

MONITORING AMPHIBIANS



NOTE: The following directions are included in this curriculum for those educators wishing to help their student's gain field sampling experience. Please read over the entirety of the document before attempting to collect field samples or refer to the Omaha Zoo website for a training video. It is extremely important to follow the procedure carefully.

Objectives:

Students Will:

Learn how to collect data in the field by testing water quality and/or testing amphibians for Chytrid fungus.

Discover what affects water quality and how this impacts animals.

Background:



Although Chytrid fungus naturally occurs in some environments and has been around for thousands of years, it has only been discovered recently by scientists.

The Chytrid fungus has had detrimental effects on the number of amphibians in recent decades, wiping out entire populations. Areas that have been largely affected by this fungus are Central America and North America. Scientists believe that Chytrid was originally introduced to this area of the world by African clawed frogs in the early 20th century, when many of these frogs were shipped over from Africa, to use as pregnancy tests for women. The African clawed frogs were immune to the fungus; however, the newly exposed amphibians did not have a built up resistance.

Over the last two decades, scientists have begun to discover the impacts of Chytrid on amphibian populations. There is still a great deal to learn. Currently one of the largest tasks facing herpetologists is monitoring where the disease is spreading.

This requires sending scientists into the field to catch amphibians, collect DNA samples from their skin, and then test the samples for Chytrid fungus; very time consuming!

Many herpetologists believe environmental factors have amplified the effects of Chytrid fungus on amphibian populations as well. Amphibians have been compared to the canary in a coal mine (these songbirds died in the presence of odorless gas, warning miners of the danger) because of their sensitivity to man-made environmental change. Amphibians are commonly referred to as bio-indicators. "Since amphibians feel the affects of pollution before many other forms of life, their rapid decline tells us that one of earth's most critical life support systems is breaking down." (Dr. Simon Stuart of the World Conservation Union) It is estimated that up to 122 species of amphibians have gone extinct since 1980. By monitoring water quality scientists can better understand what environmental factors are negatively impacting amphibians and the good news is that you can help!

Water Quality and Amphibians

Temperature – May determine the species found in a particular water source or at a particular time of year.

pH – Fresh water can become more acidic via contamination from acid rain and snowmelt. Some species are more tolerant of acidic water; however, many experience deformities or increased deaths.



Dissolved oxygen – This is the amount of oxygen that is present in the water. Low dissolved oxygen in bodies of water means that tadpoles or amphibians that spend their entire life in water could suffocate.

Turbidity – Refers to how clear water is; which is affected by suspended particles of sand, silt and algae. Although it is normal to have sediments in the water, high concentrations may mean that the water has been polluted by runoff from construction, roads and so on.

Nitrates - Come from fertilizer and pesticide runoff from yards and agriculture and can cause abnormalities in amphibians.

Materials:

General Supplies

- GPS
- Digital camera
- Amphibian field guide for the area
- Waders
- Nets

Water Quality Testing

A means to collect the following data:

- Temperature
- pH
- Dissolved oxygen
- Turbidity
- Nitrates

Chytrid Sampling Kits

(These kits can be purchased at www.omahazoo.com or by calling Omaha's Zoo Education Department at (402) 738-2092.)

Cotton swabs (important - use sterile swabs on 2mm-diameter plastic without adhesive)

2-mL tubes with screw-caps

70% ethanol

(This can be purchased directly (70% reagent alcohol) or can be made up from 100% alcohol diluted with deionized or distilled water only)

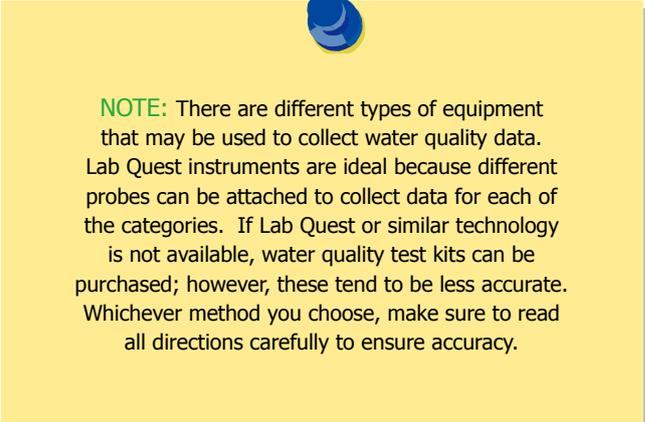
Powder-free nitrile or vinyl gloves (it is important that the gloves are powder-free)

4 zip-loc bags per group

1 alcohol-resistant permanent marker per group

Data forms (1 for each sample collected) and clip boards

* The amount of materials that you need from above depends on the number of samples you wish to collect in the field.



NOTE: There are different types of equipment that may be used to collect water quality data. Lab Quest instruments are ideal because different probes can be attached to collect data for each of the categories. If Lab Quest or similar technology is not available, water quality test kits can be purchased; however, these tend to be less accurate. Whichever method you choose, make sure to read all directions carefully to ensure accuracy.

Preparing the Sampling Kits:

1. Divide the class into groups of 2-3 students and assign each group a number.
2. Decide the amount of samples that you would ideally like each group to collect (depending on the location of sampling and the time of year, 5-10 samples per group will suffice).
3. Count out the number of tubes that you will need for each group and fill them with 1 mL of 70% ethanol. Screw the caps on tightly to ensure that there are no leaks. Divide the tubes up for each group and place each set in a zip-loc bag.
4. Careful not to contaminate the tip, count out the number of cotton swabs that each group will need (the same as the number of tubes). Divide them up for each group and place each set in a zip-loc bag.
5. Each kit should also contain 2 empty baggies. One for the tubes with a sample and one for the disposal of gloves and other trash.
6. When the kits are completed, each group should have the following:
 - a. 1 permanent marker
 - b. Data forms (each sample collected needs to have its own data form)
 - c. Clip board
 - d. 4 zip-loc bags, one bag for each of the following:
 - Cotton swabs • Tubes without samples • Tubes with samples • Trash

BEFORE YOU BEGIN - IMPORTANT NOTES:

Remember, amphibians are **sensitive animals** and they can become stressed easily! Avoid squeezing too tightly or dropping them. When you have finished swabbing the specimen, return it to the location where it was found, away from foot traffic.

If you move to a new geographic location (for example – moving from one pond to another) it is extremely important to clean all equipment to avoid spreading the Chytrid fungus (this includes shoes, waders, nets, and so on). Equipment can be cleaned with bleach or it can be left out to dry completely (Chytrid requires moisture to survive). However, if equipment is cleaned with bleach, it must be rinsed well and allowed to dry prior to reuse so that there is no residual bleach.

AMPHIBIAN SAMPLING PROTOCOL

Procedure:

1. Students that are handling amphibians should wear clean nitrile or vinyl powder-free gloves. Gloves should be disposed of and replaced with fresh ones before handling a new amphibian.
2. To obtain the sample, have one person hold the amphibian, while another group member gently but firmly swabs the ventral surface 25 times. For large animals, you may swab the ventral surface 20 times and the feet and webbing 5 times.
3. Place the swab (cotton side down) in the tube and break off the wooden applicator so that it fits in the tube. Secure the lid to prevent any contamination from leaking.



4. Label the tube according to the directions below.
(It is extremely important to properly label each tube and then record this same label on the data form for the sample.)

The label should include:

- State
- Name of site or abbreviation
- Group #
- Sample #

5. Record the tube label on the data form and fill out all other information for that particular sample. This is extremely important for being able to keep track of the tubes as well as important environmental conditions.



Example: Sample #1 collected by group #1 at the Wildlife Safari Park in Nebraska would have the label NE-WSP-1-1

6. Place the tube in the Ziploc bag for samples.

Sample tubes should be kept in bags, in groups determined by how the least contamination to data would be caused if a sample should leak (for example - samples from the same geographic location should be in the same bag; samples from different locations would be in different bags).

7. Before the amphibian is released, take a picture of its dorsal side.

Digital cameras will assign each picture a number. Record that number on the Field Observation Form for the corresponding amphibian. This will help you keep track of the data once you are back in the classroom.

8. Release the amphibian in the same location that it was found.
9. Have the other members of the group collect and record water quality data on the location where the amphibian was found.

Amphibian Database:

BACK IN THE CLASSROOM upload the pictures and data collected onto Omaha's Henry Doorly Zoo Amphibian Database. Here, researchers and other participants will be able to compare and analyze data. To join the database, follow the steps below.

- STEP 1: Create a free Google Account by going to www.google.com/accounts if you do not have one already.
- STEP 2: Once you have created your Google account, go to <http://biofinity.unl.edu/HDZ/amphibian> and log in.
- STEP 3: You will be taken to another screen where you will need to click "Join an existing lab." Look for Omaha's Henry Doorly Zoo Amphibian Database and click on "Join this lab" next to it.
- STEP 4: Once you have submitted your request to join the lab, you will have to be approved by Omaha's Zoo staff to proceed.
- STEP 5: To enter new data go to <http://biofinity.unl.edu/HDZ/amphibian/create>.

Once on the database, you will be able to fill out a new "occurrence" for each individual amphibian that was swabbed. See Figure 1 for an example of entering a new amphibian into the database. After you have submitted data for a new amphibian, the database will give the option to upload a picture of the corresponding amphibian. This will help the researchers verify the species.

For more in depth information on how to utilize the various database features, please download the user manual from www.omahazoo.com/conservation/amphibian.

WEBSITE VIEW:

Add a new amphibian to the Henry Doorly Zoo's Amphibian Database

* denotes required information

Tell us about your school

* School:
* Teacher's Name:
* Teacher's Email:

Tell us about the amphibian you saw

Common Name:
Chytrid Swab #:

Tell us when you saw the amphibian

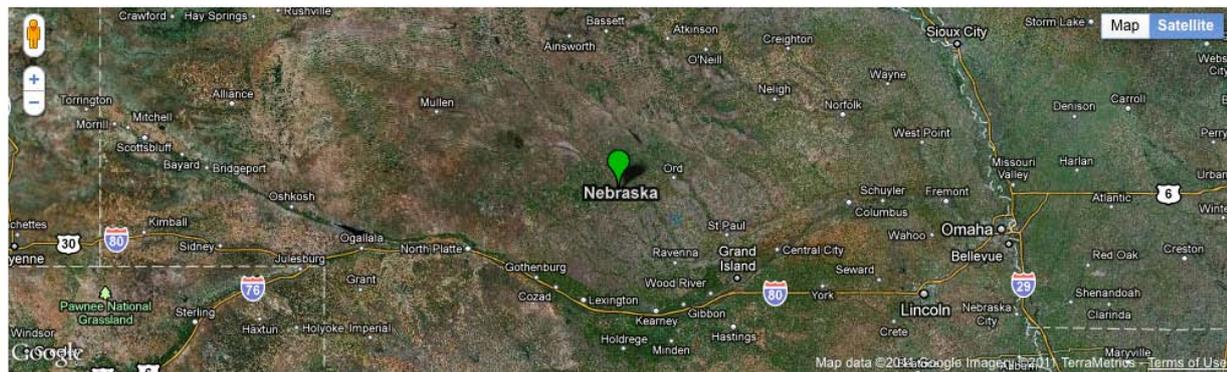
* Date: MM/DD/YYYY 12 00 AM

Tell us about where you saw the amphibian

Drag the marker on the map to automatically set the latitude and longitude of this location.

Latitude^[*]: 0.0 Expressed in **decimal degrees (DD)**, ranging from -90.0 to 90.0. Alternatively, use **degrees:minutes:seconds (DMS)**:
 0 0 0.0 N

Longitude^[*]: 0.0 Expressed in **decimal degrees (DD)**, ranging from -180.0 to 180.0. Alternatively, use **degrees:minutes:seconds (DMS)**:
 0 0 0.0 E



Lookup location details such as elevation, municipality, county, state/province, and country based on the current latitude and longitude.

FIGURE 1. Note that the database utilizes Google Maps. When entering latitude and longitude into the database, the green cursor will automatically go to that specific location. Or, simply move the green cursor by clicking and dragging with the computer mouse.

If the amphibian was observed in water, please provide additional information on the water body.

Water Body: If applicable, the name of the water body in which the location occurs.
Water Temperature: * C (degrees Celsius) If applicable, the temperature of the water where the amphibian was observed.
Water PH:
Water Nitrates:
Water Dissolved Oxygen:
Water Turbidity:
Were Fish Present?:
Was Vegetation Present?:

If any additional details are necessary to describe this location, provide them below. It is OK to leave this blank.

Save * On the following page you will be able to upload any pictures you took of the amphibian.

FIELD OBSERVATION FORM

Group Members: _____

Teacher Name: _____ School: _____

Teacher Email: _____ Date: _____ (mm/dd/yyyy) Time: _____

LOCATION INFORMATION

State: _____ County: _____

Collection Location Name (if available): _____

Latitude, decimal degrees: _____ Longitude, decimal degrees: _____

Remarks, Landmarks: _____

Habitat Description (wetland, ditch, pond, etc.): _____

Air Temperature, Celsius: _____ Water Temperature, Celsius: _____

WATER QUALITY

pH: _____ Nitrates: _____

Dissolved Oxygen: _____ Turbidity: _____

Were fish present?: No Yes

Was vegetation present?: No Yes

FROG SPECIES FOUND

Common Name: _____ Picture #: _____

Chytrid Swab #: _____

(Swab # should include the State, name of site or abbreviation, Group #, Sample #. For example, NE-WSP-Group#-Sample#)

After collecting all of your data, please log on to <http://biofinity.unl.edu/HDZ/amphibian> to enter information and pictures into the database.

Shipping samples:

Samples to be shipped should be in a box or mailing tube, not a bubble wrap envelope. The Post Office machine can exert enough force on samples in an envelope to cause the tubes to leak. Make sure to mail the data collection forms with the tubes so that the herpetologists have all of the pertinent information. You may choose to make copies of the forms to keep for the class.

Ship to: Omaha's Henry Doorly Zoo
Attn: Education Department
3701 S. 10th St.
Omaha, NE 68107