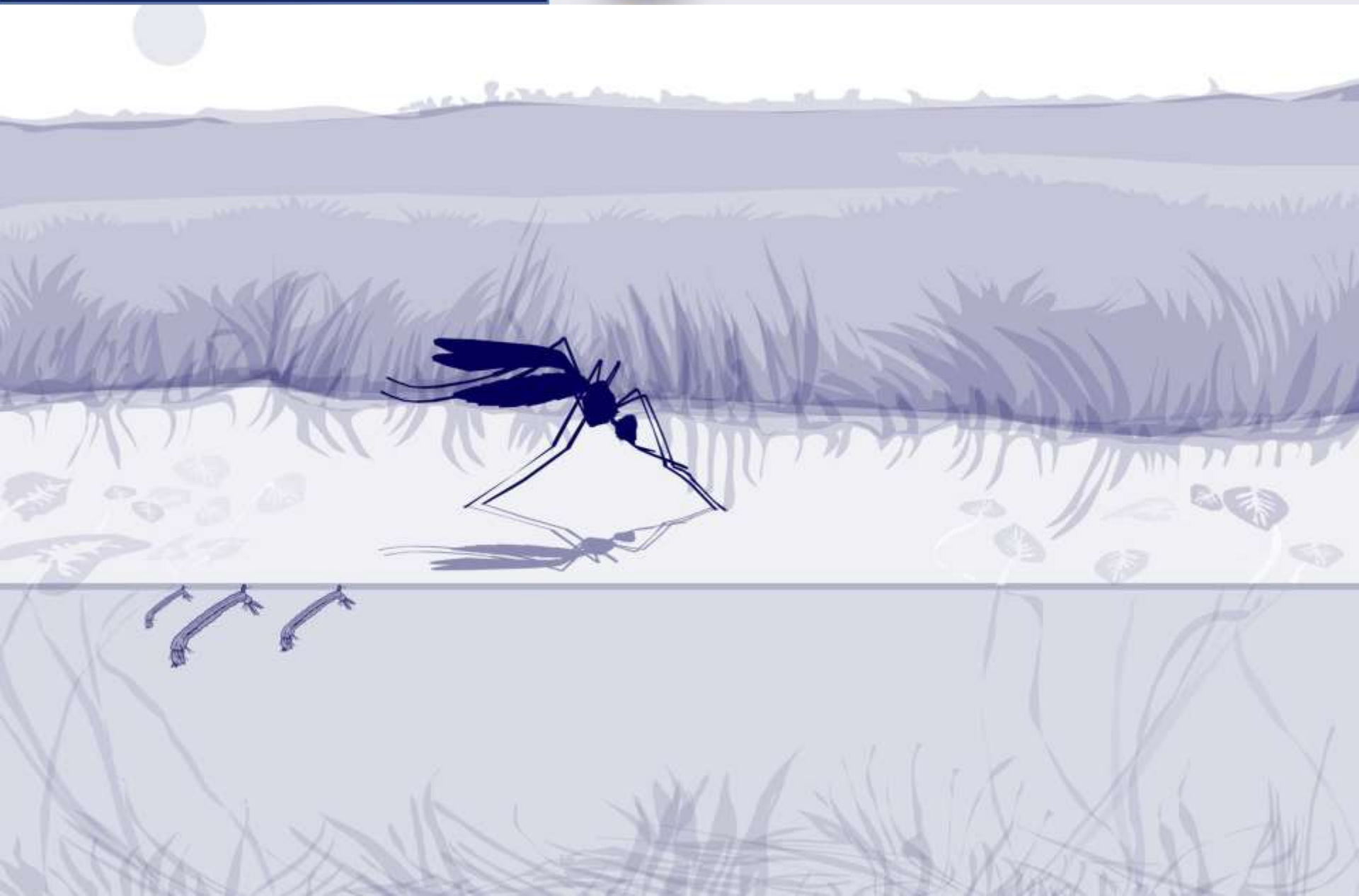


Overview

This module:

- **Reviews the selection of a GLOBE hydrology site**
- **Reviews the water sampling technique used in GLOBE hydrology protocols**
- **Provides a step by step introduction of the protocol method**

Learning Objectives

After completing this module, you will be able to:

- **Identify mosquito larvae in the breeding ground sampled at the study site**
- **Understand the importance of representative sampling**
- **Compare the number of mosquito larvae in each genus or species in different habitats**
- **Explore relationships between the larvae, genus/species, climatic factors and disease**
- **Collaborate with other GLOBE schools in collection and analysis of data**
- **Report and visualize data using the GLOBE website**

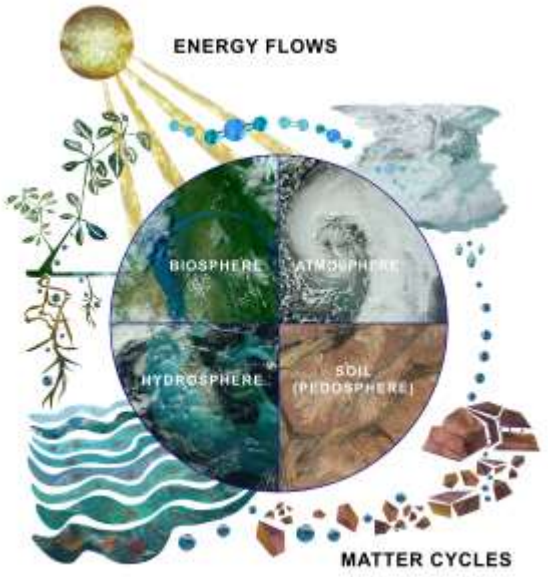
Estimated Time Needed to Complete Module: 1.5 hours

The Hydrosphere

The hydrosphere is the part of the Earth system that includes **water, ice and water vapor**. Water participates in many important natural chemical reactions and is a good solvent. Changing any part of the Earth system, such as the amount or type of vegetation in a region or from natural land cover to an impervious one, can affect the rest of the system.

The hydrosphere is home to many organisms, including the eggs and immature forms of mosquitoes.

Current measurement programs in many areas of the world cover only a few water bodies a few times during the year. GLOBE Hydrosphere protocols will allow you to collect valuable data to help fill these gaps and improve our understanding of Earth's natural waters- and, in the case of the Mosquito Protocol, improving human health.



The Earth System: Energy flows and matter cycles.

Hydrosphere Protocols

The Mosquito Protocol is one of the hydrosphere protocols used by GLOBE to describe the status of a water body.

Mosquitoes are common insects that **occur in many places around the world particularly in the tropic and sub-tropic regions.** Mosquitoes play an important role in ecosystems. They are food sources for many species of fish, birds, amphibians and reptiles. Male mosquitoes are pollinators and so help to make fruits and vegetables.

There are over 40 genera and over 3500 known species. However, three of the these genera, *Anopheles, Aedes*, and *Culex*, have species that transmit diseases that impact people including malaria, chikungunya virus, dengue fever, Zika virus, and West Nile virus.

Identifying the breeding areas of mosquitos that are disease vectors for humans is an important component of local disease management and eradication.

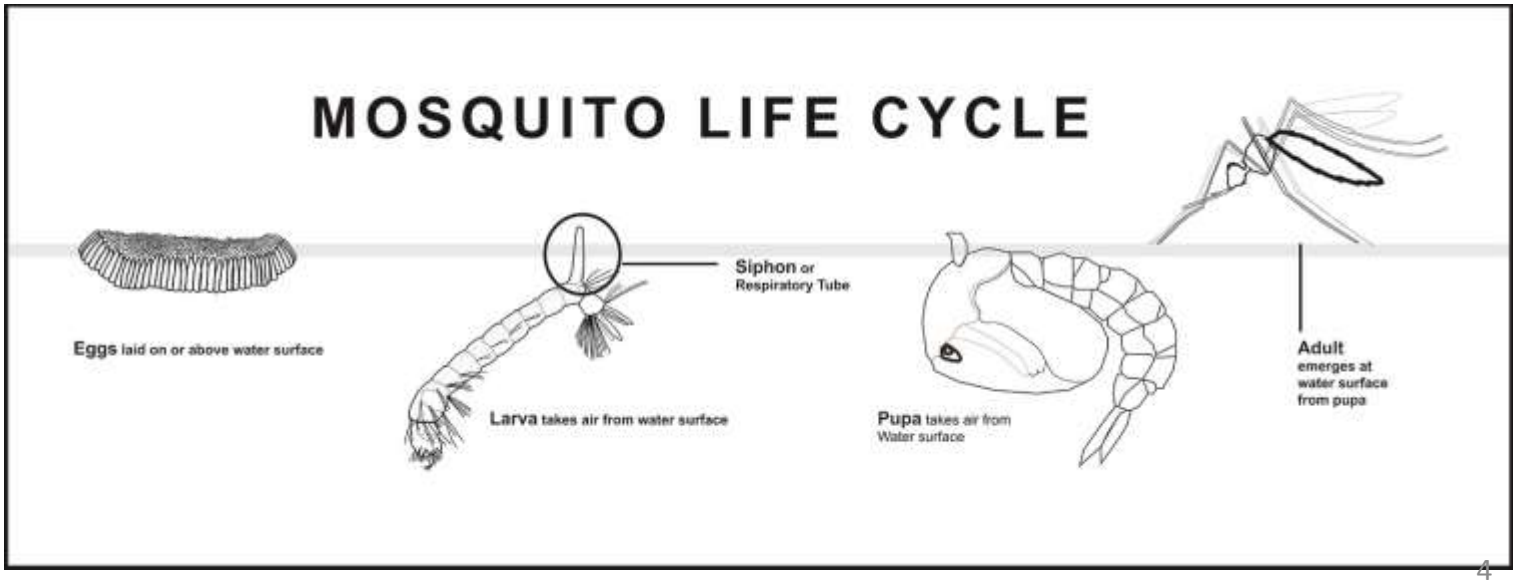
GLOBE Hydrosphere Measurements

- Alkalinity
- Conductivity
- Dissolved Oxygen
- Freshwater Macroinvertebrates
- Mosquito
- Nitrates
- pH
- Salinity
- Water Temperature
- Water Transparency

Hydrosphere-Biosphere Connection

The egg, larvae and pupae stages of the mosquito life cycle are dependent on water. The GLOBE mosquito larvae protocol focuses on collecting and **identifying mosquito larvae in standing water.**

Note that handling of eggs and the larvae is safe: the eggs and larvae do not transmit pathogens that result in disease. Only the bites of female mosquitoes transmit pathogens that can cause disease.



Mosquitoes and Climate Change

Climate models predict global average warming in the range of from **2 to 4 °C** (2–8 °F) by 2100. Rising temperatures may spread insect-borne diseases into areas where reports of infection have been relatively rare.

In the Earth system, “Everything is connected to everything else,” and changes in climate also have metabolic consequences for organisms, including *Aedes aegypti*, the mosquito that transmits the viruses responsible for **yellow fever, dengue, chikungunya, and Zika .** As the temperature rises, **nearly everything about the biology of the *Aedes aegypti* mosquito speeds up when it comes to spreading disease.**

Bill Reisen, entomologist at University of California Davis, explains. “With higher temperatures you have more mosquitoes feeding more frequently and having a greater chance of acquiring infection. And then the virus replicates faster because it's hotter, therefore the mosquitoes can transmit earlier in their life.” The thermodynamics of mosquitoes are "driven by temperature."

Read more here.

Image: CDC.

Why Collect Mosquito Data?

The life cycles of mosquitoes is closely related to their environment. By collecting mosquito and environmental data, students can contribute to better understanding the the relationship between mosquitoes, the pathogens they may carry, and the environment. This kind of information can be used locally to determine when outbreaks of disease such as malaria or dengue most likely will occur, or when chemical or other controls will be most effective.

Globally, there is a major effort to use data from satellites to predict the onset, decline, and spread of vector-borne diseases. Reliable ground-based data are helpful for the development of realistic computer models based on satellite data.

In many parts of the world, sufficient "ground-truth" data are simply not available- so GLOBE observations are critically important to tracking and controlling disease.

Mosquitoes and Zika Virus

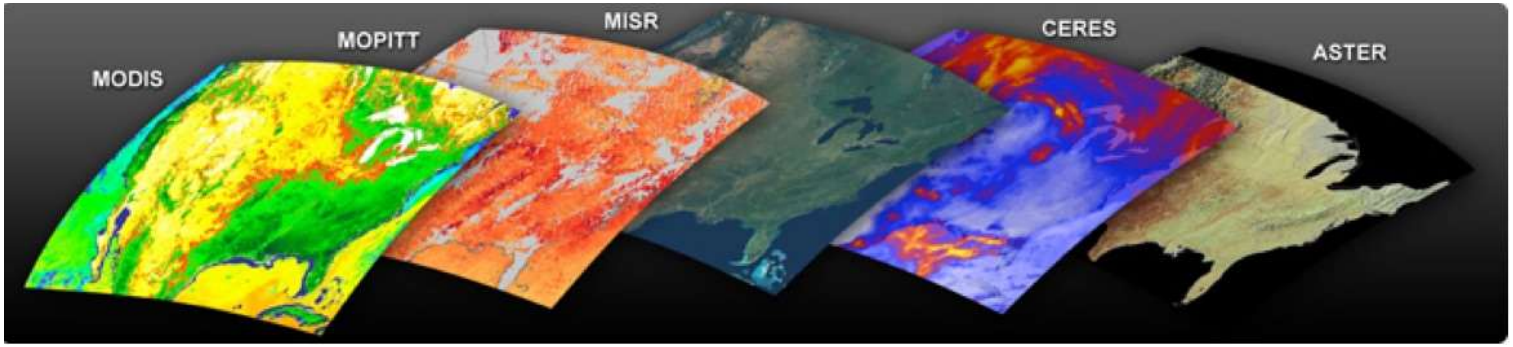
The explosive spread of Zika throughout the Americas is raising questions about the best ways to predict and control outbreaks of mosquito-borne diseases. Zika is transmitted by the mosquito ***Aedes aegypti***. This mosquito has become a serious health problem because it has evolved and adapted to human environments. They preferentially breed in containers that contain standing water.

Satellite Data and Ground Verification

Remotely sensed data collected by instruments on planes and satellite can be used to estimate the probability of mosquito breeding and disease transmission.

Terra Instruments

Terra collects data about the Earth's bio-geochemical and energy systems using five sensors that observe the atmosphere, land surface, oceans, snow and ice, and energy budget. Each sensor has unique features that enable scientists to meet a wide range of science objectives. Because all five instruments are on the same satellite making simultaneous observations, scientists are able to compare different aspects of Earth's characteristics over time.



NASA's Terra and Aqua satellites carry sensors used by researchers to measure mosquito-favoring environmental conditions on Earth. Image Credit: NASA. Read more here

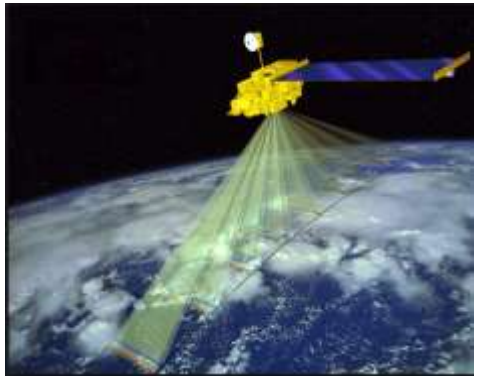
How Your Data Can Help

You Can't See Mosquitoes from Space

"I don't see mosquitoes from satellites, unfortunately, but I see the environment where mosquitoes are," says Felix Kogan of the NOAA Satellite and Information Service in this video about his work. "Mosquitoes like warm and moist environments and this is what I see from the operational satellites."

Seasonal patterns of temperature and precipitation may be altered by climate change where you live. These changes could affect the movement of insects such as mosquitoes. Climate change can affect the spread of mosquito borne diseases such as Zika, malaria and Dengue fever. Other factors such as land use are important factors contributing to the spread of diseases. These factors contribute to providing suitable habitat for mosquitoes to breed and grow, and how the disease is spread between people. Through ground-based observations, GLOBE students are able to augment broad scale satellite-based research with highly targeted local ground-based observations at a high level of granularity.

Read more here



Can't quite see mosquitoes from here. Terra from space. Image: NASA.

Summary so Far…

Seasonal patterns of temperature and precipitation may be altered by climate change where you live. These changes could affect the movement of insects such as mosquitoes. As well, it could affect the spread and intensity of mosquito borne disease. Other factors such as landscape change and land use contribute to providing suitable habitats for mosquitoes.

Let's do a quick review before moving onto data collection! Question 1

Mosquitoes are part of the biosphere, but the protocol for mosquito larvae is found in the _____GLOBE Investigation area because it is the habitat of mosquito larvae.

A. Land cover
B. Hydrosphere
C. Lithosphere
D. Earth System

What is the Answer?

Let's do a quick review before moving onto data collection! Answer to Question 1

Mosquitoes are part of the biosphere, but the protocol for mosquito larvae is found in the _____GLOBE Investigation area because it is the habitat of mosquito larvae.

A. Land cover

B. Hydrosphere- ☺ Correct!

C. Lithosphere

D. Earth System

Were you correct?

Let's do a quick review before moving onto data collection! Question 2

Which mosquito genus is responsible for the transmission of Yellow Fever, Dengue Fever, Chikungunya and Zika virus?

A. *Aedes*

B. *Anopheles*

C. *Culex*

D. All of the above

What is the Answer?

Let's do a quick review before moving onto data collection! Answer to Question 2

Which mosquito genus is responsible for the transmission of Yellow Fever, Dengue Fever, Chikungunya and Zika virus?

A. ***Aedes - ☺Correct!***

B. *Anopheles*

C. *Culex*

D. All of the above

Were you correct?

Let's do a quick review before moving onto data collection! Question 3

What is the projected impact of a warming climate on mosquito populations?

A. It is projected that mosquito species will increase their range to areas where they are currently not found

B. It is projected that mosquito species will replicate more slowly, because their metabolism will slow down because of the heat

C. Both A and B

D. None of the above

What is the answer?

Let's do a quick review before moving onto data collection! Answer to Question 3

What is the projected impact of a warming climate on mosquito populations?

A. **It is projected that mosquito species will increase their range to areas where they are currently not found- ☺ Correct!**

B. It is projected that mosquito species will replicate more slowly, because their metabolism will slow down because of the heat

C. Both A and B

D. None of the above

Were you correct?

Let's do a quick review before moving onto data collection! Question 4

Why is the the mosquito larvae data collected by GLOBE important?

A. In many parts of the world, there are insufficient ground validation measurements, so the observations are critical to tracking and controlling disease using satellite data.

B. Students can collect data that can be used locally to predict outbreaks of mosquito-borne disease, such as malaria, dengue fever or zika.

C. Students can develop their own models to understand the specific ecological needs and tolerances of local mosquito populations, so that local breeding site eradication measures can take place.

D. All of the above

What is the answer?

Let's do a quick review before moving onto data collection! Answer to Question 4

Why is the the mosquito larvae data collected by GLOBE important?

A. In many parts of the world, there are insufficient ground validation measurements, so the observations are critical to tracking and controlling disease using satellite data.

B. Students can collect data that can be used locally to predict outbreaks of mosquito-borne disease, such as malaria, dengue fever or zika.

C. Students can develop their own models to understand the specific ecological needs and tolerances of local mosquito populations, so that local breeding site eradication measures can take place.

D. All of the above - ☺ Correct!

Were you correct? Let's now look at the data collection procedures in the protocol.

Mosquito Protocol: What do you need to start?

When	**Ideally, 2x a month**
Where	Neighborhoods, school grounds, parks, wetland sites
Time Needed	1-2 hours
Prerequisites	Hydrosphere Study Site definition (can be done at the same time)
Key Instruments	Dipper, magnifying glass or app, Stereomicroscope is useful
Skill Level	Intermediate
References you need	*Mosquito Larvae Protocol* *Site Definition Sheet* *Mosquito Larvae Sampling Field Guide* *Mosquito Larvae Data Sheet* *Mosquito Identification Key*

Assemble Field Equipment

- GPS or GLOBE Mosquito App
- Compass
- Measuring tape
- Dipper, Net or Bulb Syringe (baster)
- Bucket
- Plastic zip bags
- Permanent marker and pencil
- White plastic plate
- Forceps
- Rinse bottle
- Paper towels
- Camera (phone camera is good)
- Ethanol alcohol
- Hand lens, magnifying glass or magnifying attachment for mobile device

Start your Fieldwork with Safety Steps

Safety is important when conducting the Hydrosphere protocols. While you will need to use your judgment in selecting only hydrosphere study sites that are safe to access and sample, additional precautions are needed:

- Students should wear protective gloves and goggles when handling water samples and chemicals to avoid danger from splashes.

- For the mosquito protocol, it is important to protect students from exposure to biting mosquitoes. Ask your students to wear clothes that cover the body so little bite area is exposed. Apply insect repellent. The best time to collect samples is at the heat of the day, near solar noon, when mosquitoes are least active. Women who are pregnant or are planning to become pregnant should not participate in this activity.

- Be aware that the eggs and larvae are not disease vectors, mosquito-borne disease is transmitted through the bite of the adult female mosquito.

***SAFTEY** be sure students wear gloves and goggles during your investigations*

Identifying potential mosquito breeding sites

Mosquitoes lay their eggs in places where water can collect. These can be natural places such as puddles or ponds, or artificial places such as flower pots or plastic bottles. How you collect your sample will depend on what type of place you are sampling. If you are sampling containers, you will either pour the water through the net if the container is small enough to do this. Or, if the container is big, you will use a net to gather a sample from the water in the container.

If you are sampling a pond, puddle, along a slow moving stream, or some other place that is not a container, you will dip a net in the water to collect the mosquito larvae. You will collect 5 samples waiting 5 minutes between sample collection. This is to make sure you get an accurate representation of how many larvae are in the water. With containers, you are gathering most or all of the larvae in the containers.

Whether you are collecting in containers or non-containers, you will need to create a site definition and describe the current site conditions. After collection, you will identify the mosquito larvae **using keys and count the number of larvae within each genus or species.**

Site Selection: Containers

There are a variety of indoor and outdoor containers that can be sampled around homes, schools or other buildings. You may have other types of containers in your community as well!

Indoor containers

Outdoor containers

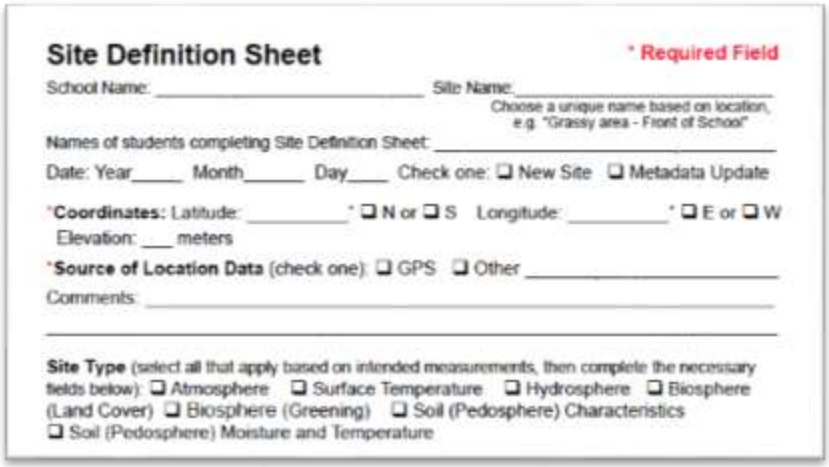
Site Selection: Non-container sampling sites

Sampling sites include habitats include ponds, streams, marshes, puddles along streets or in yards, or agricultural areas (e.g., rice fields). These are places where you cannot lift the container and pour the water into a net or container. If you are using a repeat sampling scenario, find a site that is easy for students to visit. The site should be large enough so that it does not quickly dry up and sampling can be done on a regular (twice monthly, if possible) basis.

Site Definition

Complete a site definition using the *Site Definition Sheet*.

- NOTE: If you are collecting from the same site throughout the seasons and year, you only need to do a site definition once. Then you do current site conditions each time you collect mosquito data (next slide).
- If you are collecting mosquito data at a location only once, you still need to create a hydrosphere site definition and then the current site conditions.



Site Definition Sheet * Required Field

School Name: _____ Site Name: _____
Choose a unique name based on location, e.g. "Grassy area - Front of School"

Names of students completing Site Definition Sheet: _____

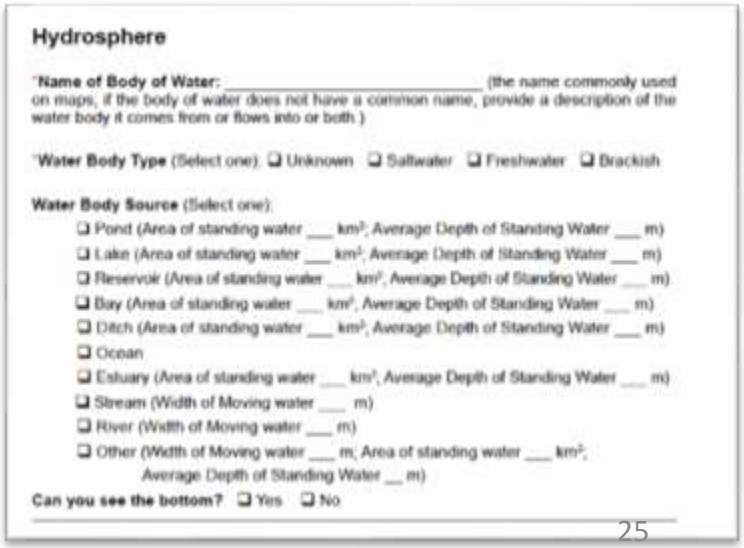
Date: Year____ Month____ Day____ Check one: ❑ New Site ❑ Metadata Update

*Coordinates: Latitude: _____° ❑ N or ❑ S Longitude: _____° ❑ E or ❑ W
Elevation: ___ meters

*Source of Location Data (check one): ❑ GPS ❑ Other _____

Comments: _____

Site Type (select all that apply based on intended measurements, then complete the necessary fields below): ❑ Atmosphere ❑ Surface Temperature ❑ Hydrosphere ❑ Biosphere (Land Cover) ❑ Biosphere (Greening) ❑ Soil (Pedosphere) Characteristics ❑ Soil (Pedosphere) Moisture and Temperature



Hydrosphere

*Name of Body of Water: _____ (the name commonly used on maps; if the body of water does not have a common name, provide a description of the water body it comes from or flows into or both.)

*Water Body Type (Select one): ❑ Unknown ❑ Saltwater ❑ Freshwater ❑ Brackish

Water Body Source (Select one):
- ❑ Pond (Area of standing water ___ km²; Average Depth of Standing Water ___ m)
- ❑ Lake (Area of standing water ___ km²; Average Depth of Standing Water ___ m)
- ❑ Reservoir (Area of standing water ___ km²; Average Depth of Standing Water ___ m)
- ❑ Bay (Area of standing water ___ km²; Average Depth of Standing Water ___ m)
- ❑ Ditch (Area of standing water ___ km²; Average Depth of Standing Water ___ m)
- ❑ Ocean
- ❑ Estuary (Area of standing water ___ km²; Average Depth of Standing Water ___ m)
- ❑ Stream (Width of Moving water ___ m)
- ❑ River (Width of Moving water ___ m)
- ❑ Other (Width of Moving water ___ m; Area of standing water ___ km²; Average Depth of Standing Water __ m)

Can you see the bottom? ❑ Yes ❑ No

Current Site Conditions-

- After creating a site definition, you need to describe the current site conditions.

1. If collecting water transparency, follow the *Water Transparency Protocol*
2. Estimate the maximum water depth. <0.5m or >0.5m
3. Estimate the perimeter of water body if puddle, pond or lake OR width if drainage ditch, stream or river:
   - ☐ < 1m, ☐ 1-10m, or ☐ >10m
4. Estimate the area of observation site in shade.
   - ☐ 0%, ☐ 25%, ☐ 50%, ☐ 75%, or ☐ 100%
5. Record whether the site has vegetation or algae.

Examples of Descriptive Data

Record if and what type of odor the water has.

☐Normal/none, ☐fishy, ☐sewage, ☐chemical, ☐petroleum, ☐other.

If there is surface oil on the water, identify the type.

☐None, ☐slick, ☐sheen, ☐globs, ☐flecks, ☐other

If you have not measured the turbidity of the water estimate whether it is ☐clear, ☐turbid, or ☐very turbid.

Season:

☐dry, ☐wet, ☐spring, ☐summer, ☐fall, ☐winter

How to Collect Mosquito Larvae: Containers

1. Locate all water containers in and around the house/school.
2. Write a unique identification number (ID) on each water container. Students can use a permanent marker to label the containers. Write the corresponding container IDs on your *Mosquito Larvae Protocol Data Sheet*.
3. Complete the information about the containers in the *Mosquito Larvae Protocol Data Sheet* (ID, container type, water level, lid, lid type, container color, and cleaning frequency).

#	Habitat Type	Container	Water Level	Lid	Lid type	Container Color	Cleaning Frequency	No. of Genera	No. of Mosquito Larvae Species
	☐ Artificial ☐ Natural	☐ Small jar ☐ Large jar ☐ Cement tank ☐ Plastic tank ☐ Other	☐ 0-25% ☐ 25-50% ☐ 50-75% ☐ 75-100%	☐ Absent ☐ present	☐ Wood ☐ Metal ☐ Plastic ☐ Nylon ☐ Cloth ☐ Other	☐ Dark ☐ Light	☐ None ☐ 1-2 times/week ☐ > 2 times/week	☐ Anopheles ☐ Aedes ☐ Culex ☐ Other	☐ Absent ☐ Present *Anopheles spp.* *Aedes spp.* *Culex spp.* *Other*

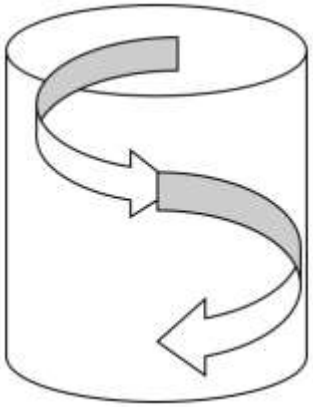
Why are these data important? Some species, such as *Aedes aegypti* show a preference for small containers, and **human-created environments provide breeding sites.** By identifying and mitigating these human-created breeding sites, GLOBE students can contribute to eradicating mosquito-borne disease.

Mosquito Larvae Sampling Method: Small Containers

Pour sample water in container through net into a bucket. In the photo to the right, GLOBE students found an abandoned bucket that collected rainwater and created a sheltered habitat where mosquitoes could lay their eggs.

Mosquito Larvae Sampling: Large Containers

Large water containers are water containers that can hold 500 L or greater of water. Examples include large water jars, water pools, and cement tanks. Sample large water containers by dipping the net in the water, starting at the top of the container, continuing to the bottom in a swirling motion and sampling all edges of the container. Alternatively, a dipper can be used to skim the top of the water surface.



Mosquito Larvae Sampling: Non-Containers

Using the mosquito dipper or net, skim the surface of the water. The net is maintained at an acute angle with respect to the water surface, see figure below.

- Take 5 samples. Wait 5 minutes between each sample
- Document the number of times you sampled and report this to the GLOBE website.

Record:

- Length of dipping net in cm.
- Diameter at opening of dipping net in cm.

Mosquito Larvae Sampling

After collecting a sample with a net, use a squirt bottle with water to gently remove the debris caught in the net into a bucket.

Mosquito Larvae Sampling

After removing all debris from the net, either) place sample in labeled plastic bags.

Leave air in bags so that larvae can breathe, and keep bags cool and in the shade. If they warm up in the sun, the larvae may die.

Identify the larvae soon after collection. If left overnight, any pupae in the sample may become adult flying mosquitoes.

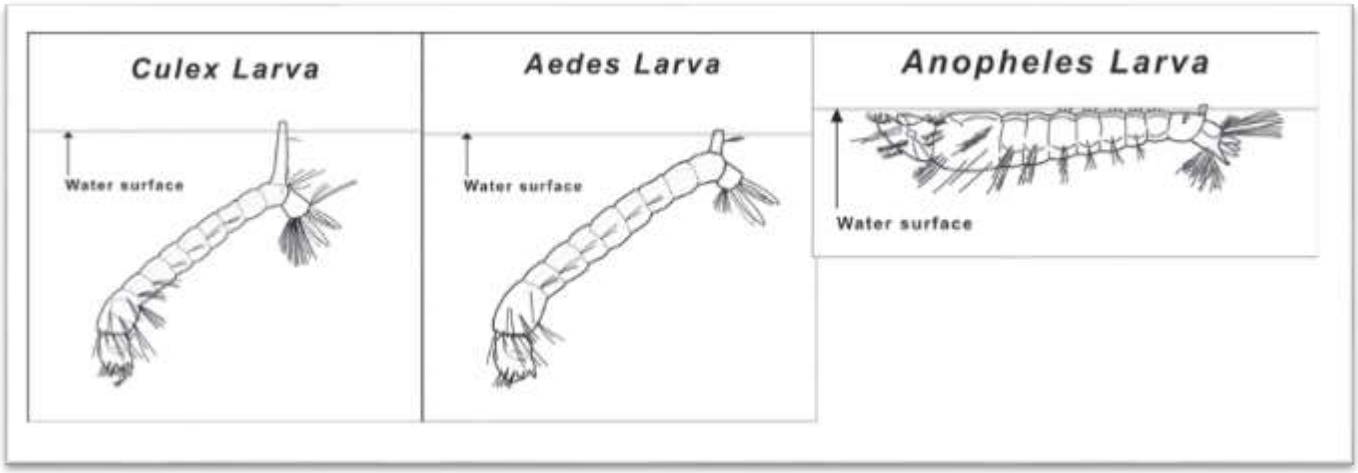
If you find adult mosquitoes in your sample bag, shake the bag to drown the adult mosquitoes and dispose of the sample by pouring on the ground.

Mosquito Identification

Use a hand lens or magnifying glass to examine what is in the water to identify to genus level. A stereo microscope may be needed to identify to the species level.

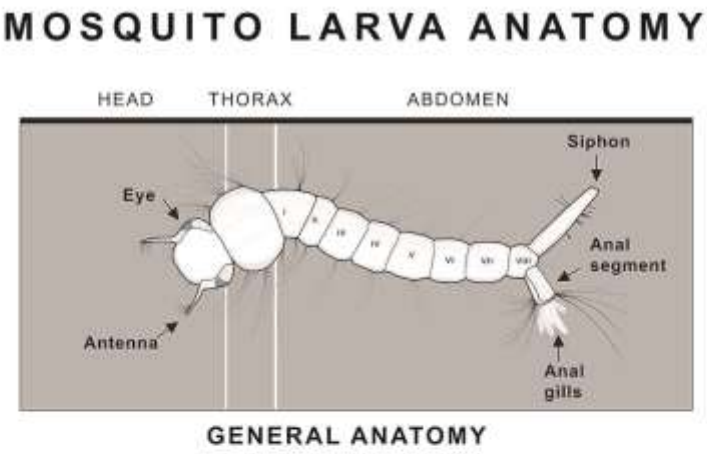
Mosquito Larvae Identification

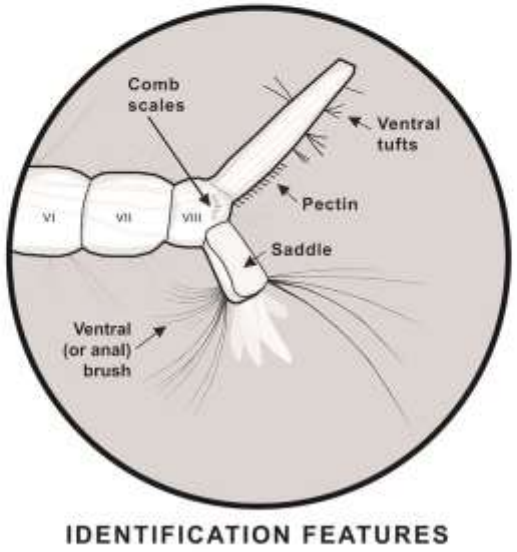
Use the *Mosquito Identification Key or other identification key* to identify the genus. You can place the larvae in vials to see how they suspend from the surface of the water to help with the identification.



Mosquito Larvae Identification

Familiarize yourself with the general anatomy of the mosquito larvae and the key features that distinguish those genera or species that are found in your locality. In particular, key features are often found on the anal segment and the siphon. Consult with mosquito experts or mosquito identification keys for your locality.





Recording your Data

After you sort and identify your larvae, you count how many are in each taxon (genus or species). You can use ice cube trays to place the larvae of the same taxon as you are sorting and identifying. You decide whether you will report genus or species to the GLOBE website.

Genus	Species	Count

Let's do a quick review before moving onto data entry: Question 5

How many samples should you take when sampling a non-container water body?

A. 3 samples, 3 minutes between each sampling with the net

B. 5 samples, 5 minutes between each sampling with the net

C. 1 sample, just like when containers are sampled, but split the sample into as many bags as you need.

What is the answer?

Let's do a quick review before moving onto data entry: Answer to Question 5

How many samples should you take when sampling a non-container water body?

A. 3 samples, 3 minutes between each sampling with the net

B. 5 samples, 5 minutes between each sampling with the net-☺ Correct!

C. 1 sample, just like when containers are sampled, but split the sample into as many bags as you need.

Were you correct?

Let's do a quick review before moving onto data entry: Question 6

Which of the following a part of the mosquito larva you would look at first to determine the genus or species of your specimen.

A. Anal segment and siphon

B. Thorax

C. Eyes

What is the answer?

Let's do a quick review before moving onto data entry: Answer to Question 6

Which of the following a part of the mosquito larva you would look at first to determine the genus or species of your specimen.

A. Anal segment and siphon ☺ Correct!

B. Thorax

C. Eyes

Were you correct?

Let's do a quick review before moving onto data entry: Question 7

What precautions will you want to make to ensure student safety in the field?

A. Protective gloves and goggles, as in all hydrosphere protocols

B. Clothing that covers and limits exposed skin surfaces

C. Application of insect repellent, with permission

D. Sample the larvae at or near solar noon

E. All of the above

What is the answer?

Let's do a quick review before moving onto data entry: Answer to Question 7

What precautions will you want to make to ensure student safety in the field?

A. Protective gloves and goggles, as in all hydrosphere protocols

B. Clothing that covers and limits exposed skin surfaces

C. Application of insect repellent, with permission

D. Sample the larvae at or near solar noon

E. All of the above ☺ Correct!

Were you correct? Let's now look at GLOBE data entry and visualization.

Submitting your data to GLOBE

- Download the Data Entry app from the **App Store**
- **Live Data Entry**: These pages are for entering environmental data – collected at defined sites, according to protocol, and using approved instrumentation – for entry into the official GLOBE science database.
- **Email Data Entry** : If connectivity is an issue, data can also be entered via email.
-

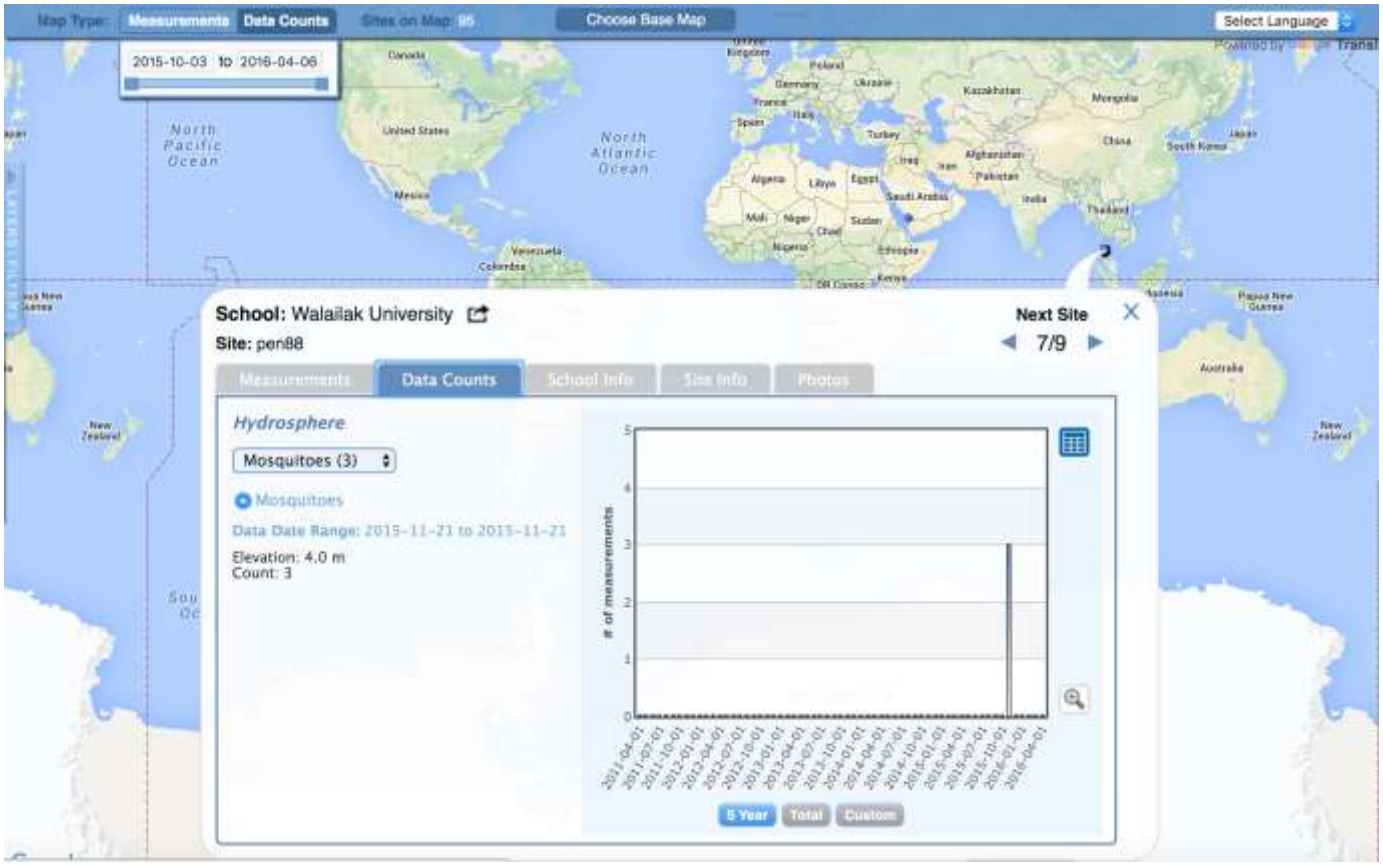
Visualize and Retrieve Data-1

GLOBE provides the ability to view and interact with data measured across the world. Select our visualization tool to map, graph, filter and export data that have been measured across GLOBE protocols since 1995. Here are screenshots steps you will use when you use the visualization tool, using water pH as an example. The Mosquito Protocol is new and so we look forward to seeing your data!

Link to step-by-step tutorial on using the GLOBE Data Visualization Tool

Visualize and Retrieve Data-2

Select the date for which you need pH data, add layer and you can see where data is available.



Review questions to help you prepare to conduct the Mosquito Protocol

1. **How many samples of a non-container site should you take?**
2. **What are some of the safety precautions that you need to take when conducting the Mosquito Protocol?**
3. **Ideally, how often can you do the mosquito protocol?**
4. **What are some of the types of data that you will report to GLOBE when conducting the GLOBE Mosquito Protocol?**
5. **If you were designing a research investigation to identify the environmental conditions that support successful Mosquito breeding seasons, what other GLOBE data sets might you consult?**
6. **True or false: mosquitoes can hatch and develop in open container environments as well as natural water bodies.**
7. **Name three genera of mosquitoes that can potentially transmit pathogens that cause disease in humans.**
8. **Which genus of mosquitoes carries Zika? Malaria?**

Are you ready to take your quiz?

You have now completed the slide set. If you are ready to take the quiz, sign on and take the quiz corresponding to Mosquito Larvae Protocol.

When you pass the quiz, you are ready to take Mosquito Larvae measurements!

Frequently Asked Questions (FAQs)

What is the mosquito life cycle?

Adult → eggs (2 -3 days) → larvae (4 -5 days) → pupae (1- 2 days) →Adult

How do you identify which one is the *Anopheles*, *Aedes* or *Culex* larvae (identify with unaided eyes)?

We can see the characteristics of mosquito larvae: In the water, *Anopheles* larvae cling parallel with the water surface. On the other hand, *Aedes* and *Culex* larvae cling at an angle of 45◦ with the side of the container. *Aedes* larvae have shorter siphons, *Culex* larvae tend to have have longer siphons.

What do the male mosquitoes feed on?

Male mosquitoes feed on any sugar source, including flowers, fruit, nectar and other insects.

At what seasons of the year are greater percentages of mosquito larvae found?

Most often they are found in the rainy season or shortly after the end of the rainy season.

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eTraining Feedback

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