Lyn Murphy
Tioga Junior High
Tioga, La.

Grade Level
Middle School (6-8)

Duration
Two months
(approximately five minutes daily)

Subject Areas
Environmental Science
Chemistry

Materials List
- **Tetratest kits** (ammonia and nitrites)
- Safety goggles
- Turkey baster (for water samples)
- Computer with graphing software (MS Excel)
- Data sheets BM #4 and 5
- PowerPoint presentation to introduce the subject "My Fish Ride a Cycle"
- Native fish tank
- *Gambusia* sp. (or other small native fish)
- Timer (or clock)

Grade/Benchmark/GLE
Science
7/SE-M-A7/41
HS/ESS-H-B1/13 (earth and space)

BM = Blackline Master

My Fish Ride a (Nitrogen) Cycle

Focus/Overview:

Students will investigate the nitrogen cycle by testing the water in a newly set up (with fish) aquarium. As the fish eat and produce nitrogenous wastes (nitrite and ammonia), students will discover the changes in nitrite and ammonia levels that occur as the biological filter is established and the *Nitrosomonas* spp. and *Nitrobacter* spp. bacteria colonies begin to convert the nitrites and ammonia to the less- toxic nitrates. Students will test daily for approximately two months or until nitrites and ammonia levels have decreased. They will graph their results and analyze the data to predict trends.

Background Information:

Please refer to BM #1.

Learning Objectives:

Students will:
- Investigate the nitrogen cycle as they monitor nitrogen and ammonia levels of the aquarium.
- Use evidence to make inferences and predict trends.
- Compare their findings to typical illustrations of the nitrogen cycle in science texts.

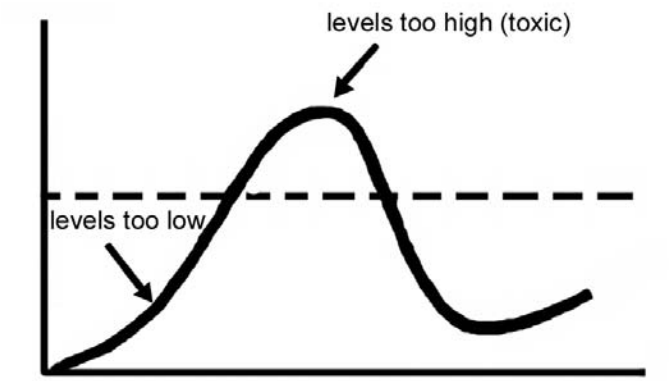
Procedure:

1. Attention-grabber. Ask students how many have had an aquarium and how many have had difficulties maintaining healthy fish. Explain that one of the problems in an aquarium is a high level of ammonia, which is toxic to fish. Show students the PowerPoint presentation.

2. Testing instructions. Demonstrate the procedure for testing the water. Point out the testing instruction sheets for nitrites (BM #2) and ammonia (BM #3), which will be posted near the fish tank. Make a testing schedule that assures all students will participate in testing the water. Have students record data in their own notebooks (BM #4 and #5) and on the classroom data sheet.

- Students begin testing as soon as fish are added to the water. *Gambusia* sp. or other small native fish will be used.
- Testing continues until nitrite and ammonia levels have dropped.
- Students will use their data, either on graph paper or on the computer using Excel, to graph their results.

Example Graph:



- Students will analyze the data they have collected to infer why ammonia and nitrite levels have dropped. The teacher will lead a discussion, asking students to explain why this is important to do before the paddlefish eggs arrive.

Assessment:

- Students will draw their fish tank nitrogen cycle, comparing the results of what they observe a land version of the nitrogen cycle.

Extension:

- Students test for nitrates, as well as ammonia and nitrites. They add plants to the tank and then test whether the presence of plants changes the nitrate level over the course of several days.

Blackline Master #1

My Fish Ride a (Nitrogen) Cycle – Teacher Background Information

One of the difficulties in raising fish in an aquarium is that it is a closed system. Therefore, nitrogenous wastes (wastes containing nitrogen compounds) can build up to toxic levels, which can stress or kill fish in the tank. One of the two filters that we use in the Native Fish in the Classroom program is a biological filter. As ammonia builds up in the tank, *Nitrosomonas* spp. bacteria begin to grow on the surface of the "bio-beads" and/or bottle caps of the biological filter and on the surface of the sponge-like physical filter. These bacteria convert the ammonia to nitrites, which are then converted to nitrates by *Nitrobacter* spp. bacteria. Nitrates are much less toxic to fish. Once these bacteria are well established in the biological filter, the cycle is completed, and the system becomes stabilized so that toxic levels of ammonia and nitrites will not build up.

Since it is desirable that the system is stabilized with a well-established biological filter prior to receiving paddlefish eggs, *Gambusia* sp. or other small bait fish can be used in the tank to start the nitrogen cycle and the production of the *Nitrosomonas* spp. and *Nitrobacter* spp. bacteria in the biological filter.

The fish produce nitrogenous wastes. Decaying, uneaten food also contributes to the nitrogenous wastes in the tank. These byproducts raise the level of ammonia compounds (NH_3 or NH_4^+) in the tank. When students graph the daily ammonia and nitrite data, they will see a rise in the ammonia level. It will then drop as *Nitrosomonas* spp. bacteria begin to grow in the filter, then the nitrite (NO_2) level will begin to rise. *Nitrosomonas* spp. bacteria through an anaerobic process, convert the ammonia to nitrite. Eventually, students will see a peak in the nitrite level, which will begin to fall when *Nitrobacter* spp. bacteria begin to convert the nitrite to nitrate (NO_3) through an aerobic process call denitrification.

If levels of nitrite reach levels toxic enough to cause fish kills of the *Gambusia* sp., baking soda can be added to the tank (1/4 lb. or less). This should increase the alkalinity and the pH. A higher pH encourages the growth of nitrifying bacteria, as well as providing a food source.

Nitrates are much less toxic to fish than nitrites and ammonia and usually should not require any action. Ammonia at 0.1 parts per million can cause hemorrhaging and destruction to mucus membranes and will cause damage to gills. At 0.1 parts per million of nitrite, the nitrite can bind to the oxygen-carrying component in the blood (hemoglobin). Since plants use nitrates, students could experiment with adding plants to the tank.

Chemical Reactions

NH_4^+ (ammonia)	+	2 H_2O (water)	=	NO_2^- (nitrite)	+	8 H^+ (hydrogen ions)
NO_2^- (nitrite)	+	H_2O (water)	=	NO_3^- (nitrate)	+	2 H^+ (hydrogen ions)

Blackline Master #2

How to Test for Nitrites (NO_2^-)

Materials Needed:
Safety goggles
Tetra*test* test kit (nitrite)
Test vial with stopper
Turkey baster
Reagent bottle 1
Reagent bottle 2
Tetra*test* color chart

Be sure to follow all instructions carefully!

1. Put on safety goggles.
2. Use the turkey baster to draw a sample of water from the native fish tank.
3. Rinse the test vial with the water to be tested. Empty the rinse water into the sink, not the fish tank!
4. Fill the test vial to the 5-ml mark with water to be tested.
5. Hold reagent bottle 1 upside-down (important!) while adding seven drops to the test vial.
6. Let the vial stand for 10 seconds, then add seven drops from reagent bottle 2. (Hold reagent bottle 2 upside-down while adding.)
7. Cap and shake the vial gently.
8. Allow five minutes for the development of the color.
9. Hold the vial and the color scale vertically and match the color of the test solution with the closest color on the chart.
10. Record the value of the nitrite level on the class data sheet and on your own data sheet.
11. Empty the test solution into the sink.
12. Rinse the test vial with tap water.

Blackline Master #3

How to Test for Ammonia (NH_3/NH_4)

Materials needed:
Safety goggles
Tetra*test* test kit
Test vial with stopper
Turkey baster
Reagent bottle 1
Reagent bottle 2
Reagent bottle 3
Tetra*test* ammonia test color chart

<u>Be sure to follow all instructions carefully!</u>

1. Put on safety goggles.
2. Use the turkey baster to draw a sample of water from the native fish tank.
3. Rinse the test vial with the water to be tested. Empty the rinse water into the sink, not the fish tank!
4. Fill the test vial to the 5-ml mark with water to be tested.
5. Hold reagent bottle 1 upside-down (important!) while adding 14 drops to the test vial.
6. Close and shake the vial gently.
7. Open the vial, hold reagent bottle 2 upside-down while adding seven drops to the test vial.
8. Close and shake the vial gently.
9. Open the vial, hold reagent bottle 3 upside-down while adding seven drops to the test vial.
10. Close and shake gently.
11. Set the timer for 20 minutes. Wait 20 minutes for the development of the color.
12. Hold the vial and the color scale vertically and match the color of the test solution with the closest color on the color scale. Read the value of the ammonia level and record it on your data sheet and the class data sheet.
13. Pour test solution into the sink, and rinse the vial with tap water.

Blackline Master #4

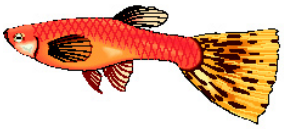
Ammonia (NH_3/NH_4) Testing Data Sheet

Date	Ammonia (mg/l)	Additional Observations (fish activity, water conditions)

Blackline Master #5

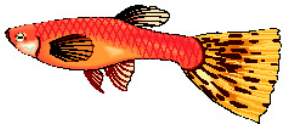
Nitrites (NO_2) Testing Data Sheet

Date	Nitrites (mg/l)	Additional Observations (fish activity, water conditions)



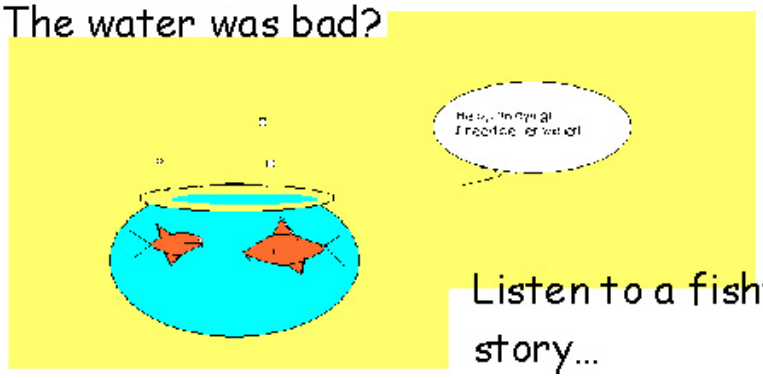
My Fish Ride a Cycle

The Nitrogen Cycle, That Is!

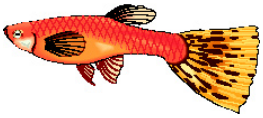


Have you ever had an aquarium where the fish were sad?

The water was bad?



Listen to a fishy story...



Hi, I'm Gus, the guppy.
I've got something to tell you.

You see, we fish are always surrounded by water.
(I know, you knew that!)

We get our food and our oxygen in the water.

But, it's also our toilet.
(Yuck! Would you like to live in your toilet?)

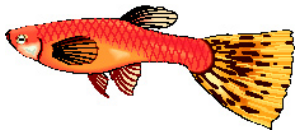
Things can get pretty icky, since we can't flush!

In rivers and lakes, that's not a problem.
They're bigger and rain helps dilute our waste.

In an aquarium, there's no place for the waste to go.

Some really poisonous stuff starts showing up in our water - nitrites and ammonia.

We can't live in that!

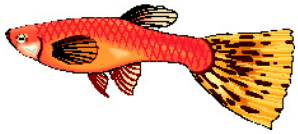


So what can you do to keep me healthy?

Change some of the water.
(Hey, I liked some of that water!)

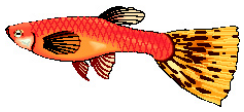
Add chemicals. (Who's paying for that?)

Let some friendly bacteria do the work.
(Hey, I like that, too! Put someone else to work!)



So, here's what you can do to see how friendly bacteria can get to work:

•First, the tank will be set up (all 45 gallons of water). Use bio-beads and bottlecaps in the filter.



•Make sure that all the chlorine is gone.

•Add lots of little fish like Gambusia sp.
Feed your fish.

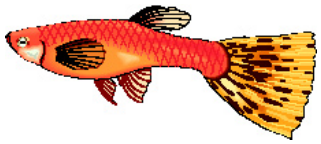
•Test the water every day for nitrites and ammonia.

•Record your results.

After about two months, graph your test results.

Be prepared to discuss as a class what has happened to the nitrites and the ammonia levels.

Also discuss the appearance of the water and the filter.



And, one last question from me, Gus,
"Are your fish happy and healthy?"