OBSERVATION: The First Necessity of Science

Learning Outcomes

In this laboratory exercise, you will observe the behavior and external and internal anatomy of an animal. After completing this exercise, you should be able to:

- **Record observations (does it move, is it colored (if so, what color), translucent, opaque, etc.)**
- **Label, to the best of your ability, the structures that you encounter & speculate on their possible function**
- **Refine your skills using dissecting and compound microscopes**
- **Describe the organization of cells and extracellular proteins**
- **Calculate (and understand how to calculate for ANY scope) the field diameter at various magnifications**

Introduction

Ezra Pound (an American writer, 1885-1972) wrote about an anecdote involving a fish, a student, and Louis Agassiz (1807-1873), a Swiss-born ichthyologist who emigrated to the U.S. in 1846. This anecdote provides the basis for this lab. Pound's recounting (1934. *ABC of Reading)* goes as follows:

A post-graduate student equipped with honours and diplomas went to Agassiz to receive the final and finishing touches. The great man offered him a small fish and told him to describe it.
Post-Graduate Student: "That's only a sunfish."
Agassiz: "I know that. Write a description of it."
After a few minutes the student returned with the description of the *Ichthus Heliodiplodokus*, or what ever term is used to conceal the common sunfish from vulgar knowledge, family of Heliichtherinkus, etc., as found in textbooks of the subject.
Agassiz again told the student to describe the fish.
The student produced a four page essay. Agassiz then told him to look at the fish. At the end of three weeks the fish was in an advanced state of decomposition, but the student knew something about it.
Today, we will test your powers of observation, exploration, and investigation by asking you to observe live organisms.

Fish

Fish are the most speciose group of Vertebrates. They develop from jelly-like eggs, rather than the hard-shelled eggs of many other vertebrates. Immature fish look like the adults but are often more inconspicuous. Many fish are brightly colored for a variety of reasons: to attract mates, to camouflage themselves amid brightly colored vegetation, or to warn predators of their toxic secretions.
What can you observe about their color: Are they multi-colored or monochromatic?
What about their behavior: Are they bold or shy? Do they keep hidden in vegetation or are they out in the open?
How do they move: using tails, appendages (fins) or both? Does one provide thrust while the other steers?

Assignment

*Working in pairs or groups of four, record, as many observations of your animals as you can. Record, and quantify when possible, **all** your observations: include drawings and as much information as you can gather during this time.*

Live Fish (15 minutes):

1. Carefully watch living individuals and record their behaviors. Staying still while you watch your animals is vital but not easy. If you move you may frighten them and change their behavior.

Preserved crickets:

1. Use a dissecting microscope (see the next page for hints on microscope use) to examine the external anatomy of preserved specimens. Think about the structures you observe and how they might function:
   - *What structures allow the cricket to move, to eat, to respond to the environment, etc.?*

2. Use the dissecting microscope to examine the internal abdominal anatomy of a female.
   - *Can you see eggs or developing offspring?*
   - *Is there anything connected to the mouth?*

Histology Slides:

1. Examine three of the available slides and record observations about the cells you observe. Make sure to view them at 100X and 400X.
   - *Can you see individual cells?*
     - *Describe their shape.*
     - *Do they have discernable organelles or apical decorations?*
     - *Do they occur in multiple or single layers?*
     - *Are they organized around some white space?*
   - *Can you see extracellular fibers?*
     - *Describe their organization.*

Use the following dissection guide to examine your cricket. You will probably not know all the terms used in this guide and that's OK. This is just to give you some practice dissecting and identifying structures of a dissected animal.

Obtain a cricket. Remove the insect's wings by cutting them off at the base. Fasten the animal to the wax, **dorsal** side up, by putting a pin through the head and the last **abdominal** segment. Now comes the crucial step-opening the animal.

Using fine, sharp scissors and forceps loosen a tergite near the tip of the abdomen and insert the tips of the scissors. Cut along the lateral edge of the tergum. Cut forward to the **thorax**. Raise the **dorsal** flap and use a dissecting needle to work it free of underlying tissues. Be careful not to damage the dorsal aorta which lies underneath. Now continue your cut forward through the thorax. When at the front of the thorax, make a new cut across the body to the other side. Make a similar cut in the last abdominal segment. Now work the dorsal flap of the exoskeleton free and lay it over and pin it down. Flood the tray with saline to float the organs and

keep them from drying. You will now systematically work your way down through the animal, exposing different organ systems. Use figure 9 as a guide to identification.

Circulatory System
Insects have open circulatory systems, as do all arthropods. Vessels move the hemolymph from one body region to another where the fluid enters the spaces between tissues and gradually percolates back.

If your removal of the exoskeleton was done carefully, you should see the tubular heart on top of the body mass in a small depression. Hemolymph enters the heart from the surrounding spaces through minute lateral openings called ostia and is pumped forward by peristaltic waves through a dorsal aorta to the head. There it leaves the aorta and enters the tissue spaces, collectively called the hemocoel. Hemolymph gradually flows back to the pericardial space and enters the ostia to complete the circuit.
As you look at the organs, a chalky white fat body may fill the hemocoel. In females, the fat body can be replaced by swollen oviducts holding up to 600 eggs. Remove this material carefully to reveal the respiratory and digestive systems.

Respiratory System
Insects have a unique respiratory system, the tracheal system which conveys oxygen directly to the tissues.

As you work though the fat body, you should occasionally see glistening white tubes. These are tracheoles of the tracheal system. They branch from main tracheae coming from the spiracles that you saw on the external lateral surface. This system of tubules branches into fine tubes that directly take away carbon dioxide and bring oxygen to every tissue in the body. The hemolymph does not serve as an intermediate carrier as blood does in many other animals. The finest tubules have fluid in them so that gas exchange occurs via a fluid medium. In larger insects, air sacs associated with the tubes help to ventilate them (see fig. 32). When the insect uses muscles for movement, the sacs are compressed and air is forced out and drawn in as the muscles relax. An interesting sidebar on respiratory systems is a problem beekeepers encounter. A parasitic mite (a chelicerate) parasitizes bees. It invades and lives just inside the spiracles in the tracheal system. This interferes with the respiratory system and can kill a whole bee colony.

Digestive System
Insects have a complete digestive system showing typical tube-within-a-tube architecture.
As you reveal the digestive system, note the different regions along its length. The foregut has three regions: the esophagus, crop (a storage area), and the gizzard (proventriculus) where chitinized plates on the inner wall grind the food into a fine pulp. Food leaves the foregut and enters the midgut consisting of a stomach with six fingerlike gastric caeca which secrete digestive enzymes. The hindgut or intestine leaves the stomach and passes back to a rectum and anus. The rectum is a water-reclamation organ and produces a relatively dry fecal material. When insects molt, the lining of the foregut and hindgut are shed along with the exoskeleton.

Excretory System
The excretory system of insects is unique and consists of structures called malpighian tubules. These can be seen radiating like threads from the middle of the hindgut. The cells in the walls of these tubules absorb nitrogenous waste materials from the hemolymph that bathes them, forming uric acid. Crystals of uric acid enter the hindgut and are compacted with fecal material before defecation.

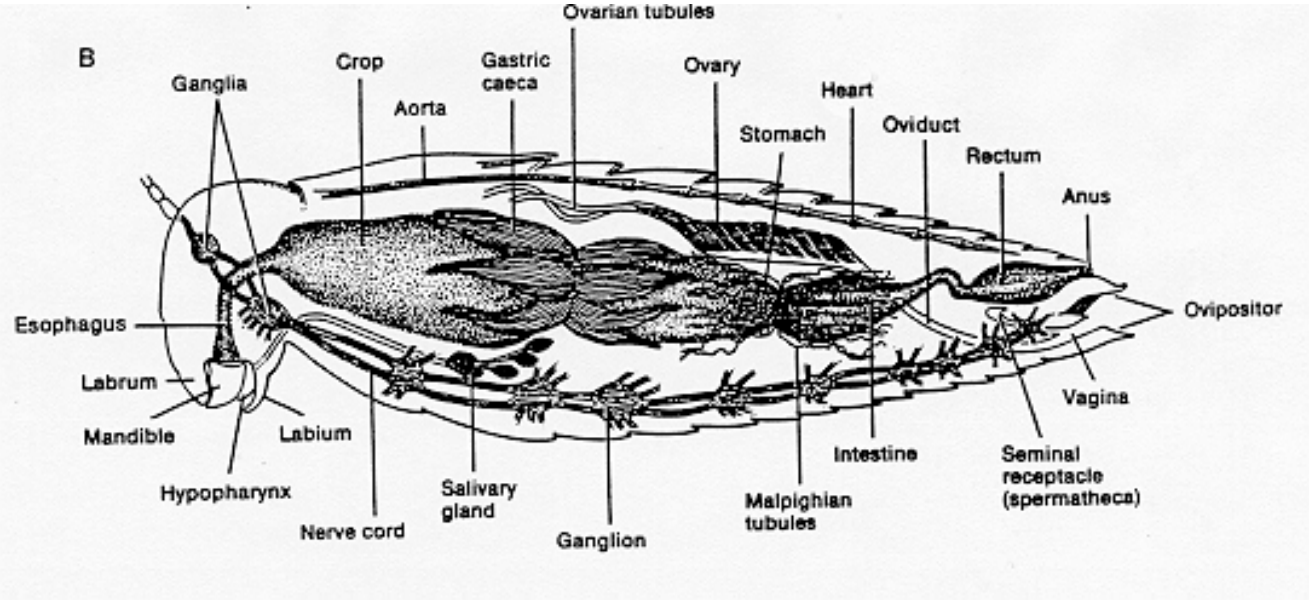
Reproductive System

The sexes are separate in insects and show sexual dimorphism (sexes look different). Based on your observation of external anatomy, you should know if your insect is male or female. If the gonads are not enlarged and filling the body cavity, then remove the digestive tube to find them. You may be able to follow the ducts leaving the ovary or testes and proceeding to the genital opening.

Nervous System

After removing the digestive and reproductive systems, you should be able to see the internal floor of the body cavity. Passing from **anterior** to **posterior** on the **midline**, you should be able to see two whitish nerve cords which form three paired ganglia in the thorax and five smaller ganglia in the abdomen. Ganglia are collections of nerve cell bodies and are where integration of nerve signals takes place.

Figure 9



Hints on Microscope use:

In order to conduct this lab you need to be able to use microscopes, both compound and dissecting. Please know and follow the following safety directions:

1. Always carry a microscope with both hands.
2. Never turn a microscope upside down; the lenses can fall out.
3. Grasp the plug, not the wire, to unplug a microscope or a light.
4. Always return your scope to the cabinet at the end of lab.
5. You will have the same microscope for the entire quarter. Please be sure to take note of the scope number and its location in the cabinet.

****Before the end of the period – be sure you know the diameter of the field of view for the compound microscope and for all objectives for your compound microscope.**

Field of View and estimating the field diameter

1. Place a clear plastic ruler with millimeter markings (1 cm=10mm) across the opening in the microscope stage. If the ruler is thin enough, you can gently slip it under one side of the stage clip mechanism. Using the 4X objective lens, position the ruler so that one millimeter mark is at the very left edge of the field of view. Count across to the right edge. If the final mm is not complete, estimate the portion of it in the field of view. For example, if you count 7 full mm, and about one-quarter of the next one, record the field diameter as 7.25 mm.

2. Don't try to use the ruler to estimate field diameters for the 10X and 40X objective lenses; the ruler is not precise enough for that. Instead, use the following equations to calculate the diameters.

   Field Diameter (FD in mm) at 100X Total Magnification = 40X/100X * FD (in mm) at 40X Total mag
   FD (mm) at 400X lens = 40X/400X * FD (mm) at 40X

   Record these values on both the worksheet and something that you will **always** bring to lab with you (syllabus, notebook cover, something like that) so that you can use them in future exercises.

   **An alternative method is to use your measured FD at 40X to find the Field # of your scope (Eq 1). Then you can plug that number into the general equation on the right (Eq2):

   Field Diameter (mm) = Field # (a Constant, but unique to each scope) / Objective magnification

   FD = Field # / Mag

   Eq1: 5mm = Field # / 40X
   5mm * 40X = Field #
   200 = Field #

   Eq2: What is the Field Diameter (FD) at 400X?
   FD = 200 / 400X
   FD = 1/2 mm or 0.5 mm

3. Now, when you view an item, you can estimate how much of the field of view it covers, and then calculate its approximate size. For example, if I calculated a field diameter of 7.25 mm when using the 4X objective (at 40X Total Magnification) lens, and a specimen I examined using that lens extended about 2/3 of the way across the field of view, then its approximate length would be 2/3 * 7.25 mm = 4.8 mm.

MORE hints on Microscope use:

Beyond safety, you must learn how to view images through microscopes. If you have never looked through a microscope before, take time to ensure that you can see images and that you know how to focus those images for your own eyes.

1. **Using your dissecting microscope:** Can you see the cricket? Notice the tabs on the scope that show you what to turn to change the focus and change the amount of magnification. Notice also that you can light the specimen from above or below; experiment with the lights to see which gives you better illumination.
2. **Using a compound microscope:**
    a. Examine the slides provided. Start with the microscope on low power (a lens that magnifies four times) and focus, using the coarse focus first and then the fine focus.
    b. Once the slide is in focus for your eyes, move the objective lens to the next medium power (a lens that magnifies 10 times) and focus, using the coarse focus first and then the fine focus.
    c. Now, move the objective lens to high power (a lens that magnifies 43 times or more) and use only the fine focus knob to focus the image.
    d. Move the iris diaphragm while you look at the slide. Notice how when the light is too bright you can't see details but that cutting down the amount of light can, up to a point, improve the image you see.