"There's Something Fishy"
The Nitrogen Cycle

Science in the Real World
Microbes In Action

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At a Glance

Description

This lab gives students a hands-on approach to the nitrogen cycle. The students observe the daily changes in different nitrogen compounds in an aquarium that has been newly setup. When an aquarium is initially set up, the levels of three nitrogen compounds (ammonia, nitrite and nitrate) fluctuate as nitrification occurs as part the nitrogen cycle. These levels will eventually stabilize as bacteria become established in the aquarium.

Time Requirements

This activity will require three weeks of daily testing. (The tests only take a few minutes.)

Curriculum Placement

This exercise would fit well into an Ecology unit.

Equipment

1 dry aquarium
1 air pump
1 filtering system (optional)

Materials

new aquarium gravel
live aquatic plants
live fish (amounts will vary based on size of tank)
1 bottle of tap water conditioner
1 can of fish food
Ammonia (NH_3) Test Strips
Nitrite (NO_2) Test Strips
Nitrate (NO_3-) Test Kit

There's Something Fishy
The Nitrogen Cycle

Viewing the Nitrogen Cycle in an Aquarium

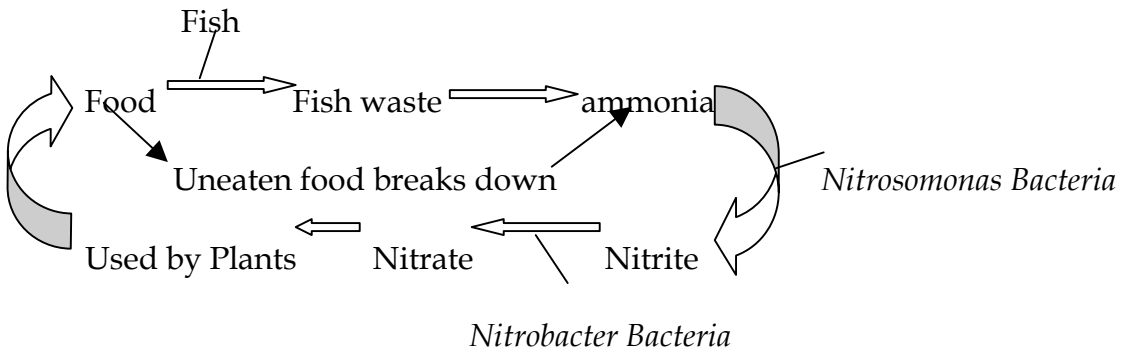
Background

All living creatures consume food and produce waste. Nitrogenous wastes (wastes containing nitrogen) are produced when proteins are consumed. Nitrogenous wastes can be harmful to our bodies, and this waste needs to be released quickly to avoid self-poisoning. Humans and other mammals convert their nitrogenous waste into urea, which is then excreted in urine when we urinate. This system works well for mammals, but what about fish? Fish excrete their nitrogenous waste by excreting ammonia through their gills. Unlike mammals, fish have to swim in the same place that they release their wastes, so how do they keep from becoming sick? Fish are protected from low levels of ammonia by a mucus covering made by their skin, but in high levels of ammonia they can lose their protective mucus covering and they are likely to become sick or even die! In their natural environments the poisonous nitrogenous wastes are quickly diluted in the large volume of water. But in an artificial environment like an aquarium, the nitrogen compounds can quickly rise to toxic levels (especially in smaller volume tanks, such as a fish bowl).

Many people have experienced this problem when trying to start up a new aquarium. Often the nitrogenous wastes build up quickly and kill the fish. Sometimes the fish are able to survive the initial shock of being placed in a new tank, and seem to do ok. What is the secret? Factors like the size of your aquarium, the number of fish, feeding frequency and changing the water can all affect the levels of nitrogen compounds. So if you don't want to have to change the water in your fish tank every few weeks, what can you do?

Well, you could buy costly additives to remove toxic compounds from your tank, but there is a much more inexpensive way—let bacteria do the work for you!! Bacteria? Yes, bacteria can consume just about every known food source that you can find, including what other organisms might consider waste or poisonous. These bacteria are abundant in nature, and given the right food (ammonia fish waste) they will multiply and begin to convert ammonia to a slightly less toxic compound called Nitrite. Then a different group of bacteria can start to multiply to consume Nitrite and produce Nitrate. Nitrate is less toxic than both Nitrite and Ammonia and can be consumed by plants. This process of changing harmful ammonia into nitrate is called the Nitrogen Cycle. The diagram below illustrates this relationship.

The Nitrogen Cycle



Purpose

1. To test for, observe and record daily changes in the amounts of three nitrogen compounds as they relate to the nitrogen cycle in a newly setup aquarium.
2. To test for, observe and record daily changes in the pH of a newly setup aquarium.
3. To understand how bacteria can clean the water by consuming and converting toxic compounds into less toxic forms.

Procedure- Day 1

1. Rinse new gravel and place in bottom of empty aquarium to a depth of 3-5 cm.

2. Fill tank with tap water and apply water conditioner to remove the chlorine.

3. Add air pump (aerator) and live aquatic plants. A filter is helpful, but not necessary.

4. Using your test kits, measure the levels of ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3^-) in the tank and record the amounts in your table.

5. Float a bag of fish in tank for 15-20 minutes to acclimate fish to the temperature of the water. The type, number and size of the fish may vary, but do not exceed 8 cm of fish per gallon, and ask for a hardy species at the store when you purchase them.

6. Measure NH_3, NO_2, and NO_3^- levels of the water that the fish came in, record on your table.

7. Place fish in tank. After the fish have been in the tank for 5 – 10 minutes, measure the levels of the three nitrogen compounds in the aquarium water.

8. Feed fish. Be careful not to overfeed.

9. Repeat the three tests every day for the next three weeks. Make sure to run the tests at the same time daily, before the fish are fed.

Data Table 1- Test Results

Time	Date	Ammonia Level	Nitrite Level	Nitrate Level
Day 1- Before fish				
Day1- Fish Water				
Day1- After Fish				
Day2				
Day 3				
Day 4				
Day 5				
Day 6				
Day 7				
Day 8				
Day 9				
Day 10				
Day 11				
Day 12				
Day 13				
Day 14				
Day 15				
Day 16				
Day 17				
Day 18				
Day 19				
Day 20				
Day 21				

Results and Analysis

1. In your own words, describe the nitrogen cycle.

2. What role does each organism play in the nitrogen cycle?

    a. Fish--

    b. Nitrosomonas--

    c. Nitrobacter--

    d. Plants--

3. On a sheet of graph paper, graph the results of your data over the last three weeks. Include all three nitrogen compounds on one graph. Also include a separate graph of pH. Be sure to include correct labels for your axes, a key (legend) and a title for each of your graphs.

4. Answer the following questions based on your graphical analysis.

    a. Which nitrogen compound increased first?

    b. How can you explain this?

    c. Which nitrogen compound increased second?

    d. How can you explain this?

    e. Which nitrogen compound increased last?

    f. How can you explain this?

5. Are there any odd results? Explain.

6. Where do you think the bacteria came from in your initial tank setup?

7. Now that you understand a little about the concerns of starting a new aquarium, what would you do to help your friend set up his new tank that would give it a better chance of surviving? Explain your answer.

8. How can humans use this knowledge of bacteria and the nitrogen cycle?

Teacher's Guide

Instructional Objectives

At the end of this activity, the students should be able to:

1. Demonstrate the following laboratory skills:
   a. Comparing and contrasting
   b. Following the directions to complete a variety of tests
   c. Constructing a graph

2. Demonstrate the methods of scientific inquiry by:
   a. Perform an experiment according to given directions
   b. Gathering and organizing data
   c. Analyzing data
   d. Applying understanding of a specific principle to a more general purpose

Sources of Supplies

Available at most major pet stores (prices are an estimate):

Description	Quantity	Price
1-gallon fish aquarium, with air pump	1	$ 10.00
Gravel- 5 pound bag	1	$ 4.00
Live plants	2	$ 2.00 each
Water conditioner	1	$ 5.00
Aqua Lab- Toxic ammonia test strips	1	$ 6.00
Aqua Lab- Nitrite test strips	1	$ 6.00
Aquarium Pharmaceuticals Nitrate test kit	1	$ 6.00
Tropical fish flakes	1	$ 4.00

Teacher's Hints and Troubleshooting

Preparation/ Procedures

- You may prepare the aquarium prior to the class, or allow students to do it, but remember to take measurements of ammonia, nitrite, and nitrate 3 separate times on day 1.
  Avoid having many students placing their hands in the tank water for testing. The lotions and oils on skin can kill fish. Using a cup with a handle (coffee cup) to pull the water out is best.

- You may substitute an aquarium and air pump that you already own, but be sure to use fresh unused gravel as used gravel may already contain bacteria and skew your results.

- You should alter the amount of fish accordingly based on the size of your tank. The number and size of the fish may vary, but do not exceed 8 cm of fish per gallon, and ask for a hardy species at the store when you purchase them. This lab was tested with a 1-gallon tank and 2 danios fish (about 2cm in length).

- You will want to use the same fish tank for all of your classes that you want to participate in this lab, therefore, before you start consider how to divide the tasks. To involve more than one class you could allow one class to be responsible for measuring one aspect (for example, nitrite level). This way they can see the changes in one substance, and could get the other information from other classes.

- Make sure to test the levels of nitrogenous compounds at the same time each day, and prior to feeding. If not, this test will give odd results.

- We used Aqua Lab brand toxic ammonia and nitrite tests. These worked well. We used the Aquarium Pharmaceuticals brand nitrate test. This test was harder to perform and read. Directions need to be followed exactly for all tests to insure accuracy.

- Avoid placing the tank in <u>direct</u> sunlight. This will cause an overgrowth of algae.

Results and Analysis- Answer Key

9. In your own words, describe the nitrogen cycle.
   Answers will vary.
10. What role does each organism play in the nitrogen cycle?
    a. Fish--*Creates the ammonia through waste products.*
    b. Nitrosomonas--*Converts ammonia to nitite*
    c. Nitrobacter--*Converts nitrite to nitrate*
    d. Plants--*Plants consume the nitrate*
11. On a sheet of graph paper, graph the results of your data over the last three weeks. Include all three nitrogen compounds on one graph. Also include a separate graph of pH. Be sure to include correct labels for your axes, a key (legend) and a title for each of your graphs.
12. Answer the following questions based on your graphical analysis.
    a. Which nitrogen compound increased first?
       Should be ammonia.
    b. How can you explain this?
       It is present in the waste of the bacteria.
    c. Which nitrogen compound increased second?
       Nitrite
    d. How can you explain this?
       Nitrosomonas bacteria have developed and are converting the ammonia into nitrite.
    e. Which nitrogen compound increased last?
       Nitrate
    f. How can you explain this?
       Nitrobacter bacteria have developed and are converting the nitrite into nitrate.
13. Are there any odd results? Explain.
    There may be odd results due to test kits and/or human error.
14. Where do you think the bacteria came from in your initial tank setup?
    They were on the fish
15. Now that you understand a little about the concerns of starting a new aquarium, what would you do to help your friend set up his new tank that would give it a better chance of surviving? Explain your answer.
    Have the friend take a few pieces of gravel from an exsisting tank and place them in the tank. This will introduce the bacteria directly to the tank.
16. How can humans use this knowledge of bacteria and the nitrogen cycle?
    Humans could use these bacteria any time they need to balance nitrogenous compounds.