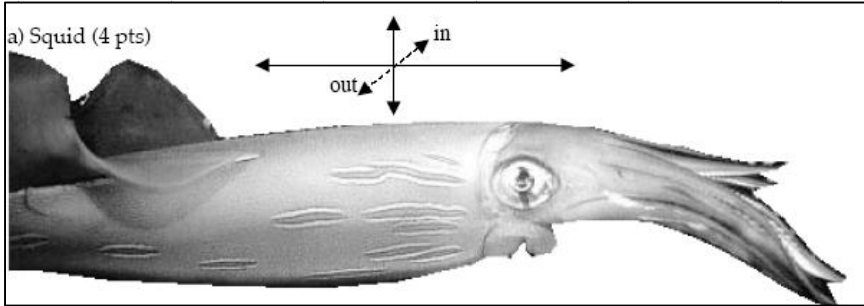


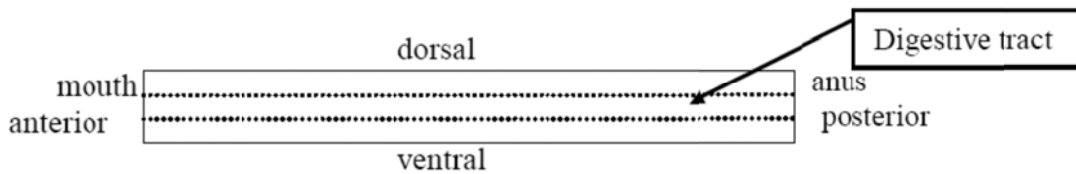
Name _____

Pre-Lab: Animal Diversity II

- 1) On each of the figures below, label the axes with dorsal, ventral, anterior, or posterior as appropriate. Note: dashed lines indicate axes that extend out of the plane of the picture.



- 2) Consider the hypothetical worm shown below:



What would you have to do (stretch, fold, etc.) to this worm to transform its body plan (including anterior, posterior, dorsal, ventral, mouth, anus, and digestive tract) it into an animal with a body plan like the squid?

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Animal Diversity : Dissection of the Trout

(*Salvelinus fontinalis*)

Objectives

- Examine the internal and external anatomy of trout.
- Compare and contrast the trout and the squid

Introduction

Phylogeny is the evolutionary history of organisms: their lines of descent, the branchings of these lines, and thus the relationships between organisms. Much of our understanding of animal phylogeny has come from comparative studies of the anatomy and embryology of present-day animals. Our concepts concerning their ancestral history and relationships have been extended, refined, and sometimes changed as a result of physiological, cellular or molecular studies.

Just as our understanding of animal phylogeny benefits from a study of anatomy, our understanding of anatomy is enhanced by an understanding of evolutionary principles. The form and function of all features of an organism are determined by: 1) the selection imposed by the organism's environment, and 2) the genetic/morphological/physiological constraints imposed by the general architecture that the organism's lineage has developed over the course of its evolutionary history. Regardless of their particular phylogenetic group, all living animals have the same basic requirements and must perform the same basic functions.

Animals may meet these problems in different ways because of differences in size, structure and environment. Within a single class, for example Mammalia, one may find animals as different in appearance as a mouse and a whale, although internally much of their machinery will be similar. You will also see examples of "convergence", where animals from different phylogenetic backgrounds and different basic architecture appear similar in many ways. As you work through the two lab periods devoted to phylogeny, keep examining animals with a view to both their phylogenetic history and the selection pressures exerted by their environments and try to build up a fuller picture of why animals are what they are today. Reading appropriate sections of your textbook will help guide the way.

We will have available in lab for dissection two different animals, the brook trout (*Salvelinus fontinalis*) and the squid (*Loligo pealii*), representing two major phyla: Chordata (Craniata in Five Kingdoms) and Mollusca, respectively.

GENERAL HINTS AND INSTRUCTIONS FOR DISSECTIONS

Preparation: Wash your animals in cold running water to remove slime and/or reduce fumes from the preservatives. Spray preserved specimens (squid) with humectant periodically, and rinse whenever fumes become annoying. **KEEP ANIMALS MOIST WITH WATER DURING DISSECTIONS** — dried out organs and tissues are impossible to dissect and maneuver. You should wear gloves to protect your hands.

Tools:

• **Scalpel:** This is the first tool that most people grab. It is the most dangerous one -both to the user and to the animal. The danger is, if you have a sharp scalpel, you can easily cut through

important structures before you realize what you've done. Thus, you should only use it when the scissors don't work. You should also be sure the blade is sharp; change it frequently.

- **Scissors:** These are the best tools for cutting through skin, etc. You can feel the different tissues better and are less likely to cut something important than you are with the scalpel. Be sure these are sharp; trade in dull ones immediately.

- **Pick:** Your 'best friend' once inside the animal. This can easily be used to pull apart and cut the connective tissue that holds organs to each other, but it is unlikely to break anything important unless you push really hard. You should use this most of the time.

All dissection instructions must be left in lab. **The lab must be left really clean**—rinse your pans, pins, and instruments, dry them carefully, and return them to designated places; clean up benches and sinks.

ANATOMICAL GLOSSARY

- **Anterior** or **rostral:** towards the head end.

- **Posterior** or **caudal:** towards the tail end.

“Your nose is anterior of your belly button. Your chin is posterior of your nose.”

- **Dorsal:** toward or near the back.

- **Ventral:** toward or near the belly.

- **Median:** in or near the plane in the middle of the body.

“Your belly button is ventral of your intestines.”

- **Proximal:** near the base or site of attachment.

- **Distal:** near the tip.

“Your fingernails are on the distal ends of your fingers.”

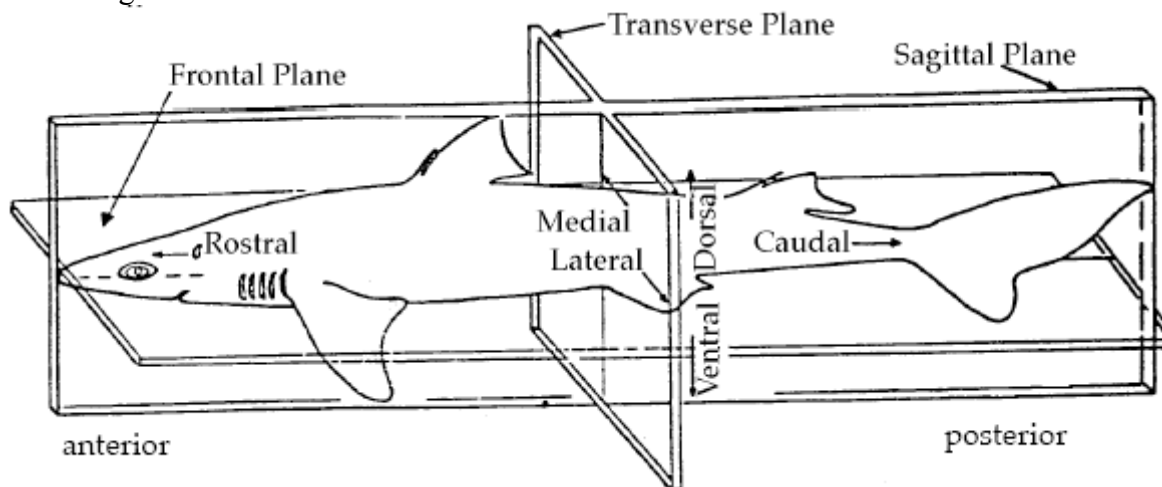
- **Sections** through the body are called:

Sagittal: dividing the animal into left and right sides

Frontal: dividing the animal into dorsal and ventral parts.

Transverse: dividing the animal into anterior and posterior parts.

See this figure:

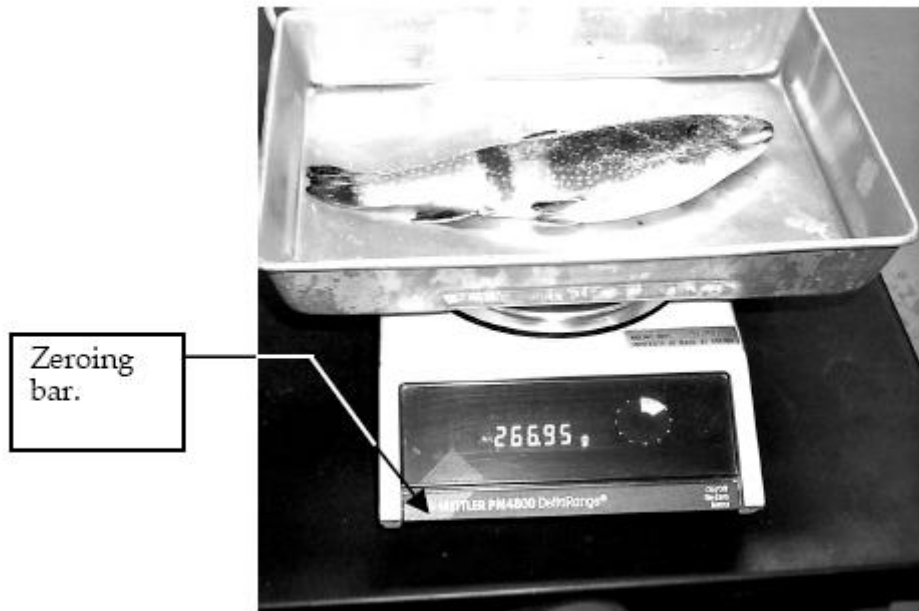


Part I Dissection of the brook trout (*Salvelinus fontinalis*)

This dissection should take you one lab period.

Note: Since later steps may destroy some structures, you should take notes and draw sketches as you go.

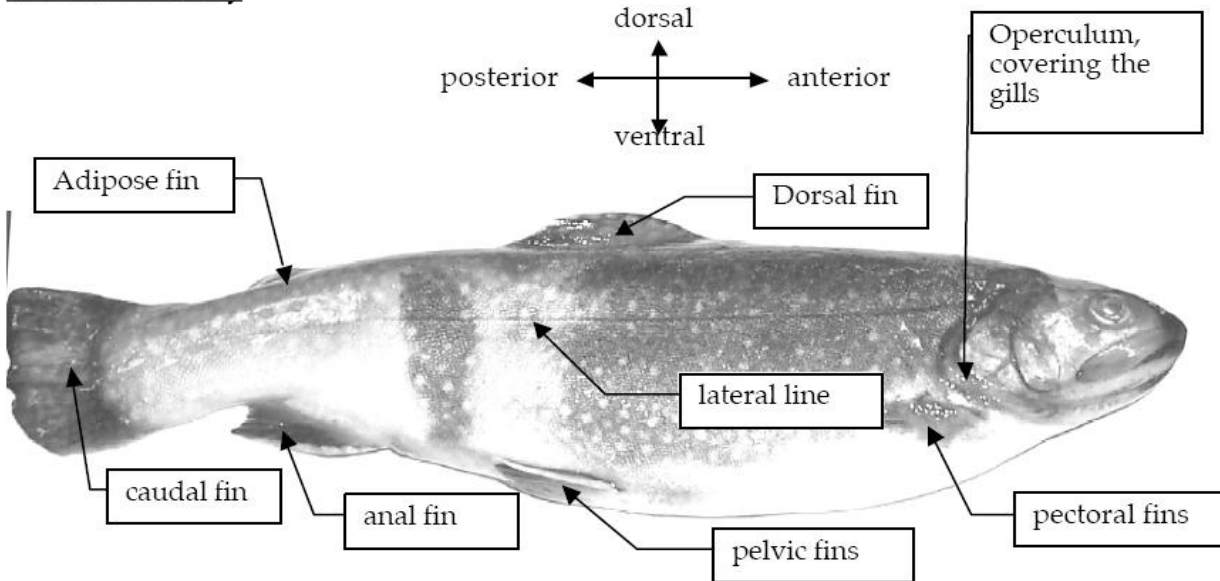
- 1) Put on gloves so that your hands won't smell of fish.
- 2) Obtain a fresh brook trout from your TA. These were raised at a fish hatchery in western Massachusetts and shipped fresh to UMass. Rinse it gently in cold water to remove any slime.
- 3) Bring your fish to the scale. Make sure there is a paper plate on the scale to keep the scale from getting wet. Zero the scale before putting the fish in the tray by pushing down on the big bar at the front of the scale. This is shown below:



- 4) After zeroing the scale without your fish on it. Put the fish on the tray and record the weight.

Weight of fish in grams _____

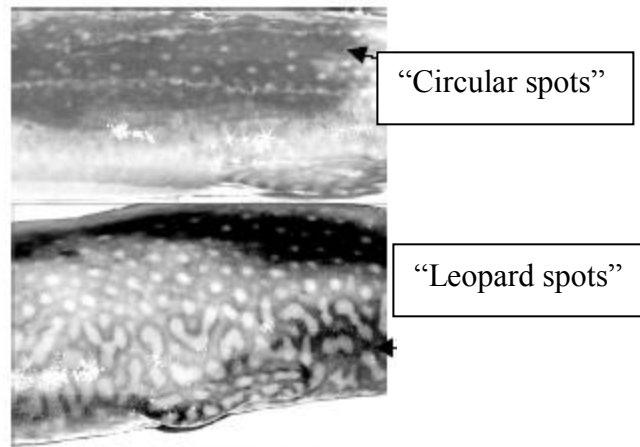
External Anatomy



- a) the lateral line is a sensory organ that the fish uses to sense vibration as well as to feel objects and other fish along its sides. In some fish, this plays a crucial role in schooling.
- b) the pectoral and pelvic fins are homologous to human arms and legs, respectively and are used in turning and stopping the fish.
- c) the dorsal and anal fins are used to keep the fish from rolling.
- d) the caudal fin provides most of the forward movement of the fish and controls direction.

Count the number of rays (bone-like spikes) in the dorsal fin. _____

5) Note the pattern of spots on the fish, especially on the dorsal side. Briefly describe them, using the photo below as a guide.

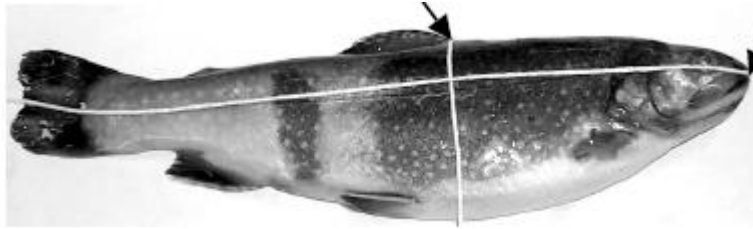


Spot pattern _____

6) Measure the length and girth of your fish as shown below. Use a string to measure the fish and then measure the string and record the value in your notebook.

- length (from tip of 'nose' to tip of tail along lateral line) _____ cm

- circumference (all the way around fish at anterior end of dorsal fin) _____ cm



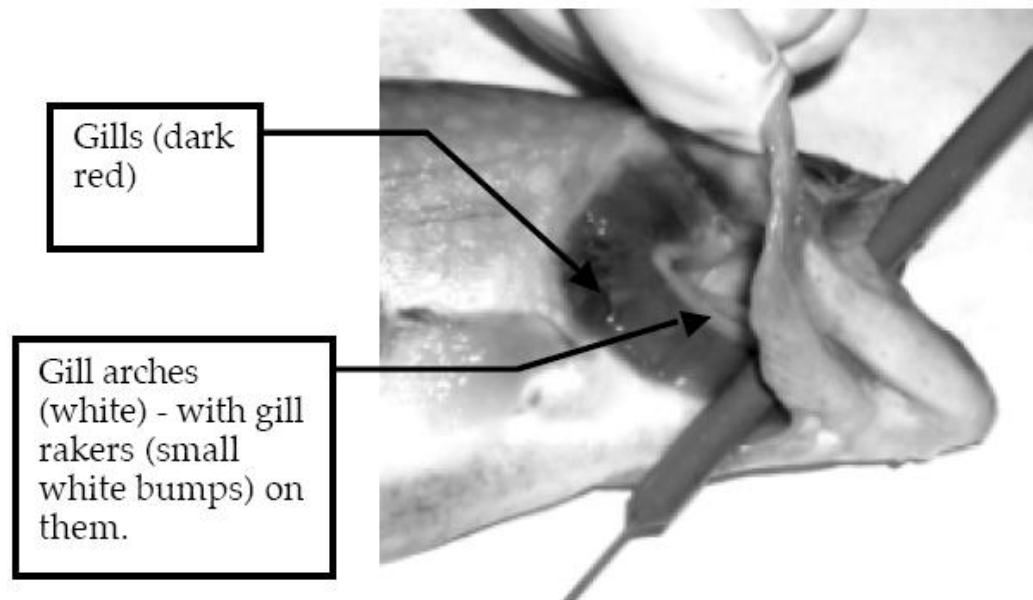
7) Measure the length of the lower (ventral) jaw as shown below. Be sure to open the jaw as wide as you can (be careful of the teeth).



Jaw length _____ cm

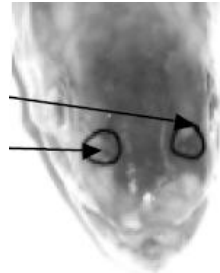
Respiratory System:

1. Lift open the operculum and look in at the gills and gill rakers as shown below.



2) Stick a blunt probe through the mouth as shown on the previous page to determine the flow of water over the gills and rakers. Observe the live fish in the tanks in the lab to see how the mouth and operculum function in respiration. What are the steps in a fish's "breath"?

3) The nares are circled in the picture below. They are used in smell sensation in the fish and bear a superficial resemblance to nostrils on a human. In a human, the nostrils are also used in breathing. As best you can, carefully dissect the area around the nares and determine if these can function in respiration in the fish.



****Stop and draw the respiratory system; consult the end of this exercise for assignment details.**

Internal anatomy

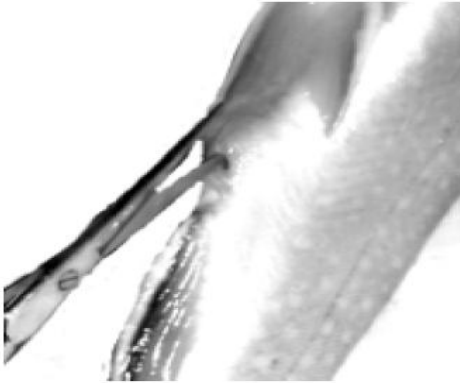
4) Open the body cavity of the fish. You must do this very delicately or you will make it impossible to study the internal organs. This is the most critical part of the dissection. Hold the fish with its ventral side up and locate the anus - it is the opening just anterior of the anal fin.

This is shown below:

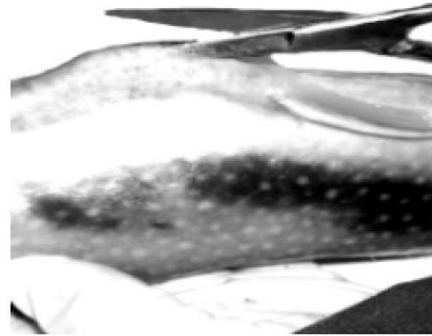


Insert the tip of a pair of scissors into the anus and cut towards the head; **DO NOT USE A SCALPEL HERE!** Be sure to cut as shallow a cut as you can so as not to disturb the internal organs. Cut as far anteriorly as you can - at least as far as operculum. You may have to go back over your cut to be sure it is deep enough. When it is deep enough, you should be able to open the sides of the body cavity to expose the internal organs.

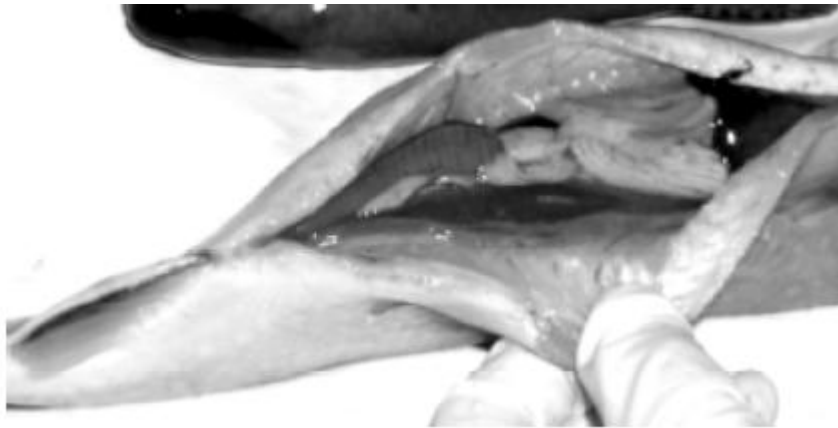
Starting to cut:



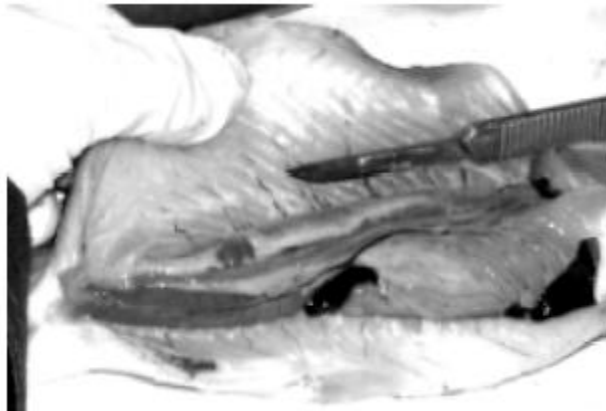
Cutting:



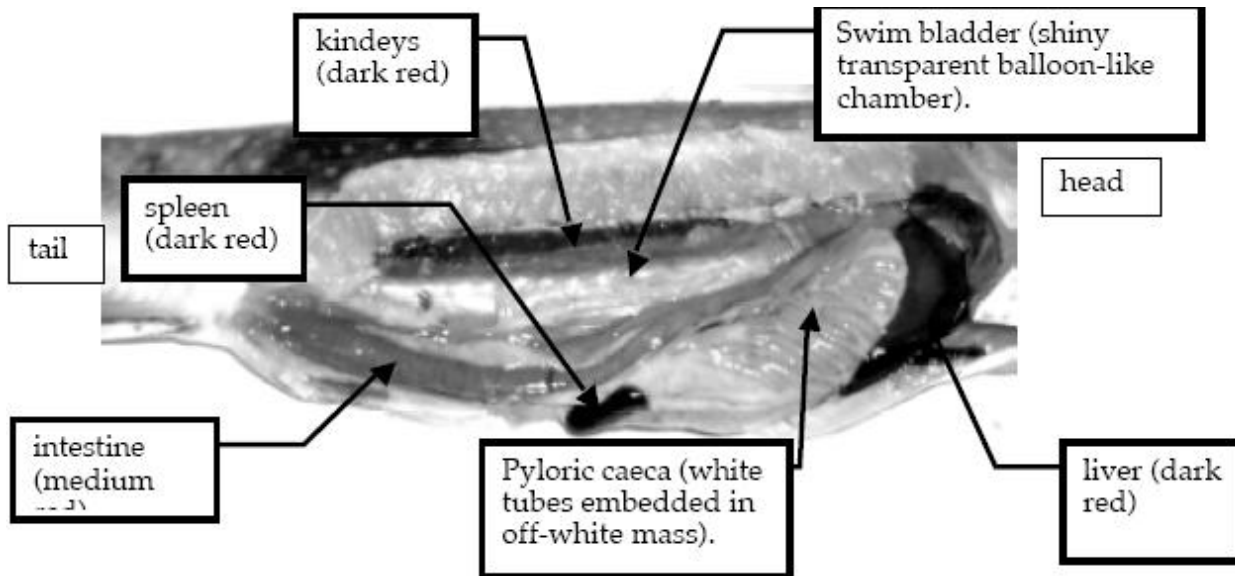
When you open it up, it should look like this:



5) On one side, carefully cut dorsally from the anterior and posterior ends of your first cut all the way to the lateral line. Lift open the 'filet' you have cut, carefully scraping any internal organs free with a scalpel.



Cut across the dorsal side of this 'filet' and remove it, completely exposing the body cavity. It should look like this:



6) The kidneys lie along the dorsal end of the body cavity as shown in the previous diagram.

7) If the body cavity contains eggs, then you have a female; if not you have a male. Eggs are spherical and vary in size and color - the smallest are 0.5 mm and white, the mature large eggs are 3-5 mm and clear with a white spot. Record the sex of your fish.

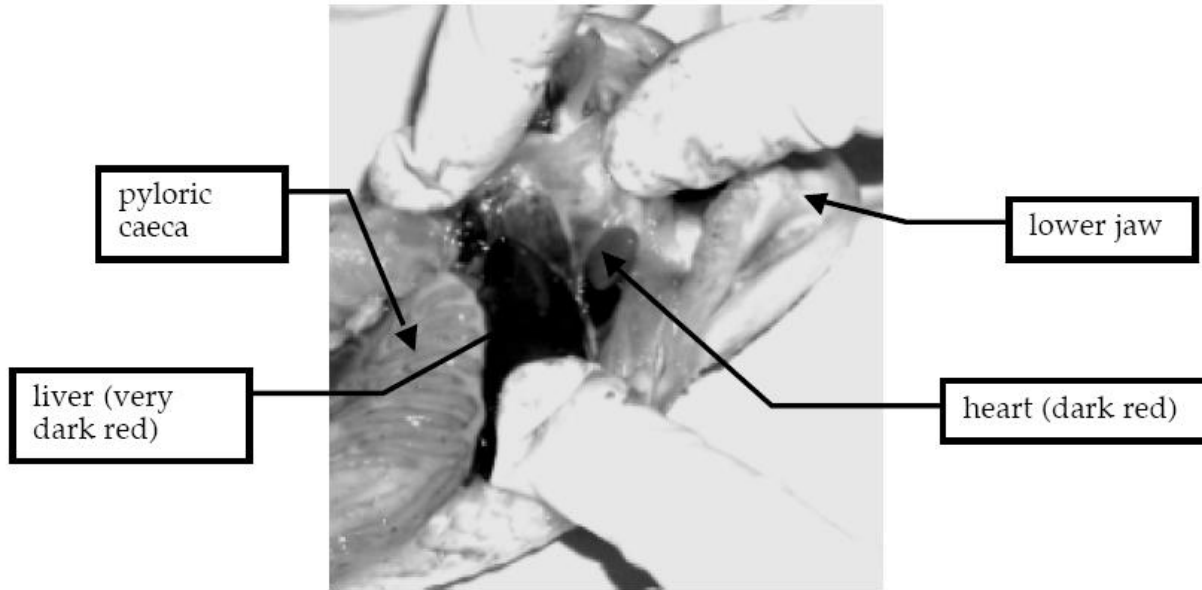
8) In some fish, the swim bladder is filled with air that the fish swallows and forces from the intestine to the swim bladder (this is a physostomous swim bladder). In other fish, the gas in the swim bladder is produced from the blood by a specific organ and the swim bladder is not connected to the intestine (this is a physocleistous swim bladder). By careful observation, try to determine whether the trout has a physostomous or physocleistous swim bladder.

9) Carefully remove the gills from the side of the fish that is facing up. Take a small fiber from one of the gills and look at it under low power in the compound microscope. Sketch the pattern of blood vessels in one of these gill fibers. Estimate the diameter of the smallest vessel you can see.

Approximate diameter of smallest vessel _____ μm

Circulatory System

10) Locate the heart - it is the dark red object about the size of the fingernail on your smallest finger and is located right in the 'throat' of the fish (anterior to the liver and right between the gills); it looks like a heart-shaped kidney bean.



11) Carefully remove the heart and weigh it. Heart weight _____g

** Stop and draw the circulatory system; consult the end of this exercise for assignment details.

12) Make a smear of the blood cells. This is a tricky procedure; it may take more than one try.

a) Clean off 2 microscope slides with ethanol and dry them. Squeeze a drop of blood from the heart onto a slide, near one end of the slide.

side view:

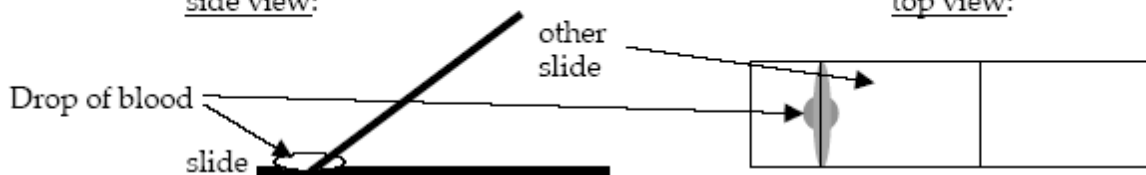


b) Take another slide and touch it to the drop until the drop spreads out along the edge of the slide:

the slide:

side view:

top view:



c) Quickly drag the second slide across the first to smear the blood out to a thin layer.

d) Let the smear dry completely.

You will now stain the smear with Wright-Giemsa Stain to make the white blood cells more visible.

You must wear gloves when staining.

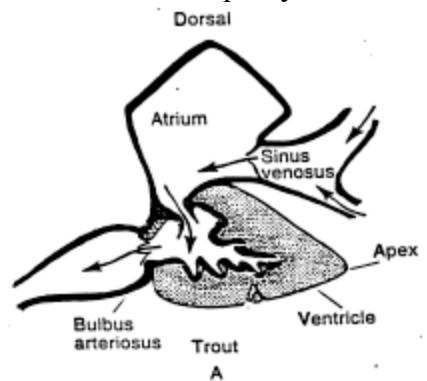
- e) Dip blood smear in Fixative Solution 5 times, one second each time. Drain excess fixative with a paper towel.
- f) Dip into Solution I, 5 times, one second each time. Drain excess solution with a paper towel.
- g) Dip into Solution II, 5 times, one second each time. Drain excess solution with a paper towel.
- h) Rinse slide in beaker of water.
- i) Allow slide to air dry.
- j) Examine under the compound microscope.

Look for red blood cells under the microscope. They will be visible as very faintly red dimpled discs (like a cookie with a dent in the center) at high power. Note that, unlike human red blood cells, these have nuclei. Estimate their diameter.

minimum red blood cell diameter _____ μm

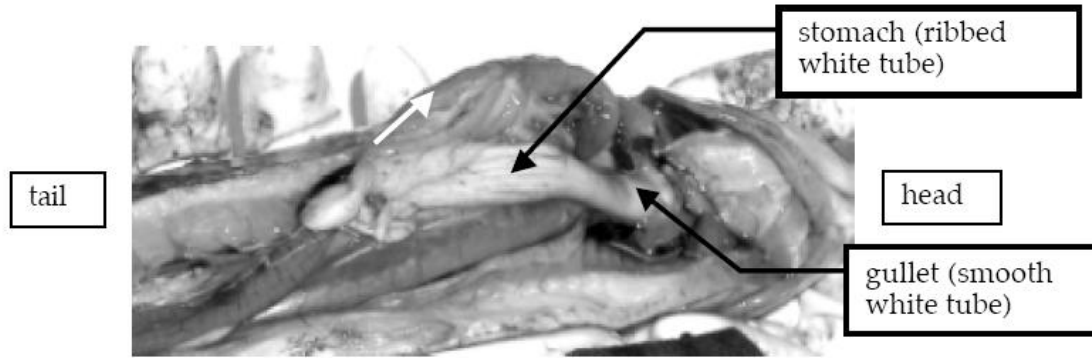
Look for the white blood cells under the microscope. They will look like figure 1.31 in the Lab Atlas. Draw one of the white blood cells you see and use figure 1.31 to identify it as best you can.

13) Shown below is a diagram of a fish heart. Carefully cut the heart open across its length; which part of the diagram below does the muscular part you found correspond to?



Digestive system

14) With a blunt probe, find the gullet (the tube that connects the pharynx to the stomach; the 'throat' in a human) and trace the path of food into the stomach. You can identify the stomach because it has a ribbed texture visible from the outside (the ribs run the long way along the length of the stomach).



15) Carefully trace the digestive tract. Be sure to note the following:

- e) the positions along the tract of the gullet, intestine, mouth, pyloric caeca, anus, and stomach.
- f) are the pyloric caeca all one long tube, a set of parallel tubes, or a set of 'dead end' branches of the digestive tract?
- g) the internal textures of the various parts of the digestive tract

16) Carefully remove the digestive tract from the fish.

** Stop and draw the digestive system; consult the end of this exercise for assignment details.

Tissue Structures

In general, it will be easier to observe internal structures in *thin* sections. With a fresh razor blade, try to cut the thinnest pieces you can so that you will be able to observe them clearly.

17) In all fish, digestive enzymes are secreted into the pyloric caeca. In some fish, nutrients are absorbed from the pyloric caeca into the blood. Any organ that is involved in absorbing nutrients into the blood will be highly vascularized (have lots of blood vessels in it). Look at a sample of the pyloric caeca and the surrounding tissue under the microscope. Do you see many blood vessels (they would be red like those you saw in the gills)? Based on this, are the pyloric caeca in the trout likely to be involved in nutrient absorption? Observe the pattern of the blood vessels if you find any. How does this pattern compare to the pattern you found in the gills?

18) Similarly, take a sample of the intestine. Is it likely to be involved in nutrient absorption? Sketch the pattern of the blood vessels if you find any. How does this pattern compare to the pattern you found in the gills?

19) Pool the data you have taken with your TA. Your TA will help you with this and will check off that you have done this.

For the lab practical, identify the following parts: Lateral line, dorsal fin, pectoral fin, anal fin, caudal fin, adipose fin, pelvic fin, gills, gill arches, gill rakers, operculum, nares, mouth, anus, kidneys, swim bladder, intestine, liver, pyloric caeca, heart, spleen, stomach and gullet.

Be able to draw the respiratory system, the circulatory system and the digestive system of the trout including the flow of food, blood or oxygen. Also be able to answer all questions in the lab manual.

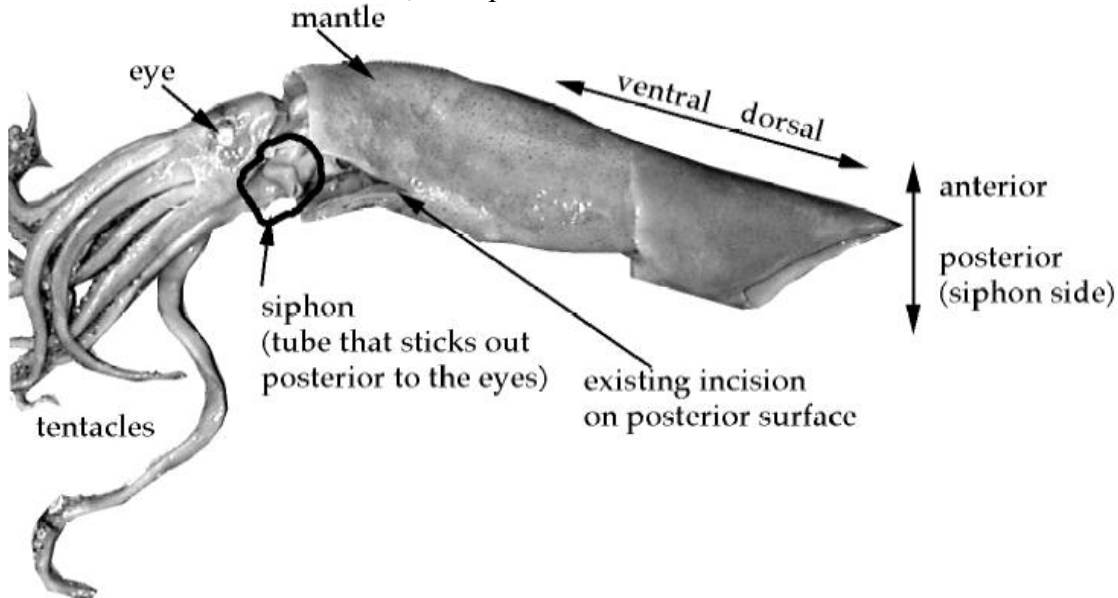
Lab assignments: Have your TA check off your drawings for the exterior, the respiratory, circulatory and the digestive systems for the trout. Take these home as part of your study guide for the lab practical.

Clean up

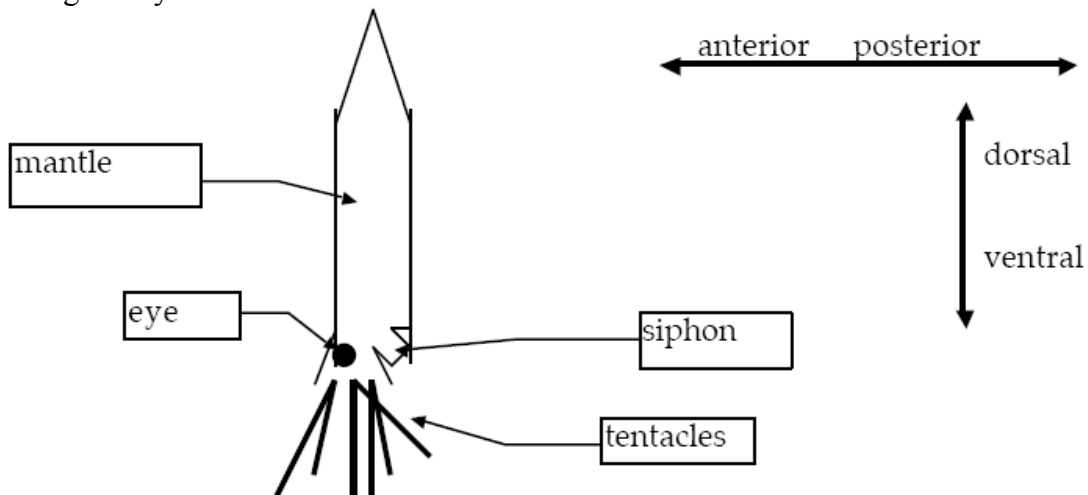
Place your fish and any fish parts into the bag labeled “fish”. These will be recycled. Other trash goes into the barrel. Wash all of your dissection tools and tray out well with soap and water and leave to dry on the counter.

Animal Diversity : Dissection of the squid (*Loligo pealii*)

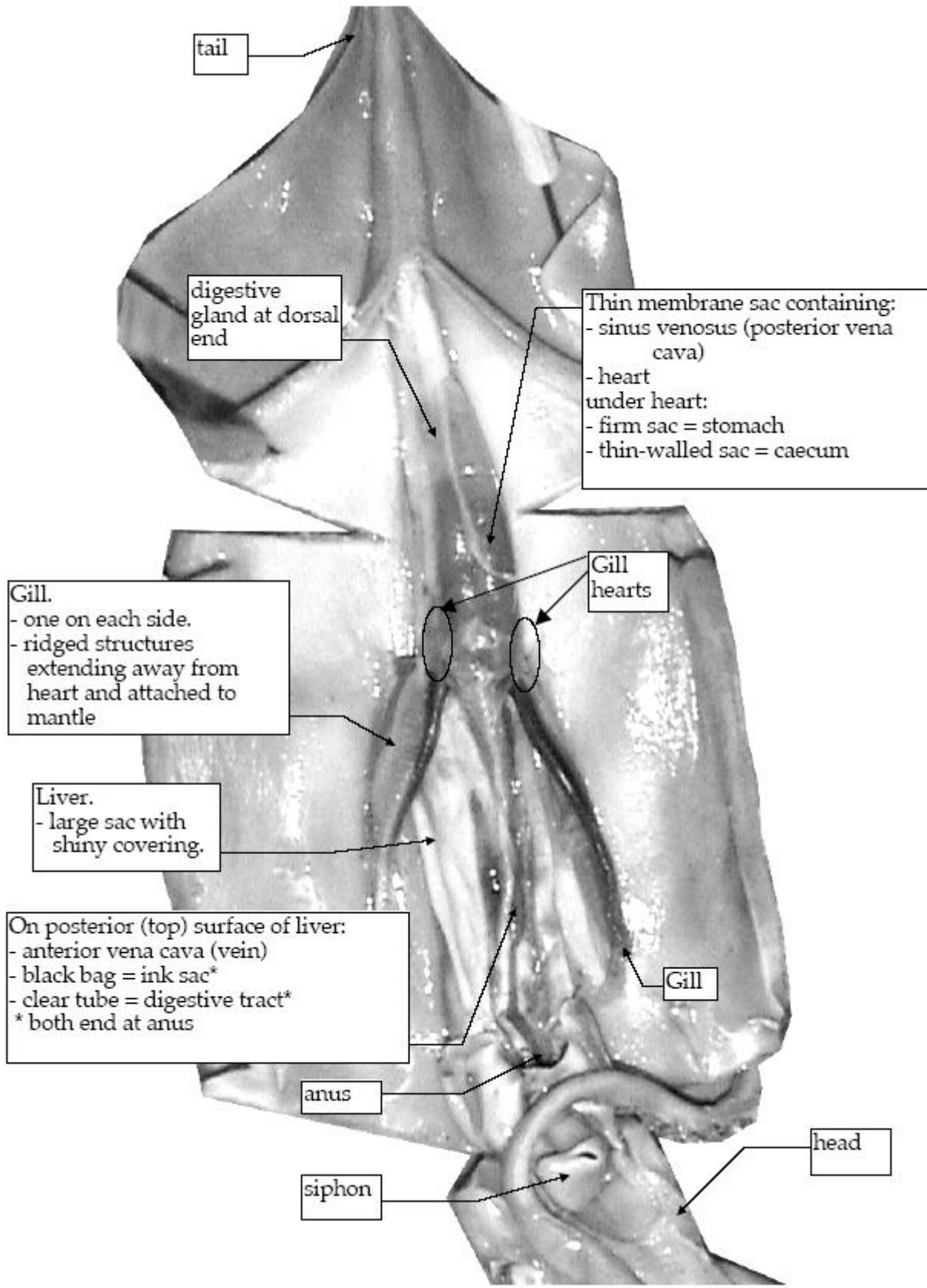
- 1) Obtain a preserved squid from your TA.
- 2) Find the siphon. It is a fleshy tube at the base of the head. The side with the siphon is the posterior side. The tentacles are the ventral side; the tip of the tail is the dorsal side.



Thus, the squid would look like this if it were in the same orientation as the shark shown under the “anatomical glossary”:

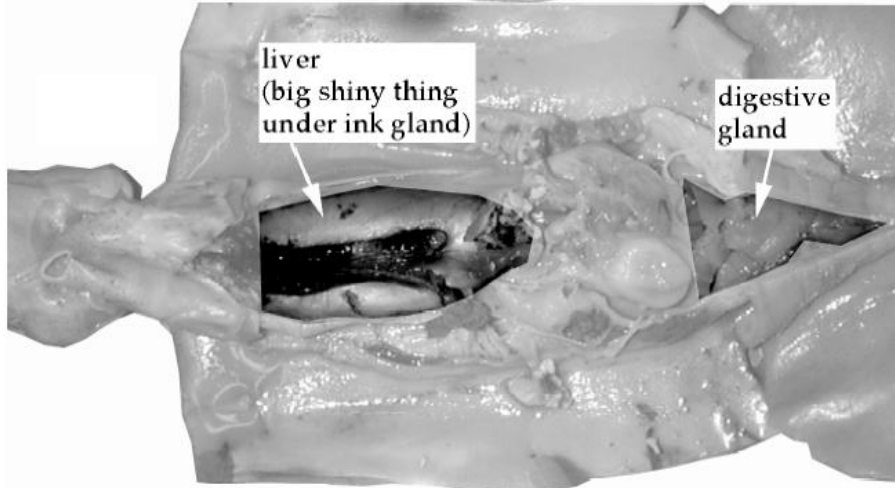
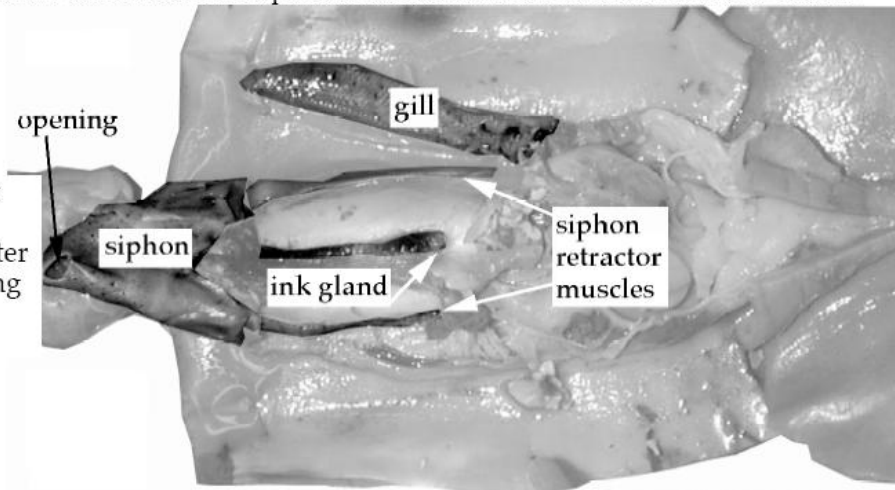


- 3) Lay the squid on its anterior side - siphon up.
- 4) You will see a started incision in the mantle, continue this to the dorsal end and peel the sides of the mantle apart. You will see something like the following page.

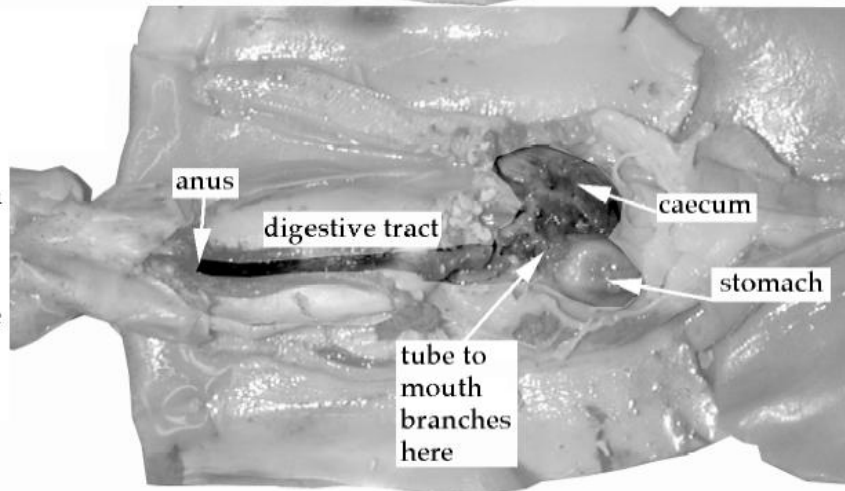


- 5) Trace the red and blue blood vessels as best you can.
- 6) Using a pick, gently pick open the sac that contains the heart, etc. The stomach and caecum are very fragile. Remove the heart. The siphon retractor muscles look like rubber bands.

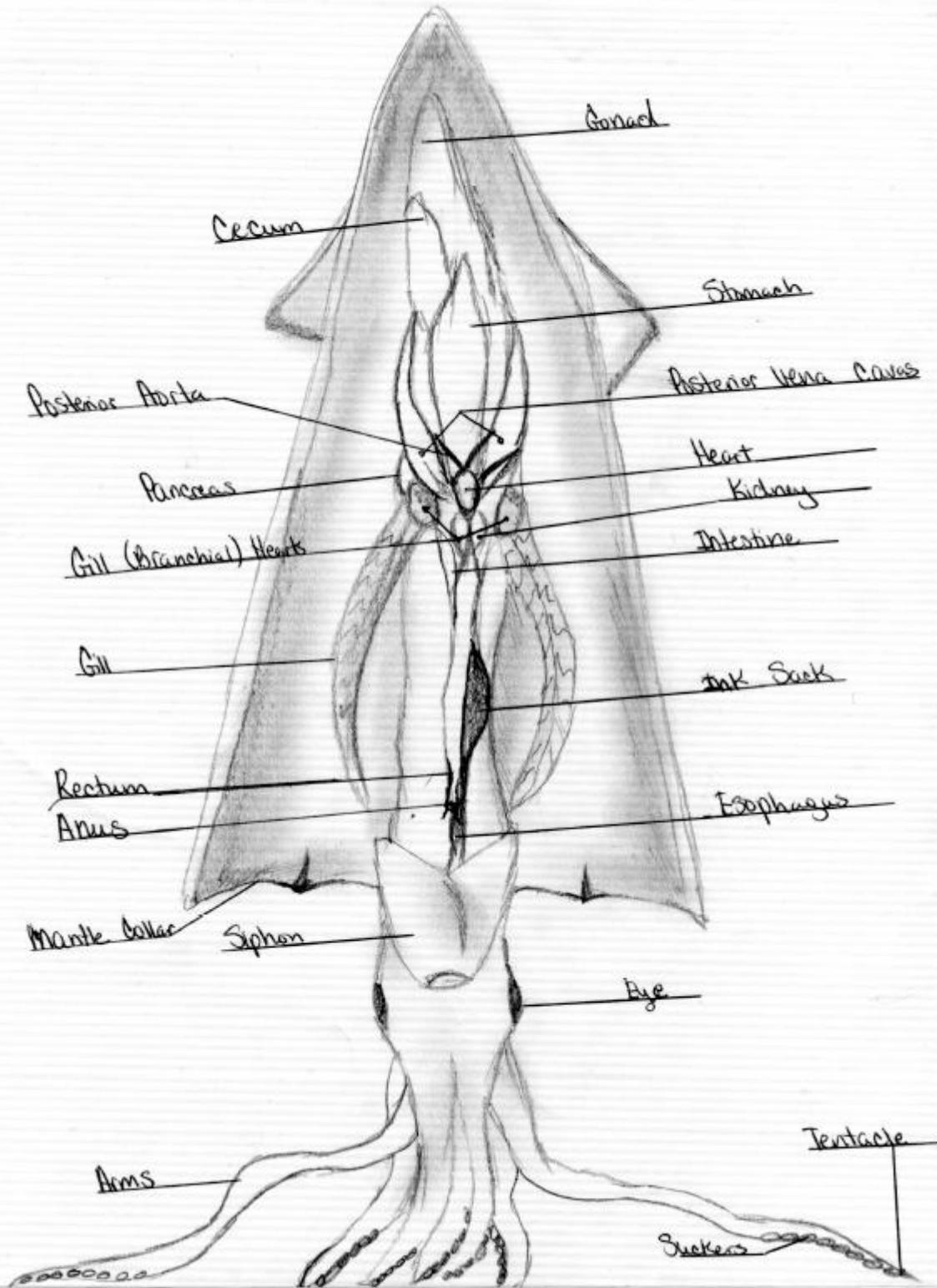
Look carefully at the siphon. The squid squirts water out of the opening in it.



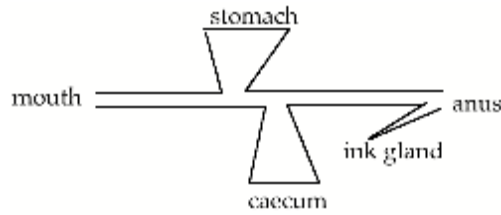
Try to trace the digestive system as it is arranged in the squid. Slit it open and examine the textures inside the stomach and caecum.



Plain Squid



If the digestive system were stretched out straight, it would look like this:

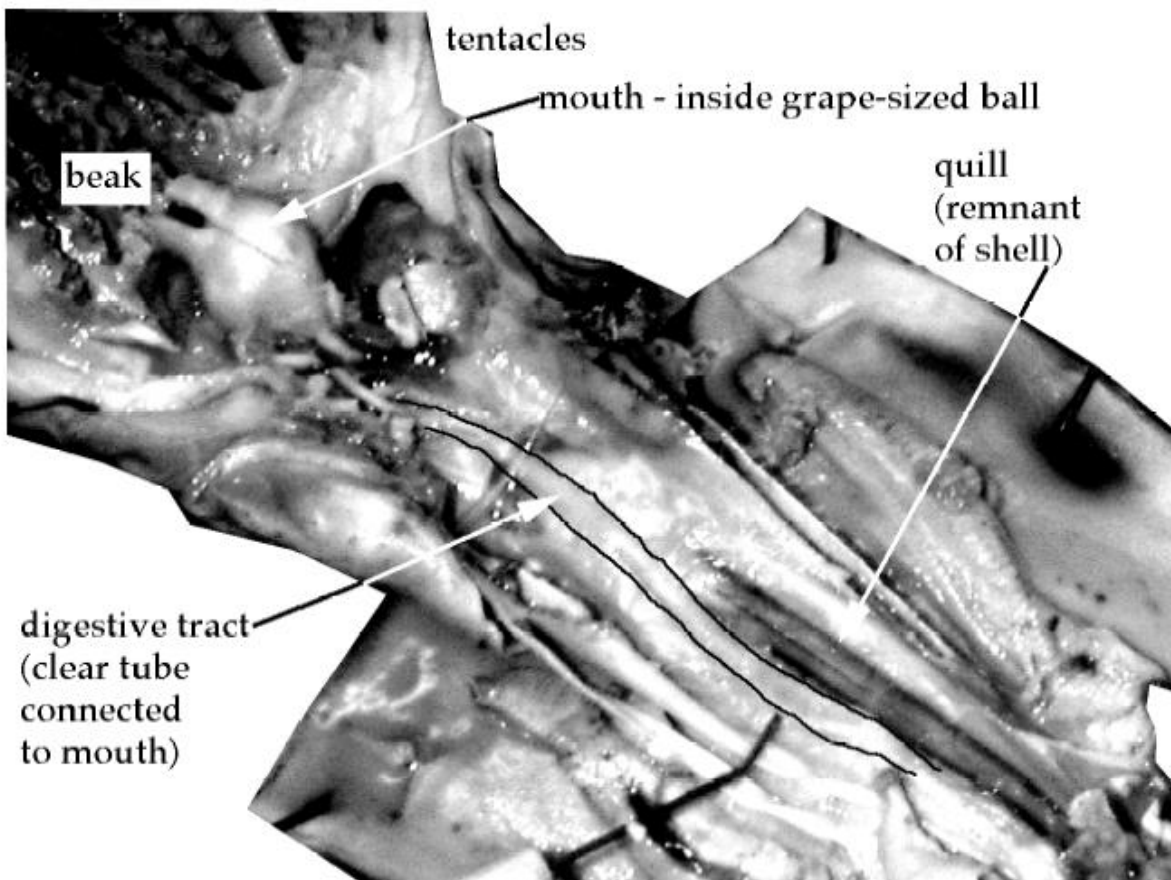


caecum = a blind pouch in the digestive system where food is digested and/or absorbed

ink gland = squids squirt black ink when threatened. It blinds the attacker and inhibits its sense of smell.

7) Carefully remove the liver. Lift it starting from the dorsal (tail) end. Using a pick, detach the connective tissue and the digestive tract (clear tube) as you lift out the liver.

8) Cut through the middle of the head from the posterior side. Do this slowly and spread the sides apart to reveal the mouth and beak. Trace the digestive tract. Cut open the mouth to examine the beak and 'teeth' (radula).



9) Cut open the eyes and look at the lens (it will be firm & clear - you can look through it) and retina (black light-sensitive membrane at the back of the eye).

10) Try to look at the gills of the fresh squid under a microscope. How do they differ from the fish gills?

11) If you have a fresh squid, try to make a blood smear from the heart or sinus venosus. What kinds of blood cells do you see?

12) Clean up. You are done with the dissection.

Clean up

Place your squid and any squid parts into the bag labeled “squid”. These will be disposed of separately. Other trash goes into the barrel. Wash all of your dissection tools and tray out well with soap and water and leave to dry on the counter.

For the lab practical, be able to identify: parts of the eyes, tentacles, mouth, beak, siphon, mantle, heart, stomach, caecum, gills, liver, ink sac, anus, anterior vena cava, posterior vena cava.

Be able to draw the respiratory system, the circulatory system and the digestive system of the squid including the direction of the flow (of food, blood or oxygen, etc). Also be able to answer all questions in the lab manual.

Lab assignments: Have your TA check off your drawings for the exterior, the respiratory, circulatory and the digestive systems for the squid. Take these home as part of your study guide for the lab practical.