Animal Diversity I, II, & III

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 three 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 and Mollusca, respectively.

You will have two lab periods to complete this assignment; the Lab Practical Exam will take place during the third session. You will be responsible for identifying all parts mentioned in the following pages. You will not need to know the data (weight, etc.) for your or any other fish.

GENERAL HINTS AND INSTRUCTIONS FOR DISSECTIONS

Bring your Lab Atlas to Lab it has many useful photos.

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.

Posting pictures as a study guide: you can post pictures of key samples to your section's blog on your Blackboard site.

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.

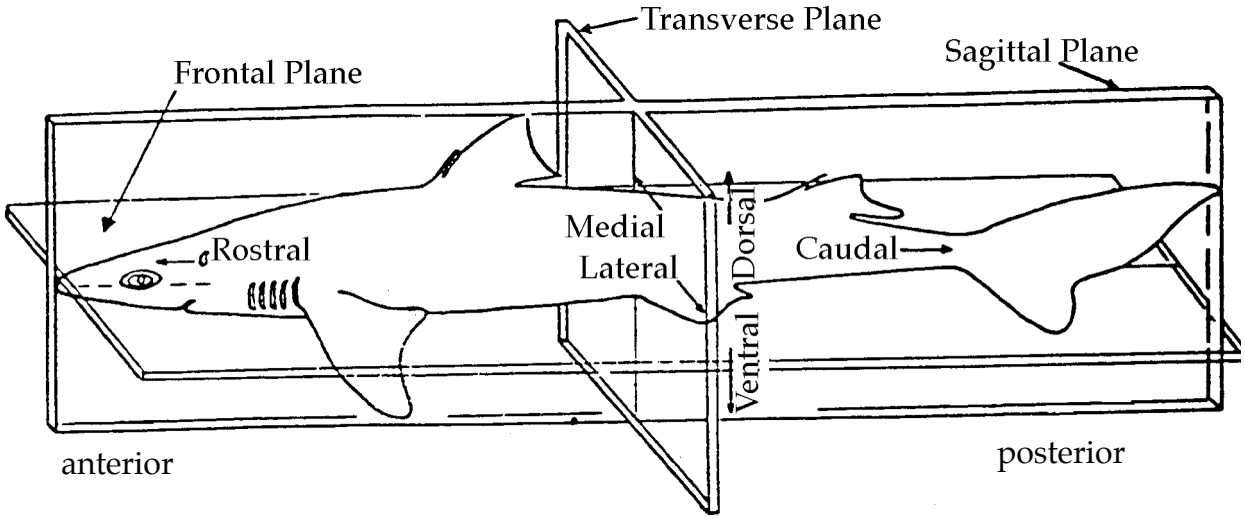
• **Pick:** Your 'best friend' once inside the animal. This can easily be used to pull apart and cut the connective tissue that holds organs to each other, but it is unlikely to break anything important unless you push really hard. You should use this most of the time.

All dissection instructions must be left in lab. **The lab must be left <u>really</u> <u>clean</u>**—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*)

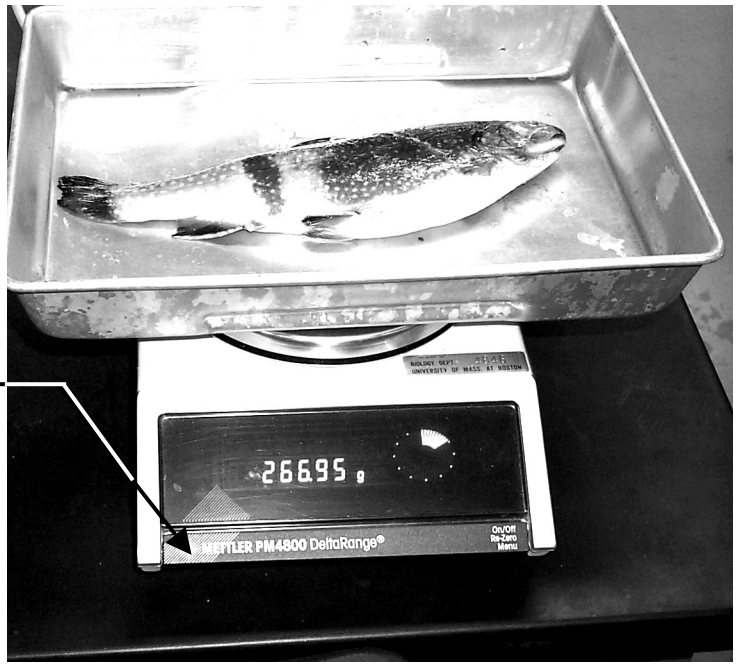
This dissection should take you one lab period. You will enter most of the data and observations you make into a database which will grow from year to year. In the future, we will use this database to draw conclusions about the fish.

Note: These steps are designed to take you through the trout dissection in order to prepare you for the lab practical exam; the lab practical is described at the end of this section. You should keep these questions in mind as you do the dissection. 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.

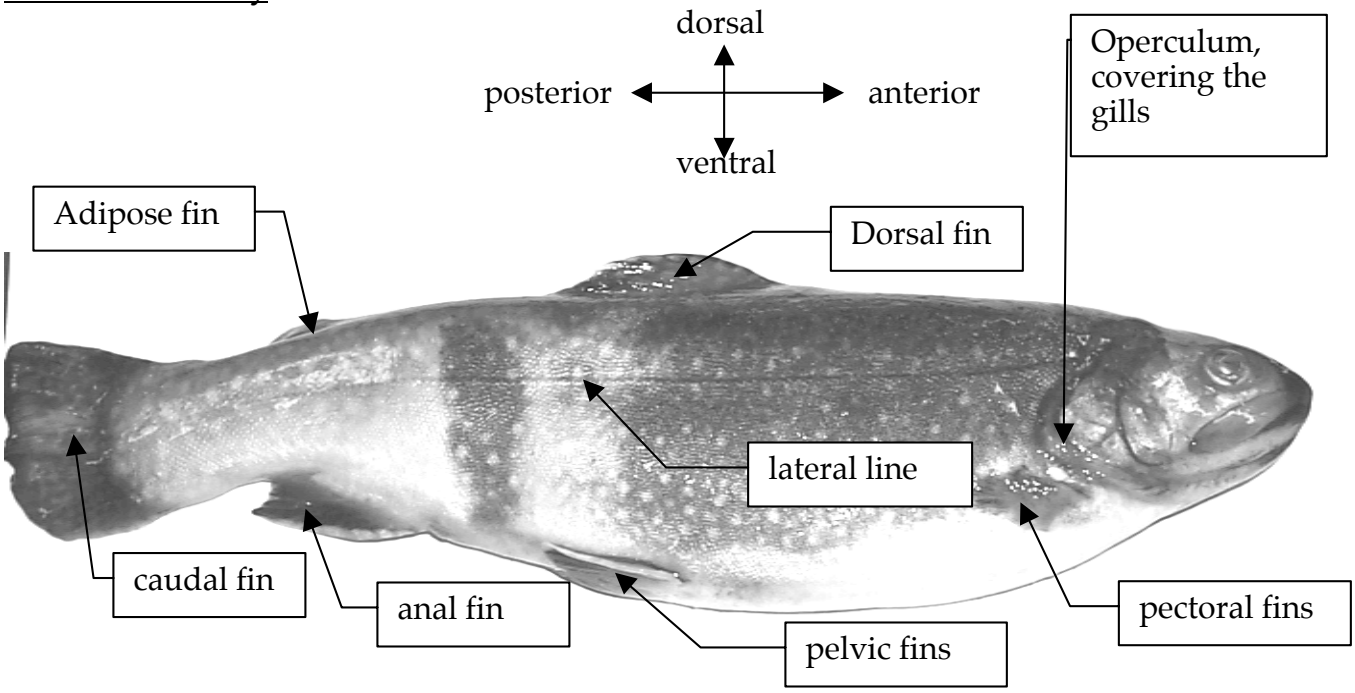
3) Bring your fish to the scale. Make sure there is a styrofoam cafeteria tray on the scale to keep the scale from getting wet. Zero the scale before putting the fish in the tray by pushing down on the big bar at the front of the scale. This is shown below:



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

Weight of fish_____grams

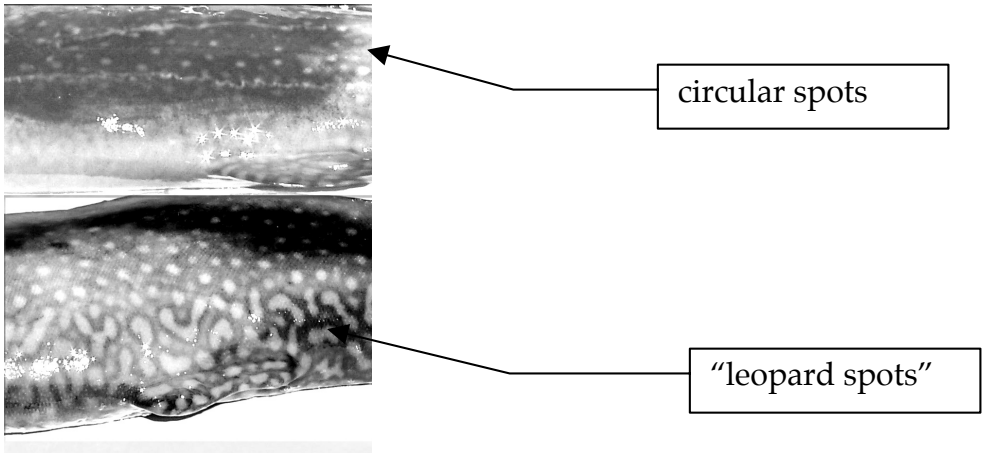
External Anatomy



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.

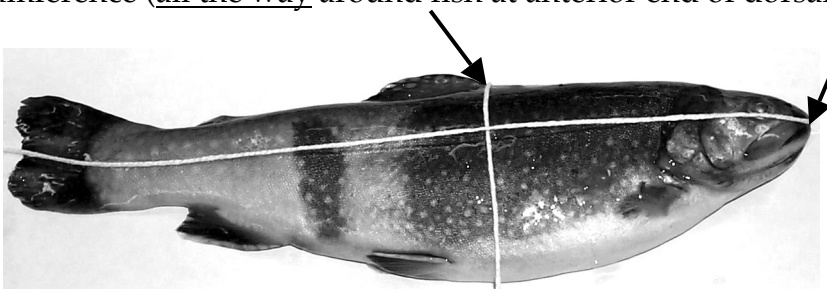


Spot pattern_____

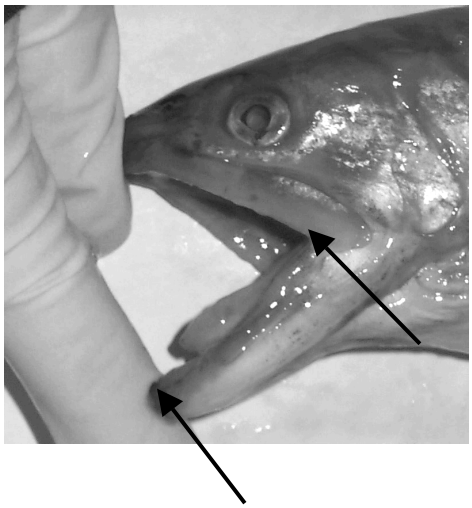
6) Measure the length and girth of your fish as shown below. Use a string to measure the fish and then measure the string and record the value in your notebook.

– 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



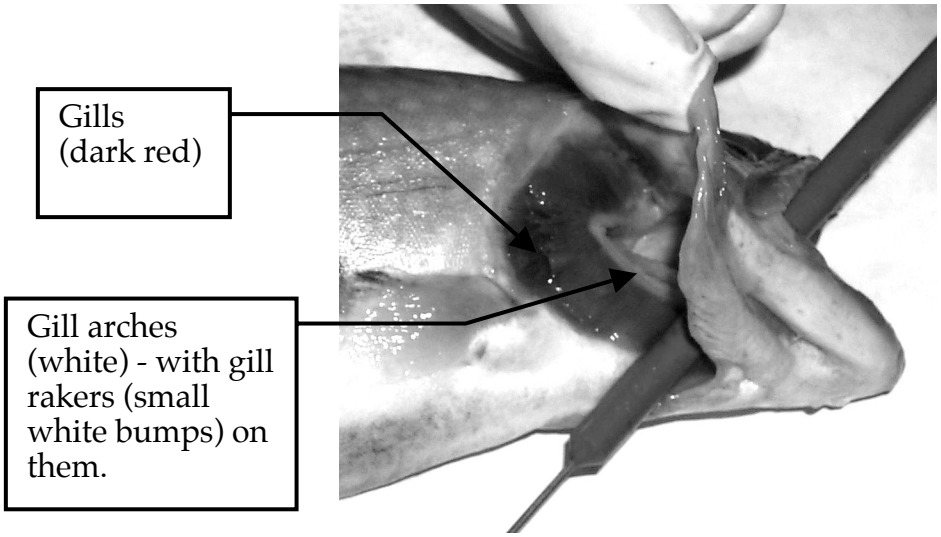
7) Measure the length of the lower (ventral) jaw as shown below. Be sure to open the jaw as wide as you can (be careful of the teeth).



Jaw length_____cm

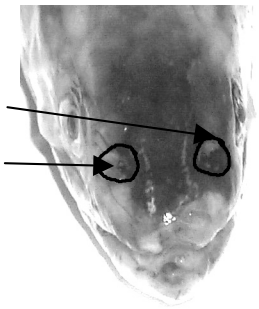
Respiratory system.

8) Lift open the operculum and look in at the gills and gill rakers as shown below:



9) 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"?

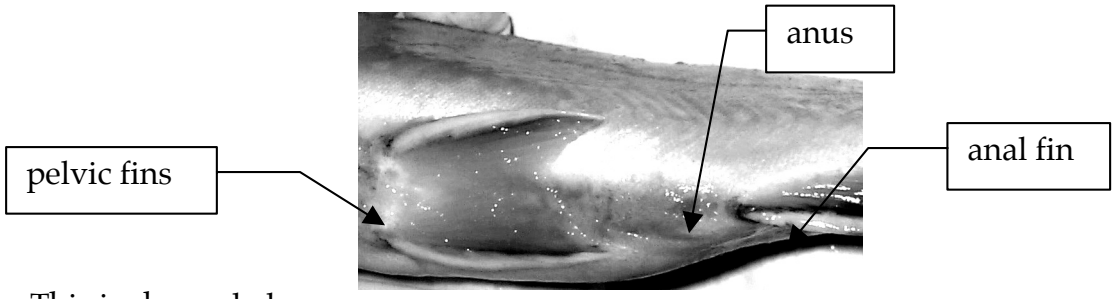
10) The nares are circled in the picture at the right . They are used in smell sensation in the fish and bear a superficial resemblance to nostrils on a human. In a human, the nostrils are also used in breathing. As best you can, carefully dissect the area around the nares and determine if these can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



** Stop and draw the respiratory system; consult the requirements for the Study Guide for details.

Internal anatomy

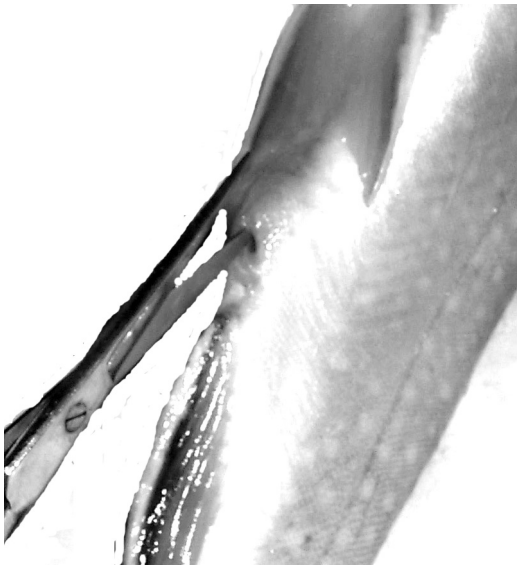
11) Open the body cavity of the fish. You must do this very delicately or you will make it impossible to study the internal organs. This is the most critical part of the dissection. Hold the fish with it's ventral side up and locate the anus - it is the opening just anterior of the anal fin.



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.

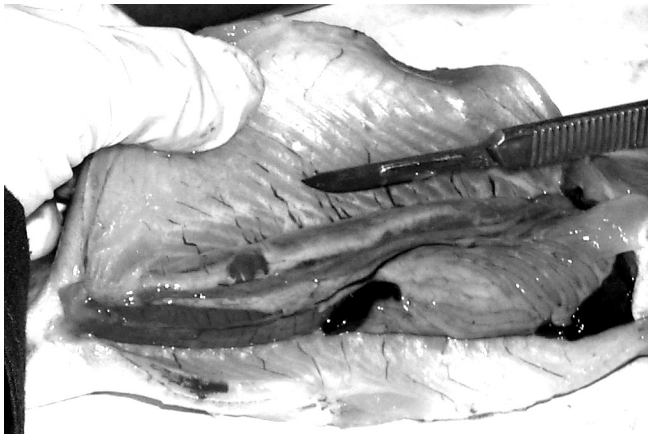
Starting to cut:



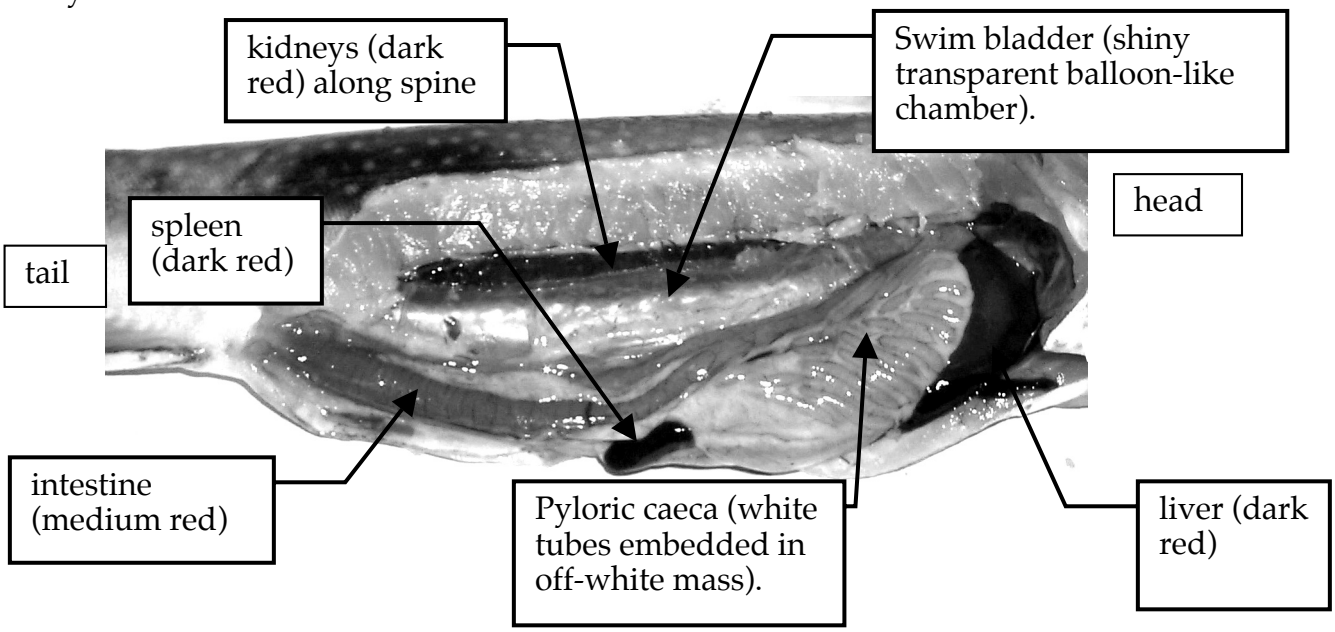
Cutting:

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

12) 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:



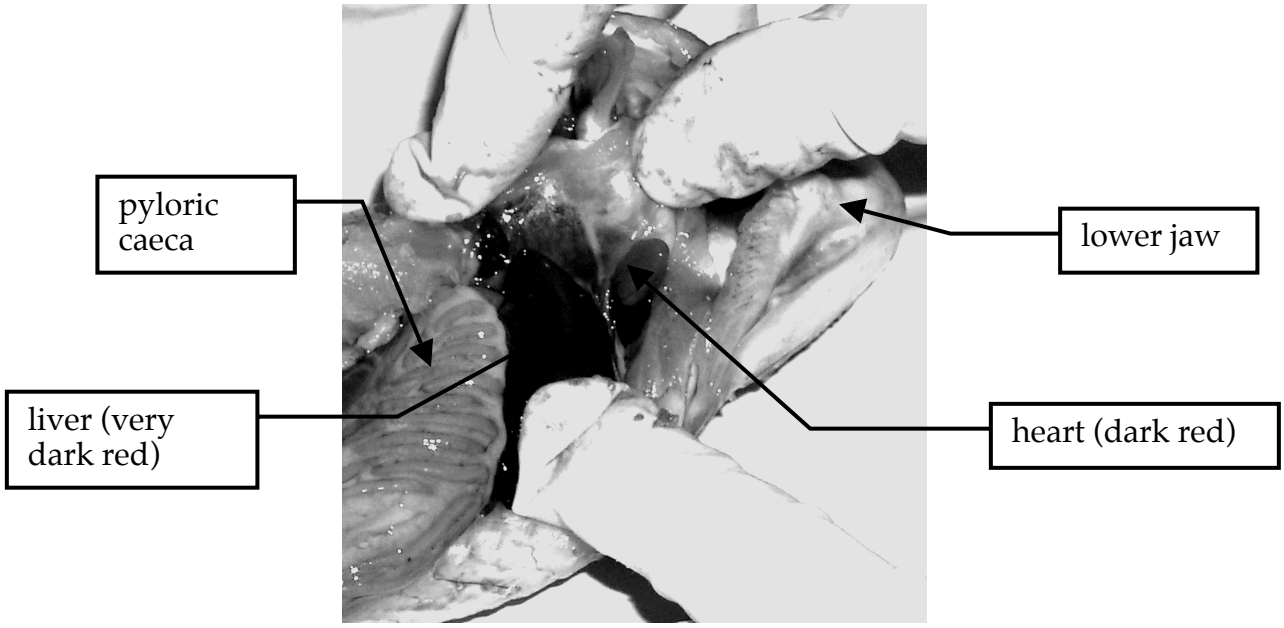
13) If the body cavity contains eggs, then you have a female; if not you have a male. Eggs are spherical and vary in size and color - the smallest are 0.5mm and white, the mature large eggs are 3-5mm and clear with a white spot. Record the sex of your fish.

14) 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.

15) 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.

Circulatory System

16) Locate the heart - it is the dark red (but not as dark as the liver) 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.



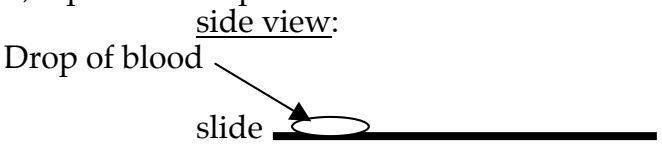
17) Carefully remove the heart and weigh it. Heart weight_____g

** Stop and draw the circulatory system; consult the Study Guide section for more details.

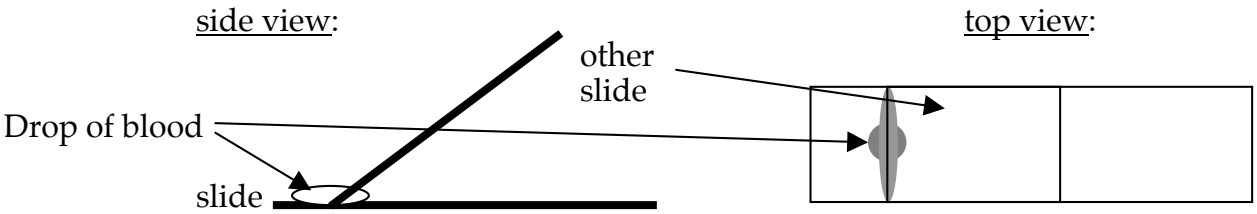
18) Make a smear of the blood cells. This is a tricky procedure; it may take more than one try.

a) Wipe the slide with ethanol (alcohol) and let it dry before starting.

b) Squeeze a drop of blood from the heart onto a slide, near one end of the slide.



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



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

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

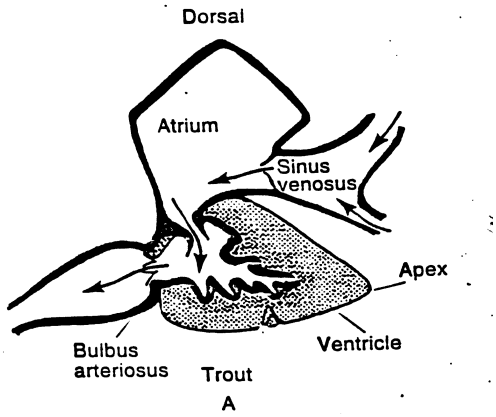
approximate fish red blood cell diameter_____μm

Look at a prepared slide of a human blood smear. Notice the differences in the forms of the cells and the diameter of the human RBCs.

_____μm

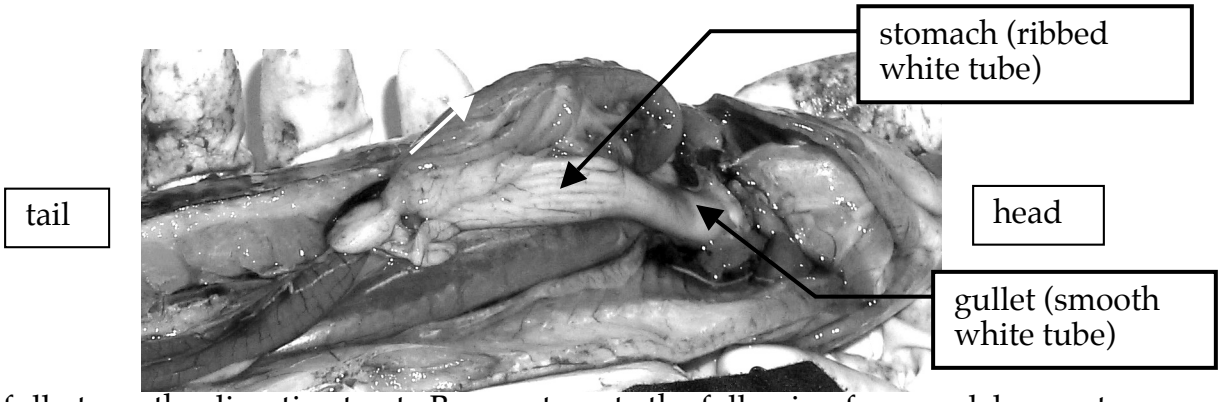
Look for the white blood cells under the microscope. They will look like figure 1.31 in the Lab Atlas. Draw one of the white blood cells you see and use figure 1.31 to identify it as best you can.

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



Digestive system

20) 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 tell the stomach because it has a ribbed texture visible from the outside (the ribs run the long way along the length of the stomach).



21) Carefully trace the digestive tract. Be sure to note the following for your lab report:

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

22) Carefully remove the digestive tract from the fish. Put some of the stomach contents on a slide and look at them under the microscope. What are they?

** Stop and draw the digestive system; consult the Study Guide section for 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. You may find it easier to see these structures in the dissecting microscope rather than the compound microscope.

23) 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 should be highly vascularized (have lots of blood vessels in it). Look at a sample of the pyloric caeca and the surrounding tissue under the dissecting microscope or with the "naked eye". Do you see many blood vessels (they would be red like those you saw in the gills)? Compare this with a highly vascularized structure like the intestine. Based on this, are the pyloric caeca in the trout likely to be involved in nutrient absorbtion? Observe the pattern of the blood vessels if you find any. How does this pattern compare to the pattern you found in the gills? Interestingly, a large collection of other evidence suggests that the caeca are absorbtive; why is this surprising?

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

25) 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.

To prepare for the lab practical, you should make a table of each of the following parts of the fish and the physiological system(s) to which each belongs.

Parts:	gill	heart	stomach	gullet	intestine
	Anus	nares	siphon	caecum	gill heart
	Liver	kidney	spleen	swim bladder	ink gland

Systems: respiratory circulatory digestive

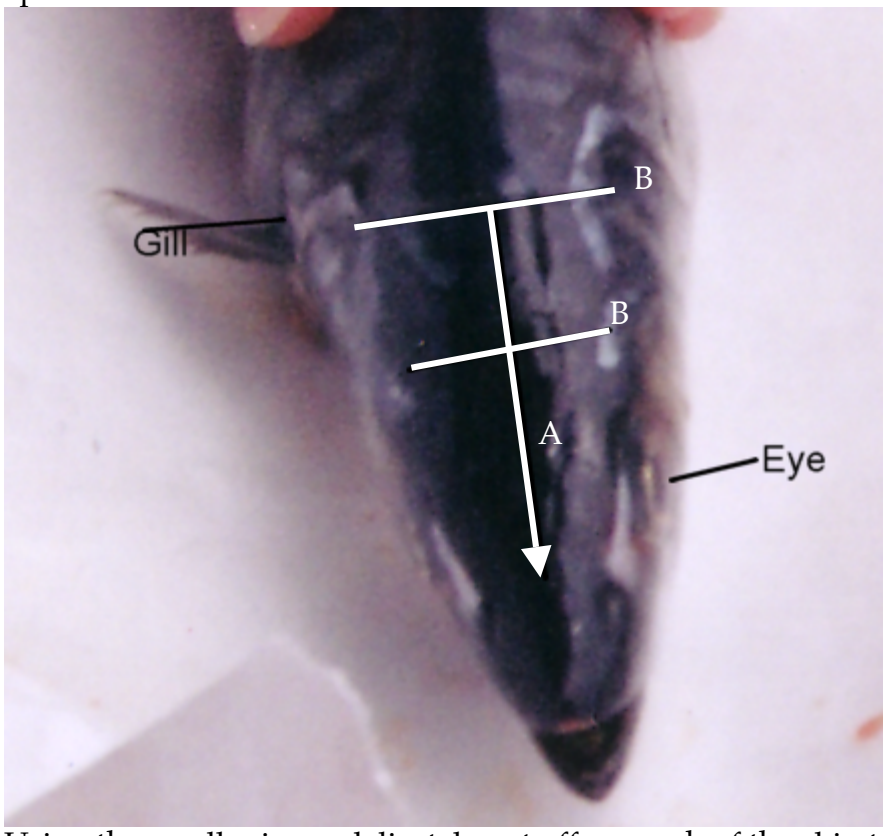
Brain and Associated Structures

WARNING: The brain is very soft and jello-like so it can be destroyed easily. In order to avoid this, each step must be done very carefully.

26) Hold the fish with its dorsal side up and make the following three cuts with the scalpel (Be careful not to cut past the cranium).

a) Start anterior to the gills and cut down the middle until you pass the eyes
b) Make one cut intersecting the center cut, from left gill to right gill. Do another similar cut between the eyes and the gills.

The Three Scalpel Cuts:

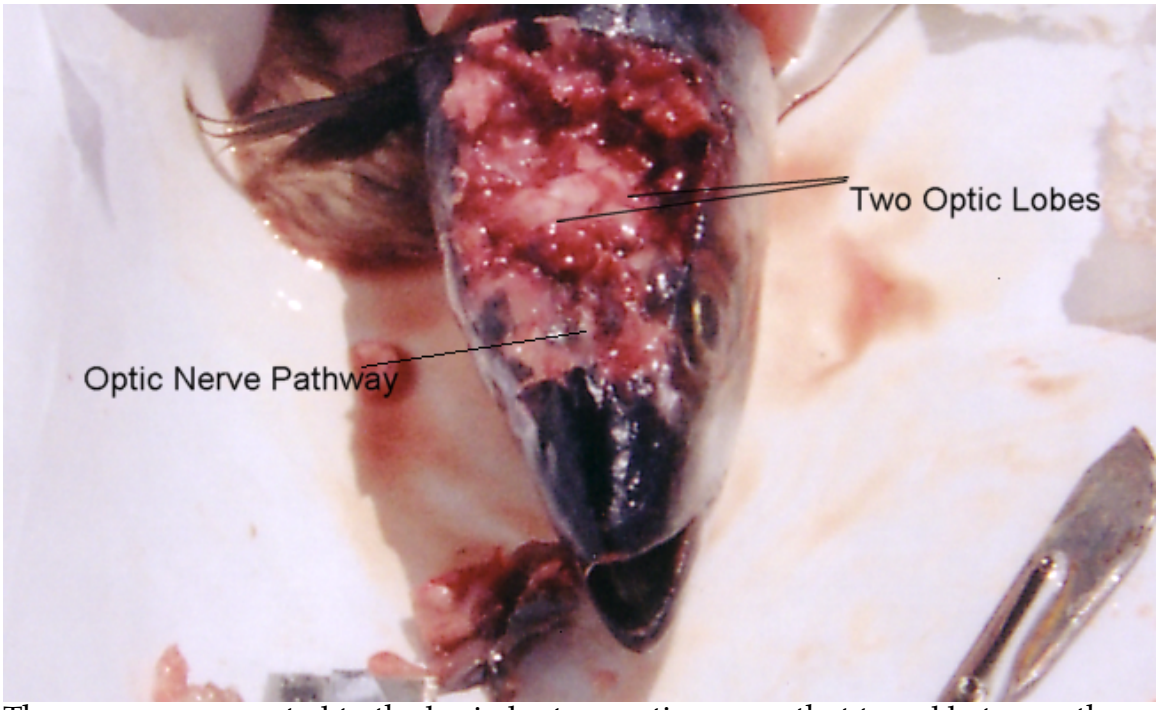


c) Using the small scissor, delicately cut off as much of the skin to get a clear view of cranium bone (Don't worry about peeling off all the skin).

27) Using the large scissor, crack and cut the cranium bone anterior to the eyes (Make sure you hear the bone cracking). This will allow you to cut the cranium from the eyes to the gills.

28) **While cutting the cranium bone, make sure the part of the scissor inside the cranium is as far away from the brain as it can possibly be.** Following the center cut (the cut in step 26a), and by using the small scissor carefully cut as far posterior as the cranium will allow. While doing this cut make sure to crack as much of the cranium as you can (make sure to get a clear view of the internal eye). Continue to crack the skull until you have a clear view of the brain and inner eyes.

Inner Cranium



The eyes are connected to the brain by two optic nerves that travel between the eyes. The nerves connect to the optic lobes by the optic chiasma. The optic lobes are very large and it's the majority of the brain. The trout is a visual feeder and relies on its vision for food. Posterior to the lobes is a little part of brain called the cerebellum . If you scoop out the brain, you will notice the cranium entrance of the spinal nerve.

BE SURE TO ENTER YOUR DATA INTO THE DATABASE (ask your TA for details)

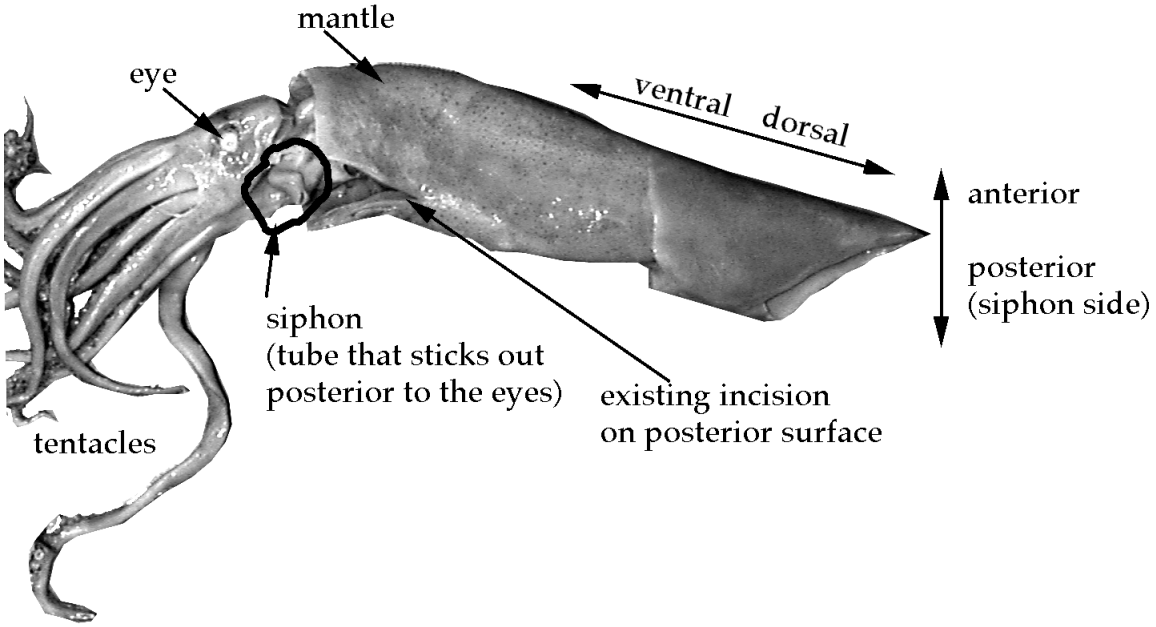
STOP HERE AT THE END OF DAY 1 AND CLEAN UP.

DAY 2

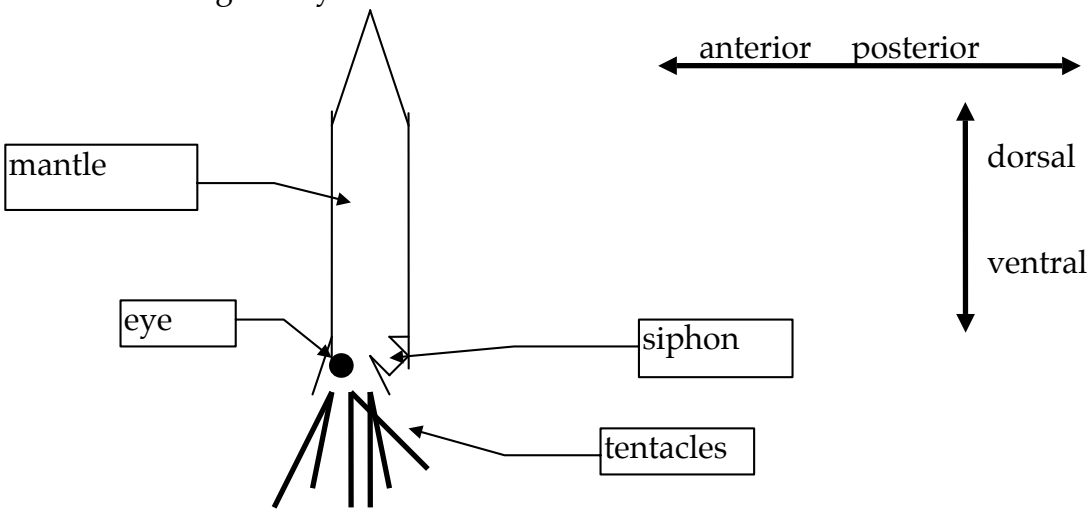
Part II: Dissection of the squid (*Loligo pealii*)

1) Obtain a preserved or fresh squid from your TA. Since some structures are more visible in one form than the other, you should be sure to look at BOTH.

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.

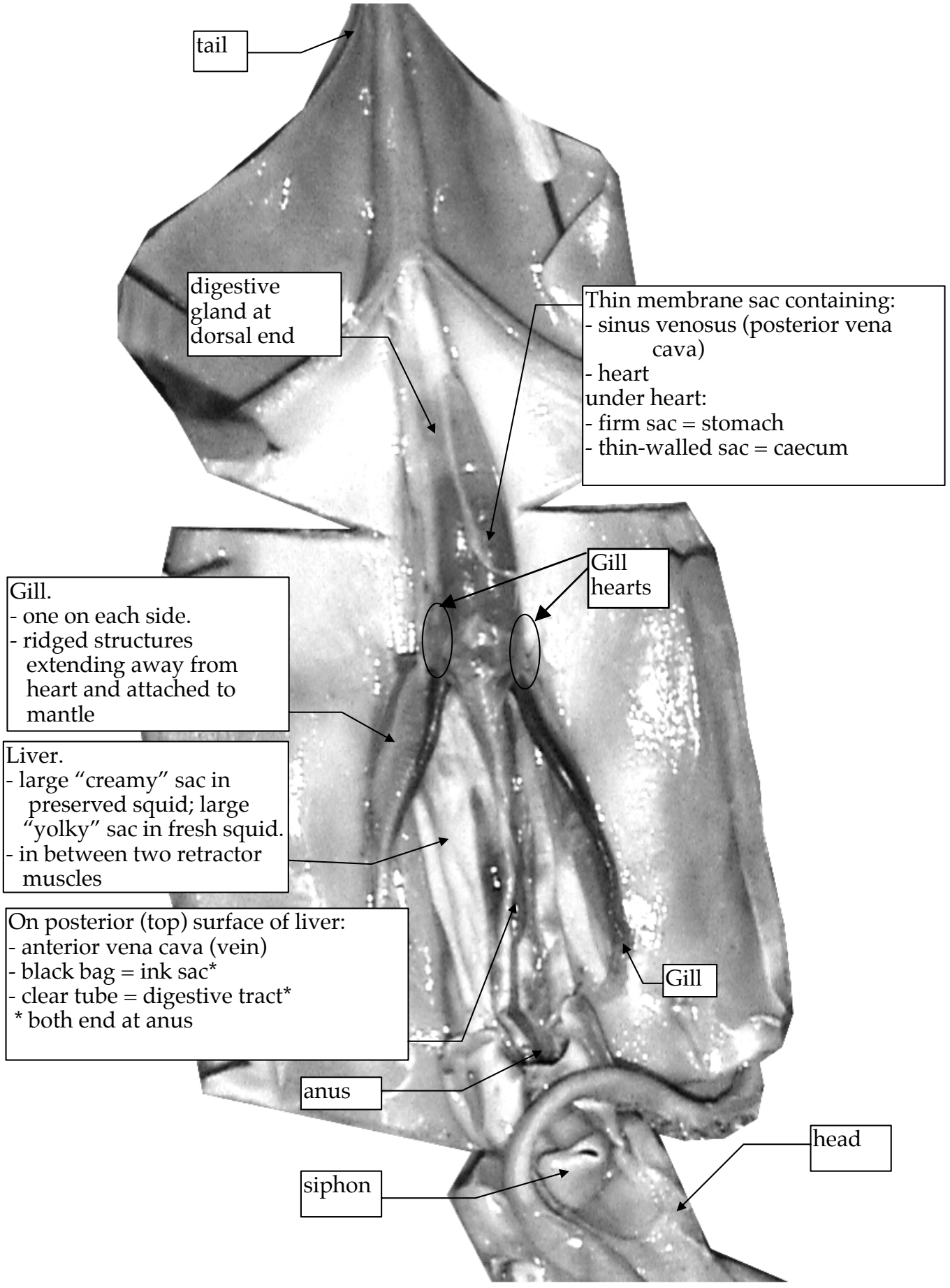


Thus, the squid would look like this if it were in the same orientation as the shark shown under the "anatomical glossary":



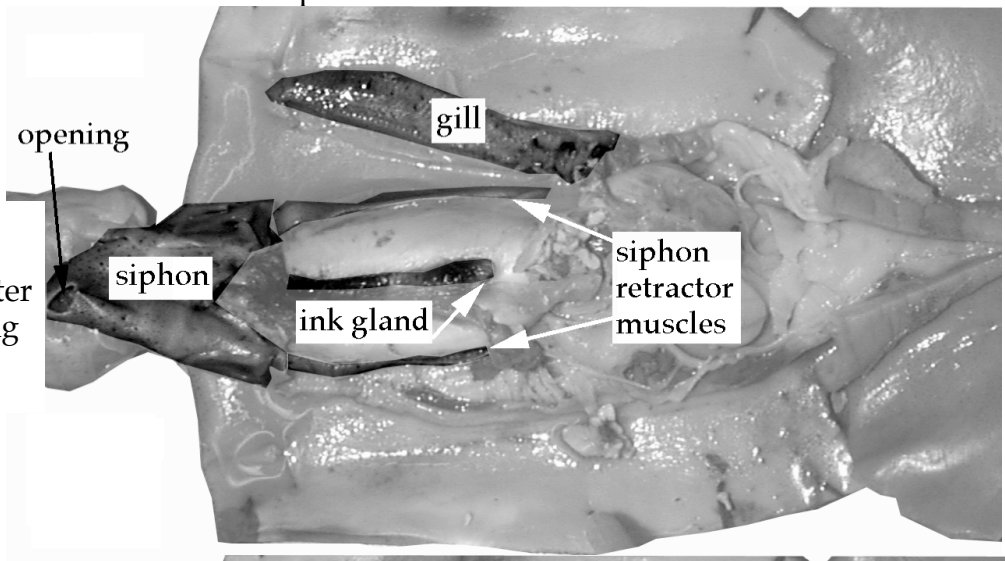
3) Lay the squid on its anterior side - siphon up.

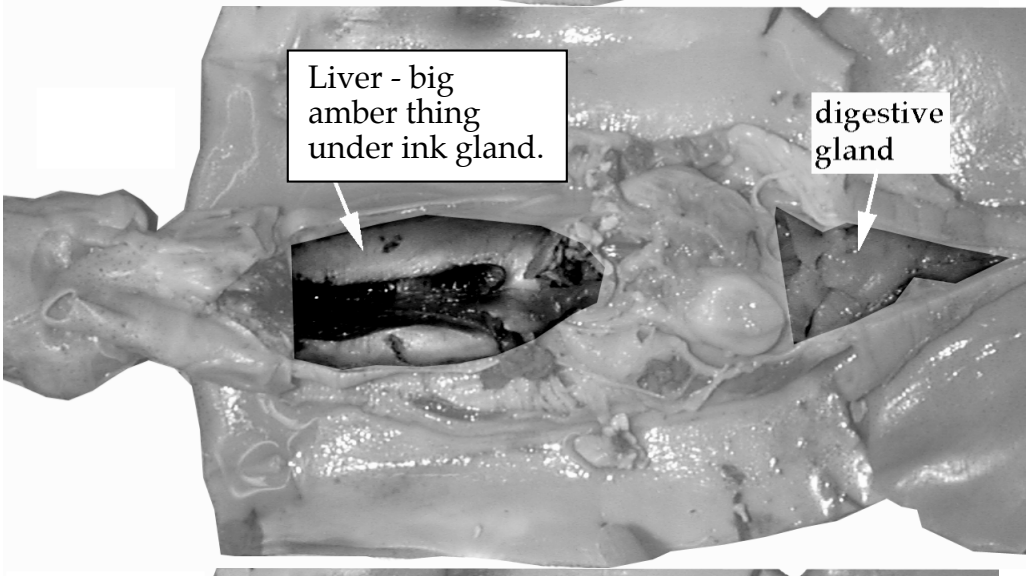
4) You will see a started incision in the mantle, continue this to the dorsal end and peel the sides of the mantle apart. You will see something like the following page. Note that the details of the squid are harder to see than those of the trout.


tail
digestive gland at dorsal end
Thin membrane sac containing:
- sinus venosus (posterior vena cava)
- heart
under heart:
- firm sac = stomach
- thin-walled sac = caecum
Gill hearts
Gill.
- one on each side.
- ridged structures extending away from heart and attached to mantle
Liver.
- large "creamy" sac in preserved squid; large "yolky" sac in fresh squid.
- in between two retractor muscles
On posterior (top) surface of liver:
- anterior vena cava (vein)
- black bag = ink sac*
- clear tube = digestive tract*
* both end at anus
Gill
anus
head
siphon

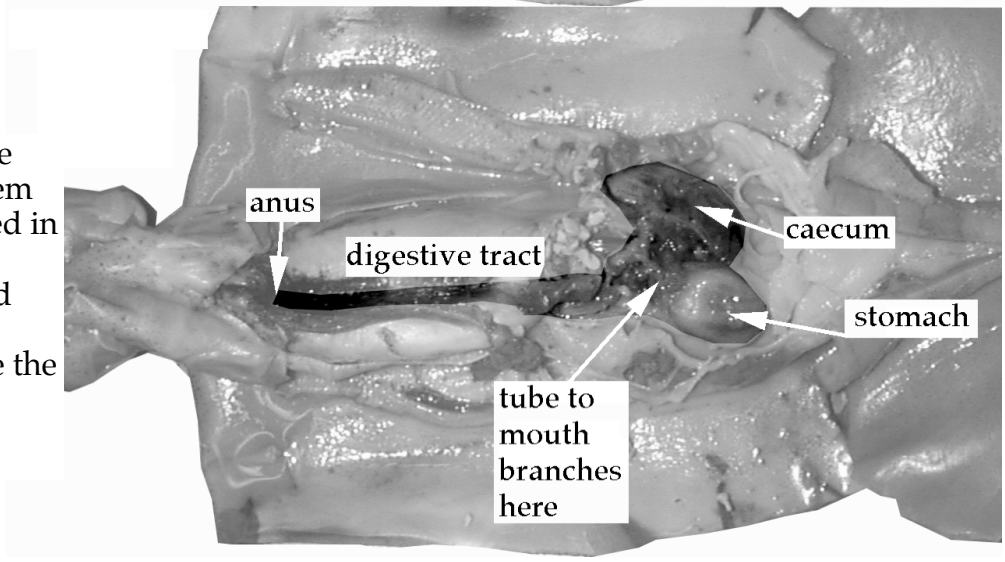
5) Trace the blood vessels as best you can.
6) Using a pick, gently pick open the sac that contains the heart, etc. The stomach and caecum are very fragile. Remove the heart. The siphon retractor muscles look like rubber bands.

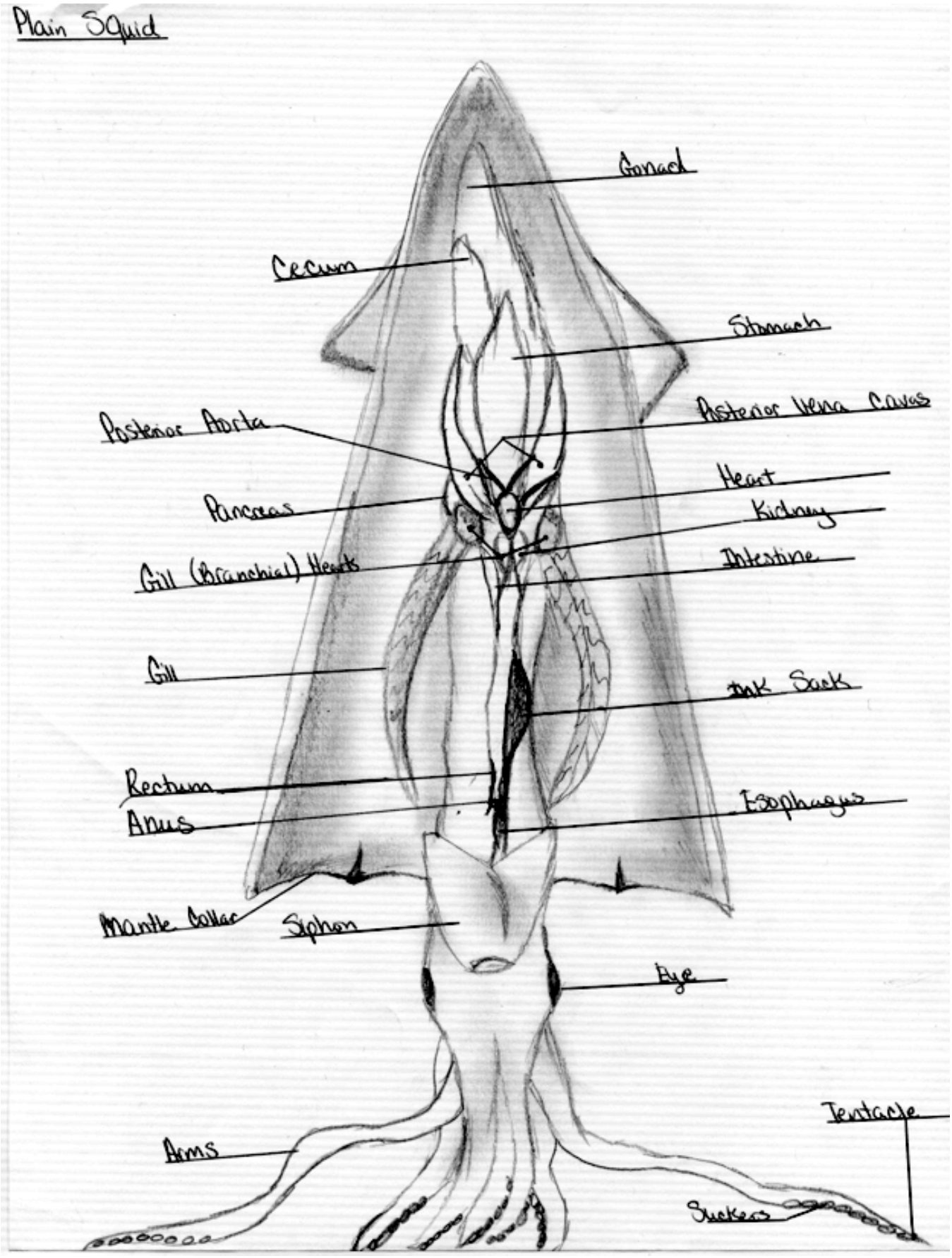
Look carefully at the siphon. The squid squirts water out of the opening in it.



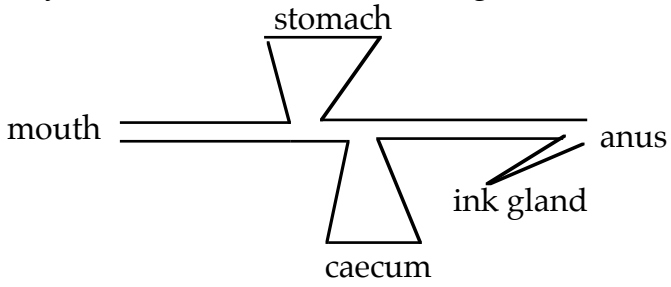


Try to trace the digestive system as it is arranged in the squid.
Slit it open and examine the textures inside the stomach and caecum.




Plain Squid
Gonad
Cecum
Stomach
Posterior Aorta
Posterior Vena Cavas
Heart
Pancreas
Kidney
Gill (Branchial) Heart
Intestine
Gill
Ink Sack
Rectum
Anus
Esophagus
Mantle Collar
Siphon
Eye
Tentacle
Arms
Suckers

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



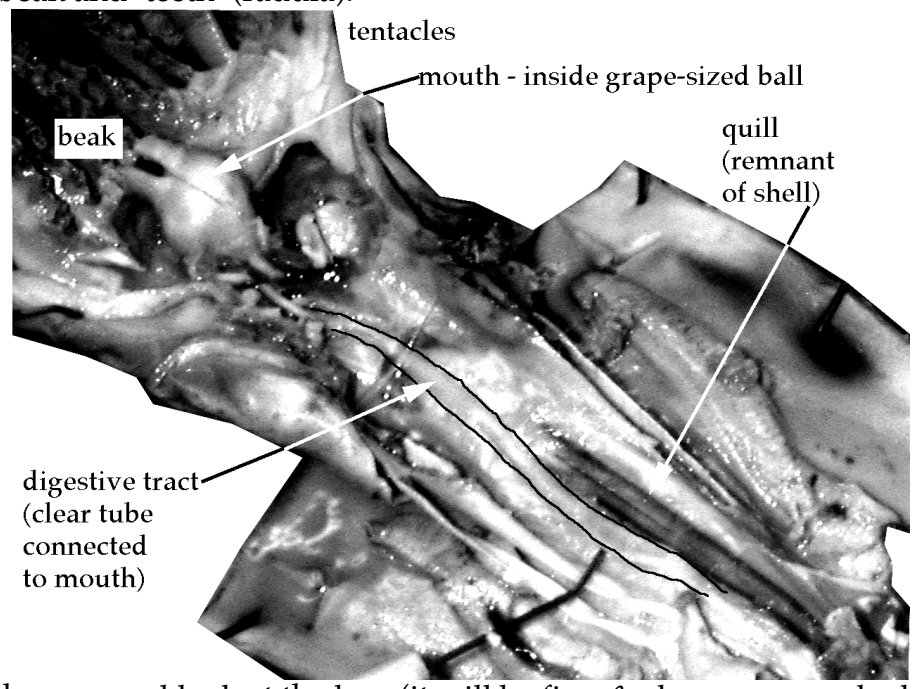
caecum = a blind pouch in the digestive system where food is digested and/or absorbed.

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

Examine the stomach contents under the microscope; what do you think they are?

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) Try to make a blood smear from the heart or sinus venosus. What kinds of blood cells do you see?

10) Clean up. You are done with dissection.

Part III: Discussion
Discuss the answers to the lab report questions.

Lab report:
- Must be typed; handwritten reports will not be accepted. Hand-drawn and labeled drawings are fine; photographs are not acceptable.
- Due at the start of the lab session you are currently in during the week indicated on the syllabus. This is a firm deadline.
- Although you will perform these activities as a group, each member of the group must turn in an individual lab report. Each person's report must be in his or her own words as much as possible.
- Your lab report must contain answers to the following questions.

1) The answers to the questions we asked during the dissection of the trout. Be sure to include answers as well as a brief justification of your answer that is based on what you observed in lab.
   a. are the nares involved in respiration?
   b. are the pyloric caeca involved in absorbtion of nutrients?
   c. are the intestines involved in the absorbtion of nuteients?
   d. is the swim bladder physostomous or physocleistous?

2) An analysis of the data you took when dissecting the trout. For your trout:
   a) What % of the total body weight is the heart? Given that fish are cold-blooded animals, would you expect this percentage to be higher or lower in humans? Justify your answer.
   b) Compare the diameter of the red blood cells from humans and fish.

3) The answer to one of the following questions. You will receive a summary of the class data for discussion and discuss it in lab. Your conclusions must be based on all the class data, not just the data from your own trout.
   a. Fisheries biologists often use another measurement to estimate the weight of a fish without weighing it. Which of the following is the best estimator of fish weight and why?
      - length
      - circumference
      - jaw length
   b. Which (if any) of the following is the best predictor of sex in trout?
      - weight
      - circumference/length ratio
      - length/weight ratio
      - spot pattern
      - ratio of jaw length to overall length

Lab Practical Exam

In the third lab session, you will take the lab practical exam. During this exam, students will rotate through a series of stations each of which will have a trout or squid sample that you saw in lab previously. You will have a set amount of time at each station (usually 2-3 minutes). During that time, you will answer a question based on that sample. No notes will be allowed and no communication will be allowed between any students during this exam.

You will be responsible for all the parts mentioned in the preceding pages - what are they and in which system(s) do they function? If some parts were not found by your lab section (especially in the squid dissection), you will not be responsible for them; your TA will let you know when this is the case. You must also know all the external anatomy of the squid and trout mentioned in the lab manual as well as the major body axes.

At a typical station, you would find a familiar trout or squid sample with a pin pointing to a particular part of the sample and a question. Typical questions could include:

- To which phylum does this sample belong?
- Which part of the organism is this?
- To which system(s) does this part belong?
- Which system(s) is this not a part of?
- Where does the food/H_2O, blood go from here?
- Which body axis is this?
- etc.

You will rotate through the stations at the direction of your TA. When the time is up for a particular station, you will have to move on to the next station even if you are not finished with the current station. There will be 10-20 stations, each worth 3-5 points. Part credit may be awarded in certain circumstances.

The first two lab sections are designed to prepare you for this exam. Experience has shown that using the lab time to prepare a study guide as described below will provide the best preparation for this exam. Because you will have to recognize the animal parts and put them into their various contexts, you should make your own drawings of these samples.

The policies and procedures will be the same as those listed in the Plant Lab Practical.

Study Guide

The following is a guide to help you prepare your Study Guide.

1) A rough sketch of each animal at various stages of dissection with parts appropriately labeled.

2) Diagram the respiratory system of each animal; this need only be a rough schematic. Indicate the direction of air or water flow.

– For the trout, be sure to include:
mouth gills gill slits gill rakers nares (if applicable)
know how water is forced over the gills

– For the squid, be sure to include:
mantle gills siphon
know how water is forced over the gills

3) Diagram the digestive system of each animal.
- For the trout:
  - be sure to include:
    - gullet, intestine, mouth, pyloric caeca
    - anus, stomach, the direction the food flows
  - know the differing textures of the different parts of the digestive tract listed above.
- For the squid:
  - draw it as it is arranged in the squid; be sure to include:
    - stomach, caecum, digestive tract, anus
    - mouth, ink gland, the direction the food flows
  - compare the textures of the insides of the stomach and caecum to the digestive organs in the trout

4) Diagram the major parts of the circulatory systems of each animal. Indicate the direction of blood flow.
- For the trout (you will need to use *Campbell* as a reference since it is impossible to trace the vessels in the fish),
  - be sure to include:
    - heart, gills, "rest of body" (other organs)
- For the squid, you will not be able to draw the complete system. Although it is closed, you can't see it all. Draw what you can, including:
  - hearts (all 3), sinus venosis, gills
  - whatever else you can find

<u>Tips from Bio 112 Spring 2008 Students</u>

On the course evaluation in Spring 2008, I asked students the following two questions:

1) Did you do all the drawings listed in the Animal Study Guide? Of the 122 students who responded, 71 (58%) said yes.

2) Whether or not you did the drawings, do you think that doing the drawings helped (or would have helped if you had done them) you to learn the material for the Lab Practical Exam? Here, 75% said yes.

Conclusion: as with the Plant Study Guide, your fellow students did the drawings and found them useful; that is why I recommend it highly.