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Locomotor Responses of Flesh Fly Larvae to Light

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This exercise provides students an introduction to the scientific process, animal behavior and the basic principles of scientific writing. Flesh fly (*Sarcophaga bullata*) larvae are used to investigate locomotor behavior to light. Students are introduced to the biology of flesh flies and based on this information, formulate predictions and hypotheses with respect to the locomotor behavior of the larvae to white light and different wavelengths of light. Students use the Chi-squared goodness of fit statistic to test their hypotheses. This lab exercise has been used in general biology courses for majors and non majors, employing both traditional and investigative approaches.

Keywords: animal behavior, flesh flies, *Sarcophaga bullata*, scientific process

Introduction

The major objectives of this exercise are to provide students an introduction as to "how scientists do science", an introduction to the study of animal behavior and the basic principles of scientific writing. Flesh fly (*Sarcophaga bullata*) larvae are used in this exercise to investigate their locomotor behavior with respect to light. This exercise is designed for two, three hour lab periods. During the first lab period students are introduced to the biology of flesh flies including their life cycle, morphology, and basic physiology using both living and preserved specimens. The use of flesh flies in forensics to estimate the time of death is also discussed. Based on information provided by the instructor as well as that obtained by the students via print and/or the Internet, the students are asked to formulate predictions and hypotheses (null and experimental) as to the locomotor response of the larvae to the presence or absence of white light. To test their hypotheses, the students carry out a series of controlled experiments in which the larvae are placed in the center of a Plexiglas "racetrack" lined with moist toweling. After a 3 minute time period, the number of larvae found on either side of the center line and at the end of the racetrack are recorded under two conditions: (1) when the apparatus is covered with a light-tight box and (2) when half of the race track is covered with a piece of aluminum foil and the other end of the race track is illuminated by a high intensity light source. Using the same apparatus, the students are required to design experiments which allow them to determine how the locomotor response of the flesh fly larvae varies with respect to four different wavelengths of light. During the second lab period students analyze and graph their data. They are introduced to the Chi-squared Goodness of Fit Test and calculate chi squared values for their data using an Excel based "Chi-squared calculator". This lab exercise has been used in an organismal biology course for majors and in a general biology course for non majors, employing both traditional and investigative approaches.

Student Outline

Introduction

One of the basic characteristics of life is the capacity for response to environmental change. An organism reacts to a specific change in its environment, a **stimulus**, by carrying out some activity that we call a **response**. The repertoire of responses that characterizes an organism is called its **behavior**. Living organisms have evolved a remarkable diversity of behaviors, which for convenience of discussion, we will group into two categories: **learned behavior**, that array of responses acquired by an organism in the course of its experience and **inherited** or **innate** behavior, which is a part of the organism's genetic endowment.

Animal behaviorists (biologists who study animal behavior) investigate what animals do, and how and why they do it. They are interested in accurate observation and description of an animal's behavior, in the mechanisms and programming which underlie it, but most importantly, in the reasons an animal behaves as it does. This approach emphasizes the evolutionary and adaptive values of a behavior to the species. The study of animal behavior relies on observation and experimentation in both the animal's natural environment and the laboratory.

Even the mere detailed description of the behavior of an animal is a difficult task not only because it requires incredibly careful observation over a long period of time, but also because our language lacks adequate words to accurately convey what has been seen. Almost all words have some sort of human connotation, imply some type of human motivation and purpose, all of which may well be irrelevant to the behavior of other animal and we must constantly guard against unwarranted attribution of human characteristics to nonhuman beings and things (**anthropomorphism**). For example, an anthropomorphic explanation of why a bird sings on a beautiful morning might be because it is happy. An animal behaviorist's explanation of the same event might be that the bird is singing to communicate two things to other members of the population: availability for mating and territorial occupation. In practice it is often very difficult for anyone to absolutely avoid the appearance of anthropomorphism when describing animal behavior.

Although it is extremely important to observe the behavior of animals in their natural environment, a great deal can also be learned about their behavior when studied under laboratory conditions. The experimental environment can be controlled very precisely and this allows the experimenter to vary only the conditions which are to be studied and to keep all other factors constant. Thus results from such an experiment can be argued to be due to the factor under study.

In this laboratory you will investigate the locomotor behavior (movement from one place to another) of *Sarcophaga bullata* with respect to the presence and absence of light and to different (colors) wavelengths of light. The locomotor response of *Sarcophaga bullata* to environmental stimuli is an innate (inherited) behavior in which the stimulus appears to trigger a fixed response that does not vary according to the previous experience of the organism. This type of innate locomotor response is most commonly found among invertebrate animals (e.g. protozoa, worms, insects, crustaceans) whereas learned types of behavior are more frequently found among vertebrates (animals with backbones - fish, amphibians, reptiles, birds, and mammals).

Innate locomotor responses can be described by two terms, **taxis** and **kinesis**. A **taxis** is an automatic movement **directly** toward or away from the stimulus. For example, a moth flying toward a light is a classic example of a taxis (in this case, a phototaxis - one caused by light). If it can be demonstrated that a cockroach actually avoids light by moving directly away from it, this behavior can also be described as a phototaxis. A taxis is said to be positive if the movement is toward the stimulus, negative if away from it. In the above examples, the moth would be described as exhibiting a positive phototaxis and the cockroach a negative phototaxis.

A **kinesis**, on the other hand, is **random** movement, caused by a stimulus but not necessarily oriented by it. For example, it is observed that a shrimp selects a dark habitat in preference to a lighted one. If it can be demonstrated that light initiates random movements of the shrimp which eventually, by chance, carries it into the darkness where the movement stops, this sort of behavior would be called a photokinesis. A kinesis is said to be positive if the movement is toward the stimulus, negative if away from it. In the above example the shrimp would be described as exhibiting a negative photokinesis.

As already noted, a prefix is added to the terms kinesis or taxis to describe the nature of the stimulus. Some common stimuli include:

photo = reaction to light - note that darkness is NOT the stimulus; darkness is simply the absence of light.
thermo = reaction to temperature
hygro (hydro) = reaction to moisture or humidity
thigmo = reaction to contact (touch)

Note: It is often difficult to be certain whether the response of the animal is a kinesis or taxis. In these cases you simply state that the animal displayed a negative or positive response to the stimulus in question. For example, if it is unclear whether an insect's movement toward an area of high humidity is a taxis or kinesis, you would simply state that the insect displayed a hygro-positive behavior.

Scientific Investigations

The general approach of scientists, including biologists, is known as the **scientific method**. By its use, scientists strive to collect information through systematic observation coupled with equally systematic testing. So much has been said about the powers of the scientific method that many suspect it involves some formula too complicated for ordinary people to understand. It does not. The scientific method is simple, and is used to some extent by almost everyone every day. As the English biologist T. H. Huxley (1825-1895) put it, the scientific method is nothing but trained and organized common sense (Huxley 1900).

Even among scientists, however, there is wide disagreement as to what is meant by the "scientific method." Some textbooks list a series of six or seven steps involved in the scientific method. Such a formal and highly structured description is unrealistic and no research scientist follows any such formalized ritual in performing his experiments. Most scientists will readily admit that every stage in the scientific process requires not just careful thought but a large measure of intuition and good luck.

1. Observation and Questions

Scientific investigations generally begin with **observations** about some particular occurrence. Once observations have been made, **questions** are formulated.

2. Hypothesis Formulation

Once the question has been identified it is possible to formulate **hypotheses**, which are simply tentative explanations **based on existing observations or data** that are put forth to account for observed phenomena. Hypotheses must be (1) **testable**; there must be some way to check the validity of the idea and (2) **falsifiable**; their must be some observation or experiment that could refute the idea if such an idea is actually not true. Although a key predictive test may demonstrate that a hypothesis cannot be true or may indicate that it must be modified, such a test can never definitively prove, once and for all, that the hypothesis is true - simply because we can never be certain that we have examined all of the relevant evidence. However, repeated successful tests of a hypothesis provide strong evidence in favor of the hypothesis. Objective scientific knowledge is built up gradually, with emphasis on repeatability of experimental results and a constant review and revision of the past conclusions that have been drawn. All conclusions are constantly subject to review and replacement by new ones that better explain the observations.

Biologists often need to compare a distribution of observed data with an expected distribution in order to test a hypothesis. For example, a biologist may have observed that honeybees seem to visit red clover flowers more often than white clover flowers. The biologist might hypothesize that bees have a preference for one color of clover flower over another. To test this hypothesis, the biologist could put individual bees into experimental chambers containing an equal number of randomly arranged red and white clover flowers, and record the colors of the flowers visited. The **null hypothesis** for this experiment would be that the bees have no preference. This would be the null hypothesis because it is the logical alternative to what the biologist expects to happen. The **experimental hypothesis** would be that bees do have a preference because that is what the biologist expects to happen, based on previous observations. An approximately equal number of visits to flowers of both colors, an indication that the bees are randomly visiting flowers, would cause the biologist to accept this null hypothesis and reject the experimental hypothesis. Consistent visits to flowers of one color versus the other would cause the biologist to reject the null hypothesis and accept the experimental hypothesis. The conclusion would be that bees prefer red flowers, for example, if a greater number of bees visit red flowers than visit white flowers.

When should the biologist reject or accept the null hypothesis? It is unlikely, due to chance alone, that an exactly equal number of visits to both flower colors will be the outcome of the experiment every time it is conducted. This is true even if the null hypothesis that bees have no preference is correct. Because of this, an objective basis of deciding which hypothesis to accept or reject is required.

3. Experimentation

Controlled experiments are performed to test alternative hypotheses. A controlled experiment consists of two randomly selected groups of organisms, the **experimental group** and the **control group**. The control group is treated exactly the same as the experimental group except in **one respect** (i.e., the factor [variable] being studied). The control group provides the standard against which changes in the experimental group are measured.

4. Statistical Analysis

Statistics is a branch of mathematics (probability theory) that helps the research biologist in three ways. First, statistics are used to guide the experimenter in the most efficient and unbiased way to set up a group of experiments, i.e., the **experimental design**. Second, statistics are helpful in summarizing data, especially data sampled from a population (e.g. mean, variance, standard deviation, etc.). A third use of statistics offers standardized mathematical methods of determining whether the results from an experiment will allow the experimenter to accept or reject a hypothesis. This is often referred to as **hypothesis testing**.

5. Publication

When a scientist has collected sufficient data to support a particular hypothesis, he or she then reports the results to other scientists, usually at a scientific meeting or in a scientific publication (journal). If the data are sufficiently interesting or the hypothesis important, the observations or experiments will be repeated in an attempt to confirm, deny, or extend them. Hence, scientists always report the methods that they used in gathering and analyzing data as well as their conclusions.

Classification of Sarcophaga bullata*:*

In this exercise you will study the behavior of *Sarcophaga bullata*, or flesh flies, which can be classified as follows:

Kingdom: Animalia

Phylum: Arthropoda. Invertebrates having a hard **exoskeleton** composed of chitin and **jointed appendages** includes the spiders, insects, crustaceans, centipedes, millipedes, horseshoe crabs, etc.

Class: Arachnida. Body divided into cephalothorax (head fused to thorax) and abdomen; four pairs (total = 8) of legs attached to cephalothorax ¬includes scorpions, spiders, mites, and ticks

Class: Crustacea. Crabs, shrimp, lobsters, crayfish, and woodlice

Class: Insecta. Body with distinct **head, thorax,** and **abdomen**; thorax normally with two pairs of wings and six legs includes butterflies, beetles, bees, grasshoppers, etc.

Order: Diptera - **True Flies.** Possess only **one pair of wings** (front wings); the hind wings are reduced to small knobbed structures called halters

Family: Calliphoridae - Blow Flies = Blue and Green Bottle Flies

Family: Sarcophagidae - **Flesh Flies**

Genus: *Sarcophaga*

Species: *Sarcophaga bullata*

Note: The species of an organism consists of a two part name, the genus and the specific epithet. For example, the species of flesh fly being used in these experiments is *Sarcophaga bullata* (genus = *Sarcophaga*; specific epithet = *bullata*). Often textbooks will list the specific epithet as the species of the organism. This is incorrect. For example, the species of the flesh fly is **not** simply *bullata*, rather *Sarcophaga bullata*.

Biology of Flesh Flies

1. Flies

Insects (Class Insecta) are the largest group of arthropods whether measured in terms of number of species or numbers of individuals. More than 70% of all the named animal species are insects. There are about 90,000 species in the United States and Canada. Insects live in nearly every possible habitat on earth including land, fresh water, and the oceans from the tropics to the arctic. The true flies (Order Diptera) constitute one of the largest orders of insects. The true flies can be distinguished from other fly-like insects (e.g. dragonflies, stoneflies, etc.) by the fact that they possess only **one pair of functional wings** (= the front wings) and the hind wings are reduced to small knob-like structures called halteres. Some flies are serious pests of humans and animals (e.g., mosquitoes, black flies, horse flies) whereas many of the blood sucking flies, and some of the scavenging flies such as the house flies and blow flies, are important carriers of disease. The microorganisms causing such diseases as yellow fever, sleeping sickness and dysentery are carried by other species of flies. In addition, many flies are scavengers, others important predators or parasites of various insect pests, and still others aid in the pollination of useful plants.

2. Life Cycle of Flesh Flies

All insects, including flesh flies, go through a series of changes (metamorphosis) that convert an immature insect into the adult form (Fig. 1). Flesh flies, like all true flies, go through the process called **complete metamorphosis** in which they pass through the following four stages in the order listed: **egg, larva (= maggot), pupa, and adult**. The adult male flesh fly deposits its sperm into the female's reproductive tract during copulation. The fertilized eggs remain in the female's reproductive tract where they develop into tiny, worm-like larvae. The female flesh fly deposits the living larvae (maggots) in decaying flesh of dead animals or feces. As is the case with most insect larvae, the flesh fly larvae are specialized for feeding and growth. The larvae molt (shed their exoskeleton) several times and grow much larger. Finally, after they have completed their larval development, they enter an inactive stage called the **pupa**. During the pupal stage most of the old larval tissues are destroyed and new adult tissues and organs develop from small discs of cells that were present in the larva but never underwent much development. Once this tissue development is complete, the adult flesh fly emerges from the pupa case. The life cycle from egg to adult takes about 20 days. The larval and pupal stages of the flesh fly life cycle are when most growth and development occurs. The adult stage is specialized for active **dispersal** and **reproduction**.

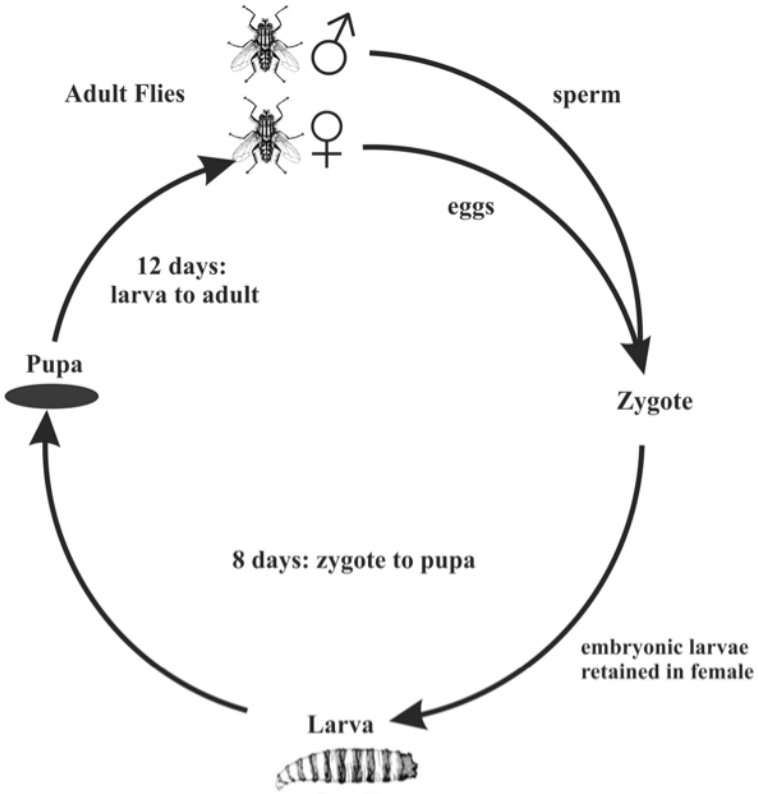

Figure 1. Life Cycle of *Sarcophaga bullata*

3. Biology of Flesh Fly Larvae

Fly larvae can be distinguished from those of all other orders of insects by the lack of any jointed appendages on the thorax, by their slender shape, and active directional movement.

The mouthparts of flesh fly larvae consist of an oral (mouth cavity) containing hook-like structures called **oral hooks** which aid the larvae in moving food (carrion) into the mouth cavity. The larvae have a gas exchange system known as a **tracheal system**. The air enters through small openings in the body wall known as **spiracles**, and is carried from the spiracles by a system of branching tubules called **tracheae.** The tracheae branch repeatedly until the finest tubules reach individual cells of groups of cells. At the end of the tracheae, are the moist cell membranes across which respiratory gas exchange occurs. The blood (called hemolymph) of flesh fly larvae is composed of liquid plasma and several different types of blood cells. The blood does not transport oxygen.

4. Biology of Adult Flesh Flies

Adult flesh flies are segmented animals with a rigid exoskeleton and jointed limbs. The fly body is divided into 3 sections, the first being the **head**, the second the **thorax,** and the third the **abdomen**. The well developed **compound eyes** are located on the side of the head. Each compound eye is composed of thousands of individual units which can detect light but also have lenses that focus light and form images. Two large **antennae** project from the head and function as sense organs. They are sensitive to touch, smell, chemicals, moisture, and temperature. The mouthparts of the adult flies consist of an extendible beak-like structure which liquid food is sucked. The thorax consists of three segments, each having a pair of legs. The thorax is almost entirely filled with muscles that operate the legs and wings. The adult flies as well as the larvae have a gas exchange system known as a **tracheal system**. The air enters through small openings in the body wall known as **spiracles**, and is carried from the spiracles by a system of branching tubules called **tracheae**. The tracheae branch repeatedly until the finest tubules reach individual cells of groups of cells. At the end of the tracheae are the moist cell membranes across which respiratory gas exchange occurs. The blood (called hemolymph) of flesh fly adults and larvae is composed of liquid plasma and several different types of blood cells. The blood does NOT transport oxygen.

Light as a Stimulus

Light is a type of energy known as electromagnetic energy (radiation). Electromagnetic radiation travels in waves. The distance between the crests of electromagnetic waves is termed the wavelength and is measured in nanometers. The entire range of radiation is known as the electromagnetic spectrum. Each type of radiation in this spectrum has a characteristic wavelength and energy content. These two characteristics are inversely related; i.e. the longer the wavelength, the lower the energy content. The portion of the spectrum which is detected as various colors by the human eye is referred to as visible light and ranges from about 400 to 700 nanometers. White light is composed of all wavelengths (colors) in the visible light spectrum (Fig. 2).

Humans see colors because objects contain pigments that selectively absorb some wavelengths of visible light and reflect or transmit others. What we recognize as an object's color is composed only of those wavelengths of light that are **transmitted** or **reflected**. If a pigment absorbs all wavelengths, it appears black.

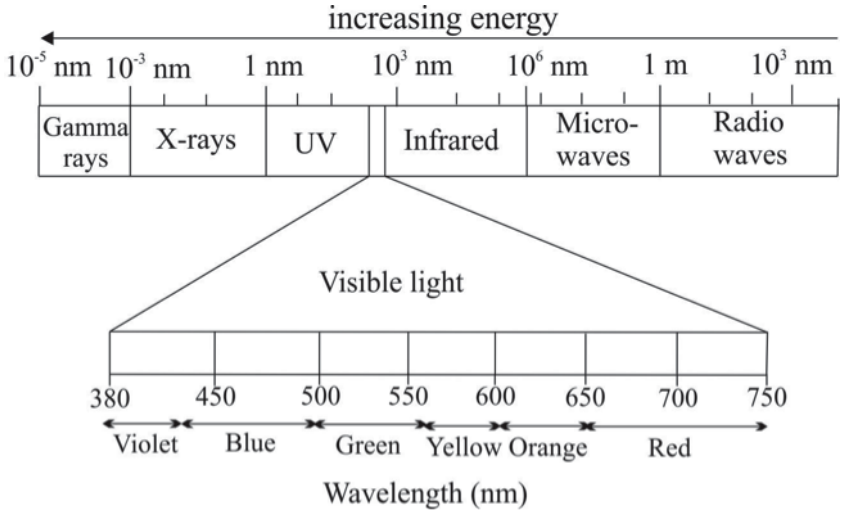

Figure 2. Electromagnetic Spectrum

Procedure

Observation of the Stages of Sarcophaga bullata *Life Cycle*

1. Adults

   Obtain a petri dish containing a freeze dried adult flesh fly and observe it under a stereomicroscope. Make a labeled sketch of an adult flesh fly.

   Body Divisions: head, thorax segments, abdominal segments
   Compound eyes
   Mouth parts
   Wings
   Exoskeleton
   Jointed appendages = legs, antennae, and bristles

2. Larvae

   Place a living larva in a small plastic petri dish and observe it under a stereomicroscope. To increase contrast between the specimen and the microscope stage, use the piece of black plastic as a background on the microscope stage. If you need to move the larva to see a particular structure, use the small paint brush provided. **Be careful not to damage the specimen**. Make a sketch of the living larva. Identify and label the following structures:

   Anterior (= front)
   Posterior = (rear)
   Dorsal (= top/back) top surface
   Ventral (= bottom/belly) surface

Head region
Oral hooks = hook like structures within the oral (mouth) cavity.
Spiracles = yellow structures located both on the side of the head and also sunken in the cavity at the posterior end of the larvae
Tracheal tubes = tiny, transparent tubes within the body of the larvae. Note: These tubes may be very difficult to see unless the light illuminates the larva at just the right angle.
Partially digested food in digestive tract = visible as a black sac-like structure just below the skin at the anterior end of the larva. Contractions of the digestive tract are often visible.

3. Larva Dissection (optional)

Using a pair of fine dissecting scissors make an incision on the dorsal portion of the head and cut toward the posterior end of the larva. Place the dissection under a stereomicroscope and using a pair of fine tipped forceps, expose the tissues of the larva. You should be able to observe the digestive tract and the masses of very fine tracheal tubes of the respiratory system.

4. Pupae

Obtain a petri dish containing a flesh fly pupa and observe it under a stereomicroscope. Make a sketch of the pupa.

5. Demonstration

Observe the living larvae, pupae, and adult flesh flies on display in the insect cage. Note the larvae feeding on the decaying liver!

Laboratory Experiments

1. General guidelines for experiments

Each pair of students will carry out a series of experiments to test the locomotor response of *Sarcophaga bullata* larvae to the presence or absence of white light and to different wavelengths of light. The following guidelines must be followed:

- ***Handle the larvae with extreme care!*** Use a camel's hair brush to transfer the larva from the culture (the container labeled "**Experimental Larvae**") to a small plastic cup for transporting. When you have completed the experiments return the larvae to the container marked "**Used Larvae**" on the instructor's desk. **At the end of the experiment, return the cups to the instructor's desk.**
- Observations of larval behavior should be recorded during the experiments and reported as data in your lab report.
- ***Use the same 10 larvae in all experiments. If a larva is lost or stops responding during one experiment, replace it with a new one.***

2. Formulation of Hypotheses - Locomotor Response of Larvae to Presence or Absence of White Light

Based on your limited knowledge of the biology of flesh fly larvae (refer to introductory material at the beginning of the lab outline), formulate a null and experimental hypothesis concerning the locomotor response of flesh fly larvae to the presence or absence of white light. Write the following information on a 3x5 card and turn it into your instructor at the end of lab. Be sure to put your name on the card.

- Null hypothesis:
- Experimental hypothesis:
- If the null hypothesis is accepted, then you would predict that:

- If the null hypothesis is rejected, then you would predict that:

3. Control Procedure: (Work in Pairs)

a. Moisten a strip of cloth towel (available at the TA desk) by wetting under the faucet, then squeezing to remove excess water. Place the cloth strip in the plastic racetrack so that its surface is evenly covered (Fig. 3). Keep the cloth strip moist by wetting between experiments.

b. Place a moist piece of filter paper in the bottom of two cups and place one cup **at each end** of the racetrack. These cups will serve to catch any larvae which reach the end of the track.

c. Place **10** larvae in the center of the racetrack. The center is marked by a line on the top sides of the racetrack.

d. Place a light-tight box over the apparatus

e. **After 3 minutes** record the number of larvae found on either side of the center line and in the cups. If an animal is directly on the center line, flip a coin to determine on which side to score it. Remember to include those larvae on each side of the center line which ended up in the cups! **Record your pair data in Table 1 and your class data in Table 2**.

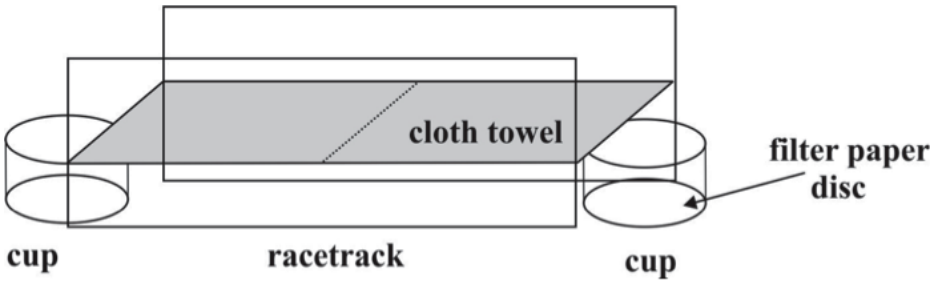

Figure 3. Racetrack: Control Set-up

4. Experimental Procedure (Work in Pairs)

a. Set up the racetrack as outlined in steps 1 and 2 above.

b. Cover one half of the racetrack with a piece of aluminum foil so as to make the track as light tight as possible without interfering with the movement of the larvae on the track.

c. Cover the face of a high intensity light source with a white filter. Place a light source at one end of the racetrack (Fig. 4). Note: The white filter changes the light intensity but **not** the light quality (wavelength).

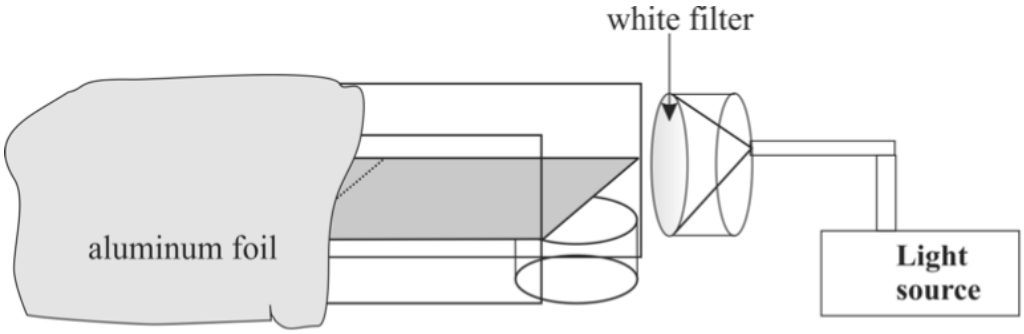

Figure 4. Racetrack: Experimental Set-Up

d. Turn the light source ON to the **low power** setting. Align the light source so that the face is level with the surface of the track and the light is directed straight down the track. Adjust the distance between the light source and the track so that the light intensity measured at the **center** of the track (marked with a black line) is approximately **9 foot candles** (fc).

NOTE: To operate the digital light meter, (1) place the sensor head facing the light source. (2) push the on/off switch downward to the 199.9 position on the gray foot candle (fc) scale, (3) make sure the response time (fast/slow) switch is set to the FAST position on the gray scale, (4) the light intensity to the nearest 0.1 fc will be shown on the LCD.

e. Measure the light intensity at the center of the track *and* at the lighted end of the track record the values in Table I in the row labeled "Trial 1".

f. Place **10** larvae in the center of the track. The center is marked by a line on the top sides of the racetrack.

g. After **3 minutes** record the number of larvae found on either side of the center line and in the cups. If an animal is directly on the center line, flip a coin to determine on which side to score it.

h. Record the number of larvae which ended up on the high light side of the race track and the number which ended up on the no light side of the race track. Remember to include those larvae on each side of the center line which ended up in the cups! **Record your data in Table 3**.

i. During the 3-minute trial, **observe and record your observations of the locomotor behavior of the larvae. Do they show a kinesis or taxis to light and is this response positive or negative?**

j. At the **end** of the 3 minute trial, using the electronic thermistors provided, record the **surface** temperature (°C) at both ends of the race track. **Record these values in Table 3**.

k. Run another **two**, 3 minute trials, using the same 10 larvae. For each trial record the number of larvae which end up on the light end of the racetrack and the # of larvae which ended up on the no light end of the racetrack. At the end of each run r***ecord the light intensity (fc) at both ends of the racetrack at the end of each trial to the light intensity is 9 fc at the center of the racetrack before running the experiment, to record the surface temperature (°C) and light intensity (fc) at both ends of the racetrack at the end of each trial***. **Record all data in Table 1.**

l. Record your **pair data** in Table 3 and the **class data** in Table 4.

5. Locomotor Response to Different Wavelengths (colors) of Light Procedure: (work in pairs)

a. Using the equipment provided, carry out experiments which will allow you to determine how the locomotor response of the flesh fly larvae varies with respect to four different wavelengths (colors) of light. For example, is the response of the larvae the same for all wavelengths of light? Experiments testing the response of the larvae at different wavelengths should result in data that provides insight into the wavelengths that provoke the stronger positive or negative locomotor response.

b. There is no "correct" experimental design. **However, those experiments with the best experimental design will be the ones which produce data that will allow you to accept or reject your hypothesis and are repeatable.** You will accept or reject your hypothesis based on the data collected, NOT on what you think should have happened. Your TA will lead the class in a discussion as to the best experimental design.

c. General Guidelines:

- Before starting the experiments, consider the question you are asking and formulate your hypotheses - both null and experimental. ***Write down the hypotheses to this second experiment on the same 3x5 card used for the light/no light experiment. Turn the card into your TA at the end of lab. Be sure to put your name on the card.***
- Use the same 10 larvae as were used in the light/no light experiments.
- Replace any larvae which are lost or stop responding during the experiments with new larvae.
- Keep the cloth towel moist at all times.
- In all experiments, maintain the light intensity at the **center** of the racetrack approximately 9 fc by regulating the light intensity setting on the light source and the distance the light source is from the center of the race track. **Record the exact light intensity at the center of the racetrack used for each experiment.**
- In all experiments, the light intensity at both ends of the racetrack should be relatively similar. Why? Record these values for each experiment.
- Record the temperature at the center and at both ends of the racetrack for each experiment.
- Be sure your experimental approach includes the appropriate controls and that you minimize the effects of other variables or stimuli.
- Record your observations of the larval locomotor behavior during the experiments.
- Run **3**, 3 minute trials for each experiment. The number of trials you complete will be based on the amount of time you have to complete the experiment. The more trials you are able to run will increase the precision of your results.
- Record your pair data in **Table 5** and the class data in **Table 6**.

6. Assignment

Next week in lab you will be analyzing and graphing the data which you collect in this week's lab. These results will be the basis for a formal lab report worth 30 points that you will submit in three weeks. Therefore it is essential that you begin to search library resources for information concerning the biology of flesh flies. This information will be required for the Introduction section of your report as well as the Discussion section. You should focus on finding the answers to the following questions:

a. Where are flesh flies found in the "wild"?

b. The importance of flesh fly larvae to humans.

c. How are flesh fly larvae used in forensics?

d. Can flesh fly larvae detect light through specialized cells or organs?

e. Is the rate at which flesh fly larvae develop influenced by environmental factors such as light and temperature?

f. What is the diet of flesh fly larvae? Does the diet of members of the genus *Sarcophaga* vary? If yes, describe their diets.

g. Are *Sarcophaga* larvae parasitized and preyed upon by other insects?

Table 1. Locomotor Response of *Sarcophaga bullata* Larvae to No Light Gradient (Control): **Pair Data**

Trial	# larvae on left side of center line	# larvae on right side of center line	Temperature (°C) left side	right side
1				
2				
3				
Total			χ^2 =	χ^2 =
%				

Table 2. Locomotor Response of *Sarcophaga bullata* Larvae to No Light Gradient (Control): **Class Data**

Student Pair	**Left End** of Racetrack			**Right End** of Racetrack		
	# larvae	% larvae	χ^2 temp. (°C)	#larvae	% larvae	χ^2 temp. (°C)
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
Total						
Range						

Table 3. Locomotor Response of *Sarcophaga bullata* Larvae to Presence or Absence of White Light: **Pair Data**

Trial	# larvae on left side of center line	# larvae on right side of center line	Temperature (°C) left side	right side
1				
2				
3				
Total			χ^2 =	χ^2 =
%				

Table 4. Locomotor Response of *Sarcophaga bullata* Larvae to Presence or Absence of White Light: **Class Data**

Student Pair	**Light End** of Racetrack				**No Light End** of Racetrack			
	# larvae	% larvae	χ^2 light intensity (°fc)	χ^2 temp. (°C)	# larvae	% larvae	χ^2 light intensity (°fc)	χ^2 temp. (°C)
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
Total								
Range								

Table 5. Effect of Light Wavelength on the Locomotor Behavior of *Sarcophaga bullata.* **Pair Data**

Trial	# larvae red end	# larvae blue end	Temperature (°C)		Light Intensity (fc)	
			red end	blue end	red end	blue end
1						
2						
3						
Total			χ^2 =	χ^2 =	χ^2 =	χ^2 =
%						

Table 5, cont. Effect of Light Wavelength on the Locomotor Behavior of *Sarcophaga bullata.* **Pair Data**

Trial	# larvae red end	# larvae green end	Temperature (°C)		Light Intensity (fc)	
			red end	green end	red end	green end
1						
2						
3						
Total			$\chi^2 =$	$\chi^2 =$	$\chi^2 =$	$\chi^2 =$
%						

Trial	# larvae blue end	# larvae green end	Temperature (°C)		Light Intensity (fc)	
			blue end	green end	blue end	green end
1						
2						
3						
Total			$\chi^2 =$	$\chi^2 =$	$\chi^2 =$	$\chi^2 =$
%						

Trial	# larvae red end	# larvae orange end	Temperature (°C)		Light Intensity (fc)	
			red end	orange end	red end	orange end
1						
2						
3						
Total			$\chi^2 =$	$\chi^2 =$	$\chi^2 =$	$\chi^2 =$
%						

Trial	# larvae green end	# larvae orange ende	Temperature (°C)		Light Intensity (fc)	
			green end	orange end	green end	orange end
1						
2						
3						
Total			$\chi^2 =$	$\chi^2 =$	$\chi^2 =$	$\chi^2 =$
%						

Trial	# larvae blue end	# larvae orange end	Temperature (°C)		Light Intensity (fc)	
			blue end	orange end	blue end	orange end
1						
2						
3						
Total			$\chi^2 =$	$\chi^2 =$	$\chi^2 =$	$\chi^2 =$
%						

Table 6. Effect of Light Wavelength on the Locomotor Behavior of *Sarcophaga bullata*. **Class Data**

Student Pair	**Red**				**Blue**			
	# larvae	% larvae	χ^2 light intensity (^{o}fc)	χ^2 temp. (^{o}C)	# larvae	% larvae	χ^2 light intensity (^{o}fc)	χ^2 temp. (^{o}C)
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
Total								
Range								

Student Pair	**Red**				**Green**			
	# larvae	% larvae	χ^2 light intensity (^{o}fc)	χ^2 temp. (^{o}C)	# larvae	% larvae	χ^2 light intensity (^{o}fc)	χ^2 temp. (^{o}C)
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
Total								
Range								

Table 6, cont. Effect of Light Wavelength on the Locomotor Behavior of *Sarcophaga bullata*. **Class Data**

Student Pair	**Green**				**Blue**			
	# larvae	% larvae	χ^2 light intensity (^{o}fc)	χ^2 temp. (^{o}C)	# larvae	% larvae	χ^2 light intensity (^{o}fc)	χ^2 temp. (^{o}C)
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
Total								
Range								

Student Pair	**Red**				**Orange**			
	# larvae	% larvae	χ^2 light intensity (^{o}fc)	χ^2 temp. (^{o}C)	# larvae	% larvae	χ^2 light intensity (^{o}fc)	χ^2 temp. (^{o}C)
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
Total								
Range								

Table 6, cont. Effect of Light Wavelength on the Locomotor Behavior of *Sarcophaga bullata*. **Class Data**

Student Pair	**Green**				**Orange**			
	# larvae	% larvae	χ^2 light intensity (^{o}fc)	χ^2 temp. (^{o}C)	# larvae	% larvae	χ^2 light intensity (^{o}fc)	χ^2 temp. (^{o}C)
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
Total								
Range								

Student Pair	**Blue**				**Orange**			
	# larvae	% larvae	χ^2 light intensity (^{o}fc)	χ^2 temp. (^{o}C)	# larvae	% larvae	χ^2 light intensity (^{o}fc)	χ^2 temp. (^{o}C)
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
Total								
Range								

Table 7. Results of chi-squared tests. Use **class data** for each test.

Experiment	Total # larvae 1st condition	Total # larvae 2nd condition	$\chi 2$	Interpretation	
				Accept Hypothesis	Reject Hypothesis
Response to no light gradient (Table 2)					
Presence vs. absence of light (Table 4)					
Red light vs. Blue light (Table 6)					
Red light vs. Green light (Table 6)					
Green light vs. Blue light (Table 6)					
Red light vs. Orange light (Table 6)					
Blue light vs. Orange light (Table 6)					

Data Display, Data Analysis

Data Table Guidelines

1. Control
   a. Complete **Table 1** Locomotor Response of *Sarcophaga bullata* Larvae to No Light Gradient (Control): **Pair Data**.
   b. Complete **Table 2** Locomotor Response of *Sarcophaga bullata* Larvae to No Light Gradient (Control): **Class Data**.
2. Locomotor response of *Sarcophaga bullata* larvae to the presence or absence of white light
   a. Complete **Table 3** Locomotor Response of *Sarcophaga bullata* Larvae to Presence or absence of White Light : **Pair Data**
   b. Complete **Table 4** Locomotor Response of *Sarcophaga bullata* Larvae to Presence or absence of White Light : **Class Data**.
3. Effect of Light Wavelength on the Locomotor Behavior of *Sarcophaga bullata*
   a. Table 5: Effect of Light Wavelength on the Locomotor Behavior of *Sarcophaga bullata*: Pair Data
   b. Table 6: Effect of Light Wavelength on the Locomotor Behavior of *Sarcophaga bullata*: Class Data
4. Chi Square Summary Table for All Experiments: Table 7
   a. You will calculate the values for this table using Microsoft Excel (Appendix A)

Graphs

1. Construct a bar graph showing your **individual** and **class** data for the response of the larvae to absence of white light (control).
2. Construct a bar graph showing your **individual** and **class** data for the response of the larvae to presence and absence of white light.
3. Construct bar graphs showing your **individual** and **class** data for the response of the larvae to various wavelengths of light (6 color combinations = 6 graphs total).

Lab Report Guidelines (Appendix B)

1. Introduction

   a. The purpose(s) or objective(s) for the experiments performed.

   b. Background information on the biology and behavior of *Sarcophaga bullata*, **larvae and adults**. Unless otherwise stated, this information must be obtained from print media, not internet sources. All information taken directly from primary print sources must be correctly cited and referenced in the Literature Cited section of the report.

   - General information concerning the biology of *S. bullata* is included in the lab exercise. Do not simply copy this information. It should be paraphrased, i.e. put in your own words.
   - Can *S. bullata* larvae detect light? If so, how?
   - What environmental factors influence the rate of development of *S.bullata* larvae into pupae and finally into adult flies?
   - What is the diet of *S. bullata* larvae? Adults?
   - What is myiasis and have cases of this process been reported for *S.bullata*?
   - What organisms prey on or parasitize *S. bullata* larvae? Adults?
   - How is *S. bullata* used in forensic entomology?
   - How has *S. bullata* been used in various fields of biological research?
   - Do *S. bullata* adults spread disease causing microorganisms? If so, which ones?

   c. Detailed statements of the null and experimental hypotheses based on your knowledge of the biology of *Sarcophaga bullata*. *You must explain what aspect or aspects of the biology of Sarcophaga bullata larvae led you to formulate* ***each*** *hypothesis*.

2. Materials and Methods

   This section should include a concise description of the materials, procedures, and equipment used in **each** experiment. There should be enough detail so that someone else could **repeat** your work. Figures that explain the methodology or drawings of the apparatus may be included if necessary. **Materials and methods are always written in the past tense. Do not simply copy word-for-word the procedures listed in the lab outline**.

3. Results

   a. Tables do not have to be reconstructed. Simply turn in the completed tables from the lab handout.

   b. All graphs must be constructed using Microsoft Excel or other graphing program. Do not use colors to when comparing graphs. Instead, use different fill patterns for bar graphs and different line types for line graphs.

   c. Written summary statements describing the results of all experiments. Interpretation of the data should not be included in this section.

   d. Your **written observations** of *Sarcophaga bullata* larval **behavior** during the experiments **with specific reference to the type of movement (taxis or kinesis)**.

   The graphs, tables, and figures should be placed at the end of the paper; before the Literature Cited section.

4. Discussion

   The following must be included in the discussion section of your report. This information must be **integrated** into your discussion, **not** simply listed as answers to questions. Credit will be deducted if the answers to these questions are simply listed in the discussion or not addressed in the discussion.

   a. Explain the logic and the statistical reasoning that allowed you to accept or reject each of your hypotheses.

   b. For the control experiments, (i.e., those with the same stimuli on both ends of the racetrack), was there a consistent pattern in the results of the chi-square tests? What do you conclude from this?

   c. The responses of the larvae to different wavelengths - red, blue and green - can be compared by ranking the relative strength of the responses. Since three wavelengths have been observed, there are three ranks. The wavelength to which most of the larvae responded positively to would receive the highest rank (rank 3) and the wavelength to which the fewest of the larvae responded positively to would receive the lowest rank (rank 1). For example, if more of the

larvae responded to blue light than to red light and green light, and more responded to green light than to red light, blue light would be ranked highest, red light lowest and green light in between (rank Does a pattern emerge when ranking the preference to wavelength of light? Do *S. bullata* have a preference for longer or shorter wavelengths of light? Does this pattern hold for each of the filters tested? How might this pattern, if there is one, be explained?

d. Based on your data do you think temperature played a significant role in your results? Explain why or why not?

e. Were there other variables that were not controlled for that should have been? How might these other, uncontrolled variables influence your interpretation of the results?

f. Why is it important to run a number of separate trials for the same experiment?

g. How are the stimuli you used in these experiments different from those to which the flesh flies are normally subjected to in the natural environment. Would you expect these differences to affect the outcome of these experiments, if so how and why?

h. In these experiments it is quite likely that all the larvae tested did not respond in the same way to the light. Discuss possible reasons why ALL the larvae in the population **may not** have shown the same locomotor response to light.

i. For these experiments, discuss possible reasons why your individual pair data differs from the class data.

j. Based on your observations, do flesh fly larvae show a taxis or kinesis locomotor response to light? Is this response positive or negative?

k. Explain **how** and **why** the reactions of the larvae to different light wavelengths may be of adaptive value to the survival of the larvae in their natural environment.

l. If you were to observe the flesh fly **larvae** in their **natural habitat**, what environmental stimuli **other than light** would be affecting their locomotor behavior at any one point in time?

m. How are oral hooks of larvae well adapted for their function?

n. Describe a brief experiment which might help you determine the location of the light receptor in the body of the flesh fly larvae?

o. Suggest ways to improve this experiment.

5. Literature Cited

Specifically, the literature cited section of this **report *must*** include the following material:

a. At least 3 citations from book chapters and/or journal articles.

b. In addition, you may include 1 citation from a **reputable** web resource

Lab Report Grading Policies

1. Point Distribution

The point distribution for each section of the animal behavior lab report is listed below.

Title:	1 pt.
Introduction:	6 pts.
Materials and Methods:	3 pts.
Results:	7 pts.
Discussion:	11 pts.
Literature Cited:	2 pt.

2. Grading Policies:

   a. The copying of lab reports from previous semesters or the current semester is considered to be plagiarism. It will automatically result in a grade of zero on the report. Even though you have worked in pairs in collecting the data for these experiments, you are expected to hand in an individual lab report written by you.

   b. The report must be word processed.

   c. Typical reports are 5 - 10 pages long excluding graphs and tables. Remember that it is the quality of the report, not the length, that is important!

   d. One point will be deducted for failure to follow the lab report format. That is, your report must be divided into the 6 sections listed above.

   e. The use of grammatically incorrect English will result in an overall lower grade on your report.

   f. Late Reports: For each day late (including Saturday and Sunday), 1 point will be deducted from your score. Reports more than 7 days late will receive a grade of zero.

   g. Do not put reports in fancy covers. Simply staple the written pages together.

Materials

- Light Meters: Dual Range Light Meter, VWR Scientific, 62344-944
- Colored Filters: Theatrical Gels can be purchased from a local sound and lighting store or online at: www.stagespot.com. The filters have specific spectral characteristics and are coded by a number.
  - Green: Rosco # 90; one layer
  - Red: Rosco #27; two layers
  - Blue: Rosco #74; one layer
  - Dark Amber: Rosco #22; one layer
- Light Sources: High intensity Tensor Lights fitted with light filters (Fig. 5).

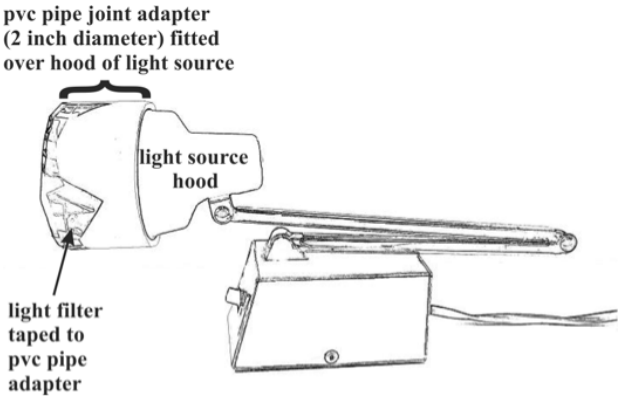

Figure 5. Light Source and Filter

Note: High intensity Tensor Lights are no longer made. We are currently investigating the use of rechargeable, LED flashlights such as the Energizer Model # RCL1NM2.

- "Racetracks" are constructed from 3 mm thick black plexiglass
  - Sides: 30.5 cm x 8 cm
  - Platform: 30.5 cm x 6.3 cm
  - Platform inserted 2.5 cm from top of racetrack
- Thermistors: Cole Parmer 90225-10 Co
- Larval Rearing Chamber: Carolina Biological, call to order separate from kit, #C674285
- Chix ™ Sports Towel: Chicopee Inc. #8470

Notes for the Instructor

Pre-Lab Introduction

1. Review how organisms are classified using flesh flies as an example. Be sure to emphasize that the species of the flesh fly is *Sarcophaga bullata* and not simply *Sarcophaga*.
2. Review the biology of flesh flies. Remind students that the introductory information in the lab exercise, should be used to formulate their hypotheses and should be the basis for the introduction of their lab report.
3. Discuss each stage of the life cycle of flesh flies using the diagram in the lab outline. Emphasize that the larval stage is primarily adapted for feeding whereas the adult flies are primarily adapted for breeding and dispersal.
   a. Emphasize that the larval stage as well as the adult stages of insects carry out gas exchange via the spiracles and tracheal tube
   b. Emphasize that for efficient movement of gases within the tracheal tubes and between the tracheal tubes and the cells, the environment must be kept very **moist**.
4. Review importance of flesh flies to humans, emphasizing their use in forensics. Be sure to show the PowerPoint slide showing the pig carcasses covered with flesh fly larvae.
5. Briefly discuss the characteristics of light. Emphasize that the white light may be broken up into its component wavelengths (colors) as it passes through or is reflected off objects.

Laboratory Procedures

1. Stages Life Cycle of Flesh Flies:

   Each pair of students will observe under a stereomicroscope and sketch the following: You may find it helpful to use the Videopresenter to show students the basic morphology and structures of the stages in the life cycle of the flesh flies.
2. Freeze-dried flesh fly adult
   a. Students can use a brush or toothpick to manipulate the fly in the petri dish while viewing under the stereomicroscope.
   b. Students should not remove the preserved specimens from the petri dishes.
3. Living flesh fly maggot (larva)
   a. Have each pair of students pick out one larva from the "Experimental Larvae" container at the instructor's desk and place it in a small plastic petri dish for observation under a stereoscopic microscope. Make sure students do not place a moist piece of filter paper in the bottom of the petri dish. The larva will stick to the plastic; thus slowing down its movement.

b. Make sure students return the larvae to the "Used Larvae" container; not to the "Experimental Larvae" container

4. Freeze-dried Flesh Fly Pupa:

   a. Have each pair of students obtain a petri dish containing a pupa from the TA desk and observe it under a steriomicroscope - not much to see!

5. Demonstration: Insect cages showing all stages in life cycle are on display in the classroom. Encourage students to observe this demonstration!

6. Students tend to spend a great deal of time doing the sketches if you let them. Emphasize that these sketches should not be works of art. All sketches they will do in this lab should be as accurate as possible but not elaborate. The purpose of these sketches is to remind the students of what they looked at in lab.

General Lab Procedures

1. Emphasize careful handling of the flesh fly larvae. It is extremely important that all larvae are returned to the "Used Larvae" containers.

   a. Remind students pick the larvae up with their fingers or use a camel=s hair brush to move the larvae into a cup for transporting. Don't let students use forceps to pick up the larvae since they tend to squeeze and damage the larvae with the forceps tips.

2. All lights in the room must be turned off except the ones on top of the microscope cabinet and the light over the TA desk. Make sure the lights above the aquaria are turned off when running the experiments.

3. Remind students:

   a. that the cloth strip on the racetrack must be kept moist at all times!

   b. to place moist filter paper discs in the cups at both ends of the racetrack as well as in the cups being used to transport the larvae from the TA desk to their lab bench.

   c. that the light intensity must be approximately **9 fc** at the center of the racetrack.

   d. to replace larvae which are lost (they can easily crawl out of the cups and fall on the floor) or become inactive with fresh larvae from the experimental culture.

   e. to wash their hands at the end of lab and wipe off the racetrack.

Experiment I: Locomotor Response of Flesh Fly Larvae to White Light Intensity

1. This experiment was designed to lead the students through the steps required to set up an experiment using the equipment provided in order to determine whether the larvae are photopositive or photonegative to white light and whether the response is a taxis or kinesis.

2. Note that each student is required to write down on a 3 x 5 card his/her hypothesis concerning the locomotor response of flesh fly larvae to two light intensities (low and high). These cards must be handed in to you at the end of lab and will be taken into consideration when the reports are graded.

3. Collect class data on the transparency provided! Be sure to take the class data transparency with you. You will need it when grading the lab reports!

4. Review how to graph the data for this experiment.

5. Make sure students do not use the colored filters for this experiment.

6. Be sure to show the students how to set up the light sources so that the center of the track has a light intensity of 9 fc. This involves using the white filter and adjusting the distance between the light source and the racetrack. In addition, you will probably have to show the students how to operate the light meters.

7. Remind the students to measure the light intensity at both ends of the racetrack after each trial and record it in the data tables.

8. Make sure the students switch the light source to the other end of the racetrack at the start of each 2-minute trial - why? Ask students why they are required to switch the location of the light source. Make sure they don't simply switch the position of the racetrack!!

9. Remind students that they are required to measure the temperature at both ends of the racetrack at the end of each trial and record these values in the tables provided.

10. Show the students how to use the digital thermometer. Be sure the thermistors are turned off when not in use.

Experiment 2: Locomotor Response of Flesh Fly Larvae to Different Wavelengths (colors) of Light

1. This experiment was designed to let the students set up their own experiments to determine what wavelength of light resulted in the strongest locomotor response of the larvae.

2. Do not tell the students how to set up this experiment! Emphasize that each pair of students is a research team which is going to test their hypothesis.
3. Make sure student take time to design an experiment that will give them valid data to be used in accepting or rejecting their hypotheses.
4. Note that each student is required to write down on a 3 x 5 card his/her hypothesis concerning the locomotor response of flesh fly larvae to three different wavelengths of light. These cards must be handed in to you at the end of lab and will be taken into consideration when the reports are graded.
5. It is your job to guide the students in setting up this experiment. Therefore it will be extremely important to circulate around the room to make sure that the students are on the right track!
6. Have the students check with you prior to starting the experiment to determine if their results will be meaningful. If this is not the case, point out why and help them design a "better" experiment.
7. The light filters slip over the light source.
    a. Although these filters will not be significantly affected by the heat of the lamp, tell students not to place the filter in direct contact with the bulb!
    b. The students will have to manipulate the light setting as well as the distance the light source is from the center of the racetrack in order to maintain 9 fc intensity at the center of the racetrack.
8. Refer to notes at the end of the agenda for hints as to how the students should set up this experiment to obtain the most meaningful data.
9. Collect class data on the transparency provided. Be sure to take the class data transparency with you. You will need it when grading the lab reports!
10. Review how to graph the data for this experiment, especially the ranking data for each color of light.

Suggestions for Making the Exercise More Student Centered (Investigative)

As stated in the Notes to Instructors, Experiment 2, Locomotor Response of Flesh Fly Larvae to Different Wavelengths of Light was designed to let the students set up their own experiments to determine what wavelength of light results in the strongest locomotor response of the larvae. So as not to influence the students' decisions as to the best experimental design, Tables 5 and 6 could be omitted and the students be allowed to construct their own tables based on the experimental design they decide to use.

Acknowledgments

We would like to acknowledge Peter Thew for his suggestions and help in preparing this manuscript.

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References for Further Reading

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About the Authors

Bill Glider earned his B.S. degree in Secondary Education from Cornell University, his M.S. degree in Botany from the University of Maine and his Ph.D. in Biological Sciences from the University of Nebraska-Lincoln in 198. He is currently a Senior Lecturer in the School of Biological Sciences at UNL. In 2002, he received the Distinguished Teaching Award from the College of Arts and Sciences. He has spent over 20 years designing laboratory curriculum for the General Biology Program at UNL and lecturing in General Biology. In addition, to General Biology he has taught Botany, Organismal Biology, and field courses at the UNL Cedar Point Biological Station. Currently, he devotes the majority of his time to teaching large lecture sections of General Biology and pursuing a number of collaborative research projects focused on methods of increasing student learning in the biological sciences and designing methods of teaching students with disabilities in both lab and lecture.

Michael Bessert earned his B.S. degree in Secondary Science Education from Concordia University-Nebraska and his M.S. and Ph.D. in Biological Sciences at the University of Nebraska-Lincoln. He is currently an Assistant Professor at the University of Wisconsin-Stout where he teaches General Biology, Genetics, and Ichthyology. His research interests include fish evolution, systematics, and conservation.

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Appendix A: Hypothesis Testing Using the Chi-squared (χ^2) Goodness of Fit Test

Biologists often need to compare a distribution of observed data with an expected distribution in order to test a hypothesis. For example, a biologist may have observed that honeybees seem to visit red clover flowers more often than white clover flowers. The biologist might hypothesize that bees have a preference for one color of clover flower over another. To test this hypothesis, the biologist could put individual bees into experimental chambers containing an equal number of randomly arranged red and white clover flowers, and record the colors of the flowers visited.

The **null hypothesis** for this experiment would be that the bees have no preference. This would be the null hypothesis because it is the logical alternative to what the biologist expects to happen. The **experimental hypothesis** would be that bees do have a preference because that is what the biologist expects to happen, based on previous observations. An approximately equal number of visits to flowers of both colors, an indication that the bees are randomly visiting flowers, would cause the biologist to accept this null hypothesis and reject the experimental hypothesis. Consistent visits to flowers of one color versus the other would cause the biologist to reject the null hypothesis and accept the experimental hypothesis. The conclusion would be that bees prefer red flowers, for example, if a greater number of bees visit red flowers than visit white flowers.

When should the biologist reject or accept the null hypothesis? It is unlikely, due to chance alone, that an exactly equal number of visits to both flower colors will be the outcome of the experiment every time it is conducted. This is true even if the null hypothesis that bees have no preference is correct. Because of this, an objective basis of deciding which hypothesis to accept or reject is required. This is the purpose of the **Chi squared Test**. (Imagine flipping a coin 50 times and tabulating the number of heads and tails each time, over and over again. It is quite likely that the observed results will not be 25 "heads" and 25 "tails" each time. If the coin is fair, the difference is due entirely to chance. If the deviation (the difference between the actual and expected results) is small, you would not be very disturbed. You intuitively and correctly, expect some deviation in any random process. But what if the deviation were large? Suppose the biologist recorded 32 visits to red flowers and 18 visits to white flowers. Can the difference between these results and the prediction of the null hypothesis (an equal number of visits to both flowers of both colors) be attributed to chance alone or to preference on the part of the bees? The chi-squared test is used to objectively distinguish between these two possibilities.

The chi-squared value is computed and the value compared to a critical value from the chi¬squared table. The critical value we use depends on the **Degrees of Freedom** and the desired **Significance Level**. The formula for the chi-squared test is as follows:

$$\chi 2 = \Sigma\ (d2/e)$$

In this formula, **d** is the **deviation** from the expected (predicted) value, **e** is the **expected** value, and Σ = "the sum of". The expression $\mathbf{d^2/e}$ alone gives a chi-squared value for each "class" or group of data in which deviation can occur. For example, in the bee experiment, there are two classes of data, white and red. Chi-squared values for each group of data are added, and the total value is compared with a table that gives information about the significance of the deviation, as we shall see shortly.

Experiment A:

Suppose you counted 66 bee visits to red flowers and 54 bee visits to white flowers. The total sample would then have been 120, and the expected ratio would have been 1:1, i.e., 60 visits to red flowers and 60 visits to white flowers if the null hypothesis is correct. The data and chi-squared calculations for this experiment are shown in Table A1 below. Study this example carefully. Ask your instructor for help if you have any questions on how to calculate the chi-squared value.

Table A1. Chi-squared Example 1

	Class 1 (red)	Class 2 (white)
Observed value	66	54
Expected values (e)	60	60
Deviation (d)	+6	-6
Deviation Squared (d^2)	36	36
d^2/e	0.6	0.6

$$\chi 2 = \Sigma\ (d^2/e) = 0.6 + 0.6 = 1.2$$

Experiment B:

In another experiment you counted a total of 32 bee visits, of which 22 visited red flowers and 10 visited white flowers. Calculate the chi-squared value for this experiment by filling in Table A2 below.

Table A2. Chi-squared Example 2

	Class 1 (red)	Class 2 (white)
Observed value		
Expected values (e)		
Deviation (d)		
Deviation Squared (d^2)		
d^2/e		

$$\chi 2 = \Sigma\ (d^2/e) =$$

Notice the great difference in size of the chi-squared value obtained in Experiment A compared to that obtained in Experiment B.

Table A3. Chi-squared Distribution

	No Reason to Doubt Hypothesis							Reason to Doubt Hypothesis		
	Deviations Insignificant							Significant Deviations		
P / C-1	.99	.95	.80	.50	.30	.20	.10	.05	.02	.01
1	.00016	.0039	.064	.455	1.074	1.642	2.706	3.841	5.412	6.635
2	.0201	.103	.446	1.386	2.408	3.219	4.605	5.991	7.824	9.210
3	.115	.352	1.005	2.366	3.665	4.642	6.251	7.815	9.837	11.341
4	.297	.711	1.649	3.357	4.878	5.989	7.779	9.488	11.668	13.277
5	.554	1.145	2.343	4.351	6.064	7.289	9.236	11.070	13.388	15.086

Significance of Chi-Square:

The chi-squared can help answer two questions:

1. How probable is it that the deviation between the observed results and the expected results is simply due to chance?
2. Should the biologist reject the null hypothesis (honeybees show no preference to red or white clover flowers) in favor of the experimental hypothesis (that honeybees do show a preference for white flower color)?

We can answer the first of these questions by comparing the chi-squared values with those shown in a standard chi-squared table, Table A3.

In brief, the table above shows the maximum chi-squared values allowable if the deviations actually found are to be considered due chance alone. The vertical column C-1 ("classes minus one") shows the number of classes of data under consideration minus one, a number often called the **Degrees of Freedom**. For both of the experiments A and B shown above, this number is 1. The p-value, on the other hand, expresses the probability that deviations producing the calculated chi-squared value are due to chance alone. Comparing the value of 1.2 (for Experiment A) with Table 4, we find that the corresponding p-value for one degree of freedom is between 0.20 and 0.30. This simply means that if we were to repeat this experiment again and again, each time we could expect a chi-squared value as large or larger than the one we actually obtained approximately 3 times out of 10 if the deviation were due to chance alone. Comparing the chi-squared value of 4.5 (for Experiment B) with Table 4, we find that the corresponding p-value for one degree of freedom is between .05 and .02. This means that each time this experiment were repeated, we could expect a chi-squared value as large or larger than the one obtained only 5 times out of 100 if the deviation were due to chance alone.

To answer our second question (i.e., should we reject our null hypothesis and accept the experimental hypothesis), we must first decide on a reasonable p-value, and then find where our chi-squared value lies with respect to that p-value. A high chi-squared value usually indicates that the experimental results deviate greatly from the predicted results. Clearly, the more the results deviate from the predictions, the more likely it is that the observed results are more consistent with the experimental hypothesis than the null hypothesis. It follows, therefore, that a high chi-squared value is more significant evidence in favor of the experimental hypothesis than is a low chi-squared value. By convention, we accept as statistically significant (and therefore as cause to seriously doubt our null hypothesis) any deviation so great that the probability of its having occurred by chance alone is 0.05 (5%) or less. In other words, any chi-squared value large enough, or larger, to yield a p-value of 0.05 is said to reflect a statistically significant deviation of the observed from the expected results - a deviation that warrants rejection of the null hypothesis and acceptance of the experimental hypothesis. A chi-squared value large enough to yield a p-value of 0.01 is said to be highly significant.

On this basis, the deviation in Experiment A was insignificant; there is no reason to reject the null hypothesis of no preference. The deviation for Experiment B is significant and the null hypothesis should be rejected in favor of the experimental hypothesis. The results of Experiment B suggest bees do have a preference for red flowers. However, the sample size in Experiment B is very small.

Factors Influencing the Size of Chi-Square Values:

From the formula for the chi-squared test, it should be clear that the larger the value of d, the larger the chi-squared value for that class of data; second, the larger the value of e (expected), the smaller the chi-squared value for that class of data. Finally, the more classes of data under consideration, the larger we should expect the total value to be. For example, if we had only two classes of data (as in the bee experiments), we expect less opportunities for deviation (and smaller chi-squared total) than if we had considered an experiment in which there were ten different data classes, and hence ten opportunities for deviation. Since the d-value and the number of classes of data were the same in both the hypothetical experiments, only the observed and expected values varied. These, of course, reflect the size of the sample. In a sample as small as that in Experiment B, we should not be

very surprised to find a large chi-squared value. In summary, the size of chi-squared values are influenced by three factors; deviation size, sample size, and the number of opportunities in which deviation can occur (i.e. the number of classes of data).

Note: A statistical test is not meaningful without an interpretation that is based on what is known about the biological process under consideration and the experimental conditions. For example, in both hypothetical Experiments A and B above, the results of the chi-squared tests alone do not explain **why** the bees behaved the way they did.

Appendix B - Writing a Scientific Lab Report

Scientific research is a group activity. Individual scientists perform experiments to test hypotheses about biological phenomena. After their experiments are completed and duplicated, these researchers attempt to persuade others to accept or reject their hypotheses. The lab report or scientific paper is the vehicle of persuasion; when it is published it is available to other scientists for review. If the results stand up to criticism, they become part of the accepted body of scientific knowledge unless later disproved.

In some cases a report may not be persuasive in nature but instead is an archival record for future generations. For example, data on the distribution and frequency of rabid skunks in a certain year may be of use to future epidemiologists in deciding whether the incidence of rabies is increasing. Regardless of whether a report is persuasive or archival, the following guidelines apply.

A **sample** journal article is posted on the Bios 103 Web Site. Read this article to familiarize yourself with the **type** of information which should be included in each section of your lab report. You are not expected to understand all of the technical aspects of the subject matter reported in this article.

1. A scientific report usually consists of the following 6 sections:
   a. Title
   b. Introduction
   c. Materials and Methods
   d. Results
   e. Discussion
   f. Literature Cited
2. Contents of each section of a lab report and/or journal article
   a. Title:

      The title should be less than ten words and should reflect the factual content of the paper. A good title is straight-forward and uses key words. For example, suppose you studied how feeding dietary supplements of vitamins A and B affected the appearance of the fur of three white rats. “Effects of Chemicals on Animals” would be a poor title for a research report dealing with this study. Why? What would be a better title?

   b. Introduction:

      The introduction defines the subject of the report. It must outline the scientific purpose(s) or objective(s) for the research performed and give the reader sufficient background to understand the rest of the report. The specific hypotheses being tested must be described.

   c. Materials and Methods

      This section should include a concise description of the materials, procedures, and equipment used in **each** experiment. There should be enough detail so that someone else could **repea**t your work. Figures that explain the methodology or drawings of the apparatus may be included if necessary. Materials and methods are always written in the past tense. Do not simply copy word-for-word the procedures listed in the lab outline.

   d. Results

      The results section should contain a written summary of the results from the experiments **without** discussing their implication. The written summary statements should refer to the figures, tables and graphs included in this section of the report. Reference is made to each figure and the important results are described. All figures, tables and graphs must have descriptive titles and should include a legend explaining any symbols, abbreviations, or special methods used. Figures and tables should be numbered separately and should be referred to in the text by number, for example:

      - Example 1: Figure 1 shows that the activity increased over the two hour time period.
      - Example 2: The activity increased over the two hour time period (Figure 1)

   e. Discussion

      In the discussion section, the data collected is interpreted in relation to the hypotheses or purposes proposed in the introduction. In the discussion section, the data collected is interpreted **in relation** to the hypotheses or purposes proposed in the introduction.

3. Literature Cited Within the Report

   This section is a listing of all articles or books referred to (cited) in the text of your report. It is not the same as a bibliography, which simply lists references

regardless of whether they were cited in the paper. Different journals require different formats for citing literature and listing literature cited.

a. Literature Cited in the Body of the Paper

All facts and opinions included in the body of the paper which are not original, must be given proper credit through a literature citation. When citing references in the text, do not use footnotes; instead, refer to articles by the author's name and the date the paper was published.

- Example 1: Fox in 1978 investigated the effects of hormones on the nest-building behavior of catbirds.
- Example 2: Hormone are known to influence the nest-building behavior of catbirds.
- Example 3: When citing references that have two authors, both names must be listed. When three or more authors are involved, the Latin *et al*. meaning "and others" may be used. A paper by Smith, Lynch, Merril and Beam published in 1979 would be cited in the text as: Smith *et al*.(1979) have shown that ...

4. Literature Cited Section of the Report

This section is a listing of all articles or books referred to (cited) in the text of your report. It is not the same as a bibliography, which simply lists references regardless of whether they were cited in the paper. Different journals require different formats for citing literature and listing literature cited. The formats for some commonly used resources are listed below:

Journal Articles

Fox, J. W. 1978. Nest-building Behavior of the Catbird, *Cumatella carolinensis*. Journal of Ecology 47:113-17.

Section of an Entire Book

Smith, C. J. 1979. Basal Cell Carcinomas. Pages 278-291 In C.D. Wilfred, ed. Histological Aspects of Cancer. Boston Medical Press, Boston.

Information From the Lab Exercise

Glider, W. V. 2011. *Locomotor Responses of Flesh Fly Larvae to Light*. Lab Exercise. University of Nebraska-Lincoln, School of Biological Sciences.

Internet Article

Lupack, Alan. *The Response of Dipteran Larvae to Light.* University of Nebraska-Lincoln Entomology Web Site. 17 January 2011. <**http:www.unl.edu/entomology**>

5. Writing Tips

Your report will be easier to read and understand if you follow these tips and try to conform to the accepted style of scientific writing that is required for scientific papers. Conformity can be a good thing if it increases communication!

a. Refer to animals and plants by their scientific names. Always underline or italicize the genus and specific epithet of organisms, for example, *Sarcophaga bullata* . The scientific name is both singular and plural. Therefore, when referring to more than one *Sarcophaga bullata*, do NOT add an "s" to the specific epithet.

b. Use the metric system of measurement.

c. Be aware that the word "data" is plural while "datum" is singular. This affects the choice of a correct verb. The word "species" is used both as a singular and as a plural.

d. Your report should be written in grammatically correct English. Every sentence must have a subject and a verb.

e. Your report should be concise and logically sound. Avoid boring the reader with copious verbiage and excessively formal writing.

f. Be consistent in the use of tense throughout a paragraph - do not switch between past and present.

g. Avoid using the first person, I or we, in writing. Keep your writing impersonal, in the third person. Instead of saying, "we weighed the frogs and put them in a glass jar," write, "the frogs were weighed and put in a glass jar."

h. Do not use slang.

Appendix C: Instructions for Using the Chi-squared Calculator

"Chi-squared Calculator" is a Microsoft Excel spreadsheet that simplifies the calculation process. Download the spreadsheet here: **http://www.ableweb.org/volumes/vol-33/glider/supplement1.htm**. When the file is opened, it will appear similar to what is shown in Table C1.

To calculate a chi-squared value for any of the experiments you performed, all you need to do is enter the total number of larvae observed in each of the two response classes you are comparing (e.g. light vs no light, green vs blue, etc.) and the spreadsheet will do the rest.

For example, turn to Table 2: Locomotor Response of *Sarcophaga bullata* larvae to No Light Gradient **Class Data** previous lab handout. These are the results of an experiment you performed last week to verify that the larvae would not

prefer one end of the race track over another in the absence of light. In this case, the two response classes you are comparing are, "# of larvae on left side of center line" and "# of larvae on right side of center line." Find the totals for each of these two classes (second line from the bottom in the table). These are your **observed values**. Enter them in cell B4 and C4 on your worksheet. When you do this, the computer will calculate the various components of the chi squared test and a chi-squared value will appear at the bottom (cell B10). Note that we are testing the null hypothesis of 'no preference' in every one of these experiments, so the **expected values** are simply the total number of larvae used divided by 2. That is, if there is no preference, we would expect 50% of the larvae to move to each side of the track. Was this the case? Record your chi-squared value in Table 7, "Chi-squared results" To interpret the results - that is, to decide whether you should accept or reject the null hypothesis - compare the chi-square value you obtained to the critical value for a test with 1 degree of freedom and p = 0.05 (see chi-squared table in Appendix A). The critical value should be 3.84. Is your chi-squared value greater than that? If so, you would reject the null hypothesis and conclude that there is a preference for one end of the race track. Complete Table 7 by performing a chi-squared test for each of the experiments listed, and interpret the results appropriately.

Table C1. Excel Chi-squared Calculator

	A	B	C
1		Class 1	Class 2
2		(insert class name here)	(insert class name here)
3		0	0
4	Observed	0	0
5	Expected (E)	0	0
6	Difference (D)	0	0
7	D^2		
8	D^2/E	#DIV/0!	#DIV/0!
9			
10	$\chi 2$	#DIV/0!	

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