Ecol 483/583 – Herpetology
Lab 5: Amphibian Anatomy
Spring 2010

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Lab objectives
The objectives of today's lab are to:
1. Learn the basic internal anatomy of the Urodela and Anura.
2. Learn differences in anatomy between these two clades.
3. Review and consolidate your knowledge of amphibian diversity.
4. Learn about statistical data analysis. **Also print out the statistical guide for the course, so that you can take notes.**

Today's lab is the final amphibian lab. It will introduce you to some basic amphibian anatomy, give you a chance to dissect a specimen, and compare anatomy of salamanders and frogs. As it is the last lab that is devoted to amphibians, you are encouraged to spend any extra time reviewing from the previous labs. Keep in mind that your lab exam is next week, so make sure that you use your time wisely.

Tips for learning the material
Anatomy is a very detailed and precise field of science. Small differences matter and every structure has a name. Although we are not learning any new species during this lab period, there is a considerable amount of material to learn, so do not blow this lab off. You should already be familiar with the external anatomy of amphibians, so today's lab will focus on the internal anatomy. Some of this will be osteology (the study of bones), and some will be soft tissues.

To learn the material, work through everything on display systematically. Pay attention to how bones and organs are arranged in each animal. Also be aware of how these animals' anatomies differ from one another. A frog has quite a specialized body shape relative to a salamander, and this will have implications for the anatomy.

Use the concept of homology to help you throughout. Most of the anatomical structures that you will be learning are not novel to each clade, but homologous among clades. This means that a frog stomach and a salamander stomach serve very similar functions, look similar (not the same), and are in a similar location within the body. This will help you to identify and learn structures, but is also useful in studying the evolution of the animals we study.

Exercise 1: Osteology

A. Cranial skeleton

Amphibian skulls are quite different from those of other tetrapods. There is a general reduction in the number of bones and, in the Anura, a lightening of the skull. Pay attention to these differences, and consider how the skulls of the three major amphibian clades - Urodela, Anura, and Gymnophiona - differ from each other.

The skull of Urodela tends to be a rather solid structure, but there is considerable variation in the degree of robustness. For example, an *Amphiuma* has a very robust skull with powerful jaws, while a *Plethodon* has a reduced skull that is far less robust (and uses its tongue for prey capture). On display today, there is the skull of a tiger salamander (*Ambystoma tigrinum*), and a copy of different skull views of *Necturus maculosus*. These will form the basis of comparison.

The easiest way to get started identifying bones in the salamander skull is to start with those that have teeth on them. In *Ambystoma*, the anterior-most bone with teeth is the **premaxilla.** It is followed posteriorly by the **maxilla**, which bears most of the remaining teeth of the upper jaw. Also notice that there are some teeth medial to the main upper tooth row – these are on the **vomer**. In some species that lack the maxilla, like *Necturus*, the mudpuppy, the vomer projects more laterally and forms part of the main upper tooth row. Now turn your attention back to the premaxilla. If you look at the skull dorsally, then the bone immediately posterior to the premaxilla is the **frontal**. This is a very large skull bone, forming a large part of the brain case. It has anterolateral projections that connect to the maxilla. Posteriorly, the frontal articulates with the **parietal**. Posterior to the parietal is the **prootic-exoccipital**, with which the first vertebra articulates. Articulated laterally with this bone is the **squamosal**, which is directed laterally and ventrally. Articulated with it is the **quadrate**, which articulates with the lower jaw, as you saw in the reptiles. **Identify all of these bones on the *Ambystoma tigrinum* skull. Also compare what you see in the *Ambystoma* specimen to what you see on the *Necturus* picture. Note the similarities and differences.**

The prootic-exoccipital forms from the fusion of two bones. What are they called? How does what you see in *Ambystoma* compare to what you see in *Necturus*?

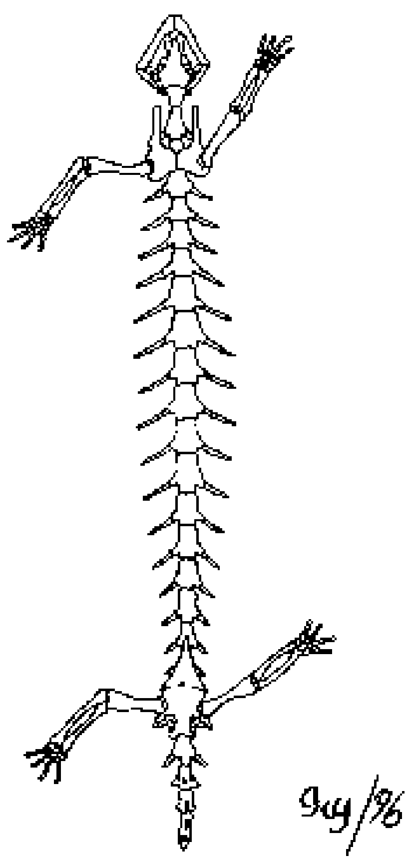
Are the frontals and parietals paired or fused in each of these two species?

Identify these bones in the other salamander skull pictures on display. Remember, you might be asked to ID a bone on one of the other specimens on an exam…

On display are several frog skulls – two are in plastic blocks and two larger ones are wired into another prep. You also have some pictures available to you. **Examine these skulls and compare them to what you saw in the salamander.** There are many similarities, but also differences with the salamander skull. The tooth bearing bones are the same: premaxilla, maxilla and vomer. The **nasals**, also present in salamanders but not identified in this lab, lie posterior to the premaxilla, as you move posteriorly in the dorsal aspect. Posterior to the nasals is a bone that is absent from the salamander: the diamond-shaped **sphenethmoid**. The pattern of fusion of bones differs between the frog and the salamander. In the frog, the frontal and parietals are fused into the **frontoparietal**. Posterior to that, are the **exoccipitals**, which articulate with the first vertebra, and lateral to those are the **prootic** bones. The squamosal articulates laterally with the prootic, and with the **quadratojugal** – there is no quadrate in the frog.

Which two bones are fused into the quadratojugal?

What differences do you see between the salamander and the frog skull? Why do you think that they are different in these places?



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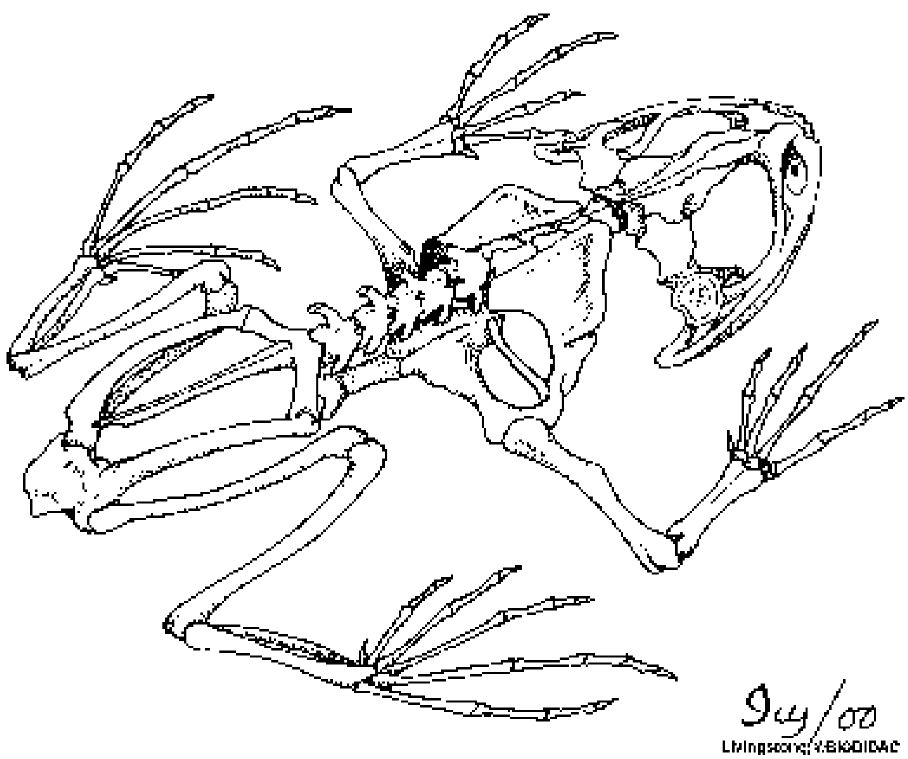
B. Postcranial skeleton

The postcranial skeleton of the Urodela and Anura is quite different as well. Salamanders have a rather generalized body plan and skeleton, with a rather typical arrangement of bones. For example, there are distinct **humerus, radius, ulna**, and **femur, tibia and fibula**, in the front and hind limbs, respectively. You do not need to differentiate the radius and ulna or the tibia and fibula in the salamander, just know where the bones are in the limbs. Salamanders have a complement of **vertebrae**, but, like most amphibians, lack well-developed ribs. Vertebrae are divided into **presacral** (those composing the body), and **postsacral** or **caudal** (those making up the tail). The vertebrae that articulate with the **sacrum** are called **sacral**. **Identify all of these bones and label them in the diagram to the left.**

How many presacral, sacral and caudal vertebrae does the *Ambystoma* salamander skeleton on display have? How many of each type of vertebra does *Taricha torosa* (pictured) have?

Species	# Presacrals	# Sacrals	# Caudals
Ambystoma tigrinum			
Taricha torosa			

In contrast, the frog skeleton is highly specialized for jumping locomotion. It looks unlike any other vertebrate. The body is made shorter and more rigid by reducing the number of vertebrae. The tail vertebrae are not recognizable as such – instead they have fused and been incorporated into the **pelvis** as the **urostyle**, a rod like bone that allows for insertion of muscles on it. The limbs are also modified. Although there are a humerus and femur, the radius and ulna have fused into the **epipodial bone** and the tibia and fibula have fused into the **compound bone**. Note that this means that the two most proximal segments of the limbs have a single bone element. The next segment, the ankle, in the hind limb is elongate. The hind limbs are massive, the front limbs are substantially shorter. **Label all of these bones on the skeleton below.**



How many vertebrae does the frog skeleton have?

Which vertebrae in the salamander are the vertebrae of the frog homologous with?

What bones is the urostyle homologous with in the salamander?

Compare the pelvic girdles and sacrums of the frog and salamander and hypothesize as to the functional reasons for those differences. Think about how these animals move and the size of the hind limbs.

Exercise 2: Soft anatomy

During this exercise, some of you will be dissecting a tiger salamander (*Ambystoma tigrinum*) and some of you will be dissecting a bull frog (*Rana catesbiana*). **Work in pairs.** Although you will be dissecting only one of these species, make sure that you take a detailed look at completed dissections of the other, done by other students. You are responsible for both sets of material. Also, take a look at examples of a male and a female of each species - you will also be responsible for knowing material for both sexes.

You will dissect the salamander and frog to learn the digestive and urogenital systems. For these systems, most vertebrates are dissected in a similar way, just the layout of the body differs and organs will be in slightly different places. You can follow the same set of instructions for a salamander or a frog. Do keep in mind, however, that these amphibians, especially the salamander, are smaller and quite delicate. Be particularly gentle with them and dissect carefully. Also, because of all of the mucous glands in amphibian skin, these specimens will feel quite slippery, more so than the snake you will dissect later in the semester. Take care not to cut yourself and stay very superficial when cutting through the skin and body wall. A deep cut will destroy some of the structures that you need to learn. **Take turns dissecting and work as a team, giving advice to one another as you go.** If you are unsure of how to proceed err on the side of caution. You can always ask the instructor for suggestions.

Dissect the specimens as follows:

1. Start by turning the animal ventral side up. Pinch the skin slightly with a pair of forceps slightly off the mid-ventral line near the cloaca. Make a small cut with the scissors though the pinched up skin. Then continue the cut anteriorly until you get to the jaw line. If the blades of your scissors are asymmetrical, make sure that the blunt blade goes inside. If you were really

superficial (which is good), you will have cut through just the skin and still have the body wall to go through.

2. Make a pair of cuts laterally from the mid-ventral cut immediately anterior to the hind limbs and immediately posterior to the front limbs. Peel the skin away from the midline. You may have to use forceps to grasp the skin because it is slippery. Separate the **fascia**, or connective tissue, that connects the skin to the underlying body wall. Once the skin is pulled back examine the body wall to see if you can make out the parallel striations of the external oblique muscle. They may be more visible laterally.

3. Skin the hind legs, particularly the legs. Observe the hind limb muscles. Those of the frog should appear quite well developed; those of the salamander much less so. **What is the function of these large leg muscles in the frog? Why doesn't the salamander need large muscles as well?**

4. Repeat your cuts through the body wall, again staying superficial so as not to damage underlying organs. You should now be able to fold away the body wall, gaining access to the body cavity and the organs you are interested in.

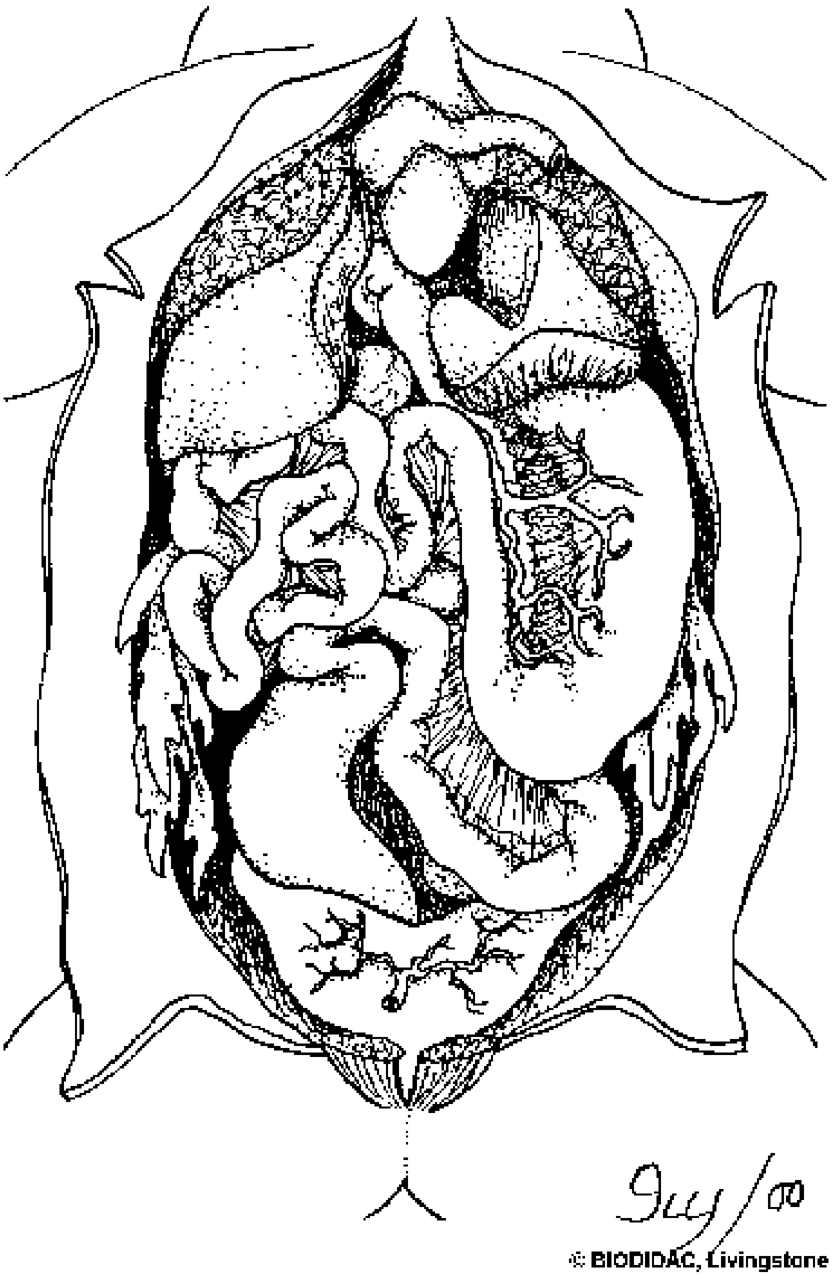
5. Yellow, finger-like projections in the body cavity are **fat bodies**. The easiest way to start identifying organs is to find the **liver**. It will be large, dark and lobular, taking up a considerable part of the abdominal cavity. The liver acts as a landmark for finding other organs. Anterior to the liver, you will find the **heart**, a dark, hard knot of muscle. To either side of the heart are the **lungs**, which will probably be deflated, but should nevertheless appear as soft, thin-walled sac-like structures. The heart and lungs are not in the abdominal cavity, but the liver is - you may have to cut (carefully) through some muscle to get to the heart and lungs. Try to trace the lungs anteriorly to find the **trachea**. One way to do this is to make a small puncture in one of the lungs and then use a **blunt** probe to find the entrance to the lung. This will be the trachea, which should lead you to the **glottis**, or the opening to the **buccal cavity** (cavity of the mouth).

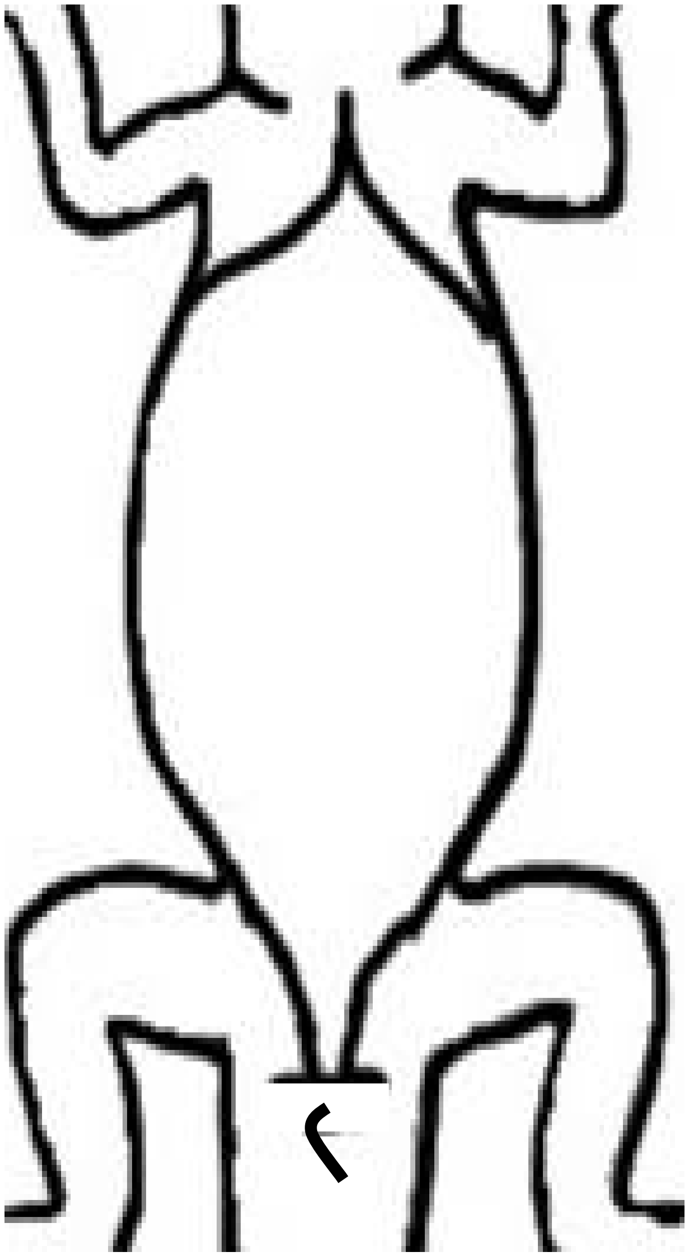
6. The **stomach** will be posterior and slightly to the left (the animal's left) of the liver. It will also be large in size and lighter in color than the liver. In the frog, it will be oriented somewhat transversely; in the salamander, more longitudinally. Once you find the stomach, trace the digestive system in both directions. Tracing in one direction will be the **esophagus**, leading to the mouth. In the other direction will be the **small intestine, large intestine**, and **cloaca**. The **pancreas** will be nestled in the bend of the stomach, near the anterior end of the small intestine. Look at the **mesentry**, a sheet of connective tissue suspending the intestines in the body cavity. Blood vessels run through the mesentry, supplying blood to the organs.

7. Finally find the **gonads** and **kidneys**. The gonads are rather discrete and well within the body cavity. **Testes** are small and "bean-like" in the frog and more leaf-live in the salamander. **Ovaries** are larger, and depending on the age of a female, may be near well-developed oviducts. **Sex your specimen – is it male or female?** The kidneys are **retroperitoneal**, meaning that they are not suspended by a mesentry, but instead are plastered against the dorsal body wall by connective tissue. They are relatively soft, dark and flat organs, not the bean-shaped organs that many mammals have. Find the **bladder** as well. It should be a sac-like structure near the cloaca and with an opening into the cloaca. The bladder is a ventrally positioned structure.

What differences in the position and orientation of the organs do you see between the frog and salamander? How does the body shape of these two animals play a role in determining position and orientation of the organs?

For the frog, label the bolded structures in the diagram below. For the salamander, draw what you see and label the structures.





Exercise 3: Metamorphosis

Although some amphibians undergo **direct development**, most undergo **metamorphosis**, where a larva metamorphoses into an adult. Substantial changes happen during metamorphosis, as the animal switches from a fully aquatic existence to a primarily terrestrial one (in most cases). The degree of differences, both morphological and ecological, between larva and adult stages differs among major amphibian clades. For example, larval salamanders look a lot more like adult salamanders, than larval frogs look like their adult counterparts.

Among the many changes that occur during metamorphosis are a loss of gills and the development of lungs (in some groups), the loss of swimming fins, a change in mouth parts, often accompanied by a switch in diet, and the development of limbs (among other changes). The switch in diet in frogs is accompanied by a change in the length of the digestive tract – frog tadpoles are herbivores, adults are carnivores (insectivores). In frogs, the changes are most obvious, with the tail being resorbed.

On display is a series of bull frogs, ranging from larva to adult. There are some intermediate stages in the midst of metamorphosis. Examine these, noting the changes that you see as you progress from larva to adult. **For each body region listed below, record the changes that you see and what the functional implications of the each change would be.**

Head:

Gills:

Front limbs:

Hind limbs:

Tail:

Gut:

Why would the digestive tract of the tadpole be of different length from that of the adult frog? Is it longer or shorter? How would this be adaptive for herbivory?

Do the front or hind limbs develop first? Why do you think this is?

Is there a change in color or pattern?