The purpose of this supplement is to provide teachers with a greater familiarity with the microorganisms that they will encounter in these activities, give them basic understanding of the roles they play in food webs, and give additional tips on performing the activities described in the article in Science and Children.

**Activity #1 on Grass Infusion Cultures**

Bacteria play an important role in decomposition since, unlike humans and most other organisms, they have the ability to digest the cellulose of plant fibers. By doing so, they can tap into an abundant energy source and reproduce. As their numbers increase, the bacteria become an abundant food source for larger microorganisms, the protozoa. These are organisms with a cell structure similar to human cells but they exist as single cells, swimming around and feeding on bacteria and each other. The two main groups of protozoa are flagellates and ciliates. Flagellated protozoa (“flagellates”) have one to several long appendages called flagella that they use for movement and eating. Ciliated protozoa (“ciliates”) have several to many short hair-like appendages called cilia that they also use for movement and feeding. Flagellates are generally smaller than ciliates and thus serve as food for them.

The basic food chain that becomes established in the grass infusion cultures is shown in Figure 1 (images of real microorganisms are given below). Unlike the plant or algae food bases that are commonly known to support terrestrial and aquatic food webs (see Activity 2), this is a decomposer-based food chain with photosynthesizing organisms playing a minor role. Bacteria break down the dead grass for food, grow in number and become food for flagellates and small ciliates. These small protozoa become food for larger ciliates and as time progresses the numbers of all three groups grow. Bacteria and protozoa die and are

![Figure 1. Microbial Food Chain in Grass Infusion Cultures. Bacteria form the food base by decomposing the grass. The thick blue arrows show the feeding interactions. The dashed arrows indicate the protozoa die and are also decomposed by bacteria.](image-url)
further decomposed by other bacteria, furthering the process. Given their size and mobility, ciliates will be the biggest attraction when making microscopic observations in this activity.

A great question we have students think about is, *where do these microbes come from?* Microbes are everywhere, often in very low numbers and in resting stages, waiting for conditions to improve. When the dry grass is added to water, the microbes “come alive” and begin growing.

*Making Observations of the Grass Cultures*

The effect of the age of the grass cultures will be apparent by sight and smell. Be sure to give the students a good amount of time to make their observations. There is a data sheet that you can copy and hand out for students to use to “collect data” on page 6 of this supplement. For the activity to have the best effect and to build some suspense, be sure to begin your microscope observations with the newest culture (Day 5) and progress to the oldest. Observe at the lowest magnification and have the students look for movement or any signs of life. You will not likely see any movement in the Day 4 or 5 cultures. It is usually in the Day 3 culture where you can see some slime and goo developing at the surface.

*Getting the Goo*

The motto for this activity is “*Get the Goo*”. Goo is great because of what it is: lots of bacteria and protozoa that have become established from grass decomposition. **Be sure to ask the students what they think the goo is and what is causing it to form.** This is a great way to have them exercise the scientific process of making observation and hypotheses.

So, a *key step in making this activity fly is to prepare a good microscope slide of some of this goo.* A couple of ways to achieve this are to:

- Suck up some slime with the eye-dropper or plastic pipette; or
- Use a small tweezer to pick up some small grass fragments that are coated with slime.

Once you have some on the slide, place a cover glass on it and view with low magnification, making sure that you focus on the gooey material on your slide. To get the best image, try adjusting the light intensity control and the iris diaphragm on the light condenser of your microscope. Once you have a nice image of the goo, give the students some time to look for signs of life. Figure 2 gives a good example of what the goo looks like at 40X magnification. Large ciliated protozoa should be easily visible swimming about and the student will be sure to let you know. The brown, grainy stuff are millions of bacteria busy decomposing the grass.

Find a spot where there appears to be a lot of activity and then switch to a higher magnification. Most compound microscopes have parfocal lenses, which means that the image stays in focus when you change between lenses of different magnification. Figure 3 shows what ciliates and flagellates look like at 100X. These will be swimming around like mad and are
sure to get some wows from the students. A great way to get the students involved is to have them count the number of different shapes of the ciliates they can see.

Figure 3. Protozoa at 100X magnification. Image A shows ciliates of various shapes and sizes. Image B shows a group of flagellates swarming around a cluster of bacteria decomposing grass fragments.

Having a microscope with a magnification of 200X or higher can really enhance this activity. Projected images of microbes can be feet in diameter to provide students with a unique encounter of cellular life. Figure 4 gives examples of what a magnification of 400X can provide. Note how three components of the food chain described in Figure 1 can be visualized.

Figure 4. Protozoa at 400X magnification. A, large ciliates and small flagellates denoted by arrows. B, two different large ciliates; the small grainy objects are bacteria. C, a large ciliate with the cilia denoted by arrows, together with smaller protozoa. D, large ciliates with the arrow denoting a ciliate undergoing cell division.
Activity #2 on Aquatic Food Webs

The microbial food chain that forms in the grass cultures (Figure 1) plays an important role in the decomposition processes in natural environments and organisms observed in the cultures are key players in natural food webs. The grass cultures, however, are considered semi-closed systems, ecologically speaking. Light can penetrate the glass and the perforations in the plastic wrap allow for gas exchange with the outside environment, but the length of the food chain is largely restricted to microorganisms: the largest protozoa are, for the most part, the end of the line. In food webs out in nature, say, in a stream or pond, the large protozoa serve as an important food source for small animals such as amphipods and zooplankton (i.e. “microcrustaceans;” daphnia or “water fleas”). These tiny animals are then consumed by larger animals such as fish. Another important difference between the grass cultures and natural aquatic environments is that there is very little photosynthesis occurring in the jars but in ponds and streams, algae are responsible for providing much of the energy input into these systems. The algae are eaten by large protozoa and by the same small animals that can consume large protozoa as described above. So, bacteria and algae together form the base of the food webs that we find in aquatic environments as illustrated in Figure 5.

![Diagram of an aquatic food web](image)

Figure 5. A basic aquatic food web. That in blue represents the food chain that is observed in the grass cultures. In red are the additional groups of organisms that we find in natural aquatic environments, how they are connected to the bacteria-based community observed in the grass cultures, and illustrating how large organisms are supported by small ones. Drawings not to scale.

When viewing the slide preparations of “mud” that you scrape from the stream or pond rock, you will see similar types of organisms that are observed from grass cultures. A key difference is the presence of algae, mostly of a diverse group called the diatoms. A key feature of diatoms is the ornate glass case they produce that houses the cell and gives each cell its shape. The number of different shapes diatoms is huge and some examples are given it Figure 6. The distinct shapes and the presence of pigments for photosynthesis make it difficult to mistake diatoms for anything else.
Figure 6. Different types of diatoms. http://en.wikipedia.org/wiki/File:Diatomeas_w.jpg
Grass Culture Data Sheet

<table>
<thead>
<tr>
<th></th>
<th>Day 5 Culture (newest)</th>
<th>Day 4 Culture</th>
<th>Day 3 Culture</th>
<th>Day 2 Culture</th>
<th>Day 1 Culture (Oldest)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you see slime or goo at the surface?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>How does it smell?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Is the water beneath the grass clear or cloudy?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Culture Comparison:** How do the cultures change as they get older?