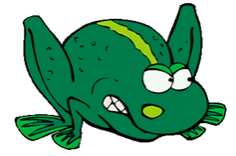


Day 1: Introduction to the Frog



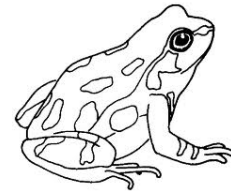
Frogs, toads, and salamanders belong to the class Amphibia (amphi = both, bio = Life) which means they are adapted to living on land, in moist places, or in water. They have a backbone, three chambered heart, fertilize their eggs externally and lay their eggs in water.

As a member of this class, the frog occupies a unique evolutionary position, retaining certain primitive features characteristic of the more advanced land vertebrates. In this unit we will explore some of these features.

The intermediate (larval) stage of a frog is called a tadpole. Tadpoles breathe by means of gills, feed and move about aided by long flexible tails, and feed on aquatic plants. This is their relationship to fish. After a time, tadpoles undergo an abrupt transition to the adult, more advanced stage, called metamorphosis.

During metamorphosis both internal and external anatomical changes occur that enable the

- ❑ Fore & hind limbs to develop,
- ❑ Gills are replaced by functional lungs
- ❑ The tail is reabsorbed
- ❑ The digestive tract is shortened to aid in the digestion of animal protein
- ❑ A tongue develops
- ❑ Eyelids appear
- ❑ The eye lens changes shape so they can see out of water.



There is one genus of frogs, *Rana*, which is used more than any other in the laboratory for student dissection. Two species are used for dissection: *Rana pipiens* (leopard or grass frog) and *Rana catesbeiana* (bull frog).

While the bull frog is native to the Southern United States, it has, however been transported to other parts of North America and has become well-established in many ponds and lakes and has become an ecological disaster for the native frog and salamanders. It out-competes the native amphibians for food and shelter and has caused the extinction of many of those fragile species.

Frogs are preferred for dissection because their organs can be compared to those of the human and they are small and inexpensive.

Before dissecting a laboratory animal a few definite rules should be observed:

1. Read the directions carefully before proceeding with the dissection.
2. Know what you are doing before you cut.
3. Try to answer your own questions before you ask for help.
4. Do neat & careful work. **Never remove an organ until you have received credit for finding it and are positive that removing it will not destroy parts still unstudied.**
5. Compare your dissection with others because animals often differ in subtle and interesting ways.

Get to know the names and proper use of each of the following dissection tools:

1. Dissecting Tray – wax is not for graffiti but to hold down the organism.
2. Pin – not for poking but for holding the organism open.
3. Forceps (tweezers) – for holding and identifying parts to see other organs.
4. Dissecting Probe – for exploring and pushing organs aside.
5. Scissors – for cutting. Use scissors for most of your incisions.
6. Baggie – Label with your period, table and name for storage.

Day 1: External Anatomy Dissection Instructions and Information Sheet



Rigor Mortis

When you first receive your frog, notice that your frog is stiff and not limp. The muscle fibers have lost the chemicals they need to contract and relax so they are fixed in one position. This is called “**rigor mortis**”. The phenomenon is caused by the skeletal muscles partially contracting. The muscles are unable to relax, so the joints become fixed in place.

More specifically, what happens is that the membranes of muscle cells become more permeable to calcium ions. Living muscle cells expend energy to transport calcium ions to the outside of the cells. The calcium ions that flow into the muscle cells promote the cross-bridge attachment between actin and myosin, two types of fibers that work together in muscle contraction. The muscle fibers ratchet shorter and shorter until they are fully contracted, or as long as the neurotransmitter acetylcholine and ATP are present.

Orientation

When dissecting the frog it is important to communicate about the location of structures. The following terms help scientists describe the locations of body parts.

Locate the following orientations on your frog

- ★ **Dorsal:** the back side
- ★ **Ventral:** the lower/belly side
- ★ **Anterior (Cranial):** the forward part towards head
- ★ **Posterior (Caudal):** the hind part towards tail
- ★ **Medial:** toward the central longitudinal axis of the body
- ★ **Lateral:** toward the longitudinal line along the sides of the body; away from the central longitudinal axis

Appendages (Legs and Arms)

Forelimb – Located at the anterior region of the trunk. It is much shorter than the hind limb and functions mainly in body support. Each forelimb consists of an upper arm, forearm, and hand.

Hand Digits – Check out the medial digit on each forelimb. If the largest digit (“thumb”) bears a “**nuptial pad**” (a swelling of the digit that is grayish in color), it is a male because he uses that pad to grasp the female during mating.

Web – A membranous sheet extending between each digit of the foot. Webbing increases the surface area for increased efficiency in swimming.

Skin

The soft, smooth, moist skin of the frog is a very complex body organ. It serves for protection, sensory reception, thermoregulation (regulating the internal temperature of the frog), and to some extent, as a breathing organ. Numerous poison and mucus glands are located in the skin. The poison secreted by these glands makes frogs distasteful to other organisms. The slimy mucus glands help reduce friction while the frog swims through the water, and help protect against desiccation (drying out).

The frog’s skin is richly colored. The general body tone blends with the surroundings, while the darker spots and blotches tend to hide the animal from predators. Leopard frogs do not contain any green pigment. Light reflected back (blue and yellow) results in the greenish color. The rest of the light is absorbed by the various pigments or scattered by the refractive granules in the skin.

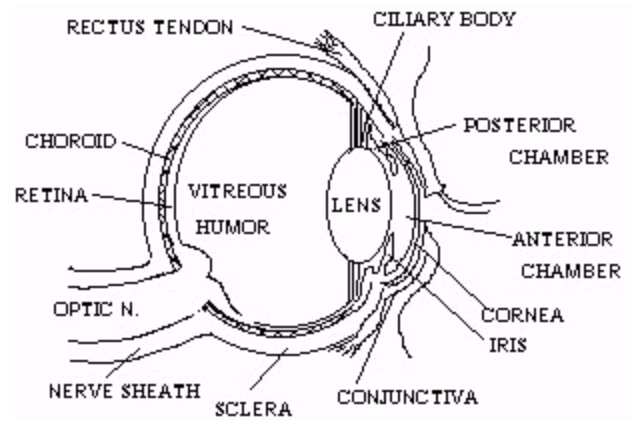
Head Region

On the dorsal anterior end of the frog you will find a pair of openings called the **external nares**. These nostrils are used by the frog to bring air into the lungs.

Brow spot – a light colored spot between the eyes on the dorsal surface about the size of a pinhead. It is a vestige of a median eye that was present in primitive vertebrates.

Eyes

Locate the frog's eyes, the **nictitating membrane** is a clear membrane that attached to the bottom of the eye that allow frogs to see while they swim. Use forceps to carefully remove the nictitating membrane. You may also remove the eyeball. If you happen to cut through the cornea and into the eyeball, you will notice the fluid of the **vitreous humor** oozing out and a hard cornified ball called the **lens**. The lens is made out of skin material not unlike a callus on your skin. Remove the lens and notice if your frog's is clear or cloudy. A cloudy lens is called a **cataract** and in humans a lens is replaced by surgery when the cataract prevents clear vision.



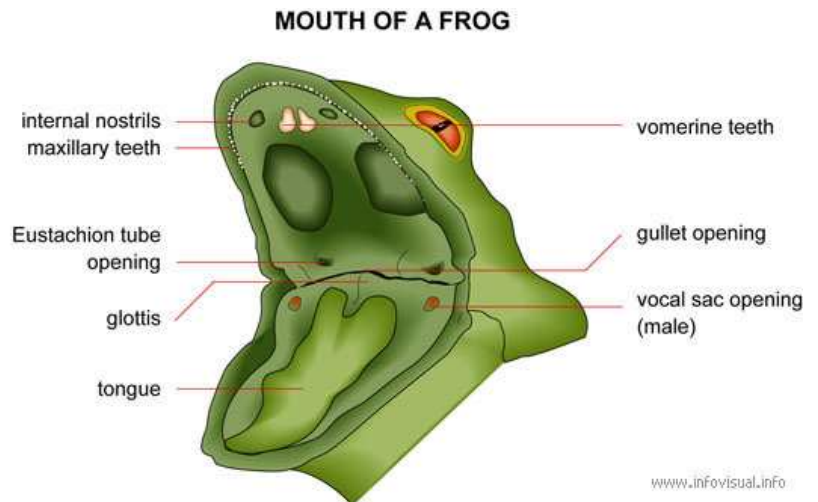
Hearing

Just behind the eyes on the frog's head is a circular structure called the **tympanic membrane**. The **tympanic membrane** is used for hearing. It works like your eardrum by vibrating when a sound wave reaches the frog.

Anatomy of the Frog's Mouth

Procedure: Pry the frog's mouth open and use scissors to cut the angles of the frog's jaws open. Cut deeply enough so that the frog's mouth opens wide enough to view the structures inside.

1. Locate the **tongue**. Notice its special attachment in front of the mouth so it can reach out and catch prey.
2. Just behind the tongue, and before you reach the back of the mouth is a slit like opening surrounded by a round muscle. (You may need to use your probe to get it to open up). This slit is the **glottis**, and it is the opening to the lungs. The frog breathes and vocalizes with the glottis. The round muscle, or sphincter, opens and closes to prevent water from entering the lungs. It functions like your epiglottis.
3. In the center of the mouth, at a crease in the back is a single opening called the **gullet opening** which leads to the **esophagus**, a tube that leads to the stomach. Use a probe to poke into the gullet into the esophagus.
4. Close to the angles of the jaw are two openings, one on each side. These are the **Eustachian tubes**. They are used to equalize pressure in the inner ear while the frog is swimming. Humans have Eustachian tubes too. When your ears “pop” when you are driving in the mountains it is through the Eustachian tubes that the air passes from your mouth to equalize the pressure on the inside of your eardrum. Insert a probe into the Eustachian tube. Observe what structure does the Eustachian tube leads to.
5. The frog has two sets of teeth. The **vomerine teeth** are found on the roof of the mouth. The **maxillary teeth** are found around the interior edge of the mouth. Both are used for holding prey, frogs swallow their meals whole and do NOT chew. Use your finger to feel the rough surface of both the Vomerine and maxillary teeth. Imagine how well adapted they are for holding flies and bugs.
6. On the roof of the mouth, you will find two tiny openings, if you put your probe into those openings, you will find they exit on the outside of the frog. These are the **internal nares** or nostrils.



Day 2: Dissection Instructions for the Frog Internal Organs

1. Dissection of the Body Cavity

Insert the point of your scissors through the skin just above the anal opening and make an incision extending to the lower jaw. Cut around the edge of the body on both sides and remove the pieces of skin. Examine the inner side of the skin and note the pattern of the blood vessels. Study the muscle layers exposed by the removal of the skin. Now, remove the muscle layers in the same manner in which you remove the skin. In the region of the thorax, it will be necessary to cut through the bones of the arms and the shoulder. Remove the muscle layers with your forceps.

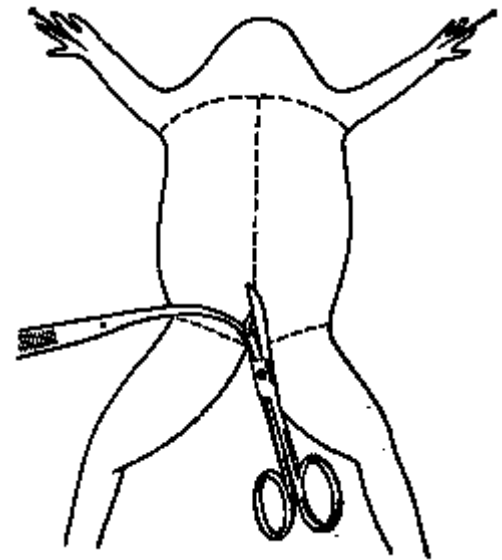
If your specimen happens to be a female, the body cavity may be nearly filled with eggs. In this case, it will be necessary to remove the **ovaries & eggs** with your forceps before proceeding further. Note that the eggs are loose in the cavity. You will find, also, the long coiled, white **oviducts** leading from the anterior end of the body cavity to the posterior end. These are the tubes through which the eggs pass and should not be confused with another tubular structure to be referred to later.

Find the large three-lobed **liver** high in the body cavity. Spread the lobes of the liver apart with your probe and find bright green **gall bladder** under the frog's right lobe. Raise the liver on the left side and notice the position of the **stomach**. Follow the **small intestine** from the lower end of the stomach to the **large intestine**. Examine the **mesentery** and the manner in which it holds the small intestine in position. Find the **spleen** in the mesentery. The **pancreas** is attached to the inner curve of the stomach, usually on the underside but can also be on the top side.

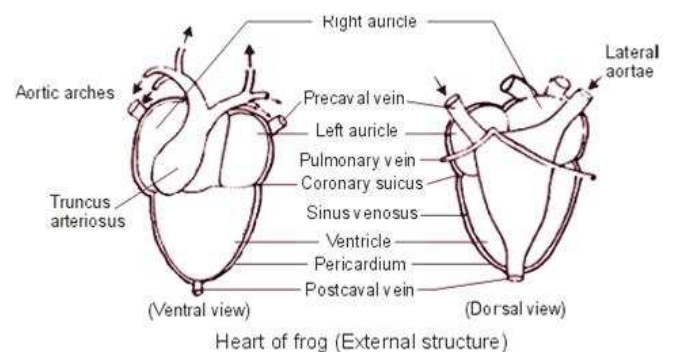
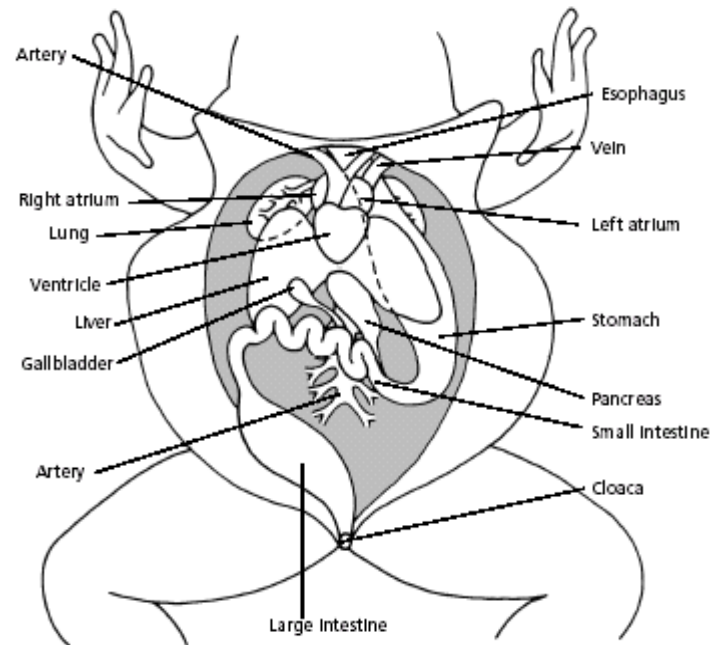
Without removing any of the above organs, locate the **lungs**, the **ventricle**, and the two **auricles** of the heart, the **pericardium**, the **fat bodies**, the **kidneys**, **urinary bladder**, and **testes**, if the animal is a male. Label all of the underlined structures in prepares drawing and sketch them in if they are not there.

The Heart

Examine the heart, which lies in a thin sac, the **pericardium**. Using your probe, find the great veins and the arteries leading to and from the heart. Remove the heart, severing the necessary blood vessels. Examine the front side of the heart with your hand lens. Three branches of the vena cava unite in a thin-walled sac, the sinus venosus, on the back wall of the heart. The sinus venosus opens into the right auricle. Pulmonary veins from the lungs join the left auricle. Examine the front and the back views of the heart in the text, and compare them with your specimen. Make a drawing of the front and the back views of the heart and label: **ventricle**, conus arteriosus (front view) and ventricle, **right auricle** (atrium), **left auricle** (atrium), vena cava, sinus venosus, pulmonary veins (back view), 3-chambered heart.



Abdominal and Chest Cavities of the Frog



Locate each of the organs below. Check the box to indicate that you found the organs.

1. **Fat Bodies:** Finger-like structures that have a bright orange or yellow color used for storing fat. If you have a particularly fat frog, these fat bodies may need to be removed to see the other structures. Usually they are located just on the dorsal side of the abdominal wall towards the medial line.
2. **Peritoneum:** A spider web like membrane that covers many of the organs, you may have to carefully pick it off to get a clear view. The pericardium covers the heart specifically.
3. **Liver:** The largest structure of the body cavity. This brown colored organ is composed of three parts, or lobes. The right lobe, the left anterior lobe, and the left posterior lobe. The liver filters out toxins and fats, stores glucose, and secretes a digestive juice called bile. Bile is needed for the proper digestion of fats.
4. **Heart:** at the top of the liver, the heart is a triangular structure that circulates blood throughout the body. The left and right atrium (**auricles**) can be found at the top of the heart. A single **ventricle** located at the bottom of the heart. The large vessel extending out from the heart is the conus arteriosus.
5. **Lungs:** Locate the lungs by looking underneath and behind the heart and liver. They are two spongy organs.
6. **Gall bladder:** Lift the lobes of the liver, there will be a small green sac under the liver. This is the gall bladder, which stores bile. (Hint: It kind of looks like a booger)
7. **Esophagus:** Return to the stomach and follow it upward, where it gets smaller is the beginning of the esophagus. The esophagus is the tube that leads from the frog's mouth to the stomach. Open the frog's mouth and find the esophagus, poke your probe into it and see where it leads.
8. **Stomach:** Curving from underneath the liver is the stomach. The stomach is the first major site of chemical digestion. Frogs swallow their meals whole. Follow the stomach to where it turns into the small intestine. The pyloric sphincter valve regulates the exit of digested food from the stomach to the small intestine.
9. **Small Intestine:** Leading from the stomach. The first straight portion of the small intestine is called the duodenum, the curled portion is the ileum. The ileum is held together by a membrane called the mesentery. Note the blood vessels running through the mesentery; they will carry absorbed nutrients away from the intestine. Absorption of digested nutrients occurs in the small intestine.
10. **Mesentery:** Thin, spider-web-like network of membranes that hold the small intestines together.
11. **Pancreas:** Located along the inner edge of the stomach, it is long, thin, and pinkish-brown in color. It is responsible for contributing digestive juices to the small intestine.
12. **Large Intestine:** As you follow the small intestine down, it will widen into the large intestine. The large intestine leads to the cloaca in the frog. The cloaca is the last stop before wastes, sperm, or urine exit the frog's body. (The word "cloaca" means sewer.)
13. **Spleen:** Return to the folds of the mesentery, this dark red spherical object serves as a holding area for blood and immune cells.
14. **Kidneys:** They are reddish brown organs located to the back of the frog and close to the spine. There should be 2 of them. They filter out cellular waste, which forms urine.

STOP! If you have not located each of the organs above, do not continue on to the next sections!

Lung Inflation: Cut the top 2mm off a micropipette and insert the tip into the glottis in the mouth. When you blow into the pipet you should see the lungs inflate if you have not damaged the lung.

Removal of the Stomach: Cut the stomach out of the frog and open it up. You may find what remains of the frog's last meal in there. Look at the texture of the stomach on the inside.

What did you find in the stomach?

Measuring the Small intestine: Remove the small intestine from the body cavity and carefully separate the mesentery from it. Stretch the small intestine out and measure it. Now measure your frog. Record the measurements below in centimeters.

The Urogenital System

The urogenital system of the frog consists of organs that function in reproduction and excretion. You will need to locate all of the structures regardless of whether your frog is a male or a female. You will need to look at other groups' frogs in order to complete the dissection. Check the box when you have located the structure. The descriptions of their locations and appearance will help you label the diagrams.

Male Frog	Female Frog
<ul style="list-style-type: none">❑ Locate the kidneys. They are reddish brown organs located to the back of the frog and close to the spine.	<ul style="list-style-type: none">❑ Locate the kidneys. They are reddish brown organs located to the back of the frog and close to the spine.
<ul style="list-style-type: none">❑ Find the vessels attached to the kidneys. These are the renal vessels (it may be difficult to determine which is an artery and which is a vein).	<ul style="list-style-type: none">❑ Find the vessels attached to the kidneys. These are the renal vessels (it may be difficult to determine which is an artery and which is a vein).
<ul style="list-style-type: none">❑ The orangish-yellow hair like structures attached to the top of the kidney are the fat bodies.	<ul style="list-style-type: none">❑ The orangish-yellow hair like structures attached to the top of the kidney are the fat bodies.
<ul style="list-style-type: none">❑ Locate the testes which are light colored oval bean-like objects at the top of the kidney.	<ul style="list-style-type: none">❑ The tube-like, noodle-looking structures to the side of the kidneys are oviducts. They may be more swollen in some females than others. Eggs can be seen as black speckled masses in the frog's body cavity.
<ul style="list-style-type: none">❑ Small tube-like structures to the side of the kidney are vestigial oviducts. In the male frog, they have no function.	<ul style="list-style-type: none">❑ At the top of the oviducts is the ostium. This is where the eggs in the body cavity enter the oviducts to be excreted. You may not be able to find it on your frog.
<ul style="list-style-type: none">❑ The tubes that extend from the kidney called ureters carry urine to the bladder, which exits the body through the cloaca.	<ul style="list-style-type: none">❑ The oviducts lead to the cloaca, eggs and urine will exit the body through the cloaca.
<ul style="list-style-type: none">❑ The flaplike structure closest to the surface is the bladder, which is empty in a preserved frogs	<ul style="list-style-type: none">❑ The flaplike structure closest to the surface is the bladder, which is empty in a preserved frogs
	<ul style="list-style-type: none">❑ The tube the extends from the kidneys to the cloaca is the ureter - it carries urine.