# Mosquito Larvae Protocol

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***Purpose***

To sample, identify, and count the number of mosquito larvae at the genus or species level in your study site or community.

***Overview***

Students will collect, sort, identify, and count the number of mosquito larvae. There are a variety of sampling strategies that can be used, depending on educational objectives and the types of mosquito larval habitats you are sampling. Students can identify mosquito larvae to either the genus or species level.

***Student Outcomes***

Students will learn to:

- identify mosquito larvae at their study site or community;
- understand the importance of representative sampling;
- compare the number of mosquito larvae in each genus or species in the different habitats;
- explore relationships between the larvae genus/species, climatic factors, and disease;
- collaborate with other GLOBE schools (within your country or in other countries); and
- report data to the GLOBE website.

**Science Concepts**

*Life Sciences*

- Organisms can only survive in environments where their needs are met.
- Organisms' functions relate to their environments.
- Organisms change the environment in which they live.
- Plants and animals have life cycles.

*Scientific Inquiry Abilities*

- Identify answerable questions.
- Design and conduct scientific investigations.
- Use appropriate mathematics to analyze data.
- Recognize and explore alternative explanations.
- Communicate procedures, descriptions, and predictions.

***Time***

Data collection: 1-2 hours per visit, per site.

***Level***

Secondary

***Frequency***

Flexible: 1 time per site; 1 time a month during rainy season for each site.

***Materials and Tools***

- ❑ *Mosquito Larvae Data Sheet*
- ❑ Mosquito Identification Key
- ❑ GPS receiver, map, or other mobile technology
- ❑ Compass
- ❑ Camera
- ❑ Pencil or pen
- ❑ Measuring tape (optional)
- ❑ Nets
- ❑ Squirt bottle
- ❑ White plate or pan
- ❑ Plastic bags
- ❑ Rubber bands

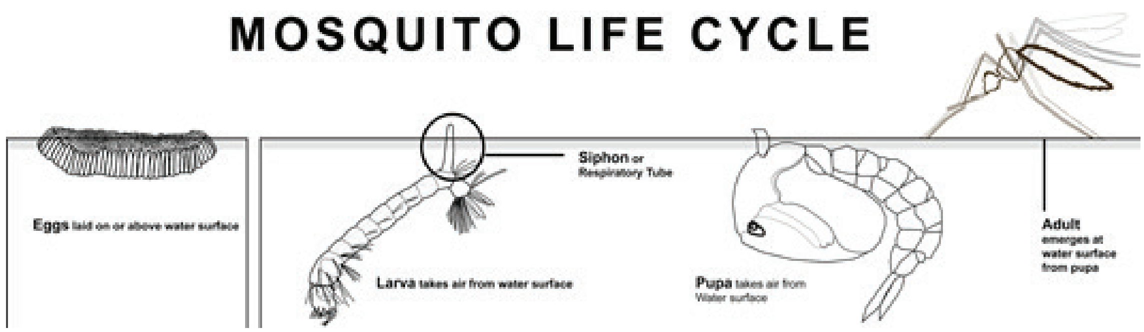
- ❑ Permanent marker
- ❑ Hand lens or magnifying glass
- ❑ Microscope (for species identification)
- ❑ Additional equipment needed if collecting water quality measurements.

***Preparation***

- Select study locations and sampling strategy.
- Practice sampling method you have selected.
- Practice identifying mosquito larvae using mosquito larvae key.

***Prerequisites***

None




## Science Background

Mosquitos are common insects that occur in many places around the world, particularly in tropic and sub-tropic regions. There are over 40 genera and over 3,500 known species. Three of these genera, *Anopheles, Aedes,* and *Culex*, have species that transmit diseases that impact people, including malaria, dengue fever, West Nile Virus, and the Zika Virus.

Mosquitoes require water to breed and grow in their early life stages. Female mosquitoes lay eggs at the surface of water in appropriate breeding sites. These include puddles, tire tracks, ponds, containers, bottles, and various objects where water can accumulate. Each mosquito species from all genera has its preferential breeding sites. Container characteristics, such as type of lids and what the container is made from, may influence female breeding selection. Eggs hatch after two days, producing aquatic larvae. From the first larval stage form to the adult mosquito (or imago), larvae pass through four stages, called "instars." The duration of the aquatic phase and each different larval phase depends on the temperature of the water.

Seasonal patterns of temperature and precipitation may be altered by climate change where you live. These changes could affect the spread and intensity of malaria, dengue fever, the Zika virus, and other disease outbreaks. 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.

The life cycles of mosquitoes are intimately related to their environment, and one of the most important contributions GLOBE students and teachers can make is to collect environmental and mosquito data. As described in the protocol, GLOBE students can identify mosquitoes at least by genus in their larval stage, which is critical to understanding the relationship between mosquitoes, the disease(s) they may carry, and the environment. This kind of information can be used locally to predict when outbreaks of disease, such as malaria and dengue fever, will occur, or when chemical or other controls will be the 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 absolutely essential for the development of realistic computer models based on satellite data. In many parts of the world, and especially in areas where malaria is endemic, sufficient "ground-truth" data are simply not available.

Most mosquitoes never travel more than a few kilometers from their breeding sites, so the local mosquito population controls local mosquito-borne diseases. Mosquitoes require standing water to breed. Unfortunately for humans, even very small amounts of water -- less than 1 $cm^3$ -- are sufficient. Although it is impossible to remove all breeding sites in areas where malaria is endemic, in some situations it is nonetheless a reasonable goal for GLOBE students to take a leadership role in reducing local breeding sites by minimizing standing water. Simply removing trash and discarded containers can have a significant impact on local mosquito populations. For example, discarded tires make ideal breeding sites for certain species of mosquitoes that prefer sites away from direct sunlight. Cleaning and covering water containers in households and neighborhoods has been shown to be effective in reducing numbers of mosquito larvae.

In addition, GLOBE schools, in collaboration with local public health officials, can help reduce mosquito-to-human contact. The basic malaria-prevention tool is insecticide-treated netting. Community-based programs based on the GLOBE model of teacher training and student implementation can be applied to increasing the use of netting, which is essential to reducing the incidence of malaria as well as other mosquito-borne diseases.

## Teacher Support

### *Advance Preparation*

*1. What types of mosquitoes are in your community and region? What types of mosquito vector-borne diseases are in your region, e.g., malaria, dengue fever, and West Nile?*

Many teachers and students have little background in the identification of mosquito larvae, and may be reluctant to begin such a class project. This is not a problem, since students find the larvae so fascinating. Students will teach themselves and each other.

In many places, local experts will be available to assist you and your students. These people can, for example, help identify mosquitoes and discuss the species of mosquitoes common in your area. For species identification, mosquito larval keys are available in printed manuals and books, and on the Internet. Select an identification key that is applicable to your locality.

Contact local experts in the area to make sure that you are not sampling at a site where other people are conducting research or in a protected area. You do not want to inadvertently disturb a long-term monitoring site or harm protected areas.

In preparation for actual data collection,students can bring in mosquito larvae they collect from the containers in their neighborhoods to identify in class. This way, they will become familiar with mosquito identification before going to the field.

*2. Review background material on the mosquito life cycle and on how to identify genus and, if applicable, species. Refer to the Mosquito Identification Chart.*

Depending on the type(s) of diseases in your area, you can talk about the role mosquitoes play in the spread of diseases that occur where you live. The Mosquito Identification Chart focuses on identifying three genera – *Aedes, Anopheles*, and *Culex* – because these are the more common genera in the spread of diseases affecting people. However, there are over 3,500 mosquito species in over 40 genera. Collecting information on any mosquito genus/species provides useful information on where different species of mosquitoes are breeding and how they are responding to changing seasons and climate.

*3. Determine whether students will conduct genus or species level of identification.*

Genus level identification of mosquitoes can be done with the trained naked eye, a handheld lens, or magnifying glass. Species level identification requires a microscope to see the distinguishing features on the mosquito head and body. If microscopes are available, then research has the possibility to target species that transmit disease. If no microscopes are available, then a wealth of genus-level research can be done. However, genus-level research may only identify possible places where disease-carrying mosquito larvae inhabit since particular species within the same genus may not transmit disease.

With the advance of mobile technology, there may be other ways to magnify the mosquito larvae without microscopes. Have your students explore ways to take photos of larvae and enlarge the photos to identify distinguishing features on the larval body.

### Site Selection

After selecting which level of identification (genus or species) you will do, you need to identify the types of water you will sample. There are two main approaches: containers or non-containers.

The type(s) of sites you select determine how to sample. The methods are discussed in the field guides.

*Containers:* You can decide to collect samples in containers inside and around your school or house. Indoor containers are categorized into two categories: 1) inside the bathroom, where there may be small or large earthen jars, cement tanks, or plastic containers; and 2) outside the bathroom, where there may be containers with water such as ant-guard, vases, plates under refrigerators, or water plant pot.

There are two categories of outdoor containers: 1) artificial containers; and 2) natural containers. Examples of artificial containers include small or large earthen jars, cement tanks, plastic containers or bottles, old cans, metal boxes, plant plates or pots, animal pans, and discarded tires. Examples of natural containers include areca husks, banana trees, coconut shells, and tree holes. Mosquito larvae are collected from all outdoor containers within 15 meters around the house or school.

*Non-Containers:* For the second approach, you can decide to sample natural habitats in your community. You can sample around your house or farther away. These 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 monthly sampling can be done.

## Site Definition and Mapping

A site needs to be defined for each area you will be sampling. Refer to the Selecting and Documenting Your Hydrosphere Study Site. If your site does not have a water body (for example you are sampling containers in your home or school) then use a descriptive phrase of the house or school as the water body name on the Site Definition Sheet. If multiple sites are being sampled in your locality, each site needs a unique site definition completed.

### *When To Go Sampling*

You will need to work with your students to identify a sampling strategy based on the types of research questions your students ask, the types of equipment you have available, and the types of seasons you have in your location. In addition, you will need to take into consideration the logistics of having students collect mosquito larvae.

Suggested sampling approaches:

1. Identify one or more sites and sample once a month throughout the year or during the rainy season and mid-way through the dry season.

2. Sample a variety of habitats that the students will visit once at the beginning of the dry season. This strategy will give you a snapshot of your location and the types of habitats that are preferred by the different types of mosquitoes. Research indicates that you will likely get mosquito larvae after the rainy season while there is still water available for breeding.

### *Supporting Protocols*

• Do you have a GLOBE weather station where students are collecting data? If not, do you have access to air temperature and precipitation data that are representative of your location? If you have access to the Internet, you could determine what is available online. Students can explore relationships between selected atmospheric data (for example, maximum/minimum temperature, relative humidity, number of rainy days, precipitation) and the quantity and types of mosquito larvae found at the study site(s).

• Taking water measurements where the mosquito larvae are being collected could produce interesting student research. For instance, students can compare relationships between pH, water transparency, salinity (electrical conductivity), alkalinity, dissolved oxygen, and temperature. (Please refer to the Hydrosphere GLOBE protocols for more information.)

• As part of the site description, your students will be collecting important habitat data. Many of the data are based on visual estimates or descriptions. Drawing a site map is very important

### *Preparing for the Field*

First, select one or more study sites before sampling.

**Students should wear appropriate field clothing such as hats, long pants, and shirts with long sleeves. Students should also apply sunscreen, and should use (and reapply, as necessary) insect repellent.**

If available, you can take folding tables or seat desks for the students to handle and count their samples in the field.

### *Managing Students in the Field*

If you have a large class, you could have students work in multiple teams. Students in each team can be responsible for different tasks. (For example, two students can hold the nets, one student can hold a plastic bag, and one student can read the instructions out loud.) Having the students work in teams will make collecting samples, sorting, and identifying quicker. Make sure there are enough sets of equipment for each team (e.g., nets, plastic bags, trays, and mosquito larval identical keys).

The most time-consuming tasks are sorting and identifying the mosquito larvae. To save time, you can divide students into two teams. Students from Team I can do the sorting, counting, and identifying the mosquito larvae using the Sorting, Identifying, and Counting the Mosquito Larvae Protocol Lab Guide. Team II can collect a second sample.

After the students collect mosquito larvae, teachers can look at the jars of sorted mosquito larvae to verify that all students identified mosquito larvae correctly. If not, gather the students and have them discuss the differences in order to identify mosquito genus or species correctly.

After all mosquito larvae collected by the teams are sorted and combined from separate jars for each genus or species, have a group of students take a look at the larvae to make sure that you all agree on mosquito identification. Then, count the mosquito larvae and report the data on one set of data sheets.

### *Measurement Hints*

• Do not sample containers or places that cannot be reached safely. If your students sample from multiple habitats, you should determine which habitats can be sampled safely and evaluate the percentage of sampling coverage for each accessible habitat.

• Record the habitats that could not be sampled.

• Students should only sort and count the number of mosquito larvae. Tadpole, small fish, and other organism should be removed from the samples and returned to the water.

• Only count the number of live mosquito larvae. You can use a small plastic spoon to collect and sort mosquito larvae to the genus level (for example, *Aedes, Anopheles,* and *Culex* spp.) and place them in small plastic cups. If you are identifying the species of the mosquito larvae, you can do this in the laboratory using microscopes. You may decide to do one or more mosquito genera. (For instance, you may decide to focus on the Anopheles larvae if malaria is prevalent in your locality.)

• If you are sampling in ponds or slow-moving streams, it is necessary to proceed slowly and carefully when searching for mosquito larvae. Approach the area to be inspected with caution; heavy footsteps will create vibrations that disturb larvae and cause them to dive to the bottom of the water column. Likewise, avoid disturbing the water, as this will have the same result. If possible, approach the area to be sampled with the sun in your face for this prevents shadows that may disturb larvae and cause them to dive to the bottom of the water column. If it is windy, sample with your net on the windward side of your study site since it is likely that most of the larvae will be found there. You may have to wait a few minutes to start sampling to make sure that the larvae are near the surface. You will be taking 5 samples with your net. Wait 10 minutes between dipping the net into the water to collect larvae.

• Voucher specimens are not required, but they may help with teaching the students how to properly identify the mosquito larvae before going into the field. (Voucher specimens are samples of the mosquito genus or species that is preserved and used for reference.) By collecting voucher specimens each time, the specimens can be compared to make sure that identifications are being done correctly each time. Voucher specimens should be preserved in 70% alcohol.

• Like scientists, students should keep field notes to carefully document what they did, in particular any deviations from the protocol. Make a photo journal of the trip, and bring parents or older GLOBE students to mentor younger students. Enjoy learning about mosquito species in the world around you!

### *Equipment Use and Maintenance*

All of the sampling materials are available commercially, but students may enjoy making the equipment using the instructions provided in the Instrument Construction section. You can also buy some parts and make others. For example, one can buy a 0.5 mm-mesh replacement net for a D-net and make the pole. This may be less expensive than buying the whole device. (Please refer to the GLOBE Freshwater Macroinvertebrate Protocol for instructions on how to make a net.)

### Student Preparation

Suggested sequence of events:

1. Conduct the learning activity to correctly identify mosquito genera (and species if identifying).

2. If identifying species, conduct the learning activity for students to learn how to use the microscopes or devise another method to magnify the larvae.

3. Identify research questions/hypotheses and the methods that will be used to answer their questions.

4. Map and document study site(s).

5. Conduct field data collection, followed by laboratory work, if necessary for identification.

6. Submit data to the GLOBE website.

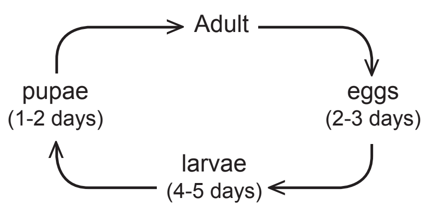
7. Conduct data analysis.

8. Present student research.

### Questions Frequently Asked

1. What is the life cycle of mosquitoes?

**A:**




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

**A:** 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 short siphons, but *Culex* larvae have long siphons.

3. What does the mosquito male feed on?

**A:** Male mosquitoes feed on nectar from flowers.

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

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

### Questions for Further Investigation

1. Are there any relationships among mosquito larvae abundance and types and the climate parameter measurements?

2. Are there seasonal variations in the numbers and types of mosquito larvae at your study site? If so, suggest possible reasons for why this is the case.