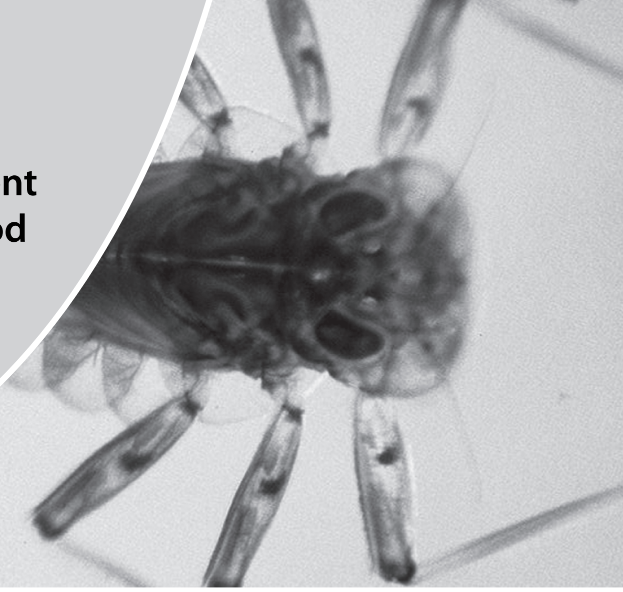
INQUIRY & INVESTIGATION

Three Simple Experiments to Examine the Effect of Sediment Pollution on Algae-Based Food Webs in Streams



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Abstract

Streams and stream macroinvertebrates are ideal natural systems for ecological inquiry. We present three simple experiments that students can use to conduct field-based investigations which illustrate the importance of algae-based food webs in streams and measure the effects of sediment pollution (scour and deposition) on stream ecological processes. Over the past 5 years, we have conducted these experiments 19 times with our students. We report on the results and reliability of these experiments and make suggestions for other educators who may want to conduct them.

Key Words: *Stream macroinvertebrates; inquiry; field experiment; sediment scour; sediment deposition.*

○ Introduction

Field-based ecological inquiry is an important component of high school science curricula; however, the scale and timing of natural processes make it difficult to conduct field-based experiments within the time constraints required of the academic year, teaching unit, or field trip. As part of a Municipal Watershed Health Education Program, we developed three field experiments and supporting curriculum to engage our students in ecological inquiry and to learn about the cause-and-effect of sediment pollution in stream ecosystems. The purpose of the experiments was to illustrate basic ecological stream processes through in-stream manipulations of substrate and observe the subsequent response of stream macroinvertebrates (hereafter "invertebrates"). Over the past 5 years, during short field trips to small streams (no more than calf-deep), we conducted these experiments numerous times with our high school students. The design of these experiments and associated curriculum meet Oregon's Next Generation Science Standards for Scientific and Engineering Practices and also address Crosscutting Relationships and several of the Disciplinary Core Ideas in the Life and Physical Sciences (Table 1; National Research Council, 2012).

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Streams and stream invertebrates are ideal natural systems for education-based ecological inquiry. Many stream ecological processes operate on relatively short timescales (e.g., hours) and across small spatial scales (e.g., individual rocks). Invertebrate behaviors such as drift (Waters, 1972; Kohler, 1985; Brittain, 1988), colonization (Fisher et al., 1982), and feeding (Zwieg & Rabeni, 2001) provide an ecological mechanism for short-term ecological experimentation of sediment pollution in streams. Sediment pollution is one of the leading causes of stream degradation in the United States (U.S. Environmental Protection Agency, 2006). However, it's difficult to quantitatively measure fine sediment in streams and to determine whether sediment levels are exceeding natural sediment conditions, so environmental scientists use stream invertebrates as indicators of sediment pollution (Relyea et al., 2012). Two major forms of sediment pollution in streams, called "scour" and "deposition," occur during the transport and settling of fine sediment. Sediment is a natural component of streams; but at excessive levels, both forms of pollution can degrade habitat used by invertebrates for feeding (Zweig & Rabeni, 2001).

Here, we present three simple experiments (one preliminary investigation and two pollution experiments) that illustrate the importance of the algae-based food web in streams and demonstrate the effect of sediment scour and deposition on substrate habitat. For each investigation, we provide the most relevant ecological theory and describe the experimental methodology. We also present example results of student-conducted experiments and assess the reliability of each investigation.

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Table 1. Summarizes the curricular activity or experimental outcome that meets Common Core Standards in the areas of *Crosscutting Relationships* and *Disciplinary Core Ideas in the Life Sciences and Earth Sciences*.

Curricular Activity or Experimental Outcome	Crosscutting Relationships	Disciplinary Core Ideas
Examine relationships between algal food-web condition and invertebrate feeding.	Patterns	Life Sciences (LS2.A): Interdependent Relationships in Ecosystems
Demonstrate causal mechanisms of sediment-related stream degradation.	Cause and effect	Life Sciences (LS2.B): Cycles of Matter and Energy Transfer in Ecosystems
Use standardized abiotic variables (i.e., rate per day) and biotic variables (i.e., invertebrates per square inch).	Scale, proportion, and quantity	NA
Identify scale-relevant sample units (i.e., individual substrate).	System and system models	NA
Illustrate the linkages between pollution, stream function, and invertebrate response.	Energy and matter: Flows, cycles, and conservation	Earth Sciences (ESS3.C): Human Impacts on Earth
Quantify the effect of functional change on the stream food web.	Structure and function	Life Sciences (LS2.C): Ecosystem Dynamics, Functioning, and Resilience
Understand the short time scales of some stream ecological processes (i.e., sediment transport, invertebrate drift).	Stability and change	Earth Sciences (ESS2.C): The Roles of Water in Earth's Surface Processes

○ Methods

Student Participants & Supporting Curriculum

Our students are from a range of classroom settings, including a 9th–12th grade Vocational Forestry program and an AP Environmental Science class. We conducted the experiments a total of 19 times in five streams around the region. The streams ranged from moderately impaired by urban land use to pristine with little human disturbance. The experiments were structured around two curricular units, either as a follow-up to a stream bioassessment unit or as a stand-alone ecological inquiry unit. After an introduction and case study on stream experimentation, we asked our students to design an experiment and describe it using diagrams and schematics. Specifically, we asked students to state a hypothesis based on one of several ecological theories (drift, avoidance, disturbance, or food webs), design methods for a 2-day experiment, create a simple diagram to explain the experiment, and create a hypothetical box plot to illustrate expected results. In the field, we gave students a demonstration of how to install and harvest the experiments. We also discussed the challenge of unbiased invertebrate counting in the field and cautioned them to minimize selection bias by standardizing search effort. While the experiments were taking place, we or other trained assistants either helped students count invertebrates or observed counting protocols and made suggestions. After the experiments, we held several in-class data-analysis sessions and students worked on the final product, a research poster created using a PowerPoint template that we provided.

Methodology & Data Analysis

We designed the investigations so that a similar set of field methods requiring minimal equipment (white plastic tub, brush, and tweezers) could be used to install and harvest the experiments during two short field trips (<1.5 hours each). The invertebrate data collected by our students were typically only counts, but some students with more identification experience chose to identify invertebrate order or family. In most streams, the experimental substrate was colonized by small mayflies (Heptageniidae and Baetidae).

The experiments followed the same basic methodology and were conducted through in-stream manipulations of substrate (smooth and flat stream rocks 20–30 cm wide) and subsequent "harvesting" of the experiment 3–5 days later. Experiments were installed in arrays of substrate placed on the stream bottom, so that each experiment had 10–20 substrate replicates and each substrate was either a treatment or a control (Figure 1). In the preliminary experiment, the treatment was substrate covered with algae and the control was substrate without algae (obtained from the stream bank). For the pollution experiments, the treatment substrate was sediment-polluted (either scour or deposit) and the controls were natural substrate with no sediment pollution. To provide space for harvesting, substrate was placed ≥0.5 m apart. Substrate was marked with crayons as either treatment or control and also with wire marking flags pushed into the stream bed (Figure 2). Experimental arrays were always installed downstream and harvested upstream. It is critical that all substrate used in the experiment is gently cleaned of invertebrates before installation in the stream. This was accomplished by placing the substrate in a tub of water and gently rubbing the rock surface with hands or a very soft brush (e.g., paintbrush) to dislodge clinging invertebrates. After 3–5 days in the stream, the experiment was harvested by quickly removing the substrate from the stream and directly placing it in a white dish-tub. An optional approach was to place a D-net directly downstream of the substrate to capture any invertebrates that drifted away during

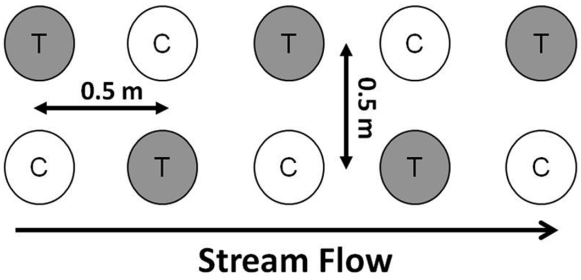

Figure 1. Diagram showing the in-stream placement of experimental arrays with treatment and control substrates. Substrate consists of rocks (20–30 cm wide) obtained from the stream. Substrate should have ≥0.5 m space in between and be installed working downstream and harvested working upstream.

Figure 2. Two experimental arrays. Note the placement of the arrays on the margins of the stream to avoid deep water and high velocity in anticipation of an upcoming rain event. It is important to make sure that substrate is placed in areas of the stream with moderate flow and no upstream obstructions to drifting insects.

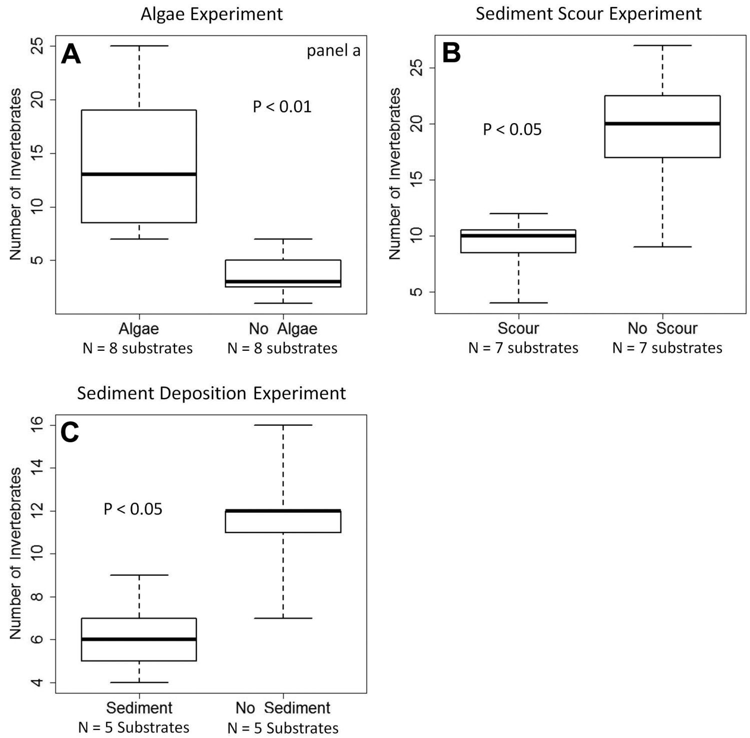

Figure 3. Example results from a typical experiment conducted by students with a colonization period of 3 days. (**A**) The algae experiment compared substrate with and without algae. The sediment pollution experiment compared control substrate to substrate with (**B**) sediment scour or (**C**) sediment deposition. Box plots show median, interquartile range, and minimum and maximum invertebrate counts per substrate.

substrate harvesting; however, we have found that either approach works equally well. With the substrate in the tub, students removed invertebrates by gently rubbing the substrate with their hands or a soft brush or by picking with no-crush tweezers. The substrate was then removed from the tub, and the remaining invertebrates were counted and returned to the stream unharmed. Invertebrate counts were used to calculate colonization rate (invertebrates per day) for each substrate. Data were analyzed by comparing colonization rates for treatment and control substrates using box plots and t-tests in the AP classes.

We evaluated the reliability of the experiments by determining how many of the student-conducted experiments generated significant results ($P < 0.05$) that confirmed the expected hypothesis. The statistical significance of each experiment was determined by using one-tailed t-tests to compare mean colonization rates for treatments and controls. For each of the three experiments, box plots were used to display a typical example of results generated by students (Figure 3).

Preliminary Experiment: Algae-Based Food Web

The algae and other microbes that grow upon stream substrate, called "periphyton," are part of the autotrophic base of a stream food web. "Scrapers" are invertebrates that feed on substrate surface, using specialized mouthparts to scrape and consume the periphyton growing there. As scrapers drift in the current or move along the stream bottom looking for food, they colonize substrate habitat with palatable periphyton (Fisher et al., 1982). Our students examined this food-web process by comparing colonization rates between substrates with and without periphyton. Substrate with periphyton was obtained from the stream and cleaned of invertebrates. Similar-sized substrate without periphyton was collected from the stream bank. Both substrates were placed in the stream in the arrays described above. In this investigation, our students tested the hypothesis that substrate with algae (treatment) will have higher invertebrate colonization rates than substrate without algae (control). The simplicity of this experiment made it useful as a practice for students and also to test whether the stream substrate and colonization rates were suitable for the pollution experiments.

Pollution Experiment: Sediment Scour

Excessive levels of inorganic fine sediment (hereafter "sediment") in streams is a widespread form of stream pollution (U.S. Environmental Protection Agency, 2006). Sediment is transported in streams by a process known as "saltation" (Schofield et al., 2004). Saltation occurs when sediment particles are carried in the current by rolling and bouncing along the stream bottom. In a process known as "scour," saltating fine sediment abrades the substrate surface and, at excessive levels, can reduce the amount of periphyton available for feeding (Schofield et al., 2004). For this experiment, periphyton scour was simulated by gently rubbing the surface of the rock with sand (obtained from the stream bank) and then rinsing the sand off the substrate. In this investigation, students tested the hypothesis that scoured substrate (treatment) will have lower colonization rates than nonscoured substrate (control).

Pollution Experiment: Sediment Deposition

Inorganic fine sediment deposited on the stream bottom is another major form of stream pollution. Deposition occurs when the sediment transport capacity of the stream is exceeded by the volume of sediment particles in the stream. As stream velocity drops after a storm event, or as a result of increased inputs from erosion, excess sediment particles settle on the substrate surface, where they stick to the periphyton (Suren, 2005; Peeters et al., 2006). Burial of the periphyton with fine sediment limits feeding access and may reduce food quality (Graham, 1990; Broekhuizen et al., 2001). In this experiment, sediment deposition was simulated by covering the substrate periphyton with a thin layer of fine sediment. This was achieved by manually covering the experimental substrate with sand and silt obtained from the stream bank or by using substrate that was already naturally covered by fine sediment as a result of being located in a depositional area of the stream. Control substrate with no sediment was obtained from the stream and also cleaned of invertebrates before placement. In this investigation, our students tested the hypothesis that the substrate covered with fine sediment (treatment) would have lower colonization rates than substrate without a covering of sediment (control). The two methods for simulating sediment deposition reflected different approaches to field experimentation. Using naturally deposited sediment reflected the actual in-stream process of deposition; however, the amount of sediment and periphyton condition was not known and could not be controlled. In the second approach, a known amount of sediment was added to the substrate, but pouring the sediment on the substrate did not mimic natural processes in the stream. The different approaches helped our students understand and recognize the limitations of field experimentation.

○ Results

General Results

From 2010 to 2014, eight classes conducted 19 experiments in five streams. Seventeen (90%) of the experiments generated statistically significant results that confirmed the expected hypothesis. Of the two experiments that resulted in insignificant results, one was in the food-web experiment and the other was in the sediment deposition experiment. Across all experiments, invertebrate colonization rates per substrate rock ranged from zero to 97, with the majority between zero and 20. Mean percentage of substrate with zero invertebrate colonization was 19% in treatments and 8% in controls and occurred even on substrates with good periphyton cover. Less than 5% of all substrate placed in the streams was lost or could not be recovered.

Food Web & Autotrophy

Thirteen food-web experiments were conducted in five streams. A typical experiment contained 10–20 substrate samples and resulted in a median of 13 invertebrates for substrate with periphyton and four without periphyton (Figure 3A). Twelve of the 13 experiments generated statistically significant results supporting the hypothesis that substrate with periphyton would have higher colonization rates. The insignificant finding was generated in a very small stream where the overall colonization rate was relatively low. A subsequent experiment in the same stream over 3 nights resulted in higher colonization rates and significant results. This simple yet reliable experiment illustrated the importance of periphyton in the stream food web and demonstrated the relatively rapid rate of invertebrate colonization of high-quality habitats. The food-web experiment was the first investigation we developed and has been an excellent introductory experiment for our students.

Sediment Scour

Four sediment-scour experiments were conducted in four streams. A typical experiment contained 10–20 substrate samples and resulted in a median of 10 and 20 invertebrates on nonscoured and scoured substrates, respectively (Figure 3B). All four experiments showed significant results, supporting the hypothesis that fewer invertebrates would colonize scoured substrate. Despite having only four trials, we believe that the consistent results observed in this investigation suggest that the experiment was also reliable. The value of this experiment was that it showed students that sediment scour has the potential to degrade the quality of substrate periphyton.

Sediment Deposition

Four sediment-deposition experiments were conducted in four streams. Both techniques for covering the substrate with sediment were used. Figure 3C shows the results from an experiment using substrate that was covered by fine sediment naturally deposited in the stream. The median numbers of invertebrates on the sediment-covered and control substrate were 6 and 12, respectively. Three of the four experiments showed significant results supporting the hypothesis that fewer invertebrates would colonize sediment-covered substrate. The experiment that generated insignificant results was due to one treatment sample with a substantially higher invertebrate count. After discussions with our students, it was apparent that the outlier was likely caused by a mix-up of treatment and control substrate in the field. However, because our students did not have field notes indicating the possibility of a mistake, it was decided that the outlier could not be dropped. Overall, this experiment also appears to be relatively reliable and illustrates the effect of sediment deposition on the nutritional quality of periphyton.

○ Discussion

The experiments we have described here provide a simple, reliable approach for engaging students in ecological experimentation and provide authentic research experiences in the natural sciences. For example, a study of mine pollution in Pennsylvania (DeNicola & Stapleton, 2002) utilized a substrate transplant design very similar to the methods employed by our students. In conducting these experiments, students were faced with a number of critical decisions that draw on fundamental research skills such as designing randomized methods, field sketching and schematics, identifying treatments and controls, and isolating variables. The data generated by these experiments were also well suited for teaching students how to analyze and interpret ecological data. Because the dependent variables were invertebrate counts and the independent variables were categorical (e.g., scour vs. no scour), our students used box plots to display and interpret results. In doing so, they considered the relative difference between means, data variance, and how to handle outliers. For example, we allowed students to remove outliers only if they had field notes indicating a problem with the sample. Perhaps most importantly, we observed the intrinsic appeal to students when their experiments were "harvested" and ecological stream processes were revealed.

Educators who plan to use these experiments should be aware of some challenges and useful precautions. In most of these experiments, the difference between invertebrates found on treatment and control substrates were relatively small, and thus it's easy to obfuscate results through methodological error. For example, failure to remove invertebrates from the rock surface before installation, inadequate rinsing of collection equipment between samples, standing upstream of the experimental arrays, or inconsistent searching for invertebrates can substantially affect results. Also, experimental arrays must be placed with enough spacing to be sampled without disturbing other experimental substrate and to ensure that the algae side of the substrate is always facing upward. Finally, because substrate is mainly colonized by invertebrates drifting in the current, avoid placing experimental arrays in areas with low stream flow or where drifting invertebrates may be blocked by in-stream obstructions such as large boulders or logs.

In some of our advanced classes, we extended the rigor and depth of these experiments by using statistical analysis to determine whether the difference between treatment and controls was significant. Our students calculated P values by hand using a standard formula for t-tests. The rigor of the methods was also increased by having students calculate colonization rates per day per square centimeter of rock surface using the formula $LW(\pi/4)$, where L is the length and W is the width of the rock (Bergey & Getty, 2006). Another method for advancing the methods was to use a "blind counting" method that allowed students to count the invertebrates without knowing whether they were counting treatment or control samples. We used the double-blind approach only for students who had completed the preliminary experiment.

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