INSECT EXPERIMENTS FOR THE CLASSROOM

SAMPLING INSECT POPULATIONS

BACKGROUND

In the broadest sense, biodiversity includes determining the number of species present and the relationships among them and their environment within a geographic region. It also includes the assessment of the impact of human activities on the environment and how those activities affect the ability of species to survive. Although the topic of biodiversity regularly appears in the news and popular press, it is often portrayed as an issue of importance primarily in tropical regions. The emphasis on the tropics in media coverage centers around two primary factors. First, until fairly recently, very few detailed insect surveys had been conducted in many tropical regions. Because of that, few detailed lists of species existed, and little information was available on the life histories, seasonal cycles, and distributions of those species. Second, due to the relatively high rate of habitat loss from deforestation in the tropics, there is a genuine fear that species will become extinct before their presence is documented allowing us no opportunity to prevent those extinctions from occurring.

In many temperate regions, we have much better information on the insect fauna due to the historical fact that most major research universities and museums have been located in temperate areas. However, in many cases, only insect species that are important as either agricultural, medical, or veterinary pests, or their predators or parasitoids have been studied in detail. In addition, although it may not be as dramatic as deforestation of tropical rainforests, the loss of temperate habitats to urban development is occurring at an ever increasing rate that can approach or surpass rainforest loss. The impact of this type of habitat loss on insect populations is only just beginning to be documented.

Although most media coverage also portrays the emphasis in studying biodiversity as occurring mainly in the tropics, many biotic surveys are being conducted in temperate regions. In the United States, many Federal and State properties are either in the process of conducting biotic surveys or are developing survey protocols. Surveys of the organisms found on school, industrial, and corporate properties in many areas are also being conducted.

Conducting biotic surveys on school grounds is an excellent way to illustrate how different sampling techniques can be used to obtain an accurate record of insect populations. The internet also allows students at different sites to share and compare data gathered from geographically dispersed locations. Some examples of internet-based programs that have a biotic survey component include:

The GLOBE Project (http://globe.fsl.noaa.gov/),
Estuary-Net (http://inlet.geol.sc.edu/estnet.html),
The Rivers Curriculum Project (http://www.siue.edu/OSME/river/),
The South Carolina Butterfly Project (http://butterfly.clemson.edu).

Collecting data on local insect populations can be accomplished through a variety of sampling techniques. In designing sampling plans for use in student surveys it should be kept in mind that many of these techniques will collect small specimens that will require microscopes for identification. Keeping detailed records is also important so that data collected can be quantified to allow comparisons among different locations (*i.e.*, Do all schools in the district with nature trails have the same number of ladybugs per m^2?), or, over time at a single location (*i.e.*, Are the number of butterflies observed in 15 minutes in a butterfly garden the same as last year?).

A. SAMPLING TECHNIQUES

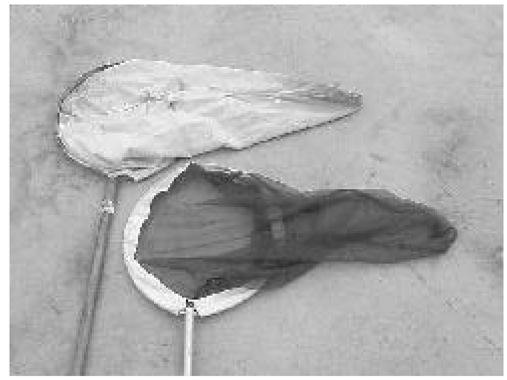
1. Transect and point sampling

In transect samples, the person taking the samples follows a predetermined sampling path and records the presence of all organisms that are to be counted within a fixed distance (*i.e.*, 1 m, 5 m) on either side of a specified length of travel along the transect. Data is generally reported as the number of organisms per unit of ground surface, calculated as length traveled along the transect times lateral distance examined (*i.e.*, If someone traveled 100 m along a transect and counted all ladybugs within 1 m on either side of the transect they would report data as the number per 200 m^2).

In the related point sample technique, fixed sample sites are established and the person making the counts moves from site to site recording the numbers of organisms observed during a predetermined period of time (*i.e.*, 5 min, 10 min) at each site. Sample sites should be chosen so that all habitat types within the study area are included. These data are reported as the number of organisms per unit observation time (*i.e.*, number of butterflies per 15 min).

2. Sweep net sampling

Collections of flying insects, or those inhabiting foliage, can be made using a sweep net. Several types of nets are available, with standard sizes being either 30.5 cm (12 in) or 38 cm (15 in) diameter. Aerial nets (net on the right) have an open mesh collection bag and are used mainly to capture flying insects, although they can be swept through light vegetation such as tall grass. Beating nets (net on the left) are made from canvas and are used to sweep through vegetation that could snag and tear a mesh bag.



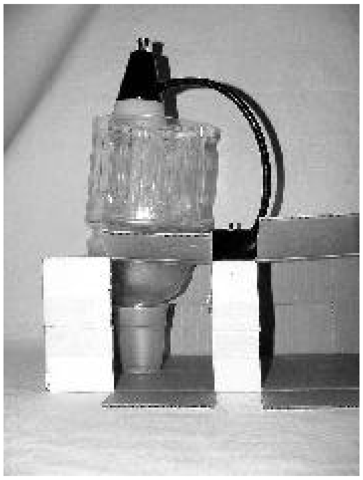
A single back and forth sweep covering a 150^0 to 180^0 arc is considered as a single sweep. The number of sweeps taken should be recorded so that samples can be quantified as the number of insects per sweep. Collected insects can be identified and counted live in the net, or placed in a container with a piece of paper towel soaked with non-acetone nail polish remover to kill them to facilitate counting.

3. Shake or beat sampling

Another technique used to collect insects from foliage is to place a shallow light colored pan, or piece of cardboard, beneath the foliage, and then either roughly shake or strike the foliage, to dislodge insects onto the collection pan (at left). To sample low growing plants, a canvas sheet can be placed on the ground under the foliage and the plants shaken over the cloth. Samples are quantified by the amount of plant material sampled (*i.e.*, cm of tree limb, number of plants, number of stems, etc.). Collected insects can be counted alive in the pan, or placed in a container with a piece of paper towel soaked with non-acetone nail polish remover to kill them to facilitate counting.

4. Leaf litter sampling

Insects that inhabit leaf litter or mulch can be surveyed by placing some of this material in an apparatus know as a Berlese-Tullgren funnel, which is designed to separate insects from the litter (see at left). A simple funnel can be constructed by removing the bottom from a 3.78 L (128 oz), or 1.89 L (64 oz), juice bottle and placing a piece of 0.6 mm (1/4 in) mesh hardware cloth inside the neck of the bottle. The inverted bottle should be supported over a container to collect the insects that come out of the litter. A simple support can be made by cutting a hole in a cardboard box. To facilitate the movement of insects from the litter, a 15W to 25W bulb placed over the funnel will cause the insects to move deeper into the litter, through the screen, and into the container.



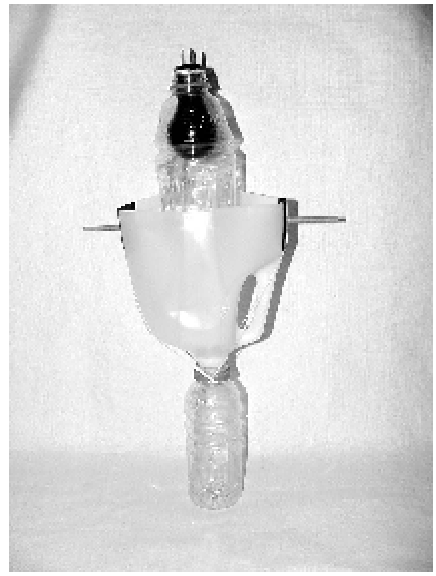
The preservative in the container can be either a 10% dish detergent solution or 75 to 80% ethanol. If a soap solution is used, the sample should be sorted the next day, or stored in 75 to 80% ethanol in an airtight container until the insects are sorted and counted. Before storing, samples should be poured into a sieve, such as a kitchen strainer, and rinsed with gently running water.

5. Light trap sampling

Although insects have good color vision, they do not see the same wavelengths of light as humans. The human eye can typically distinguish colors ranging from red (760 nm [nanometers]) to purple (380 nm), while it has been shown that most insects see colors from yellow / green (550 nm) to ultraviolet [UV] (340 nm). Since many nocturnal insects are attracted to UV, trapping or killing devices have been developed that use UV bulbs to attract insects either to a collection container (we call these light traps) or to an electric grid (we call these bug zappers). Because insects do not perceive light in the red to yellow range, we use yellow bug lights outdoors to reduce the number of insects attracted to areas around our homes. Since most humans see relatively well under yellow light, these bulbs allow us to see while not attracting many insects.

A simple light trap design is illustrated below. It's construction is also described. Traps can be built either as single units or as a series of lights that can be used to allow comparison among bulbs of different colors, from several manufacturers, or having different wattage.

The main parts of these traps consist of recycled plastic bottles (see below). The upper part of the trap is made from a wide-mouth 0.95 L (32 oz) drink bottle that houses the light bulb and serves as a baffle that insects strike as they fly toward the light causing them to fall into the trap. To make this part of the trap, remove the bottom of the bottle so that the sides end in a vertical edge. Open four 0.6 cm (¼ in) heat vents about 2 cm (3/4 in) below the molded lip beneath the cap threads, and two additional 0.6 (¼ in) holes on opposite sides of the lower end of the bottle approximately 2.5 cm (1 in) above the cut where the bottom was removed.



There are two alternatives to wiring the traps. Single traps can be constructed by placing a socket with a plug-base (as in the illustration above) into the mouth of the bottle. Depending on the type of bottle used, these may need a layer of duct tape wrapped around them so that they fit snugly in the opening. This type socket can be plugged directly into an extension cord to power the light trap, or into a screw-in plug that can be placed in an existing light fixture. A string of multiple traps can be created using sockets with a base that tightens over two pieces of 14- or 12-gauge stranded copper wire. These sockets have prongs that pierce the wire insulation as the base of the socket is tightened, eliminating the need to make individual electrical connections. The base of the socket must be placed into the neck of the bottle before attaching it to the wires. A plug is placed on one end of the wires, and wire nuts covered with electrical tape placed on the other ends so that they do not short circuit. At a minimum, sockets should be placed every 3.6 to 4.6 m (12 to 15 ft) along the wires.

Below the baffle is a funnel made from a 3.78 L (1 gal) “milk” bottle. Bottles with a neck opening that fit snugly inside the mouth of a 1.89 L (32 oz) bottle, which will be used for the collection container, work best. The bottom of the 3.78 L bottle should be removed and two 0.6 cm (¼ in) holes made on opposite sides of the cut-off end of the bottle. To attach the funnel to the baffle, insert a 30.5 cm (1 ft) long piece of 0.5 cm (3/16 in) dowel through the holes in the open end of the baffle and place a rubber band over both ends of the dowel across the opening of the baffle. This will keep the dowel from shifting. Attach the funnel by placing the dowel through the 0.6 cm (¼ in) holes.

The collection jar is a wide-mouth 1.89 L (32 oz) bottle. The mouth of this bottle is placed over the mouth of the funnel and attached by placing two rubber bands under the lip below the cap threads and extending them to the ends of the dowel holding the funnel to the baffle. A killing and preserving solution of either 10% dish detergent solution or 75 to 80% ethanol should be placed into this bottle. If a soap solution is used, collected insects should be sorted the next day to prevent them from decaying. If that can not be done, the sample should be rinsed by pouring them into a strainer, and gently running water over it to rinse off the soap. Rinsed samples should be placed in 75 to 80% ethanol in a container with an air tight lid and held until the insects are sorted and counted.

When placing light traps in the area where the study will be conducted, the bottom of the traps should be about 1 m (3 ft) above the ground and arranged so that all traps are approximately equidistant from any major component of the surroundings, such as buildings or woods. If possible, it is best to place them away from other light sources, that would interfere with insect activity. Traps should be lighted at dusk and allowed to remain lighted all night.

a. Types of light trap studies

Light traps can be used to gather several different types of data. To conduct a general survey of nocturnal flying insects, one or more single traps can be used. The most effective bulbs to use for survey trapping would be 60W 'black light' bulbs, which are available at poster shops and some hardware stores. If these are unavailable, plant grow bulbs also have a coating that produces UV wavelengths. A simple soft-white or clear bulb also can be used, although they will not attract as many insects.

In addition to conducting general surveys, there are several experiments that can be conducted to determine the attractiveness of different light sources to insects. The effect of bulb color can be examined using a string of six traps housing 25W bulbs that are available in red, orange, yellow, green, blue, and clear. In this system, the red through blue bulbs provide individual segments of the color spectrum, while the clear bulb serves as a control as it produces a broad spectrum from a single bulb. The type of bulb can be examined using either four 40W or 60W bulbs that include a 'white' soft-white, 'yellow' bug-light, 'blue' plant grow bulb, and 'purple' black light bulb. The same type of bulbs produced by different manufacturers can be examined using multiple 40W or 60W 'yellow' bug lights. The effect of wattage can be examined using by using 25W, 40W, or 60W, bulbs of the same type (*i.e.*, all soft white or all clear) from the same manufacturer. **Note**: In tests we've done, 75W bulbs generate too much heat and will melt the 0.95 L bottle used as the baffle creating a fire hazard.

Since all incandescent bulbs produce some red wavelengths, students should determine the actual wavelengths produced by the bulbs used. This can be done using a diffraction grating such as the QA SPECTROSCOPE {Science Kit & Boreal Laboratories, cat. no. 16525} that has a nanometer scale that can be used to determine the range of wavelengths produced by each bulb. Students will find that most bulbs produce a wider range of wavelengths than they would expect from the bulb color they see. By selecting the appropriate set of bulbs, this activity can be used to link a life science study with either the physical characteristics of light, or consumer education by comparing the level of attractiveness of bulbs from various manufacturers.

6. Pitfall trap sampling

Pitfall traps are a survey technique used to assess populations of insects and other arthropods living on the ground surface. Typically, traps are set up and allowed to collect arthropods for a minimum of 24 hours. The basic trap consists of a container set into the ground so that the upper rim is flush with the ground surface (see below). Since many insects are predators and will eat other insects in the trap, a solution to kill and preserve insects entering the trap is used. If traps will be active for a single trapping period of a day or two, the container may serve as the trap with the preservative placed directly into it. However, if samples will be taken

repeatedly, it saves time to place a container in the ground that will serve as a trap liner, with a second, easily removable, container placed into the first to hold the preserving solution. The removable container must fit snugly in the rim of the trap liner to ensure that all insects entering the trap enter the collection container. An inexpensive trap system uses 0.9 L (16 oz) plastic drink cups for the trap liner (left cup in figure), and 0.53 L (9 oz) cups (right cup in figure) for the collecting containers. **Note**: Dart® brand cups fit together the best. When placing trap liners in the ground it is helpful to place a second container inside the first so that any soil falling into the liner while backfilling around the liner can be easily removed.

If there is a chance that it will rain during the trapping period, a simple cover can be made out of a disposable styrofoam or plastic plate. The plate is held above the cup rim by inserting three scaffolding nails (these have double heads so the plate can be placed between the two heads to keep it in place) into the outer rim of the plate and pushing the nails into the soil so that the plate is 3 to 5 cm (7.6 to 12.7 in) above the cup rim and soil surface.

The most commonly used preservative solutions are ethylene glycol, propylene glycol, ethanol, or soap solutions, each of which has advantages and disadvantages. For traps set up to collect continuously for periods of more than 3 to 4 days, either ethylene glycol or propylene glycol work well due to their slow evaporation rates. Ethanol (75 to 80%) is an excellent preserving solution but evaporates relatively quickly, and so only works well if traps are active for 1 to 3 days. If handled incorrectly, all three of these materials also can be hazardous to both students and the environment. For studies where traps will only be active for 1 to 2 days, a 10% soap solution works well. The main drawback to using a soap solution is that the high percentage of water allows collected insects to deteriorate relatively quickly. Because of this, if samples will not be sorted immediately after traps are emptied they should be rinsed by pouring them into a strainer, and gently running water over them. Rinsed samples should be placed in 75 to 80% ethanol an airtight container until specimens are sorted and counted.

To conduct a pitfall trap sampling study, traps should be placed in all representative habitats in the area to be studied. In order to reduce variation among sites and traps, it is best to place 3 to 4 traps in each habitat type. After traps have been active for a predetermined period of time, the collected material should be returned to the classroom and specimens identified and counted.

7. Aquatic sampling

CAUTION: Since bacteriological pollution can not be detected visually, it is very important to remember to wash your hands after working in any stream. Likewise, if you scrape or cut yourself while working in a stream you should clean the wound with peroxide.

Sampling aquatic insects can be accomplished using a variety of techniques. Most adult insects found in aquatic habitats live primarily on the surface, while the majority of immature insects in these habitats live underwater. Sampling techniques range from simply looking under submerged objects (*i.e.*, rocks or logs), using different types of nets, to a variety of passive samplers. Sorting samples is usually easier if you place the sample in a white pan or on a white plastic sheet (a white trash can liner works well) so that you can more easily see the specimens. If you are only interested is collecting some aquatic organisms to observe, samples can be taken from any portion of the habitat. However, if you are going to conduct a quantitative survey of a stream or pond, samples should be taken in all representative parts of the habitat.

a. Sampling surface dwelling aquatic insects

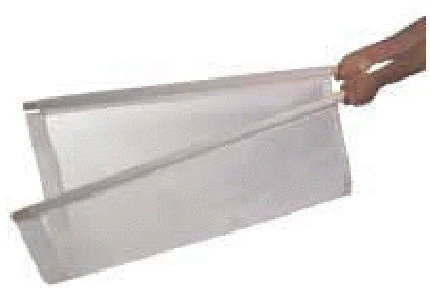
A long handled aquatic net is the best way to collect insects that live on the water surface. However, a standard (aerial) insect net can be used for this although the length of the handle will restrict how far you can reach. To use an aerial net, you will need to tie the end of the net bag to the handle so that the net forms a shallow bowl rather than a full sized net. This allows it to move faster through the water when capturing insects. Many adult insects living on the surface are predators and will bite if handled, so they should be removed from the net using forceps or scooped directly into a collection container.

b. Sampling from submerged objects

This is done by simply lifting objects, such as rocks or branches, out of the water and visually examining them for aquatic arthropods. If the insects are large they are generally easy to spot, although smaller ones may be easier to see if you wait a minute or two until they begin to move. Some, like water pennies (beetle larvae) are cryptic and do not move and require close observation to detect.

c. Kick-nets

This type of net consists of a piece of fine mesh netting stretched between two poles (1 m - 1.5 m in length) (at right). The ends of the poles are placed against the bottom of the stream, and the stream bottom upstream of the net is disturbed by “kicking” (actually any sort of rough disturbance will work) objects and debris on the bottom with your feet. The organisms that are dislodged by the disturbance will flow downstream and collect on the net. The net can then be take to the shore and the material placed into a white pan and sorted.



d. D-nets

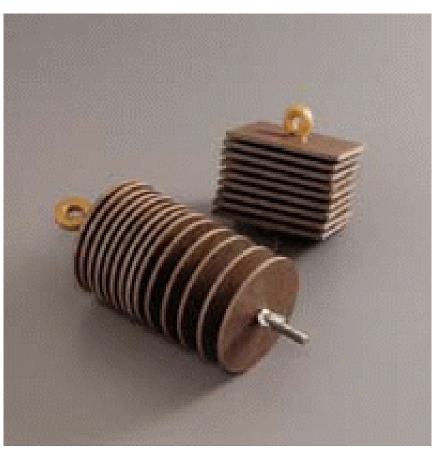
These nets have a opening with a flat side (the D part) (at left). They can be used

like a kick-net, with the flat side placed on the stream bottom so that it collects organisms dislodged when submerged objects upstream are disturbed. It also can be used more like a dip net and scraped along submerged objects to dislodge and collect organisms, or the flat side can be pulled across the bottom sediments of a pond or stream to sample organisms in that habitat.

e. Passive samplers

There are several types of samplers that can be placed into a stream or pond that can be used to sample those aquatic organisms that will colonize submerged objects. For all of the samplers described below, once constructed they should be attached to a thin nylon line and submerged in the stream or pond you wish to sample. Since it takes some time for the organisms to colonize these samplers, they should be left in place for 4 - 8 weeks before they are removed and examined. Samplers should be checked following any storm events to be sure that they have not been washed onto the bank by flooding. If you are using these samplers to conduct a survey, all samplers of a given type should be the same size so that the data can be easily quantified.

A Hester-Dendy sampler can be made by drilling a hole in the centers of four to six, 7.5 cm [3"] - 10.2 cm [4"] pieces of 0.64 cm [1/4"] thick Masonite (see at left). An eye bolt is placed through the holes with each piece separated from the next by a nut to create a space between adjacent plates. After the sampling period, the organisms



can be removed in the field or after they are returned to the lab. To remove organisms, remove the individual plates from the eye bolt and remove all visible organisms using forceps or your fingers and place them in a white sorting pan. Place the individual plates over a fine mesh sieve (a large diameter flour sifter works well) and gently brush them with an old toothbrush to dislodge any organisms that were not seen previously. Add these to the ones in the sorting pan. Organisms can be identified in the field, their numbers recorded, and they can be released, or they can be placed in a vial of 80% EtOH (ethyl alcohol) and returned to the classroom for identification and counting.

A similar sampler can be made by tying several pieces of tree limb / branches together and placing them in the water. In order to quantify this data, each sampler should have the same number and size pieces of branches. Removal of organisms is the same as for the Hester-Dendy sampler.

Leaf pack samplers can be made by using 1.27 cm [½ in] mesh screening to construct a box to hold leaf material. There is no standard size, but generally a box about 15.25 cm [6 in] square and 10.2 cm [4 in] deep will hold plenty of leaf material. All joints in the box, except the lid, should be securely fastened with wire or soldered. After loosely filling the box with leaf material the lid should be wired shut. At the end of the sampling period, organisms are collected by opening the screen box and placing a few leaves at a time into a large, fine mesh, kitchen sieve. With the sieve partly submerged, pick up individual leaves and vigorously swish them in the water in the sieve. Then visually examine each leaf and remove any remaining organisms before discarding. After each set of leaves is swished and examined, place the organisms into a white pan for identification and counting.

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Other activities can be found at http://entweb.clemson.edu under K-12 Education / Insects in the Classroom