Duckweed Unlimited

Using common duckweed (*Lemna minor*) to measure water quality

Overview

Concerns for the quality and contamination of surface and groundwater are ever growing as we become continually more aware of threats to our health and the health of our planet. Agricultural fertilizer and pesticide use are often suspect in water quality issues. It is important that fertilizers and pesticides be applied at proper times and at appropriate application rates.

The testing and monitoring of water is a costly venture for government and commercial laboratories and can be equally costly to study in high school environments. National and regional studies are continually under way to find reliable and accurate testing procedures and indicator species that will make water-quality monitoring economically feasible. Common duckweed (*Lemna minor*), a tiny floating aquatic plant found all over the world, is getting a lot of attention as a candidate for a water contamination indicator species.

This lab is designed to demonstrate how an indicator species can be used to monitor a water supply by testing the susceptibility of common duckweed to a variety of contaminants. Results can usually be seen within 96 hours. The lab is designed to be a basic experiment that can lead to further optional investigations.

Biological and agricultural concepts

Ecology
Indicator species
Bioassay
Water quality
Asexual reproduction
Point source contamination

The teachable moment

Both agriculture and biology teachers can use this unit when discussing the water cycle and water quality, along with issues of chemical safety and agricultural chemical runoff.

Biology teachers can use this unit when teaching about asexual plant reproduction and concepts in ecology, including the use of indicator species to monitor ecosystems.

Agriculture teachers can use this unit when teaching how crop fertilizers, pesticides or other soil amendments can move and leach through the soil and when teaching proper methods for disposing of agricultural chemicals.

Background

All living things are products of their environments. An *indicator species* is a plant or animal that points to or responds specifically to conditions in a community or habitat.

For example, the disappearance of a particular plant that is sensitive to salt would indicate that a particular soil is salty. In the Upper Midwest, excessive grazing of dry prairie regions can be diagnosed by a decrease in little bluestem, a species of grass, or an increase in ragweed, dandelion and white clover plant species. A stream dominated by a particular type of midge fly larva is generally found to be polluted with organic wastes such as sewage.

Currently there are four indicator species commonly used for freshwater environmental monitoring. They are *Pimephales promaelas,* water fleas *(Daphnia magna and D. pulex),* and *Selenastrum capricornutum* (Peltier and Weber 1985; Horning and Weber 1985, from Wang 1990 second citation). These species are used routinely in tests conducted by federal and state regulatory agencies, industries, and consulting laboratories to assay for water contamination.

Recent studies have shown that common duckweed holds great promise as an indicator species in assays determining water quality.

The EPA (Environmental Protection Agency), research institutions, and consulting labs are beginning to use duckweed as a fifth indicator species in the monitoring of effluents. Duckweed has also been incorporated into tests monitoring for hazardous chemicals and a duckweed test protocol has been published by the American Society of Testing and Materials (Wang 1991 from Wang second citation).

It is essential to remember that duckweed, like other indicator species, does not measure the amount of contamination or what it is, but

merely suggests that water quality has been affected and further methods of source contamination need to be investigated.

Duckweed refers to a group of floating, aquatic plants of the family *Lemnaceae*. Common duckweed (*Lemna minor*) is found worldwide in many aquatic surface environments and is very easy to grow in the classroom.

Duckweed plants are tiny, 2mm–4mm in diameter, and each with a simple two-part structure of a *frond* and a root. The plants grow in colonies and, after undergoing asexual vegetative reproduction, form aggregates of two or more fronds. New fronds grow roots and eventually break away from the original plant. Duckweed grows very fast, doubling in frond number every one to three days.

Because duckweed floats, it is especially susceptible to *hydrophobic* surface toxins (toxins that are not water soluble and form a layer on top of the water). Duckweed is also very sensitive to herbicides, making it more appropriate than other species in assaying for herbicides (Wang unpublished).

It is important to note that duckweed, because of its high reproductive rate, adapts quickly to changes in its environment, including environmental toxins. Duckweed's ability to adapt can interfere with test results if you take duckweed from a water source and then in turn try to test that water source for the same contamination. Duckweed is highly sensitive to toxins only if the duckweed that is used to test a water source does not come from that same water source.

Duckweed, because of its broad range of sensitivity, can be used as an indicator species to test landfill runoff, industrial effluent, groundwater quality, surface water quality, and agricultural chemical runoff (also termed "off-target chemical movement").

Teacher management

Preparation

30–60 minutes for collection of materials and mixing stock solution. This is assuming the students assist in collecting bottles before the lab begins.

Many types of contaminants can be used in this experiment, including coffee, soda, atrazine, local pond water, etc. The example given here, using detergent, is meant as a possible example experiment.

Making stock solution: Each team needs 100 ml of a 5 percent liquid detergent and 5 ml of nutrient solution. The solutions can be prepared as follows:

Preparation of 5 percent detergent solution—

# Teams	Detergent (ml)	Distilled water (ml)
1	5	95
5	25	475
10	50	950

Nutrient solution preparation — 1 T Peter's fertilizer / 1 gal water

Activity time

Initial bottle construction and laboratory setup will take two class periods. You might want to spend day 1 cutting bottles and making dilutions and day 2 setting up the experiment with the duckweed. We recommend that you construct the observation dishes on a Friday and start the laboratory experiment on the following Monday. If you decide to have students perpare the dilutions, you may want to allow for extra time to explain this procedure.

Data collection— 10–20 minutes each day for four days

Materials

Each team (two to five students) needs:

- six 2–liter plastic soda bottles with caps
- distilled water
- 100 ml of 5 percent detergent solution
- 48 two–frond colonies of common duckweed species
- grease pencil or marking pen
- hand lens (see Appendix A for information on making film canister hand lenses)
- graph paper

Classroom needs:

- several pairs of scissors
- labels or tape
- 10 ml graduated cylinder
- 100 ml graduated cylinder
- hot water (140 degrees F) or a hair dryer

Sources of materials

Many of the necessary materials are already found in most classroom science laboratories. Students can bring in their own soda bottles or collect them from other teachers or parents.

Common duckweed (*Lemna minor*) can be collected from freshwater ponds in most areas of North America. It can also be ordered from biological supply houses.

Tips and safety

If you live in a region where ponds freeze in the winter, you will want to collect your duckweed during the summer or early in the fall. Remember not to use duckweed from the same pond in which you intend to test for contamination.

To keep duckweed alive in the classroom, place in an aquarium or other glass container. Lightly aerate. Once a month change the water in the container and remove excess duckweed. Because duckweed is considered a pest in some areas, do not discard duckweed down the drain or dump into an outside water source.

If you set your duckweed under a light bank, be sure the duckweed doesn't get too warm.

See the introduction to Bottle Biology for more information about bottle cutting and construction.

You may substitute many substances to be tested in this procedure. This investigation has a lot of potential for a variety of extension experiments using different substances. Safety considerations would become necessary if toxic substances were used.

When having students count the duckweed fronds, it is essential that they count any protruding bud as a frond. This will eliminate individual bias. We recommend that you demonstrate how to count the fronds.

Each member of the team should count each team dish to ensure accuracy.

If using a powdered soap or detergent, mix the 5 percent solution as follows: 5 g powder to 95 g water (95 g water = 95 ml water).
If using a chemical (2,4–D, Roundup, etc.), the stock solution should be mixed to two times the normal field concentration, determined by the label instructions that accompany the chemical container.

If using a substance other than a chemical or detergent (coffee, soda,

gasoline) your stock solution should be at the strength normally used.

It's important that the lab is set up on a Monday so the final observation can be taken on Friday (96 hours or 4 days later).

Make sure the duckweed doesn't stick to the sides of the observation dish.

Key Terms

Bioassay: the use of a living organism to test the effects or presence of a substance

Indicator species: a species of plant or animal which is used to monitor the health of an ecosystem

Point source contamination: pollution that comes from an identifiable source, or has been concentrated in one area

Leach: the ability of a substance to move through the soil over time

Hydrophobic: incompatible with or insoluble in water

Frond: leaf structure of duckweed (see Appendix A)

Effluent: liquid or dissolved waste from a commercial, industrial or agricultural operation

References

American Society for Testing Materials, *Standard Guide for Conducting Static Toxicity Tests with Lemna gibba G3, E 1415–91.* Philadelphia, Penn. 1991.

Clark, J.R. V.D. Nicholson, R.B. Cherry, D.S. and J. Cairno. *Accumulation and depuration of metals by duckweed(Lemna perpusilla.)* Ecotoxicol Environment Section 5, pp. 87–96. 1981.

Horning, W.B., and C.I. Weber(eds.). *Short–term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms.* EPA/600/4–85/014. U.S. Environmental Protection Agency: Cincinnati, Ohio. 1985.

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Wang, W. *Toxicity Test of Aquatic Pollutants by Using Common Duckweed: Environmental Pollution (Series B)* 11: 1–14. 1986.

Wang, W. *Literature review on duckweed toxicity testing.* Environmental Research 52(1):7–22. 1990.

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Duckweed Unlimited

Using common duckweed (*Lemna minor*) to measure water quality

Introduction

Have you ever wondered how clean the water is that you drink, swim in or bathe in? Water is such an essential part of our ecosystem that our survival depends on maintaining water quality. In the following laboratory you will explore ways to test for water quality. You will see how monitoring an indicator species gives us clues about what is happening in the environment around us.

All living things are products of their environments. An *indicator species* is a plant or animal that points to or responds specifically to conditions in a community or habitat.

For example, the disappearance of a particular plant that is sensitive to salt would indicate that a particular soil is salty. In the Upper Midwest, excessive grazing of dry prairie regions can be diagnosed by a decrease in little bluestem, a species of grass, or an increase in ragweed, dandelion and white clover plant species. A stream dominated by a particular type of midge fly larva is generally found to be polluted with organic wastes like sewage.

Common duckweed (*Lemna minor*) can be used as an indicator species to monitor water quality. Duckweed toxic testing has been accepted and used by the EPA (Environmental Protection Agency) and private industrial laboratories.

Duckweed is a tiny floating aquatic plant found worldwide and has a simple two-part structure: *fronds* (leaves) and roots. The plants grow in colonies and, after undergoing vegetative asexual reproduction, form aggregates of two or more fronds. Duckweed grows very fast, doubling in frond number in one to three days. Duckweed is found to be sensitive to surface substances, *hydrophobic* compounds (substances that do not mix with water), and is especially sensitive to herbicides.

Duckweed, because of its broad sensitivity range, can also be used as an indicator species to test landfill leachate, industrial effluent, groundwater quality, surface water quality, and agricultural chemical leachate. It is essential to remember that duckweed does not measure the degree of contamination or the identity of the contaminant. It merely suggests that water quality has been affected and further methods of source contamination testing need to be investigated.

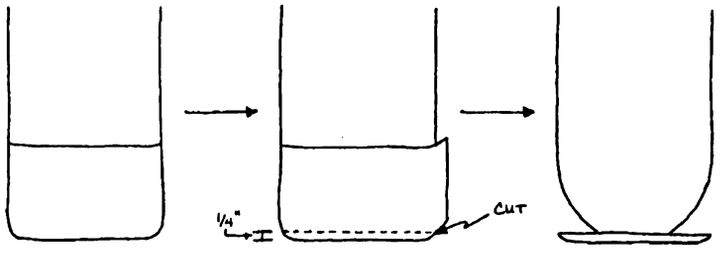
Materials

Each team (two to five students) needs:

- six 2–liter clear plastic soda bottles with caps
- grease pencil to mark bottles
- scissors to cut bottle
- hot water (140 degrees F) to remove bottle labels
- labels or tape for labels
- distilled water for dilutions
- 100 ml of 5 percent detergent solution
- 100 ml graduated cylinder
- 10 ml graduated cylinder
- 48 two–frond colonies of duckweed (*Lemna sp.*)
- hand lens for counting fronds
- graph paper
- liquid fertilizer (mixed to standard strength)

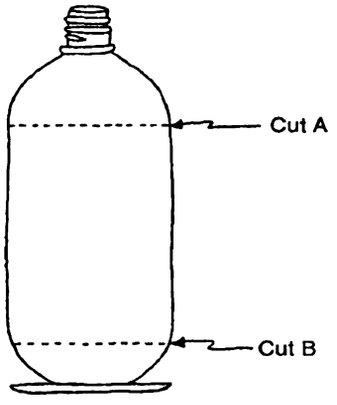
Procedure

1. Cut the colored base of the bottle to make a pedestal. Cut down the colored plastic to about 1/4 inch above the bottom. Cut around the base until the excess is removed. Make sure you leave enough of the bottle base so that the bottle sits in a stable way.

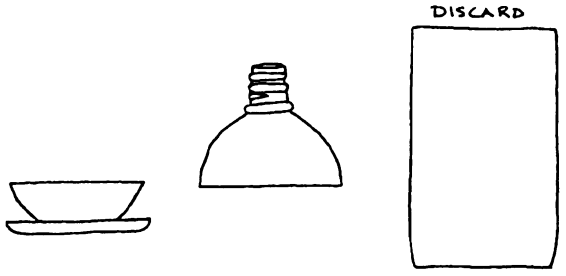


2. Mark the cutting lines:

Cut A should be made about 1/4 inch below the shoulder. Cut B should be about 1/4 inch below the hip.



3. Cut the bottle as marked. Discard the middle section. (You may want to save the middle section for future Bottle Biology projects).



4. Label the observation dishes with your team name and with the following:

   A – Control
   B – 1X
   C – 1×10^{-1} X
   D – 1×10^{-2} X
   E – 1×10^{-3} X
   F – 1×10^{-4} X

   These concentration values are listed in *scientific notation*. This style is a convenient way to express very small or very large numbers. A value of $1 \times 10^{-1} = 0.1$, $1 \times 10^{-2} = 0.01$, $1 \times 10^{-3} = 0.001$ and $1 \times 10^{-4} = 0.0001$.

5. Fill dish A with 90 ml distilled water. This is your control.

6. Fill dishes C, D, E, F with 90 ml distilled water.

7. Fill dish B with 100 ml of the 5 percent detergent solution.

8. Take 10 ml of liquid from dish B and add to dish C. Mix well.

   Note: It is important to completely rinse and drain the 10 ml graduated cylinder between dilutions, so that your dilutions will be accurate.

9. Take 10ml of liquid from dish C and add to dish D. Mix well.

10. Take 10ml of liquid from dish D and add to dish E. Mix well.

11. Take 10ml of liquid from dish E and add to dish F. Mix well.

12. Take 10ml of liquid from dish F and discard.

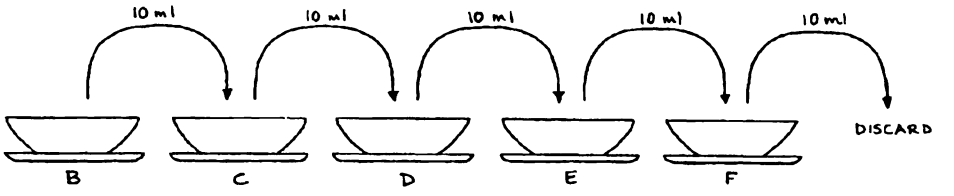
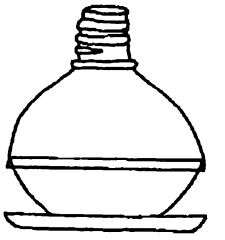


Diagram of dilutions

13. Add 5 mls of liquid fertilizer to all dishes.

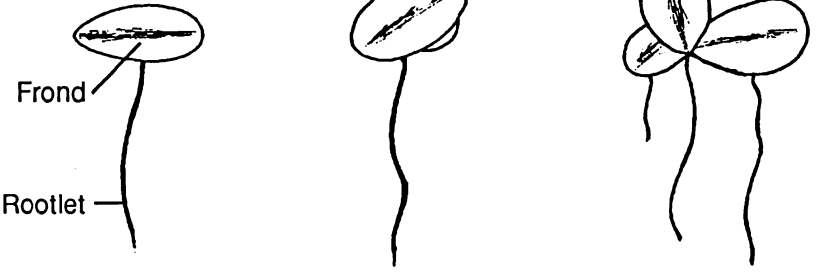
14. Place eight two–frond colonies of duckweed into each dish and cover. Try to pick plants where the two fronds are about the same size to ensure that they are at approximately the same stage of growth.



15. Place dishes under light, so they get 24 hours of light a day.

16. Observe the dishes every 24 hours for three days (96 hours), and record the following:

    - total number of fronds
    - any changes in color
    - any other changes in appearance (roots broken off, colonies broken apart, etc.)
    - general frond size in relation to the fronds in the control dish



Single-frond plant

Two-frond plant showing new frond bud

Triple-frond plant

Each team member should count the fronds in each dish to ensure accuracy.

17. After 96 hours, graph the data and write your conclusions.

Duckweed Unlimited Data

Name: _____

Day 1: Question: Does detergent affect the growth of duckweed? If so, could duckweed be used to assay for the presence of detergent in a water sample?

Predict: What do you expect to see happen to the duckweed colonies in the different concentrations? Which colonies do you expect to reproduce normally? Which colonies to you expect to reproduce more slowly, or die completely, if any?

Hypothesis: _____

_____

_____

Prepare: Construct observation dishes and add duckweed colonies.

Days 2–5: Record observations on the data tables.

Table 1: Total number of fronds

	Hours of exposure				
Dish	0	24	48	72	96
Dish A (Control)					
Dish B (1X)					
Dish C (1 x 10-1X)					
Dish D (1 x 10-2X)					
Dish E (0.001X)					
Dish F (0.0001X)					

Table 2: Other Observations

Include comments on any changes in color, roots broken off, colonies broken apart, general frond size in test dishes compared to frond size in the control dish.

0 Hours: (Beginning observations of colonies)

24 Hours:

48 Hours:

72 Hours:

96 Hours:

Conclusions:

Results and discussion

1. Did your data support your hypothesis?
2. Were there any dishes with results which surprised you? Which ones and why?
3. List at least one question that these results lead to.
4. Design an experiment that could be used to answer your question from #3.

Extensions

1. What is the ability of a substance to leach in the soil?
2. Do different soil types affect the ability of a substance to leach?
3. What is the affect of leachate on duckweed?
4. Does the rate of leaching vary over time?
5. Design a system to monitor environmental changes using duckweed.
6. Do crops affect leaching?