Lab 5: Phylum Mollusca

Objectives:

- **Understand the taxonomic relationships and major features of mollusks**
- **Learn the external and internal anatomy of the clam and squid**
- **Understand the major advantages and limitations of the exoskeletons of mollusks in relation to the hydrostatic skeletons of worms and the endoskeletons of vertebrates, which you will examine later in the semester**

Textbook Reading: pp. 700-702, 1016, 1020 & 1021 (Figure 47.22), 943-944, 978-979, 1046

Introduction

The phylum Mollusca consists of over 100,000 marine, freshwater, and terrestrial species. Most are familiar to you as food sources: oysters, clams, scallops, and yes, snails, squid and octopods. Some also serve as intermediate hosts for parasitic trematodes, and others (e.g., snails) can be major agricultural pests.

Mollusks have many features in common with annelids and arthropods, such as bilateral symmetry, triploblasty, ventral nerve cords, and a coelom. Unlike annelids, mollusks (with one major exception) do not possess a closed circulatory system, but rather have an **open circulatory system** consisting of a heart and a few vessels that pump blood into coelomic cavities and sinuses (collectively termed the **hemocoel**). Other distinguishing features of mollusks are:

- A large, muscular **foot** variously modified for locomotion, digging, attachment, and prey capture.
- A **mantle**, a highly modified epidermis that covers and protects the soft body. In most species, the mantle also secretes a **shell** of calcium carbonate.
- A **visceral mass** housing the internal organs.
- A **mantle cavity**, the space between the mantle and viscera. **Gills**, when present, are suspended within this cavity.
- A **radula**, a protrusible, rasp-like feeding organ present in most, but not all, species. In herbivorous mollusks (e.g., chitons and snails), the radula is used for scraping algae from rocks. In carnivores, the radula can be fang-like and is used for piercing prey (e.g., squids and octopods), or may be pointed and used for drilling through shells (e.g., some snails).

Of the five classes of mollusks, four (listed below) are fairly common, and the first three will be studied in the laboratory (Figure 1):

- **Class Bivalvia**, clams, scallops, and oysters; characterized by a hinged shell of two **valves** (parts) and a foot used for digging; lack a radula; marine and freshwater filter feeders.
- **Class Gastropoda**, snails, slugs, whelks, limpets, abalones, and nudibranchs; usually possess helical shells and a foot used for crawling; marine, freshwater, and terrestrial herbivores and carnivores.
- **Class Cephalopoda**, squids, octopods, and nautiloids; usually lack external shells; possess a **siphon** for jet-propulsion; marine carnivores.
- **Class Polyplacophora**, the chitons, primarily herbivorous marine species with a shell consisting of many plates (hence its name).

You will be given unpreserved organisms to dissect, and there will also be mounted slides for you to examine under the microscope. This year, you will also have living clams to work with. Careful dissection may enable you to see muscular contractions of the large foot and maybe even of the small heart! By working with living organisms, we hope that you will gain a greater appreciation for the dynamic aspects of animal organ systems.

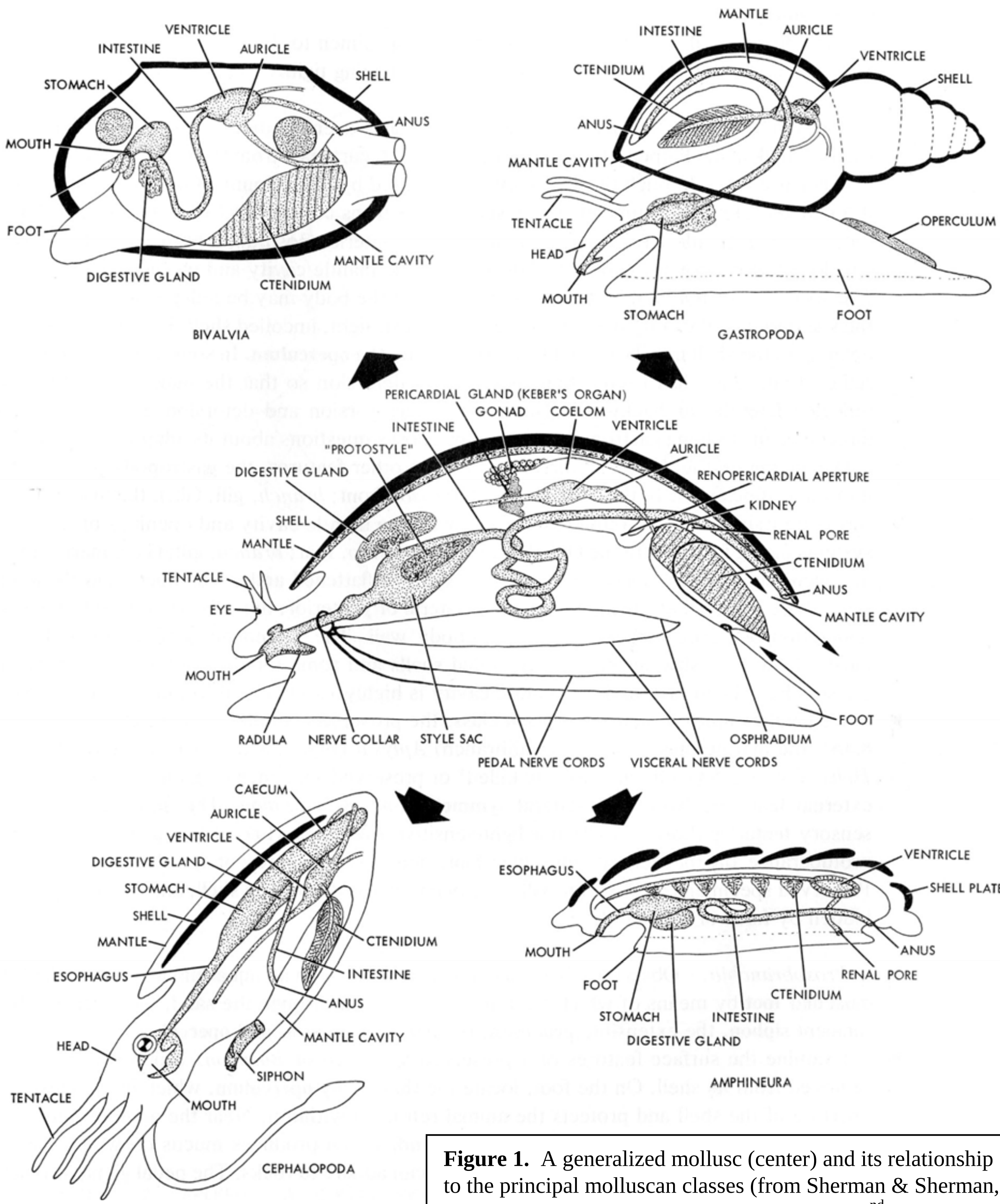


Figure 1. A generalized mollusc (center) and its relationship to the principal molluscan classes (from Sherman & Sherman, 1976, *The Invertebrates: Function and Form*, 2nd ed.).

Specimens of Mollusks

A) Class Bivalvia

Although most bivalves that you are familiar with live either attached to the substrate (mussels) or burrowed into the ground (clams), there are a small number that are free living at the surface. You are probably already familiar with the most conspicuous features of clams, mussels and scallops: their body shape, lack of a recognizable head, and two-piece shell. You will be studying the Northern Quahog, *Mercenaria mercenaria*, as a representative of a living clam. These saltwater clams are sedentary and live intertidally and in shallow subtidal areas of sand flats on the east coast of the U.S. The morphology and anatomy of modern bivalves have been much altered from those of ancestral mollusks, which had a distinct anterior end with a mouth and a posterior end with an anus (Figure 1). Consequently, as you look at the specimen, note how various features (gill, foot, mantle) have been modified for a sedentary, filter-feeding lifestyle.

Examination of External Structure

1. Examine an unopened clam in the finger bowl on the front bench. A hard exoskeleton composed of a pair of **valves**, or shells, protects the soft body. The **valves** are attached dorsally by a hinge-ligament that opens the shell when the **adductor** muscles are relaxed. The anterior elevation near the hinged margin is the **umbo**. It is the oldest part of the shell, and as your clam grew, it added shell to this base; the concentric lines ("growth rings") represent successive periods of growth. If the animal is undisturbed, the valves may be slightly agape ventrally and you can see at the posterior end the fringed edges of the **mantle**, which lines the valves. The posterior edges of the mantle are shaped so as to form two openings (**siphons**) to the inside of the **mantle cavity** (**Figure 2**).

2. Familiarize yourself with the application of the following terms to the clam: anterior, posterior, dorsal, ventral, left, and right.

Examination of Internal Structure

1. The lab TA's will open a clam for you. Keep the shell that has the soft tissues of the clam attached to it submerged in cold sea water while you examine it (you may get to see the heart beat if you do so!). First, however, examine the other valve. The shell is composed of three layers, formed by secretions from the mantle. The mother-of-pearl layer lines the inner surface of the valve.

2. Study the figures of the internal structure of the clam. Locate the adductor and retractor muscles. The **adductor muscles** (which were cut in two to open the shell) close the valves, whereas the **retractor muscles** pull in the foot. Notice the large mass of the two adductor muscles, which allow for the prolonged closure of the valves. Note that there are no muscles that *open* the shell - this is accomplished in part by the **elastic hinge ligament**, which acts like a spring and opens the shell when the adductors relax. What other force might open the shell?

3. Locate the **mantle**, which completely lines the valves and encloses the other soft tissues. Observe the relationship between the mantle and the siphons. Most of the mantle is ciliated,

except for the outer edge around the margin of the shell where glands lay down shell material. As a result of the lack of cilia, any particles that become lodged between the mantle and the shell in this area cannot be removed - instead, the particles are covered with nacre, or inner shell material (and become what bits of wisdom?).

4. The mantle encloses the soft **visceral mass** (yummy!). Inside are housed the **gonads** and much of the **digestive system** (more yummy!). The muscular **foot** (even more yummy!), which can be engorged with blood, lies ventral to the visceral mass. What types of structures allow the foot to retract and extend?

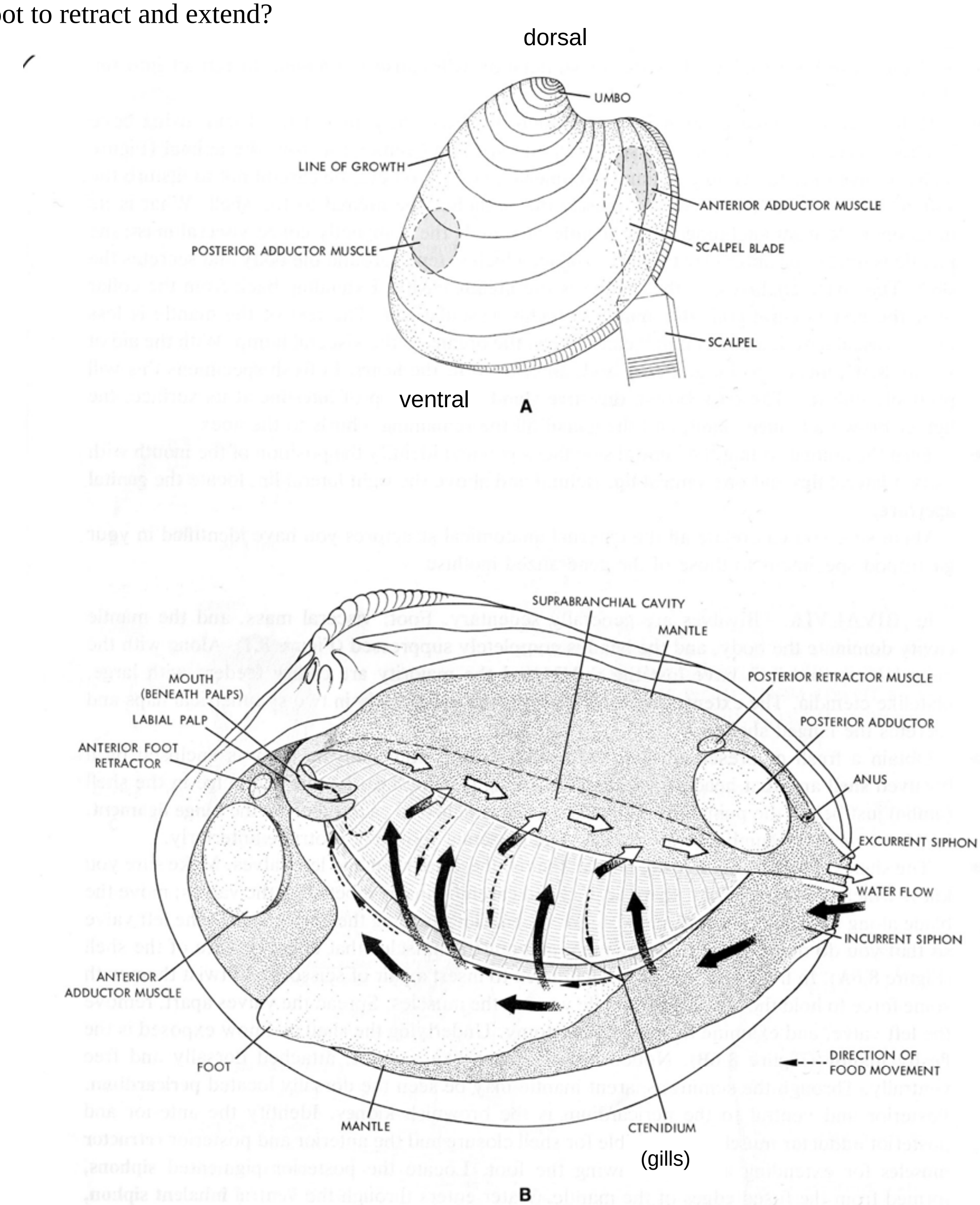


Figure 2A & 2B. *Mercenaria*. See legend on next page.
(from Sherman & Sherman, 1976, *The Invertebrates: Function and Form*, 2nd ed.)

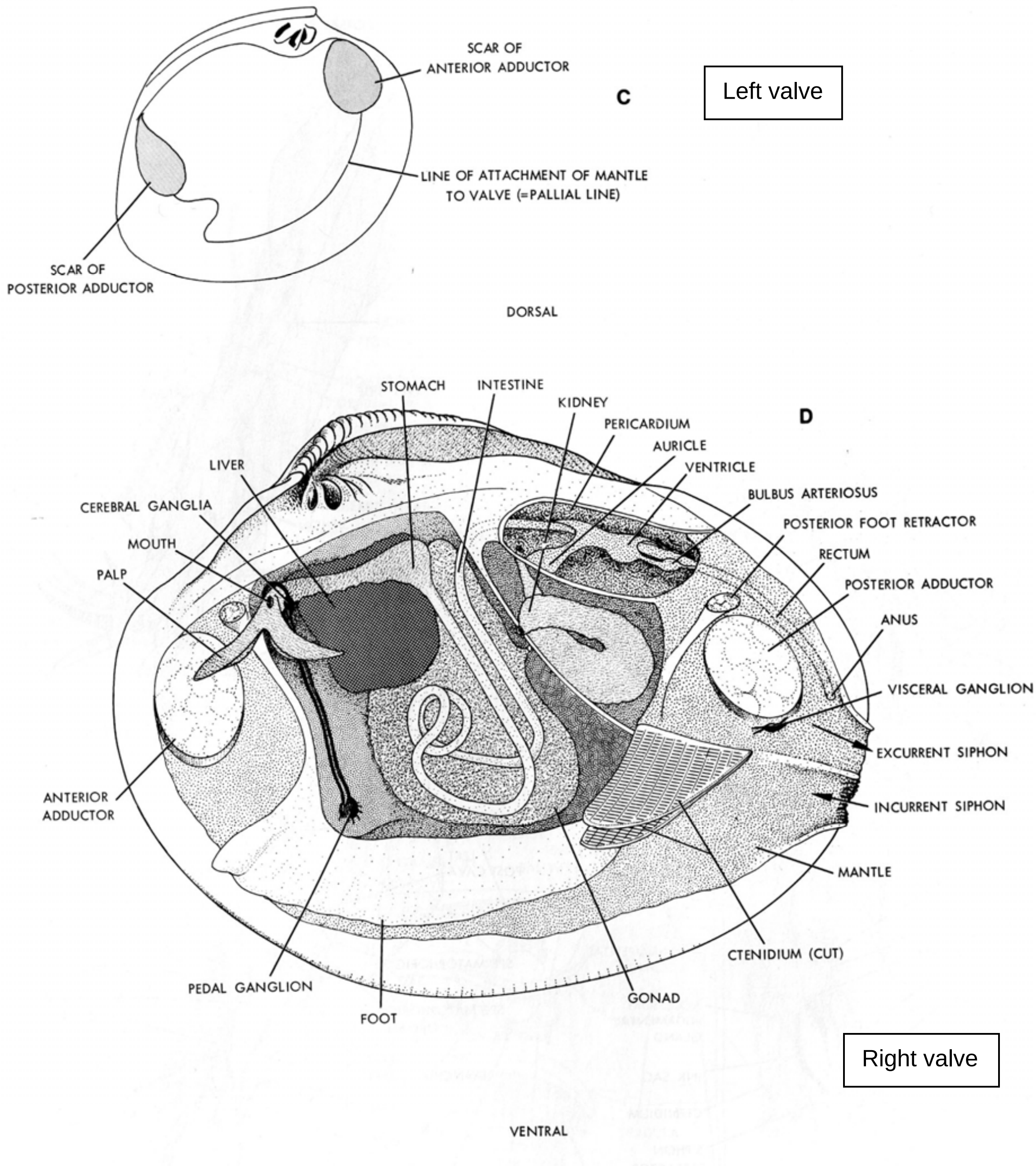


Figure 2 *MERCENARIA (VENUS).*. **A** EXTERNAL FEATURES AND METHOD OF INSERTING A SCALPEL TO CUT THE ADDUCTOR MUSCLES. **B** WITH THE LEFT VALVE AND MANTLE REMOVED. **C** VIEW OF THE INSIDE OF THE LEFT VALVE. **D** INTERNAL ANATOMY. THE LEFT VALVE, MANTLE, GILLS, AND TISSUES OVERLYING THE INTERNAL ORGANS HAVE ALL BEEN CAREFULLY REMOVED.

Gas Exchange with the Environment

5. Gas exchange occurs across the surface of both the mantle and the gills (how many gills are there?), and all are ciliated to promote water movement. ***Why is it important to keep the water moving over the surface of the gills?***

6. From the **incurrent siphon**, run a probe into the **suprabranchial chamber**. With scissors, slit the suprabranchial chamber to see the tops of the water tubes of the outer gill. Compare the living clam to the diagram in Figure 2B, to understand the path of water movement through the mantle cavity, across the surface of the gills and out through the (upper) **excurrent siphon**.

7. Carefully trim off the mantle where it attaches dorsally to the gills. First note the structure of the gills. Then cut a transverse section about 1 mm wide from the lower (ventral) part of the gill. Stand the section on edge on a slide and examine with a dissecting microscope. Identify the lamellae, filaments, and water tubes, and the partitions (interlamellar junctions) that hold the two lamellae (see figure). How many layers of tissue make up each gill? Compare your slide to the prepared slide of clam gills that is on the demonstration microscope. Why must the gills of the clam, called the **ctenidium**, be thin structures?

Nutrient Acquisition and Digestion

8. The water entering the mantle cavity usually contains suspended particles of organic matter, such as dead animals and plants as well as microorganisms. The water tubes of the gills are lined with a mucous secretion to which these small particles adhere. Cilia lining the tubes move the mucus and food to a food groove on each gill, and then to **labial palps**. The labial palps sort food particles for size and density before delivering them to the **mouth**.

9. The green, pulpy tissue exposed around the **stomach** is the **digestive gland**. The **intestine** is a long, coiled tube running throughout the foot (why do you suppose the clam has such an extensive digestive tract? Would you expect such a tract in a carnivorous mollusk?). The tissue surrounding the intestine will for the most part be yellowish or cream-colored **gonads** and muscle fibers. Sexes are separate in clams, but male and female organs are similar in appearance and cannot be distinguished in a simple dissection.

Transport/Circulation

10. Gently lift and remove the pair of gills that are present on the exposed side of your clam. Near the dorsal midline, just ventral to the hinge, is the thin-walled **pericardial sac**, which contains the **heart**. The three-chambered heart is composed of a single **ventricle** and two **auricles**.

11. Using a fine pair of scissors, slit open the pericardium to expose the heart. Observe the pumping of the ventricle if the heart is still beating. Note that the intestine runs <u>through</u> the heart.

12. Two **aortae** leave the heart - the **anterior** and **posterior**. If the heart is beating, carefully inject a small amount of neutral red dye into the ventricle and trace the path of the two aortae. Although there are vessels going both to and from the heart, the circulatory system in

the clam is usually considered to be "open" (what does this mean?). Blood is pumped out of the heart through several arteries into a series of large sinuses (parts of the hemocoel). Blood from the sinuses flows into the gills, and then back into the heart.

B) Class Gastropoda (snails, slugs, and nudibranchs), preserved specimens

1. Examine the slide of a snail **radula**. What is the function of this structure?

2. Examine the external morphology of *Busycon*, a large, marine snail better known as a conch (**Figure 3**). Pick up the coiled shell and note the large opening that allows protrusion of the foot and head. How must the body be modified to fit within such a shell? Where do you think the posterior end (anus) of the animal is located? Snails occur in both marine and freshwater habitats, and some are even found on land. In terrestrial gastropods, gills are absent, but the mantle cavity has become highly vascularized and serves as a lung. Such snails are referred to as "pulmonate".

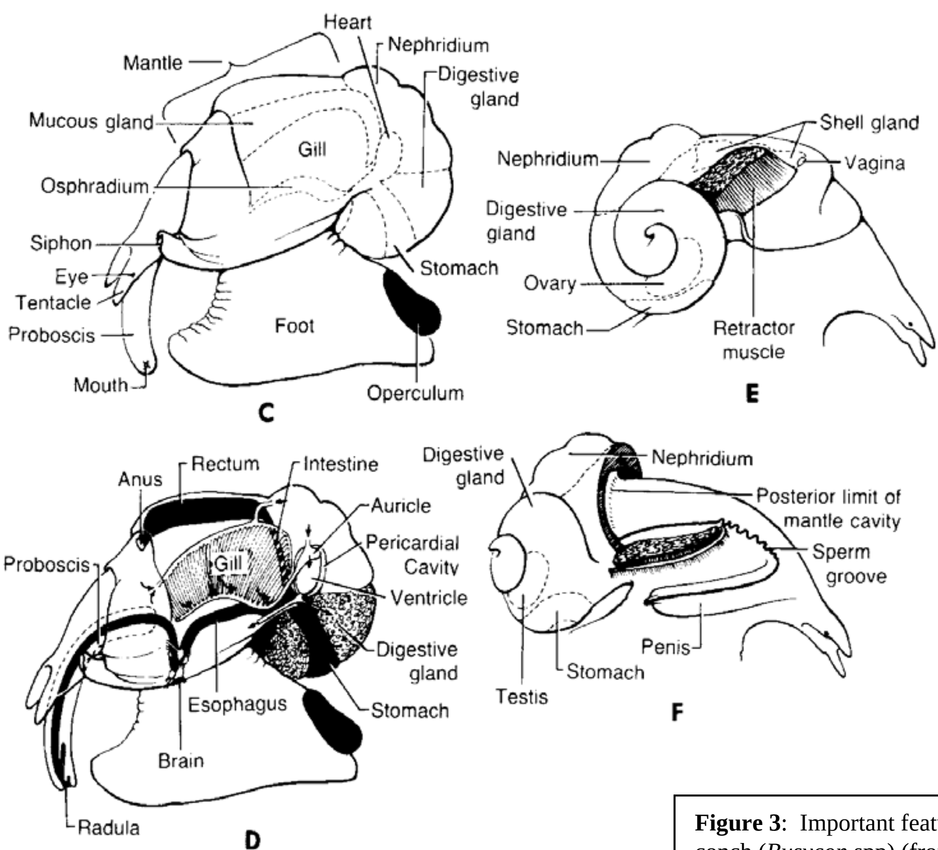


Figure 3: Important features of the marine conch (*Busycon* spp) (from Barnes 1980, Invertebrate Zoology)

C) Class Cephalopoda, fresh-frozen squid

Part A: External Anatomy.

Examine a fresh-frozen squid, which was thawed out earlier today. Unlike other mollusks, the shell of squids is not external but rather is internal (and much reduced in size). A tough, muscularized **mantle** completely surrounds the animal (**Figure 4**).

1. The head of the squid should have eight arms of about the same size, known as **grasping arms**. It should also have two longer arm-like structures called **tentacles**. What typical structure of mollusks are the arms and tentacles derived from?

2. How do the tentacles differ from the grasping arms? Use scissors to remove one of the tentacles and observe its end using the dissecting microscope. What do the specialized ends of the tentacles look like?

3. The mouth, encircled by the tentacles and grasping arms, is equipped with a **radula** that has been modified into a hawk-like beak. Note the large eyes, which function much the same way as the eyes of vertebrates

4. The term cephalopod means "head-foot." Why do you think squid got this name?

5. Rinse your squid under running water before beginning your dissection. Hold the squid vertically in the stream of water with the tentacles pointing upward so that water flows into the **mantle cavity**. Tilt the head back from the **siphon** and stand back! What happens?

How does the action of the siphon enable the squid to rapidly propel itself through water?

6. Observe the squid's skin and look for spotted areas. These spots contain cells that have color-producing pigments (called **chromatophores**), which allow the squid to change its color and pattern. Pull off a section of this thin layer of skin and observe on the dissecting microscope. **Why might it benefit the squid to rapidly change its appearance?**

7. What <u>internal</u> structure do you expect to see in the squid that functions in defense?

8. Given the above morphological and anatomical features, what sort of lifestyle do you think the squid leads? Would you expect to find them in the same habitats as snails or clams, and if not, where would you expect to find them?

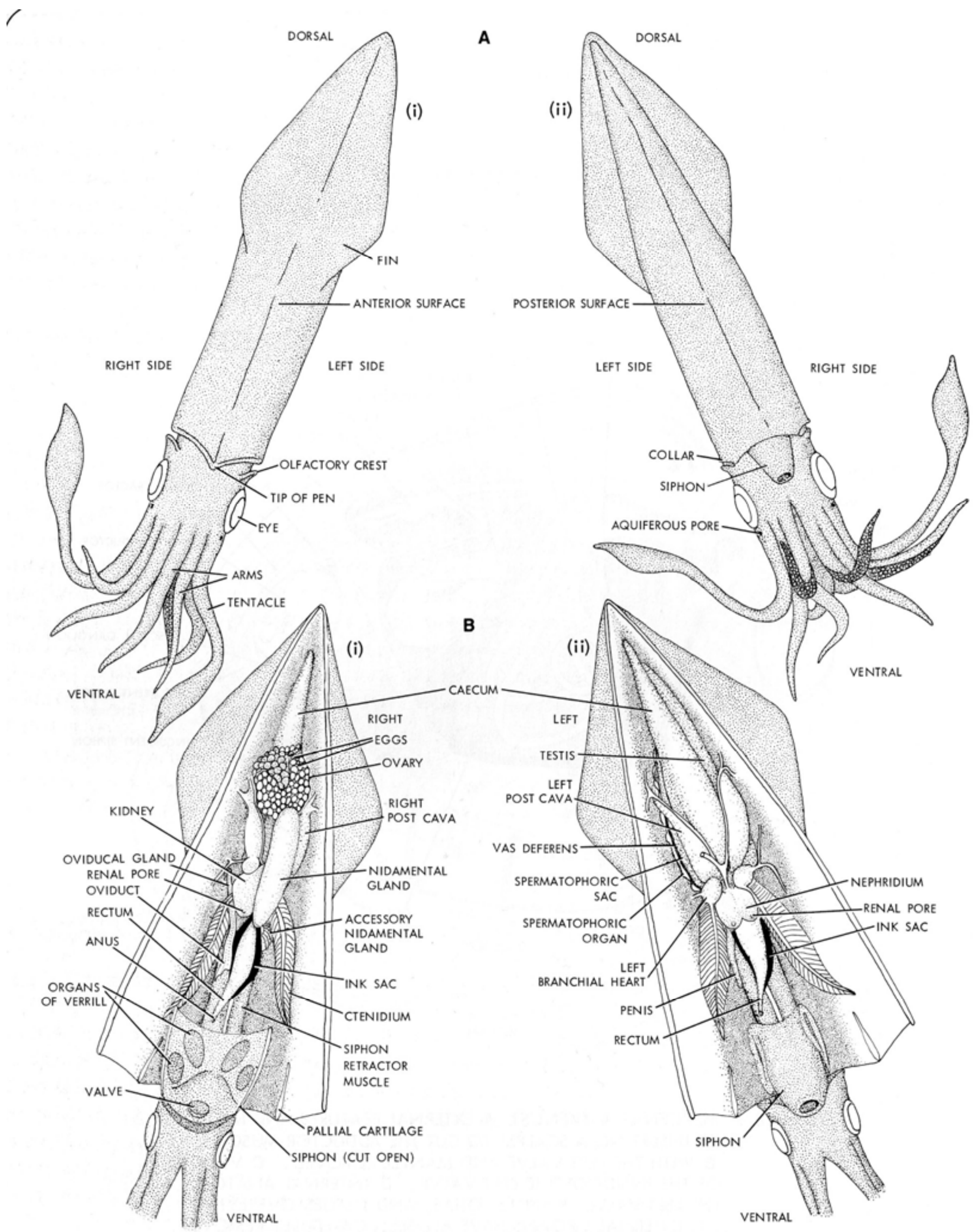


Figure 4A & 4B. The squid *Loligo.* See next page for legend.
(From Sherman & Sherman, 1976, *The Invertebrates: Function and Form*, 2nd ed.).

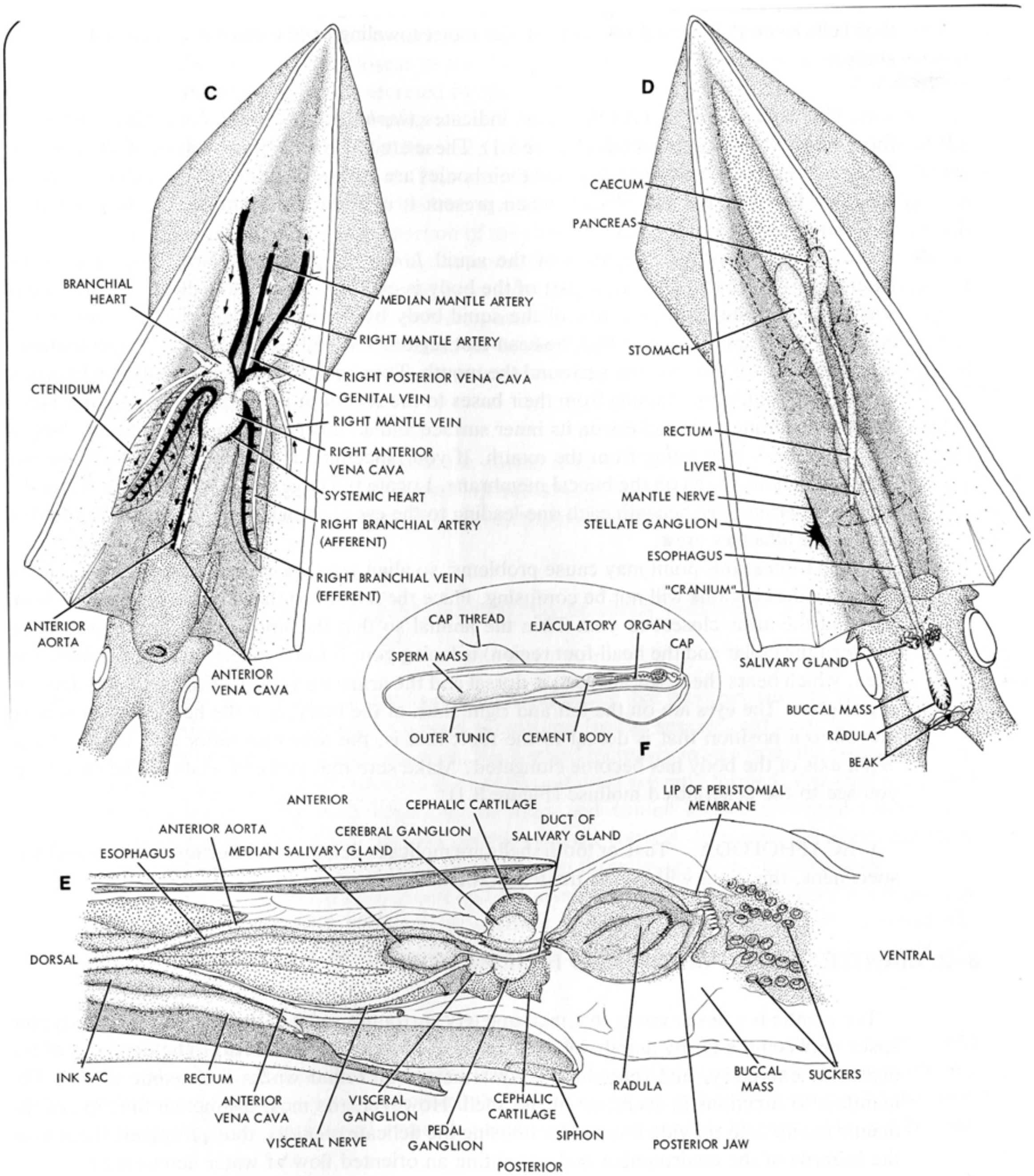
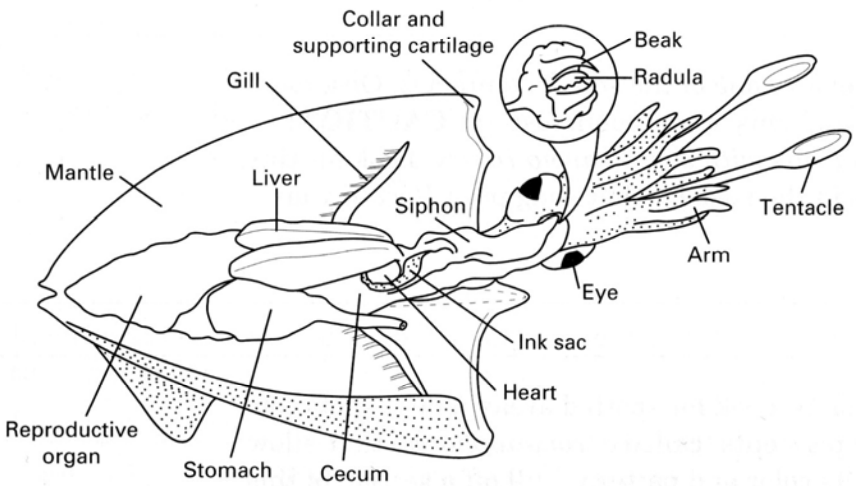


Figure 4. Squid, continued

LOLIGO. **A** EXTERNAL FEATURES: (i) ANTERIOR SURFACE; (ii) POSTERIOR SURFACE. **B** DISSECTED TO SHOW INTERNAL ANATOMY: (i) FEMALE WITH LEFT NIDAMENTAL GLAND REMOVED: (ii) MALE. **C** DISSECTED TO SHOW THE CIRCULATORY SYSTEM. **D** DISSECTED TO SHOW THE DIGESTIVE SYSTEM. **E** HEMISECTED TO SHOW THE NERVOUS SYSTEM, BUCCAL MASS, AND OTHER INTERNAL ORGANS. **F** SPERMATOPHORE.

Part B: Studying the Squid's Internal Anatomy

1. Locate the squid's siphon. Place the squid so the siphon is on top and the fins lie flat against the plate (the ventral [abdominal] side of the squid should be facing up). With scissors, make a cut along the mantle toward the pointed end of the squid. Cut only the mantle—take care not to damage the organs beneath it.



Squid

2. Pull the mantle open to study the internal organs. If you are using a dissection tray, you can use dissection pins to hold the mantle open. Notice the ridges of supporting cartilage on the inside of the mantle. This part of the mantle is called the collar. Now you can see how the siphon is attached. Locate the long tough white muscles on either side of the siphon. Carefully cut these muscles and gently pull the siphon out so you can better see the organs below it. The actions of these muscles move the siphon, changing the angle at which the water is pushed out. This propels the squid in different directions.

3. The digestive system extends from the esophagus into the stomach. Food then passes into a long pouch off the stomach called the cecum. Most absorption occurs in the cecum. From the cecum, the intestines go back towards the head and end in the anus near the mantle collar. Wastes exit from the opening of the mantle. To begin your exploration of the digestive system, first locate the liver. The large, white liver consists of two side-by-side lobes. Underneath the liver is the stomach, which is also white. Carefully remove the liver without removing the stomach or any other organs. Be careful also not to remove the ink sac, which looks like a silver pouch.

4. Next locate the mouth. At the point where all of the arms meet, look for a tiny black structure. This is part of the beak. Squid use their sharp beaks and the radula located inside of the beak to crush or rip prey apart. To access the esophagus, pry the beak apart and gently insert the probe into the beak. When you slide the probe down the esophagus, you should be able to see the probe inside of it. Remove the beak by pulling on it and observe how the two halves fit together. Sketch the beak and the radula in the space below.

5. On either side of the stomach, look for the almost transparent, feathery gills. As water circulates through the mantle, it washes over the gills. Oxygen diffuses through the gills and enters the squid's blood. Two hearts (called *branchial hearts*) pump blood from the gills. One branchial heart is located at the base of each gill (the end furthest from the head). Gently remove one gill by snipping it with scissors. Observe the gill with a stereomicroscope. Sketch the gill in the space below.

How do you think the shape and texture of the gill relate to its function?

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6. Squid have a flexible internal shell called a *pen* that gives a squid its shape. To find the pen, gently remove the rest of the internal organs. Look for the pen lying along the whole length of the mantle.

7. Cephalopods (squid, octopus, and their relatives) have very complex nervous systems and keen vision. Examine an eye with the dissecting microscope and notice the **cornea** (clear, disk-shaped structure). (From Pearson Education, 2004, *Biology: Exploring Life Laboratory Manual*).

The Squid Nervous System

Brains:

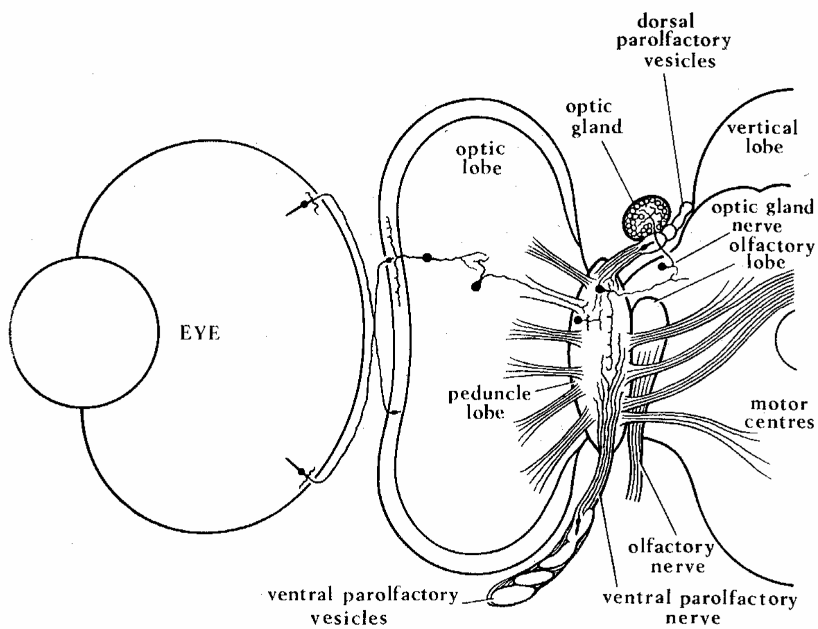
Where is the brain in the squid? How large is it relative to the size of the body? How does this size ratio compare to that of the earthworm? What does this tell you about the amount of central processing that the brain of each animal performs?

Invertebrate Vision Systems:

One striking similarity that you will observe later in the semester in the visual systems of vertebrates is in the way their eyes are structured. Because vertebrates are relatively closely-related organisms, it is not unusual that they have eyes with very similar structures. We call these types of structures **homologous** structures, because their structural and functional relatedness is based on the common history of the organisms being compared. What is really amazing is that even relatively unrelated organisms, such as cephalopods and mammals, can have very similar eye structures. We call this type of similarity **convergent** evolution.

In today's lab, make sure you examine the squid eye (Figure 5). Use the comparative eye diagram (Figure 6) to note your observations, as well as to compare the eye structures of organisms you will not dissect in lab (marine sandworm, spider).

Figure 5: Lateral view of eye and optic lobe (brain) of the squid, *Todarodes*, showing position of the optic gland (from Baumann, 1970, The extra-ocular light receptors of the squids, Brain Res.).



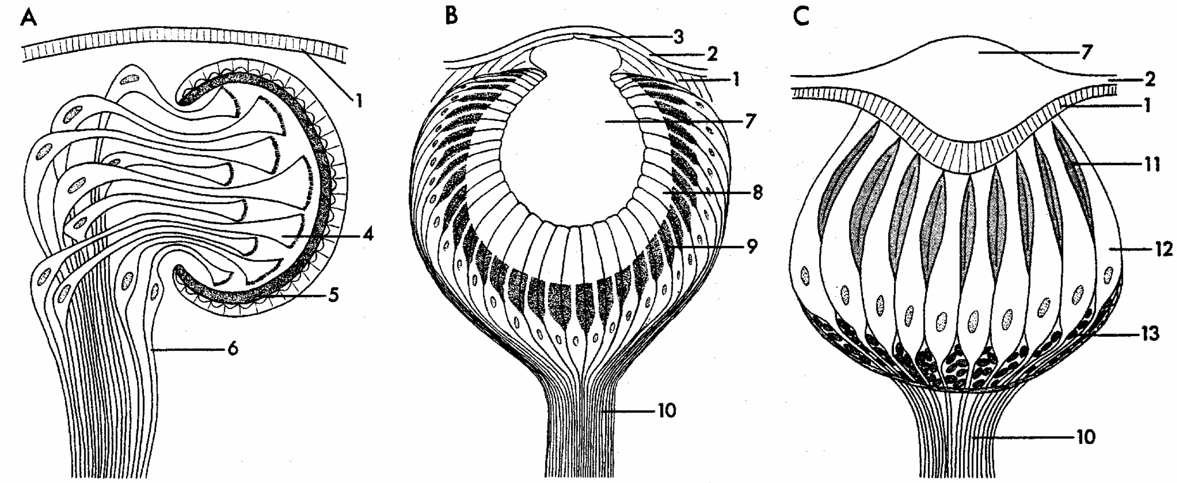
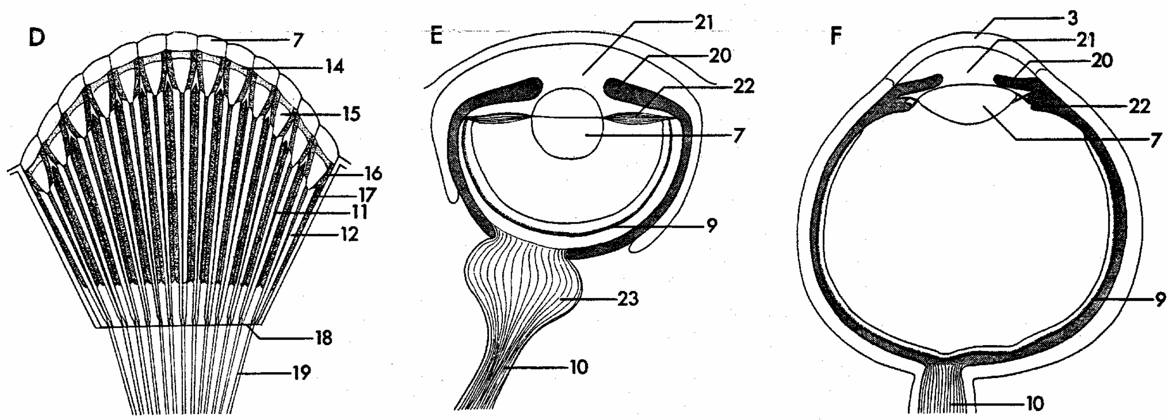


Figure 6: Comparative eye anatomy with major features indicated by number, with labels below. Use this diagram to take notes on for the squid eyes, noting things like color and indicating what features you could see in the dissection and which you couldn't (from Carolina Biological).



A. Eyespot (planaria)
B. Advanced eyespot (sandworm)
C. Simple eye (spider)
D. Compound eye (insect)
E. Image perceiving eye (squid)
F. Image perceiving eye (man)

1. Epidermis
2. Cuticle
3. Cornea
4. Sensory cell
5. Pigment
6. Nerve cell
7. Lens
8. Rod
9. Retina
10. Optic nerve
11. Rhabdome
12. Retinal cell
13. Light sensitive cell
14. Corneagen layer
15. Crystalline cone
16. Primary iris
17. Secondary iris
18. Fenestral membrane
19. Nerve fiber
20. Iris
21. Pupil
22. Ciliary process
23. Optic ganglion

The Respiratory and Circulatory Systems of the Squid

Squid have gills and closed circulatory systems (Figure 7), whereas crayfish have gills but open systems (as you will see next week in lab). What does this difference tell you about the average metabolic needs of each animal? What other large surface do squid use for gas exchange?

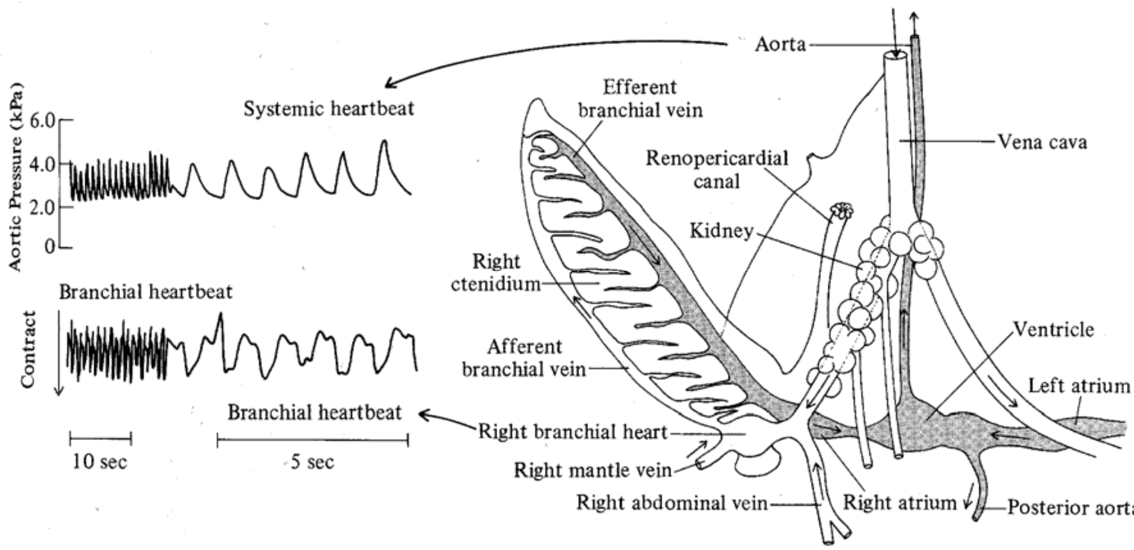


Figure 7: Circulatory and respiratory systems of a cephalopod mollusk showing the right gill (ctenidium), right branchial (gill) heart, and systemic heart and the relationship between systemic heart contractions and branchial heart contractions. What aspects of the squid lifestyle might demand that it have a higher metabolic rate and thus need two pumps to drive its circulatory system? (from Withers, 1992, Comparative Animal Physiology).