outcomes:
Instructors will assess learning outcomes by examining student diagrams, evaluating student reflection responses, and checking student answers to a multiple choice question on the activity worksheet. The connections students make in their stream food web diagrams will illustrate their understanding of how detritus fuels the food web and which invertebrate groups take advantage of the allochthonous resources. Further, students will describe these connections in a reflection paragraph. Optionally, instructors could implement a pre-test of the materials from the introduction slides and/or discussion to determine how much foundational information was internalized prior to entering the field.

Context for Students:
The context for the students can be presented as lecture slides (found in the lesson repository) or as a group discussion of diagrams and videos. Below are points instructors should make to provide foundational knowledge to students before starting the field experience.

- We know the foundation of the food web is autotrophs (primary producers), which are mostly plants (e.g. watch this video— what is the primary producer called in this food web?).
  - However, these plants are not always alive, or they might not grow within aquatic habitats but alongside them instead
- Allochthonous litter falls into aquatic habitats, and is an important resource for the food web (Evans-White & Halvorson, 2017; Frainer et al., 2021; Marks, 2019; Moore et al., 2004).
Discuss Figure 3 from Marks, 2019
Examine Figure 1 from Moore et al., 2004
Read introduction section of Little & Altermatt, 2018

- Some invertebrates are more dependent on detritus than others. Watch this video and write down which functional feeding groups might eat more detritus than others. Optional background reading is Cummins & Klug, 1979.
- Other reading on invertebrates and detritus:
  - Invertebrates contribute to nutrient cycling (Balik et al., 2018)
- During this lesson, we will explore litter types and sources in a stream (or wetland) near us, as well as what invertebrates consume them.

Lesson Content:
This lesson focuses on exploration of allochthonous inputs to a stream ecosystem and their role in the food web. Following coarse particulate organic matter collection protocol from Lamberti et al., 2017, students will collect leaves and other litter. Students will sort the litter into types in pans. These categories will be specific to the stream where the activity is taking place (e.g. leaves, twigs, and sedges; or maple leaves, beech leaves, and oak leaves). Using the litter, students will construct leaf packs that will be deployed in the stream for at least 24 hours.

During the next class period, leaf packs will be retrieved and colonizing invertebrates will be observed. Aquatic invertebrates will be identified to family and organized into functional feeding groups. Students will determine which functional feeding groups preferred which types of detritus and see how well it aligns with the foundation provided in Cummins & Klug, 1979.

Throughout the lesson, students will diagram the food web. They should make connections between the terrestrial and aquatic habitats, the allochthonous and autochthonous resources, and the aquatic invertebrates.

Procedure:
PART A (approximately 3 hours):
1. Arrive in the field. Instructor briefly discusses water safety protocol and site characteristics (e.g. restroom location, layout, poison ivy) and everyone settles in to the site (5 min)
   - Field site is ideally a wadeable stream with canopy cover, possibly in autumn to increase leaf litter mass available
2. Instructor asks the students what connections they see between the aquatic habitat and the terrestrial habitat. Do these connections change during different conditions/seasons? (15 min)
   - Students discuss leaf litter input– allochthonous detritus
   - Students begin drawing diagrams of detrital inputs to streams on worksheet
3. Sample litter (40 min)
   - Instructor gives examples of when to use different sampling equipment (e.g. core vs drift vs Hess or Ekman grab; qualitative vs quantitative) and demonstrates how to use samplers
   - Students set up drift net (with guidance from instructor)
   - Students use core sampler in multiple locations within the stream reach
4. Explore litter (40 min)
   ○ Students place detritus in pans and sort it by type (type will vary by geographic context)
   ○ Instructor prompts students to describe the texture of the litter— is it slimy?
     i. Talk about microbial colonization (peanut butter on celery analogy) and its importance to invertebrates
5. Students continue to add to their diagrams (10 min)
6. Build leaf packs and deploy (30 min)
   ○ Weigh sorted detritus into equal piles
     i. Record pre-weights, coordinates, etc. on data sheet
   ○ Stuff the piles into the mesh bags and sew or zip-tie closed
     i. Remember to add a label or color-code bags/string
   ○ Cut a 1-1.5m string, tying one end to the leaf pack (through the mesh) and one end to a sturdy riparian feature (e.g. thick root, trunk of a tree or shrub, or a stake if no riparian structure is available)
     i. Keep in mind that if flows increase, the packs and strings will need to endure
     ii. Consider adding flagging to mark leaf pack locations
   ○ Place the leaf pack on the streambed, holding it down with a rock if necessary
   ○ Remind students that leaf packs will be collected during the next lab period

PART B (approximately 3 hours):
1. Instructor reintroduces invertebrate families and functional feeding groups. Instructor prompts students to think about what types of invertebrates might rely on different types of plant material (algae vs allochthonous litter, coarse vs fine…) (20 min)
   a. Have students write down expectations (do not add invertebrates to the diagram yet)
2. Students collect the leaf packs from the stream and explore the contents (2 hours)
   a. Dividing students into teams to work through leaf packs will make this process more efficient (such as one team per litter type, one leaf pack per student or pair of students)
   b. Use trays or plastic bags to limit loss from the bags, carry them to a flat area or picnic table
   c. Open one leaf pack in each tray, removing the contents
     i. Adding a little stream water to the tray can make it easier to find the invertebrates
     ii. Sort the invertebrates into ice cube trays or cups by order or family (using a laminated guide) and
     iii. Enumerate invertebrates (students may need to use clicker counters)
   d. Record data in data sheet
     i. Instructors should modify the data sheet to fit the number of leaf packs deployed and the level of invertebrate identification
     ii. Students will need to copy data from other groups or enter into a shared spreadsheet later
3. Begin a discussion about which functional feeding groups were most prominent in the different litter types (20 min)
   a. How did these observations compare to student expectations?
4. Finish filling out the worksheet (20 min)
   a. Add invertebrates to the stream food web diagram

Outside of Class (approximately 1 hour):
1. Students should be given access to other class’ master worksheets and data sheets from the lesson repository to compare to their own data and fill out the venn diagram on the activity worksheet
2. Students should complete the reflection and multiple choice question on the activity worksheet
3. Instructors should collate student data and create a master worksheet to upload to the lesson repository for future students to use for comparison (such as example worksheet)

Recording Observations:
Part of this lesson is students working as a class to make observations, both qualitative and quantitative. Please download the full spreadsheet from the supplemental materials, fill it out during the student activity, and upload a master spreadsheet to the lesson repository for your field site. This data framework is designed to allow comparisons between classes that have all completed the activity. For example, a group in Nebraska could download and compare data from another group that completed this activity in Florida. To make these comparisons informally, we have also created a worksheet. Students will fill out the worksheet in the field, making observations about the stream they are working in. Then, they can compare their observations to other observations using the venn diagram in the worksheet. We implore instructors to upload their class data and/or a collated master worksheet to QUBES for future classes to analyze for comparisons.

Data Analysis and Interpretation:
Students will tally the number of individuals of each invertebrate order, family, or functional feeding group found in each litter bag. Then, the students will calculate the proportion of the total invertebrate density that each group comprises within each bag, and compare proportions across litter types. Instructors can be as detailed about data and analysis as they see fit for the course level, such as generally making observations or applying statistical analyses (see links to other lessons below for examples of student data activities for leaf litter breakdown in streams). Students should determine whether the invertebrate patterns observed relate to hypothesized patterns and postulate what could account for differences.

Reflection:
As students diagram the stream, its allochthonous inputs, and the invertebrates that rely on those inputs, they will reflect on how those three components are connected. Instructors can additionally ask students to write a few sentences about anything they noticed during the lesson that they didn’t notice before, any fears of rivers that were overcome during the activity, or the
sampling equipment they liked the most. Finally, the students can describe where they as humans fit into the scheme of the diagram.

**Links to Other Lessons and Concepts:**
This lesson is designed to provide a foundation for field exploration of detritus and aquatic invertebrates in streams. Instructors have the option to increase or decrease the level of detail or the amount of time spent exploring. Due to this flexible and field-based structure, we do not offer advice on organizing a data analysis activity or highly quantitative data collection. If you wish to incorporate a data focus, see the lessons below (full citations in reference section).

- For basic methods to quantify coarse particulate organic matter, see the exercise detailed in Lamberti et al., 2017
  - Or for fine particulate organic matter, see Hutchens et al., 2017
- For a more data analysis-oriented lesson on leaf litter decomposition incorporating the influence of urbanization, see McIntosh, 2021
- For a data-based exercise involving factors that influence leaf litter decomposition and stream invertebrates, see Janmaat, 2018
- For a community assembly approach to leaf packs, see the lesson plan by Doherty et al., 2011

**Further Reading:**
- Marks, 2019
- Moore et al., 2004
- Subalusky et al., 2017
- Taylor & Chauvet, 2014
- Vannote et al., 1980
- Wantzen & Wagner, 2006
- Wissinger et al., 2021

**References:**

  https://doi.org/10.1146/annurev.es.10.110179.001051

  http://tiee.esa.org/vol/v7/experiments/doherty/abstract.html


