Suggested Classroom Activities

Also see Dissection Guides, page 20 and following.

(1) Cladistics

How Do We Reconstruct Evolutionary Events in the Past Using Evidence in the Present?

Phylogenetic analysis, or cladistics, is a technique that was developed specifi cally to learn about the evolution and relationships of biological species. Scientists use large datasets and computer programs to conduct their analyses, but a simple demonstration, using five taxa (species) and five characters can demonstrate the basic principles.

Step 1. Choose your taxa.

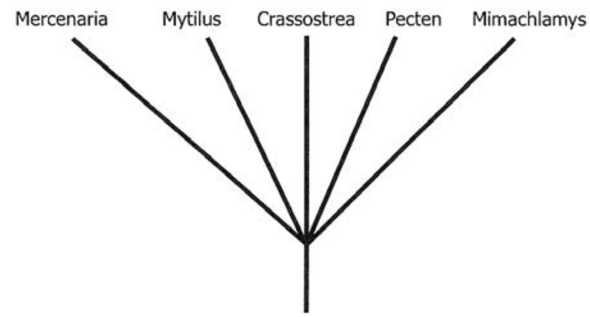
For this example, let's use:

Mercenaria	Clam	
Mytilus	Blue Mussel	
Crassostrea	Oyster	
Pecten	King Scallop	
Mimachlamys	Noble Scallop	

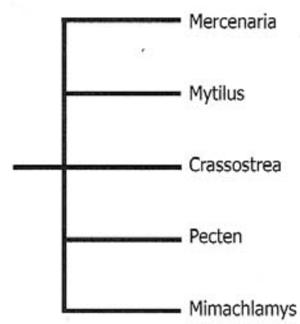
Note: We are intentionally using two scallops in this example – let's see if they turn out to be most closely related in our analysis!

Suggested Classroom Activities

Suggested Classroom Activities



OR



Without any character data at all, if we are constructing this tree manually, we can already draw the tree. Note that there are two common ways of representing the tree. Although they look different, they mean the same thing.

homologous • *Having the same structure and origin, although current function might differ.*

Step 2. Define your first character – modify the tree.

Each character must be the same feature in every species – we call these **homologous** - so that we are not trying to "compare apples and oranges." Each character must exist in your set of taxa in two or more conditions – called "states" – and at least one state should exist in more than one taxon. The simplest character states are "present" and "absent." Homologous characters are presumably those that evolved from one state to another over time within the lineage.

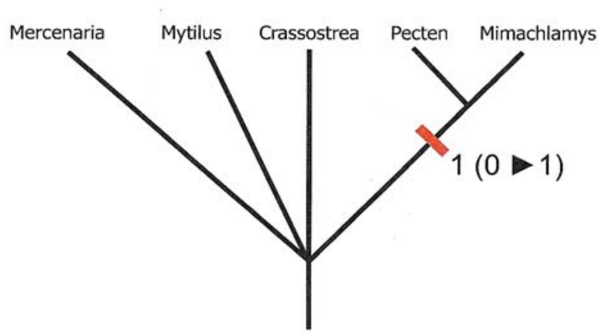
Let's use the "ears" (called "auricles") that are clearly present on the two scallops. So our first character is:

(1) Auricles: absent (0) or present (1)

We assign a numerical "code" to each state. Traditionally, the most primitive state is coded 0, and the more advanced (called "derived") states are coded 1, 2, etc. [This is determined through comparison with a taxon outside the immediate study group, called an "outgroup," but is not discussed in detail here.] We enter these codes in a data matrix or data table.

	(1) Auricles
Mercenaria	0 Absent
Mytilus	0 Absent
Crassostrea	0 Absent
Pecten	1 Present
Mimachlamys	1 Present

So we add this information to the tree by grouping the taxa according to their character states.



We indicate the character state change by a notch on the branch that means "character 1 (auricles) changes from absent (0) to present (1)."

Step 3. Define and code additional characters – refine the tree.

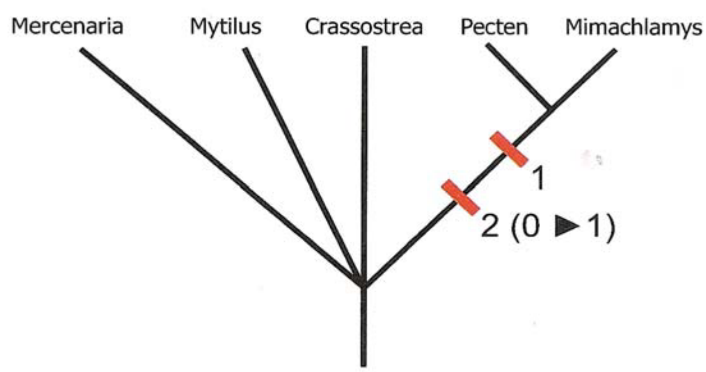
For each of these characters, we code all of the taxa, add the codes to the data matrix, and add the pattern to the tree. See if you can follow along.

(2) Radial sculpture: absent (0) or present (1)

Radial sculpture is the pattern of ridges radiating from the "beak" of the shell, called the "umbo," to the edge.

	(1) Auricles	*(2) Radial*
Mercenaria	0 Absent	0 Absent
Mytilus	0 Absent	0 Absent
Crassostrea	0 Absent	0 Absent
Pecten	1 Present	1 Present
Mimachlamys	1 Present	1 Present

Notice that the branching pattern has not changed, but we now have two characters supporting the branch (called a "clade") containing *Pecten* and *Mimachlamys*.



(3) Muscle scars: two (0) or one (1)

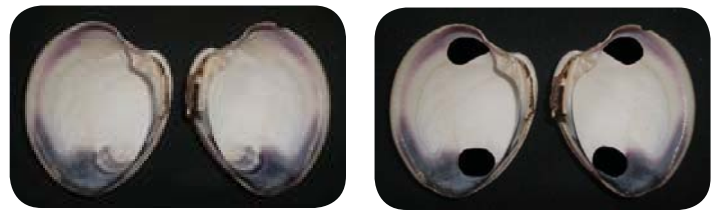
For this character, we need to look at the interior surface of the valves. Bivalve shells are held together by two muscles that attach to each valve, called an-

Suggested Classroom Activities

Suggested Classroom Activities

terior and posterior "adductor muscles." This information may be reviewed in the *Bivalve Anatomy* section. Some bivalves have lost one of the two muscles and moved the remaining one to the center of the shell. Where each of these muscles attach to the shell is represented by a scar – clearly impressed in some species, or more subtle in others. We have colored these muscle scars black in the third column for clarity.

Mercenaria



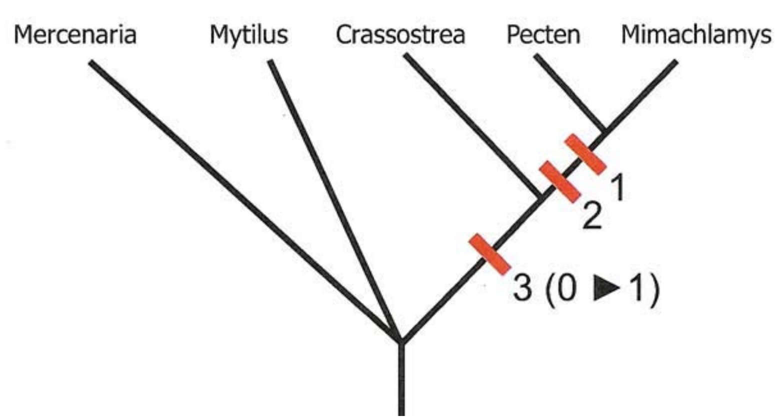
Mytilus

Crassostrea

Pecten

Mimachlamys

	(1) Auricles	*(2) Radial*	*(3) Scars*
Mercenaria	0 Absent	0 Absent	0 Two
Mytilus	0 Absent	0 Absent	0 Two
Crassostrea	0 Absent	0 Absent	1 One
Pecten	1 Present	1 Present	1 One
Mimachlamys	1 Present	1 Present	1 One



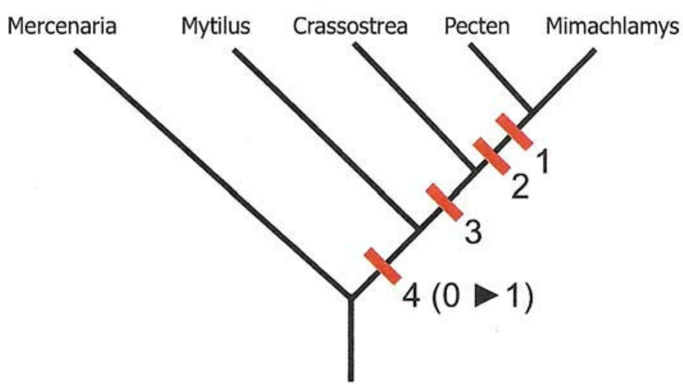
(4) 18S Positions #1-2: CG (0) or TA (1)

Molecular sequences can be used as characters too. Here are five real sequences (part of the 18S ribosomal gene):

Mercenaria	CGCCTTTACACGGCAAAACT
Mytilus	TACTTTTACATAGTGAAACC
Crassostrea	TACTCTTGCACAGTGAAACC
Pecten	TACTTTTTGATGGTGAAACC
Mimachlamys	TACTTTTTGATGGTGAAACC

Remember from the structure of DNA that: A = adenine, C = cytosine, G = guanine, T = thymine. (These letters are traditionally color coded for easier reading.)

	(1) Auricles	*(2) Radial*	*(3) Scars*	*(4) Pos 1-2*
Mercenaria	0 Absent	0 Absent	0 Two	0 CG
Mytilus	0 Absent	0 Absent	0 Two	1 TA
Crassostrea	0 Absent	0 Absent	1 One	1 TA
Pecten	1 Present	1 Present	1 One	1 TA
Mimachlamys	1 Present	1 Present	1 One	1 TA



Can you code more differences (molecular characters) from these sequences?

About Multistate characters

This example used very simple “binary” characters, that is, those with only two states. However, there are many characters in bivalves that can exist in more than two states – these are called “multistate” characters.

One example is muscle scars (see character 3 above)

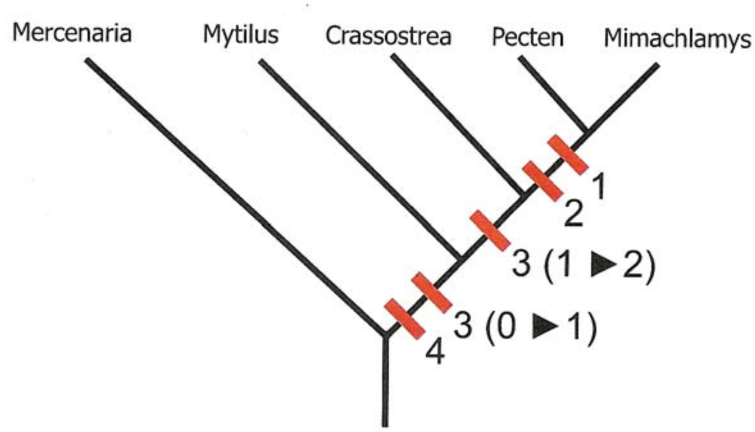
The muscle scars could have been coded as:
- Two of equal size (0)
- Two of unequal size (1)
- One muscle (2)

Suggested Classroom Activities

Suggested Classroom Activities

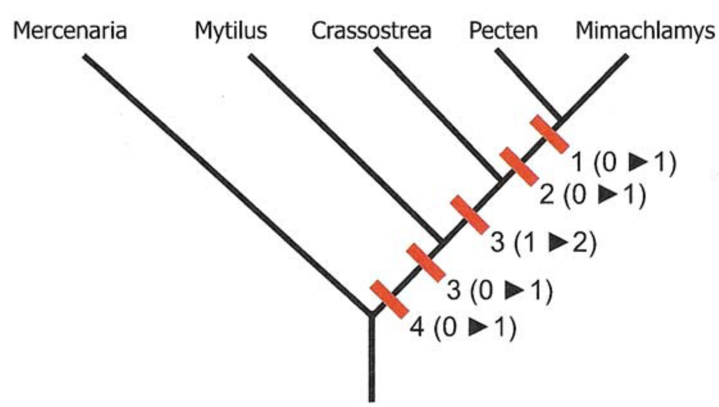
This sequence reflects the reduction (in size) of one of the muscles, presumably in the evolutionary pathway toward losing it entirely. If we substitute this coding for character 3:

	(1) Auricles	*(2) Radial*	*(3) Scars*	*(4) Pos 1-2*
Mercenaria	0 Absent	0 Absent	0 Two equal	0 CG
Mytilus	0 Absent	0 Absent	1 Two unequal	1 TA
Crassostrea	0 Absent	0 Absent	2 One	1 TA
Pecten	1 Present	1 Present	2 One	1 TA
Mimachlamys	1 Present	1 Present	2 One	1 TA



We see that this change did not change the branching pattern on the tree. Character three now exists as two changes on the tree, from 0 to 1 between *Mercenaria* and *Mytilus*, and from 1 to 2 between *Mytilus* and the other three taxa.

Here is the final tree incorporating all the branches and character changes.



We conclude that the two scallops (*Pecten* and *Mimachlamys*) are most closely related, supported by the presence of auricles and radial sculpture. They are most closely related to the oyster (*Crassostrea*), supported by the single muscle scar. These three taxa are most closely related to the mussel (*Mytilus*), supported by the molecular sequence in our dataset. We also see that muscle scars evolved (reading from the base to top of the tree) from two equal scars (in *Mercenaria*), to two unequal scars (in *Mytilus*), then to a single scar.

Can you see that changing just one number in the matrix might change the relationships portrayed on the tree? Try it!

(2) Bivalve Senses

Humans and vertebrate animals use their senses, in varying degrees, to explore their environment, avoid danger, and even learn. The five main senses are hearing, sight, smell, taste, and touch. Some species have evolved additional senses such as echolocation in bats and electroreception in sharks.

It might be surprising to learn that these senses are also found in many invertebrate animals, including bivalves. An invertebrate might not have eyes or ears that look like ours but it might have another kind of sense organ that accomplishes the same thing. Some of the senses might be absent or poorly developed but others might be more sensitive than human senses. The strengths of the senses will depend upon the type of bivalve you test but, in general, it is believed that touch is the most developed, followed by sight, smell, taste, then hearing.

Ask your students to design experiments to test the senses of bivalves living in your classroom aquarium (see Bivalve Introduction: In the Classroom for information on how to maintain bivalves in the classroom). The reactions can vary between bivalve species (adaptations), but can also vary among individuals (variation). These species adaptations and individual variations will provide clues to the evolutionary past and potential evolutionary future.

Please be creative and have fun with the experimental design and testing (but please caution your students to be gentle – these are living animals). If you have access to other types of invertebrates (snails, starfish, etc.), include those too in the tank and see how their reactions differ from those of the bivalves. Here are a few suggestions and hints to get you started:

- ***Touch***: Gently touch different parts of the bivalves (shell, edge of the mantle, siphons, etc.) and observe or measure the reactions. [Hint: Bivalves are very sensitive to touch and usually clamp shut at the slightest touch anywhere.]
- ***Smell***: Place a small piece of different kinds of food in different areas of the tank (away from the bivalves) and observe or measure the reaction. [Hint: Bivalves probably will not respond to this at all. Most bivalves are filter feeders and do not need an acute sense of smell to detect food. Contrast this with a carnivorous snail or starfish.]
- ***Taste***: Provide different foods directly to the bivalves and observe or measure the reaction. [Hint: Bivalves probably will not respond to this at all. There are very few bivalves that detect and eat food or prey near them.]
- ***Sight***: Move your hand or another solid object over the bivalve, creating a shaded area, and observe or measure the reaction. [Hint: Some bivalves have primitive eyes, actually photoreceptors that can detect light and dark, as an anti-predator mechanism. Some bivalve eyes actually have lenses, but the degree to which they can "see" in the way that humans can is uncertain.]
- ***Hearing***: Expose the tank to different frequencies and volumes of noise and observe or measure the reaction. [Hint: Bivalves do not have ears, however, sound is really vibrations in the air. Water movement is another form of vibrations, and can be detected by a bivalve in the same way that touch is detected.]

Suggested Classroom Activities

Suggested Classroom Activities

(3) Diversity

“Diversity” can be expressed in several ways:

Taxonomic diversity usually is expressed as the number of taxa (species, genera, or families) in a given area. The taxonomic category “family” (which in zoology always ends in -idae) groups bivalves with similar morphologies and lifestyles. For example, species in the family Veneridae have round or oval shells, usually relatively smooth (without spines), that are heart-shaped in anterior view, and that have siphons to live below the surface of the sand (evidenced by the pallial sinus).

There are approximately 100 families of bivalves living today, with approximately 20,000 living species. The fossil record is equally large. Using books, the internet, and/or specimens in your classroom or that you can collect on a field trip, ask your students to identify representatives of bivalve families.

Ecological diversity reflects the different ways that organisms interact with their environment.

Diversity of habitat:

- Bivalves that live below the sand (Hard-shelled Clam, Soft-shelled Clam, Freshwater Mussels, Lucine Clams).
- Bivalves that live on top of the sand (Scallops), or that cement or use a byssus to attach to rock (Arks).

Diversity of protection against predators:

- Bivalves that burrow below the sand to hide (Hard-shelled Clam, Soft-shelled Clam, Freshwater Mussels, Lucine Clams).
- Bivalves that clamp tightly closed (Arks, Hard-shelled Clam).
- Bivalves that are thick-shelled and resistant to cracking by predators (Hard-shelled Clam, Freshwater Mussels, Oysters).
- Bivalves that are thin-shelled but can swim to escape (Scallops).
- Bivalves that live gregariously, tightly crowded together on rocks (Mussels, Oysters).

Notice that some species use several anti-predator tactics. Discuss these adaptations with your students and ask them to think of anti-predator tactics of other animals or plants.

See also Activity (8) **Reading Empty Shells**.

(4) Taxonomy

(1) Notice that each bivalve species has three parts to its name. Consider the name *Mercenaria mercenaria* (Linnaeus, 1758):

(a) The first word (*Mercenaria*) is the ***genus*** name – it is always capitalized and italicized.

(b) The second word (*mercenaria*) is the ***species*** name – it is always italicized and in lower case. If the species is unknown, the species name is replaced by "sp." (not italicized).

- Genus and species names are always in Latin or are Latinized. They are also always italicized, underlined, or otherwise appear in a different font from the rest of the text.
- Note that when we refer to a "species," we use both the genus and species names (i.e., our species is *Homo sapiens*, not just *sapiens*).
- The system of two-part names is ***binomial nomenclature***.

(c) The third part to the name is the ***taxonomic authority*** – a name and date (Linnaeus, 1758) of the person or persons who first described the species. Parentheses are present around the name and date if the species is now placed in a genus different from that originally used (in our example, *Mercenaria mercenaria* was originally described in the genus *Venus*). The taxonomic authority is often omitted in sentences, but it is important in scientific contexts because very common species names, such as *albus* = white, or *variabilis* = variable, have been used more than once, often in the same genus.

A note about Common Names: In your classroom discussions, encourage your students to use the scientific names of the bivalves. Although common (non-scientific) names are often provided, and in general are easier for non-scientists to understand, they are not universal and are often misleading. This is especially true for invertebrates like bivalves; it is less of a problem for birds and mammals, which have had standardized common names for many, many years. So, whereas the name Black-Capped Chickadee can only mean one species of bird [*Poecile atricapillus* (Linnaeus, 1766)], the bivalve *Mercenaria mercenaria* (Linnaeus, 1758) is known variously as Hard-shelled Clam, Hard Clam, Northern Quahog, Cherrystone, Little-neck Clam, or Steamer Clam, depending on who is speaking and where they are from. This is further complicated by the fact that "Steamer" can also refer to *Mya arenaria* (Linnaeus, 1758), and "Quahog" to *Arctica islandica* Linnaeus, 1767. A similar case can be made about the word "mussel" - applied to marine mussels, freshwater mussels, and Zebra Mussels (each in a different family and genus).

Common names are especially important to the field of environmental conservation, in which scientists must communicate effectively with governmental officials, who are not scientists. If two groups of scientists call an endangered bivalve by two different common names, legislators will think either (1) more than one species of bivalve is being discussed, or (2) the scientists do not know what they are talking about. The American Fisheries Society has supported efforts, including publication of a checklist, to standardize names of American species of bivalves [1].

[1] Turgeon, D. D., J. F. Quinn, Jr., A. E. Bogan, E. V. Coan, F. G. Hochberg, W. G. Lyons, P. M. Mikkelsen, R. J. Neves, C. F. E. Roper, G. Rosenberg, B. Roth, A. Scheltema, F. G. Thompson, M. Vecchione, and J. D. Williams. 1998. *Common and Scientific Names of Aquatic Invertebrates from the United States and Canada: Mollusks, 2nd ed.* American Fisheries Society, Special Publication 26, Bethesda, Maryland, 526 pp. + CD-ROM.

Suggested Classroom Activities

Suggested Classroom Activities

(2) Ask your students do some research on the taxonomic authorities of bivalve species. Notice the varied professions that these people held (professor, geologist, museum curator, "naturalist").

For example, many bivalve species are credited to "Linnaeus, 1758." This is the earliest date that you will ever see as a taxonomic authority, because taxonomists have designated this work – *Systema Naturae, 10th edition,* 1758, by the Swedish naturalist Carolus Linnaeus (aka Carl von Linné) – as the beginning of modern taxonomy. Linnaeus invented binomial nomenclature, which replaced long, wordy descriptions and provided a universally recognized name for each species. Because he was the first, Linnaeus is credited with the names of many of the most common bivalves, and in fact, many of the most common animals, including:

- Hard-shelled Clam, *Mercenaria mercenaria* (Linnaeus, 1758)
- King Scallop, *Pecten maximus* (Linnaeus, 1758)
- Blue Mussel, *Mytilus edulis* Linnaeus, 1758
- Green Sea Turtle, *Chelonia mydas* (Linnaeus, 1758)
- White Shark, *Carcharodon carcharias* (Linnaeus, 1758)
- Crow, *Corvus corone* Linnaeus, 1758
- Gray Wolf, *Canis lupus* Linnaeus, 1758
- Human, *Homo sapiens* Linnaeus, 1758

Here is a list of other authors that your students might research. What species did they name?

- Thomas Say
- William Healy Dall
- Johann Friedrich Gmelin
- Michael Tuomey & Francis S. Holmes
- Peter Simon Pallas
- Jean Guillaume Bruguière
- Angelo Heilprin

(3) The scientific names of organisms have meaning, often describing characteristics of the species. Ask your students look up a scientific name of one of these bivalves in a Latin dictionary and compare that to the shell.

- For example, *edulis* means "edible," and *maximus* means "large." So, *Mytilus edulis* is the "edible mussel," and *Pecten maximus* is the "large scallop."
- Some names are named after people or places. So, *Chesapecten jeffersonius* is "Jefferson's Chesapeake Scallop" and *Crassostrea virginica* is the "Virginia Oyster."

(4) New species of bivalves are being described every year, if not every month or week. Ask your students to research new species recently described. Where have they been described from? What kinds of data (morphology, molecules, ecological traits) have been used to characterize the new species? Think about why these species have never been described until now.

(5) Create some new bivalve "species" out of paper and ask your students to name them, explain the meaning of each name, and list the key morphological features of each one.

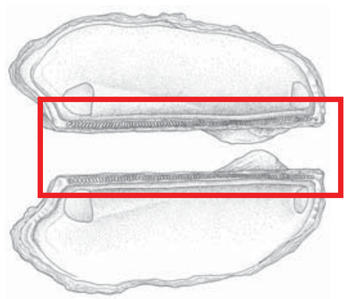
See also Activity (9) **Finding Nature's Order: Classification**.

(5) Types of Hinges

The hinge is an important feature of the bivalve shell. It joins the two valves, and - importantly - keeps them aligned when the valves close (via the adductor muscles) or open (via the ligament). It is widely used in taxonomy, and is usually characteristic at the family level. Ask your students to find the following basic types of bivalve hinges.

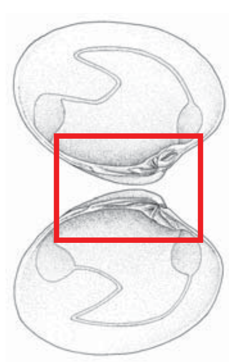
Taxodont – made up of many, small, similarly-shaped teeth. This is the most primitive kind of bivalve hinge. It keeps the valves closely aligned, but does not allow the valves to open very wide, limiting the bivalve's movement.

Example: Ark Shells



Heterodont – made up of two kinds of teeth: more central **cardinal teeth** (radiating from the umbo, or beak, of the shell) and more remote **lateral teeth**. Lateral teeth can be absent in some species. This is a more derived kind of bivalve hinge. It allows the valves to open more widely, and was essential in the evolution of siphons, swimming, and other traits that require a wider gap.

Examples: Hard-shelled Clam
Lucine Clam
Crassatella Clam
Kitten's Paw
Freshwater Mussel



Edentate – lacking teeth. Bivalves that lack teeth usually have other means of aligning their shells, such as a ligament or muscles. The Soft-shelled Clam (below) has a spoon-shaped process at the hinge line; this is a **resilifer**, a support for an internal ligament.

Examples: Blue Mussel
Soft-shelled Clam
Zebra Mussel

Suggested Classroom Activities

cardinal teeth • *Hinge teeth located directly below the umbo in a heterodont hinge..*

edentate • *Lacking hinge teeth.*

heterodont • *Having more than one kind of hinge teeth.*

lateral teeth • *Hinge teeth located far away from the umbo in a heterodont hinge.*

resilifer • *Hinge structure supporting an internal ligament.*

taxodont • *Having many, small, similarly-shaped hinge teeth.*

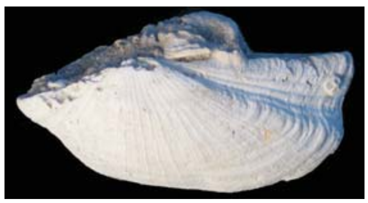
Suggested Classroom Activities

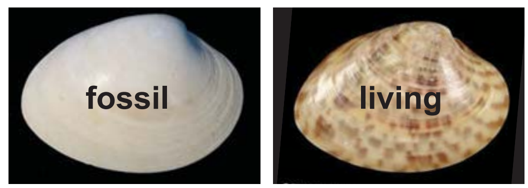
(6) Types of Fossils

The processes of fossilization occur over a very long time. Fossils of various ages show different stages of these processes.

The shells of living bivalves are composed of calcium carbonate in either of two forms: calcite or aragonite. Holding the crystals of $CaCO_3$ together is organic material (kind of like "mortar" to the crystal "bricks") that also gives the shell its color.

Young fossils (those below are from the Pliocene Epoch, approx. 5 million years old) look a lot like those of living species, only "chalkier." They are often full of sand. Their shells are still composed of calcium carbonate, but the organic material in the shell has degraded slightly. Former color patterns sometimes show under "black light" (ultraviolet light) – try it!





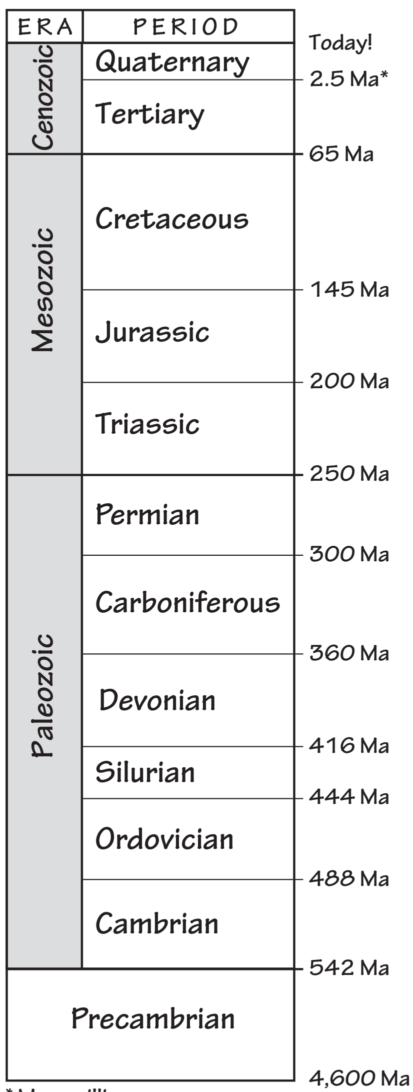
Older fossils either have some shell still attached to a rock-like interior or themselves look more or less like rock (example: Fossil Oyster, Cretaceous Period, approx. 100 million years old).

All traces of the shell have completely disappeared on the oldest fossils. This might be a mold of the inside surface of the original bivalve (like the Devonian bivalve shown here, ca. 400 million years old) or of the outside surface of the original bivalve. Sometimes we have two pieces of rock that have split to reveal the mold of a bivalve on both sides – one of these is called "part" and the other is called "counterpart."

The processes of fossilization include a long period of alteration, involving (in chronological order) recrystallization of the shell minerals, "permineralization" (during which other minerals seep into and fill voids within the shell), or complete replacement of the shell by minerals. Think of the difference between a newly cut tree trunk and a slice of "petrified wood"; the latter has had all of the wood replaced by minerals, turning it to stone!

Black light bulbs are readily available at hardware and novelty stores (especially around Halloween!). Darken your classroom or use a large box to create a "mini darkroom." Observe a young fossil under natural light and under black light. Those with smoother surfaces are more likely to show color patterns. Why wouldn't color patterns be preserved in older fossils?

Discuss with your students what characters are typically needed to identify a bivalve (such as hinge structure, pallial sinus, exterior sculpture, periostracum). Are these available or unavailable in these different kinds of fossils? In other words, how are the fossil species similar to, or different from, the modern ones? What might that mean to a paleontologist?



* Ma = million years ago

Suggested Classroom Activities

Suggested Classroom Activities

convergence • *Evolutionary change in two or more unrelated organisms that results in the independent acquisition of similar traits.*

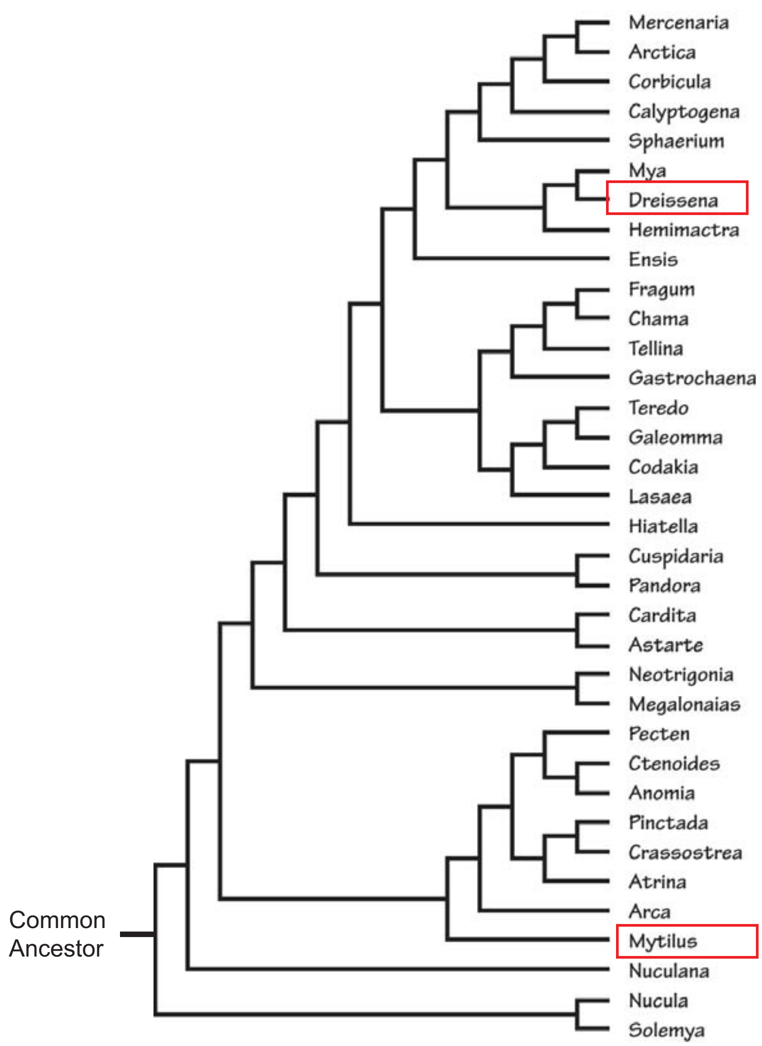
mytiliform • *Mussel-shaped.*

phenotype (adj. phenotypic) • *The observable physical or biochemical characteristics of an organism, as determined by both genetic makeup and environmental influences; what an organism "looks like."*

(7) Convergence

When is a "Mussel" not a Mussel? – The common name "Mussel" is used for many kinds of bivalves, most of them wider than tall, slender, and asymmetrical, with the umbo (the oldest part of the shell) at one end – the Blue Mussel and Zebra Mussel are examples. This is **convergence** – the evolutionary process that results in two distantly related forms looking very similar.

Here is a recent Bivalve Tree of Life that shows the positions of the Blue Mussel (*Mytilus*) and the Zebra Mussel (*Dreissena*). Notice that they are far apart on the tree; that means that they are not closely related. That in turn tells us that "mussel shape" (also called **mytiliform**) has evolved more than once during bivalve evolution. Why?



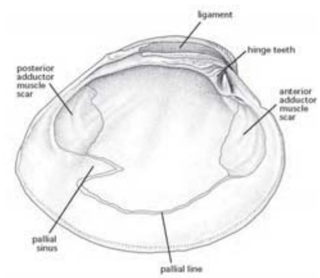
The most common explanation is that the mussel shape is conducive to a crowded population of bivalves that need to attach to a hard substratum, like the surface of a rock. If one end of each shell is pointed, the pointed ends can fit into tiny crevices, allowing more of the bivalves to fit in a small space. This is an ecological (rather than genetic) explanation for a common **phenotype** (outward appearance).

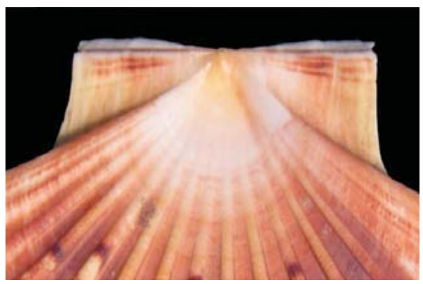
Ask your students to think of other examples of convergent evolution, such as birds and bats, or sharks and dolphins, or dolphins and the now-extinct swimming reptiles called ichthyosaurs.

(8) Reading Information from Empty Shells

The features of bivalve shells can reflect much about the lifestyle of the living bivalve that made the shell. This is especially important to paleontologists (who never see the living animals) but also for biologists, who often have only empty shells available. Ask your students to identify any of the following from the shells available in your classroom, and at the same time, ask them to think about adaptation and the relationship between function and structure.

Deep burrower – Bivalves that burrow deep in the sediment must have siphons to maintain contact with the water. Those siphons are controlled by muscles that leave scars on the shells in the form of an embayment called the **pallial sinus** (see below left). The deeper the sinus, the longer the siphons (see Hard-shelled Clam, Soft-shelled Clam). What must we conclude about a bivalve shell without a pallial sinus?





Jet propulsion – The "ears" or **auricles** of scallops (see above center) have tiny gaps through which jets of water are expelled when the valves close suddenly. This results in a kind of jet propulsion that creates the scallop's swimming movements as it claps its valves together.

Byssal attachment – Some bivalves that produce byssal threads for attachment on a hard surface have a gap in the edge of the shell even when the valves are closed (see Arks). This indicates a relatively large byssus that stays attached even when the bivalve must close tightly to avoid a predator.

Cementation – Cementing species (see Oysters) show clearly the place on one valve that has been cemented to a rock. This sometimes assumes the shape of the surface to which it is attached. For example, an oyster on the outside surface of a scallop might show the wavy surface of the scallop shell.

Predation scars – Bivalves and other mollusks have the ability to repair breaks in their shells, if the damage is not sufficient to cause death of the bivalve. Crabs might chip at the edges of the shell, but fail to break into the shell to consume it. Such a minor break can be repaired, but leaves a scar on the shell. Moon Snails (family Naticidae), Murex Snails (family Muricidae), and Octopuses (Phylum Mollusca, Class Cephalopoda) all bore holes in other mollusk shells using their radular teeth, sometimes with the help of chemicals to soften the shells. These holes have beveled edges in the case of the snail predators, and straight-sided in the case of the Octopus. Such a hole, if complete (above right), is a clear indication of the cause of death of that particular bivalve.

Epifauna – Not all features of an empty shell reveal something about the living bivalve. For example, you might think that barnacles growing on the outside of a bivalve shell means that the bivalve lived epifaunally, that is, not buried below the sediment. This is not the case, because the barnacles might have attached to the shell after its death, when the empty shells were scattered on the sea floor.

Suggested Classroom Activities

auricles • *Ear-shaped structures on the shells of scallops, one of their defining characteristics.*

epifaunal • *Living on top of the sediment, i.e., unburied; also called epibenthic.*

pallial sinus • *An embayment in the posterior part of the pallial line that indicates the attachment of siphonal retractor muscles and demarcates that part of the mantle cavity into which the siphons can retract in bivalves.*

Suggested Classroom Activities

binomial nomenclature • *The two-part scientific name – genus and species – for a plant or animal.*

(9) Finding Nature's Order: Classification

Developed by Richard Kissel, PRI's Director of Teacher Programs

Objectives: This lesson is designed for students to learn to recognize and understand the relationships between living organisms. Students will determine that related organisms have a common ancestor and/or common traits.

Age Level: Grades 6-12

Materials:

- Shells, fossils, or other items that are similar to each other, but not identical.
- Paper
- Markers/pencils
- Rulers

Time: Allow for 10-20 minutes for an introduction to the topic including new vocabulary (see below), and 30 minutes to complete the project.

Background:

Scientists have identified 1.8 million species living today. Some researchers estimate that there might be as many as 100 million! However, those species alive today are only a very small percentage of the perhaps billions of species that have lived on this Earth since life first evolved approximately four billion years ago.

Over 75% of the described living species belong to the Phylum Arthropoda, which includes such diverse organisms as lobsters, barnacles, spiders, and insects. The insects are by far the most abundant arthropods. Mollusks are the most species rich group in the sea, and bivalves comprise the second largest class of mollusks. How are the many species of insects or bivalves arranged, categorized, and classified? How does the classification scheme reflect phylogenetic (evolutionary) relationships? It can be a bewildering yet extremely interesting problem. The following activity is intended to demonstrate the importance of classification through the examination of objects and the identification of common characteristics that organisms do or do not share and the way in which subgroups are created.

Procedure:

Part I – Discussion

1. Introduce new vocabulary to your class and provide definitions (e.g., classification [Kingdom Phylum Class Order Family Genus Species], phylogeny, taxonomy), as appropriate to the grade level.

2. Facilitate class discussion on the importance and relevance of classification and determination of the relationships among living organisms. *Optional Extension*: Introduction of Carolus Linnaeus as inventor of modern classification (1758, *Systema Naturae*). Linnaeus developed **binomial nomenclature** to identify organisms by a generic (genus) and specific (species) title.

Part II – The Project

1. Divide students into groups of 3 or 4. Distribute approximately 25-30 shells (or other objects) to each group. (If you only have a few examples, conduct the project as a full-class activity.)

2. Provide rulers to students and ask them to think about ways in which objects are similar or different (size, shape, function, appearance etc.).

3. Ask students to separate the objects based on their physical characteristics according to what they think is most important (size, shape, function, appearance, etc.).

4. Move between groups, asking thought-provoking questions about why students chose to separate objects in the manner that they did. Did they separate the shells by size, and can they be further divided by shape? If they were separated first by appearance, can they be further subdivided by color?

5. After each group has subdivided their objects into groups, ask them to write a brief description of their classification scheme by: (a) describing the rules they followed to create their relationships; and (b) describing why they chose their classification scheme. Have a representative from each group make an oral presentation to the entire class following the activity.

Helpful Analogy for Struggling Students:
As an example, think of your Halloween candy. On Halloween night, after returning from trick or treating, what do you and your friends do? Many kids pour their candy out on the floor or bed, and separate the candy into piles based on how the candy items are the same, and how they are different. The chocolate goes into one pile, the Smarties™ into another, the Sugar Daddy™ into yet another pile, until all the candies are in the appropriate places. Biologists do the same thing with life forms (see also http://www.kidsbiology.com/biology_basics/classification/classification1.php).

See also Activity (4) **Taxonomy**.

Based on: Turner, T., & W. DiMarco. 1998. *Learning to Teach Science in the Secondary School*. Routledge, 325 pp., ISBN 978-0-41515-302-7.

Project adapted from *The "Nuts and Bolts" of Taxonomy and Classification* (http://jrscience.wcp.muohio.edu/lab/TaxonomyLab.html).

Suggested Classroom Activities

Dissection Guides

This section provides dissection guides for the common bivalves that you might have in your classroom, either from collecting or the fish market. If you have a different type of bivalve available, one of these guides could still be useful in your investigations.

The dissection guides are available courtesy of Professor Richard Fox, Lander University (http://webs.lander.edu/rsfox/invertebrates).

- Hard-shelled Clam, *Mercenaria mercenaria*
- Asian Clam, *Corbicula fluminea*
- Freshwater Pearl Mussel, *Actinonaias ligamentina*
- Blue Mussel, *Mytilus edulis*
- Eastern Oyster, *Crassostrea virginica*

Lander University

Invertebrate Anatomy OnLine

Mercenaria mercenaria

Quahog

with notes on *Tapes japonicus*©

24may2007

Richard Fox
Lander University

Preface

This is one of many exercises available from ***Invertebrate Anatomy OnLine***, an Internet laboratory manual for courses in Invertebrate Zoology. Additional exercises, a glossary, and chapters on supplies and laboratory techniques are also available at this site. Terminology and phylogeny used in these exercises correspond to usage in the Invertebrate Zoology textbook by Ruppert, Fox, and Barnes (2004). Hyphenated figure callouts refer to figures in the textbook. Callouts that are not hyphenated refer to figures embedded in the exercise. The glossary includes terms from this textbook as well as the laboratory exercises.

Systematics

Mollusca ^{P}, Eumollusca, Conchifera, Ganglioneura, Ancyropoda, Bivalvia ^{C}, Metabranchia ^{sC}, Eulamellibranchia ^{SO}, Veneroida ^{O}, Veneroidea ^{SF}, Corbiculidae ^{F} (Fig 12-125, 12-122)

Mollusca ^{P}

Mollusca, the second largest metazoan taxon, consists of Aplacophora, Polyplacophora, Monoplacophora, Gastropoda, Cephalopoda, Bivalvia, and Scaphopoda. The typical mollusc has a calcareous shell, muscular foot, head with mouth and sense organs, and a visceral mass containing most of the gut, the heart, gonads, and kidney. Dorsally the body wall is the mantle and a fold of this body wall forms and encloses that all important molluscan chamber, the mantle cavity. The mantle cavity is filled with water or air and in it are located the gill(s), anus, nephridiopore(s) and gonopore(s). The coelom is reduced to small spaces including the pericardial cavity containing the heart and the gonocoel containing the gonad.

The well-developed hemal system consists of the heart and vessels leading to a spacious hemocoel in which most of the viscera are located. The kidneys are large metanephridia. The central nervous system is cephalized and tetraneurous. There is a tendency to concentrate ganglia in the circumenteric nerve ring from which arise four major longitudinal nerve cords.

Molluscs may be either gonochoric or hermaphroditic. Spiral cleavage produces a veliger larva in many taxa unless it is suppressed in favor of direct development or another larva. Molluscs arose in the sea and most remain there but molluscs have also colonized freshwater and terrestrial habitats.

Eumollusca

Eumollusca, the sister taxon of Aplacophora, includes all molluscs other than aplacophorans. The eumolluscan gut has digestive ceca which are lacking in aplacophorans, the gut is coiled, and a complex radular musculature is present.

Conchifera

Conchifera, the sister taxon of Polyplacophora, includes all Recent molluscs other than aplacophorans and chitons. The conchiferan shell consists of an outer proteinaceous periostracum underlain by calcareous layers and is a single piece (although in some it may appear to be divided into two valves). The mantle margins are divided into three folds.

Ganglioneura

Most Recent molluscs are ganglioneurans, only the small taxa Aplacophora, Polyplacophora, and Monoplacophora are excluded. Neuron cell bodies are localized in ganglia.

Ancyropoda

The mantle cavity, with its gills, is lateral. The calcareous portion of the shell is bivalve, with the valves opening laterally and joined dorsally by a derivative of the periostracum.

Bivalvia C

Bivalvia is a large, successful, and derived taxon. The body is laterally compressed and enclosed in a bivalve shell. The two valves are hinged dorsally. The the foot is large and adapted for digging in the ancestral condition. A crystalline style is usually present but never is there a radula. The mantle cavity is lateral and in most bivalves the gills are large and function in respiration and filter-feeding. The head is reduced and bears no special sense organs. The nervous system is not cephalized. The group includes scallops, clams, shipworms, coquinas, marine and freshwater mussels, oysters, cockles, zebra mussels, and many, many more.

Metabranchia sC

Most bivalves are metabranchs. The gills are adapted for filter feeding and water enters the mantle cavity posteriorly.

Eulamellibranchia SO

Eulamellibranchs have gills with tissue interfilamentar connections.

Veneroida O

Shell is usually equivalve and without a nacreous layer.

Laboratory Specimens

The clam *Mercenaria mercenaria* (= *Venus mercenaria*), variously known as the northern quahog (pronounced CO hawg), hardshell, littleneck, cherrystone, or chowder clam, is a common and commercially important species on the east coast of North America where it lives in soft sediments in shallow water (Fig 12-89, 12-92, 12-110F). This species is farmed commercially and is often available alive at reasonable cost in inland supermarkets and seafood markets. When thus available it offers a practical opportunity for study of the anatomy of a living bivalve, complete with beating cilia.

Preserved or living specimens of *Mercenaria* can be used for this study. The exercise is written for living specimens and these are recommended if available. Parenthetical comments refer to a similar species, the Japanese steamer clam, *Tapes japonicus*, which has been introduced to the west coast of North America and is available in seafood markets on the west coast.

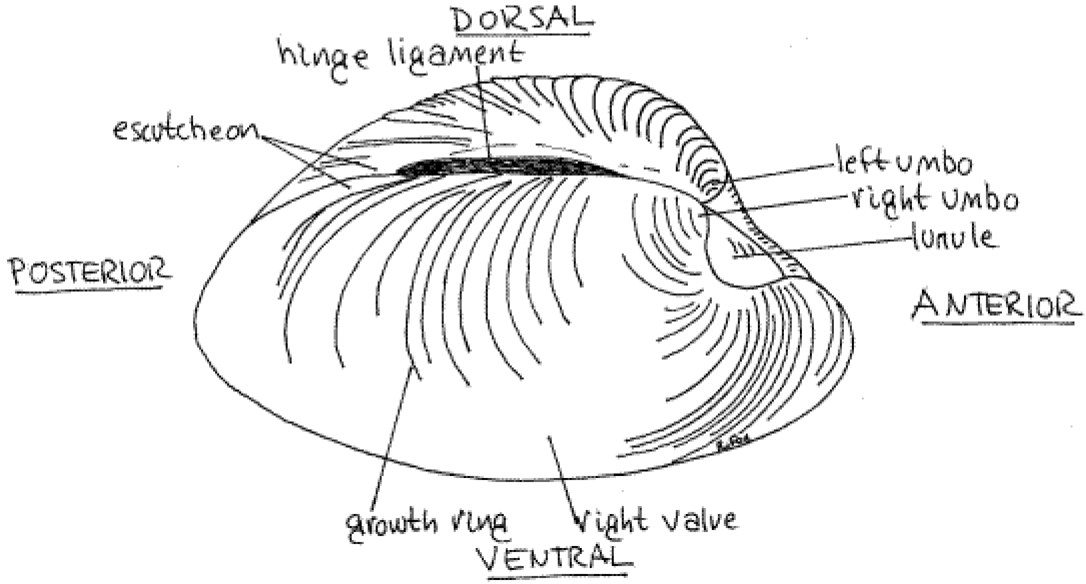
The dissection should be conducted in isotonic magnesium chloride (if living) or tapwater (if preserved. Use the dissecting microscope as needed.

External Anatomy

Shell

Study a complete, dried, empty shell. The thick chalky **shell** of *Mercenaria* consists of two similar **valves**, which fit tightly together to enclose and protect the soft parts of the animal (Fig 1, 12-92B). (The shell of *Tapes* is neither thick nor chalky). The exterior of each valve is rough and ornamented with irregularly spaced, raised, concentric **growth ridges**. (*Tapes* has, in addition, distinct **radial ridges**.)

Figure 1. Oblique view of the right side of an intact shell of *Mercenaria mercenaria*. Bivalve63L.gif



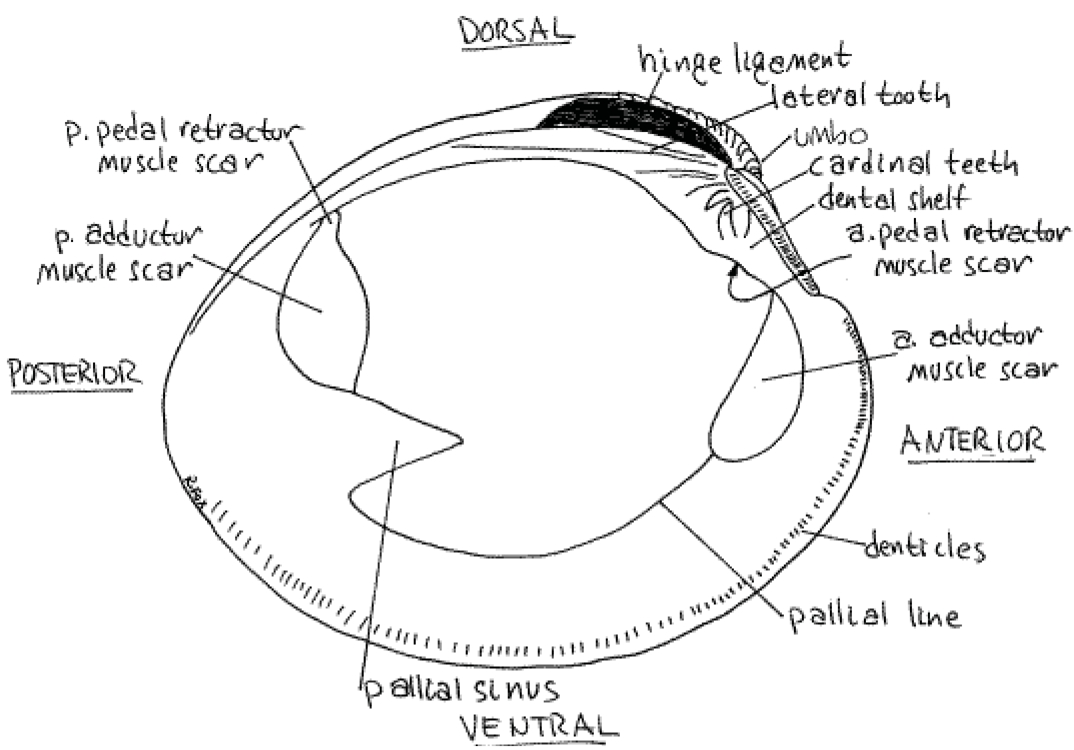
The shell is bilaterally symmetrical and the plane of symmetry passes through the hinge to divide the shell into right and left **valves**. The valves are nearly identical in size and shape, a condition referred to as **equivalve**. Some bivalves, oysters for example, have right and left valves of different size and shape and are inequivalve.

Each valve is strongly convex outwards and bears a conspicuous raised protuberance, the **umbo** (= beak), on its dorsal surface near the hinge (Fig 1, 2). The umbo is the oldest part of the valve and the concentric growth ridges are centered around it. When the valves are closed, the umbos almost touch each other on the midline. The umbos are dorsal and slightly anterior to the middle of the hinge.

The two valves are joined dorsally at the flexible (in life) **hinge** and are free to separate from each other along the ventral margin. The dark brown, proteinaceous **hinge ligament** occupies part of the hinge (Fig 1, 2, 12-92B). The hinge ligament is a good dorsal landmark. The small (in life) gap between the ventral margins of the opened valves is the **gape**. The gape of dead specimens is far wider than in life.

Use the hinge and umbos as landmarks to recognize **dorsal** and **anterior**, respectively, and, having accomplished that, decide which valve is left and which is right. Find **posterior** and **ventral** and identify the plane **of symmetry**.

Figure 2. Interior of the left valve of the shell of *Mercenaria mercenaria*. Bivalve64La.gif



Place the two valves together and look at the dorsal surface. The most obvious features are the umbos. Anterior to the umbos is a conspicuous, heart-shaped depression known as the **lunule** (Fig 1). It is a characteristic feature of Veneridae. Posterior to the umbos is a broad, flat, indistinctly defined platform, the **escutcheon**. (The escutcheon is small and obscure in *Tapes*.) The free edge of the posterior right side of the escutcheon overlaps the free edge of the left so that, even when the clam is open, this part of the gape is protected, perhaps from falling sand grains. (The overlap in *Tapes* is very slight.)

Viewed from the side, each valve is lopsided, with a longer bluntly pointed posterior end and a shorter, smoothly rounded anterior end. (The posterior end of *Tapes* is not pointed.) Valves with asymmetrical anterior and posterior halves are said to be **inequilateral**. *Mercenaria* and *Tapes* are only moderately inequilateral, mussels are strongly inequilateral.

Look at the medial side of one of the two valves (Fig 2, 12-92B). Dorsally, the margin is occupied by the hinge, which is the articulation between the two valves. The hinge ligament is a conspicuous feature of the hinge. The ligament is composed of protein (conchiolin) and may or may not be present on dry empty shells. Look at a fresh, undissected specimen and find the ligament if your empty shell does not have one. The ligament of living animals is elastic but that of dry valves is hard and brittle.

The hinge is equipped with strong **teeth** to assure the proper alignment between the valves each time the shell closes. The teeth are located on a platform called the **dental shelf** (Fig 2). Most conspicuous of these are the short, vertical, or oblique **cardinal teeth** located directly ventral to the tip of the umbo. Each valve has three cardinal teeth but one tooth on the left is obscure making it appear as if there are only two teeth on this side. (In *Tapes* all three teeth are distinct.) A **posterior lateral tooth** is located posterior to the cardinal teeth. It is a long, low, rough, irregular ridge with its long axis oriented anterior to posterior. Unlike the cardinal teeth it does not look like a tooth.

The anterior and ventral margins of the valves bear fine teeth known as **denticles** (Fig 2). (*Tapes* lacks denticles.) The denticles probably prevent shear (sideways slipping) when the valves are pulled tightly together by the powerful adductor muscles. Hold the two valves together as they would be in a living clam with its valves closed. If you have the cardinal teeth properly aligned, the valves will fit snugly together and cannot be made to slip past each other.

The large, smooth **anterior** and **posterior adductor muscle scars** are easily seen at their respective ends of the inside of each valve (Fig 2, 12-92B). In intact clams the two adductor muscles run transversely from one valve to the other and their action is to pull the valves together (adduct) and hold them closed. Two additional muscles, the anterior and posterior pedal retractor muscles, insert on the shell near the adductor muscles. The **anterior pedal retractor scar** is separated by a small space from the anterior adductor scar and is under the anterior end of the dental shelf. (The anterior pedal retractor muscle scar is easy to see in *Tapes* and is located just anterior to the dental shelf.) The **posterior pedal retractor scar** is continuous with the posterior adductor scar (Fig 2).

The **pallial line** runs from one adductor to the other and parallels the ventral margin of the valve. It is the site of insertion of pallial muscles in the mantle and is indented sharply beside the posterior muscle scar. This V-shaped indentation is the **pallial sinus** and is a recess in the line of pallial muscles for the withdrawn siphons. ("Pallial" is another word for mantle.)

The inside of each valve of *M. mercenaria* is white with posterior purple markings. The shells of this species were cut into beads and used by east coast North American Indians as money, called wampum. This is the basis of the scientific name "mercenaria" (mercenari = hired for wages).

The typical mollusc shell consists of three layers (Fig 12-91). The outermost is the periostracum composed of the protein conchiolin. This layer is thin, eroded, and insignificant in *Mercenaria*. The dull, chalky **prismatic layer** (ostracum) is the middle layer. It is exposed on the outside of the valve due to the absence of the periostracum. The innermost layer, and the one in contact with the clam, is the calcareous **lamellar layer** (hypostracum) which is smooth and glossy. (The thin, brown periostracum of *Tapes* remains intact and the prismatic layer is not evident.)

Soft Anatomy

"

Open the shell of your clam to study the soft anatomy within. The adductor muscles keep the shell closed and it cannot be opened until they are severed. If you are dissecting a preserved specimen, the valves may already be pegged open with a wooden wedge. If this is the case, you may skip the remainder of this paragraph and proceed to the instructions for cutting the adductor muscles.

If you have a living specimen or an unpegged preserved specimen, you must make anterior and posterior openings in the shell to give you access to the adductor muscles so you can cut them. This is not easy to do but the best procedure is to use a pair of pliers to pinch away the anterior and posterior edges of the shell. Do not strike the shell as it cracks easily. First refer to the empty valves to help determine the positions of the two adductor muscles and begin removing the shell closest to them.

Gain access to the anterior adductor first by pinching away the anterior edges of the contiguous valves. You will not be able to get as close to the posterior adductor muscle as to the anterior because the posterior shell does not provide a raised edge to pinch. Remove enough shell to create a narrow opening at each end.

Insert a scalpel blade into the anterior opening and feel for the adductor muscle. Use the longest and sharpest blade you have but be very careful that you do not cut anything until you find the muscle. The muscle is firm, rubbery, and unyielding whereas the other tissues are soft and do not resist gentle probing. The muscle is easy to recognize by touch. Once you have found the muscle, sever it completely with the scalpel. Do the same with the posterior adductor muscle. The pedal retractor muscles should be cut also.

Arrange the clam so the left valve is up, facing you, and you are looking at the left side of the animal. With all the muscles severed, carefully lift the left valve a little so you can see into the gape. The body will be cradled in the concavity of the right valve but a thin sheet of tissue, the left mantle skirt, adheres closely to the inner surface of the left valve. Slip the *blunt* end of your scalpel handle between the left mantle skirt and the left valve and use it to free the soft tissue from the shell. Carefully work the scalpel handle around the stumps of the adductor muscles being careful that you do not damage the mantle. Note the two pedal retractor muscles that insert on the shell beside the much larger adductor muscles. Cut them now if you did not do so earlier. When the mantle is free, detach the left valve at the hinge and set it aside.

Place the clam in a culture dish or dissecting pan with the right valve down. Cover the clam with isotonic magnesium chloride (if living) or tapwater (if preserved).

>1a. Look at the inner surface of the detached left valve for a difference in the appearance of the lamellar layer proximal and distal to the pallial line. The surface of the lamellar layer inside the pallial line, is alternately eroded and then redeposited as part of the normal metabolic operations of the animal. When the valves are closed and the clam is forced to respire anaerobically, calcium is removed from this region to neutralize the succinic acid produced by this metabolism and calcium succinate is produced. When the valves open and the clam switches to aerobic respiration the calcium is redeposited. <

Muscles

Look at the left side of the clam and find the **anterior and posterior adductor muscles** (Fig 3). The muscle fibers run transversely, across the clam, from valve to valve and their action is to adduct, or close, the valves.

Each muscle is composed of two distinct regions (Fig 3). The anterior part of the anterior adductor muscle and the posterior part of the posterior adductor muscle are **catch muscles** composed of smooth muscle fibers with abundant connective tissue. They are capable of sustained, slow contraction and in life are white. The remainder of each muscle is the **fast muscle** composed of obliquely striated fibers with abundant supplies of myoglobin and mitochondria but with relatively little connective tissue. In life fast muscle is red due to its myoglobin. Fast muscle contracts and relaxes rapidly but fatigues easily and is incapable of sustained contraction. The adductor muscles are opposed by the elastic recoil of the hinge ligament.

The pedal retractor muscles are paired. Find one of the small **anterior pedal retractor muscles** (Fig 3). It is a short cord of muscle and connective tissue running from the anterior end of the foot to the shell. The **posterior pedal retractor muscles** are similar cords extending from the posterior end of the foot to the shell. The retractors pull the foot back into the shell as is necessary before closing the gape, or, when digging, to pull the clam toward an expanded foot anchored in the sand. *Mercenaria* lack a pedal protractor muscle.

Mercenaria exemplifies the **dimyarian** condition in having two similar adductor muscles. Many bivalves, notably scallops and oysters, are monomyarian and have only one adductor muscle (the posterior). Others, such as mussels and pens, are heteromyarian and have a large posterior muscle and a reduced anterior muscle.

Bivalves do not have abductor muscles to open the valves. The adductor muscles extend across the animal from one valve to the other. When they contract they pull the valves together, close the gape, and stretch the elastic hinge ligament. When the muscles relax, the ligament returns to its original resting shape and in doing so pulls the dorsal ends of the valves toward the midline (toward each other). This, of course, means that the ventral margins of the valves move away from each other, thereby opening the shell and creating a narrow gape through which the foot can be extended.

The ligament of *Mercenaria* is outside the hinge. Some bivalves also have an internal ligament (= resilium) on the inside of the hinge that is compressed, rather than stretched, when the valves close. When the muscles relax, the elastic resilium *pushes* the valves apart, and this also opens the gape. *Mercenaria* does not have a resilium.

Mantle Skirts

The bivalve body is enclosed on the right and left by two large, thin double layers of body wall (Fig 5). These are the **right and left mantle skirts**. Each is a thin sheet of tissue, free at the ventral edge but connected with the body dorsally. The pallial muscles attach the mantle skirt to the shell along the pallial line.

Mantle Cavity

Between the right and left mantle skirts lies a large space, the mantle cavity, in which lies the body (Fig 3, 5).

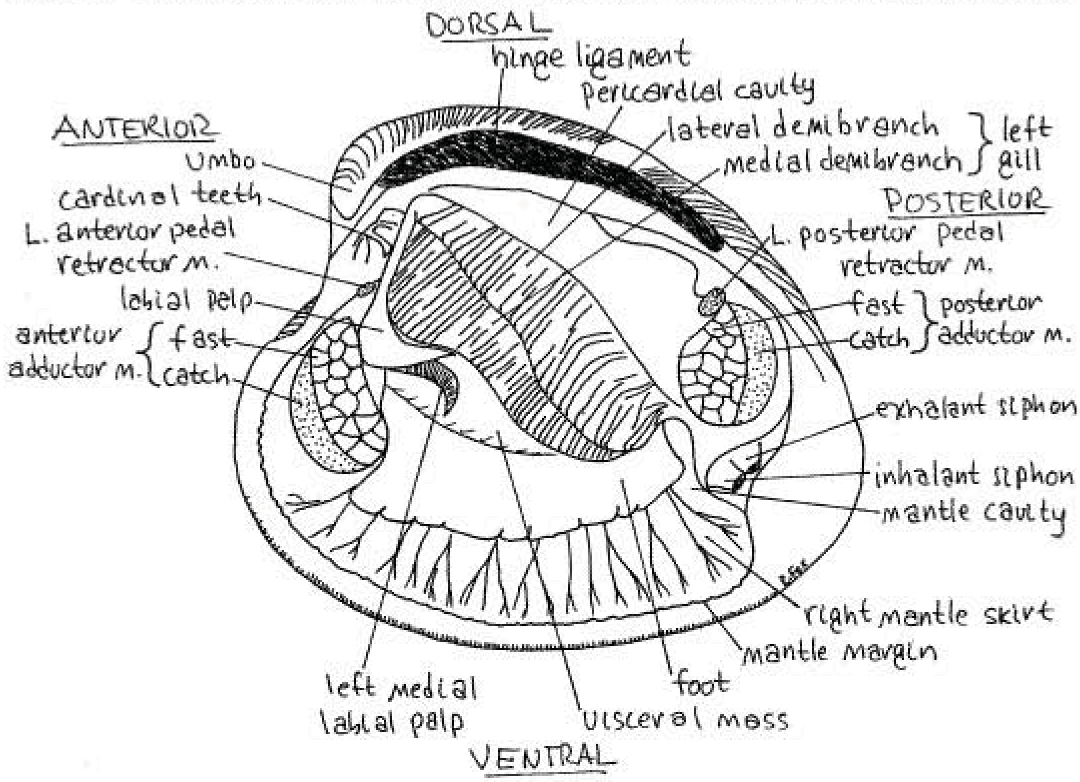
The mantle cavity is continuous with the sea and is filled with seawater. Without cutting tissue, lift the left mantle skirt and find the **mantle cavity**. It is divided into a large ventral region below and beside the body, known as the **inhalant chamber** (= branchial chamber), and a smaller dorsal region above the body, the exhalant chamber (= suprabranchial chamber) (Fig 5, 12-90). The two chambers are separated from each other by the gills. The space you see now is the spacious inhalant chamber and you cannot yet see the much smaller exhalant chamber.

Look into the mantle cavity (inhalant chamber) and make a quick identification of the most conspicuous features of the body so you can use them for landmarks later. With the left mantle skirt held out of the way, closest to you is the long, leaflike **left gill** (Fig 3, 5). Medial to the gill is the large bulging **visceral mass** with the thinner, muscular, bladelike (in life) **foot** attached to its ventral midline. Lift the foot and visceral mass to see the right **mantle cavity**, right gill, right **mantle skirt**, and right **valve**, in that order.

Mantle Folds

Study the ventral margin of the right mantle skirt with magnification. The mantle margin of *Mercenaria* and other venereid clams is composed of four folds although most bivalves have only three (Fig 4, 12-91). The homologies between the four venerid folds and the three folds of other bivalves are unclear but the extra fold probably arose through subdivision of the inner fold.

Figure 3. The left side of *Mercenaria* with the left valve and left mantle skirt removed. Bivalve65La.gif



The **outer fold**, which is adjacent to the shell, secretes the prismatic layer and periostracum (Fig 4, 12-91). The lateral side of the outer fold secretes the prismatic layer of the shell whereas the medial side secretes the periostracum. The lamellar layer is secreted by the entire lateral surface of the mantle skirt.

The **inner fold** is muscular and in *Mercenaria* is double. The wide border of the mantle, from its free edge to the pallial line, is thicker than the rest of the mantle due to the presence of the pallial muscles that insert on the shell along the pallial line. These muscles can be seen radiating across the mantle border peripheral to the pallial line. Tug gently on the free edge of the right mantle and observe that (in life) it is firmly attached to the shell along the pallial line.

The **middle fold** lies between the inner and outer folds and is sensory. It is weakly developed in *Mercenaria*.

The groove between the outer and middle folds is the **periostracal groove**. In living specimens the freshly secreted **periostracum** can be seen extending out of this groove across the exposed surface of the margin of the valve. It is a very thin, transparent, delicate, glistening membrane.

Siphons

Posteriorly, the right and left mantle skirts coalesce with each other across the midline to form the two short tubular siphons (Fig 3, 12-89). The siphons are darkly pigmented and each has short sensory **tentacles** surrounding its outer aperture. The ventral one is the inhalant siphon and the **exhalant siphon** is dorsal. These short tubes are formed by the fused right and left posterior mantle skirts. (In *Tapes* the siphons are long tubes and are pigmented only at their tips.) The siphons are elaborations of the fused right and left mantle margins. The sensory tentacles are part of the middle mantle fold, which is the sensory fold. In its life position *Mercenaria* is shallowly embedded in sediment with only the tips of the short siphons exposed above the sediment surface. It is appropriate that the greatest concentration of sense organs is on the siphons rather than around the periphery of the gape. What would you predict about the life position of *Tapes*?

The inhalant siphon brings water into the inhalant chamber. Insert your blunt probe into the external opening of the inhalent siphon and show that water entering here would enter the inhalant chamber of the mantle cavity. Water in the inhalant chamber passes through tiny openings, the ostia, in the gills to enter the exhalant chamber, from which it then exits via the exhalent siphon (Fig 12-89B).

A delicate fold of tissue, located dorsal to the inner aperture of the inhalant siphon and known as the siphonal membrane, can be extended from its retracted resting position to partially cover the inner aperture of the inhalent siphon. This deflects water that normally would have gone directly to the gill surfaces, to the ventral inhalant chamber where it stirs and resuspends the accumulated debris and pseudofeces so they can be more easily expelled by the next contraction of the adductor muscles. The siphonal membrane is easily seen on the dorsal surface of the inner aperture of the inhalant siphon.

Dorsal to the siphons the entire right and left mantle margins are fused to each other on the midline beneath the hinge. A special secretory region of the dorsal mantle, ventral to the ligament, is called the subligamental ridge. It secretes the ligament.

Gills

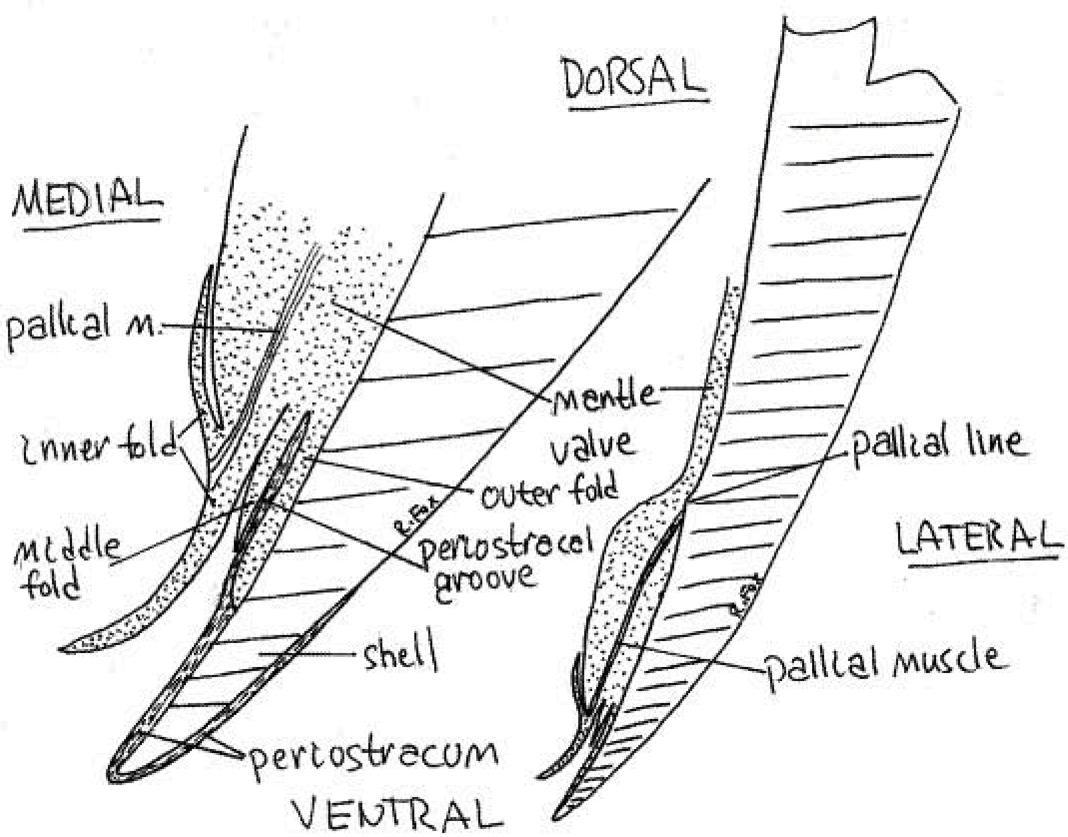
"

With scissors, remove the left mantle skirt and look at the left gill. Each gill consists of a central axis from which are suspended sheets of fused filaments. On each side is a single complete gill, or **holobranch** (Figs 3, 5, 12-96D). A holobranch is composed of two delicate, leaflike **demibranchs**, or half gills. These are the outer **lateral demibranch** and the inner **medial demibranch** (Figs 3, 5, 12-90). The two are joined dorsally along the **central axis** which is attached to the body (Fig 5, 12-90).

Each of the two surfaces of a demibranch is a **lamella** (Fig 5). One surface is the **descending lamella** firmly attached to the central axis and the other is the **ascending lamella** weakly attached to either the mantle or the visceral mass (Fig 5). In *Mercenaria*, but not *Tapes*, the connections of the ascending lamellae to the body are fragile and the margins of the gills are easily torn free of their attachments. At present you are probably looking at the ascending lamella of the lateral demibranch of the left gill.

Figure 4. Cross section through the ventral edge of a valve and the associated mantle skirt of *Mercenaria*.

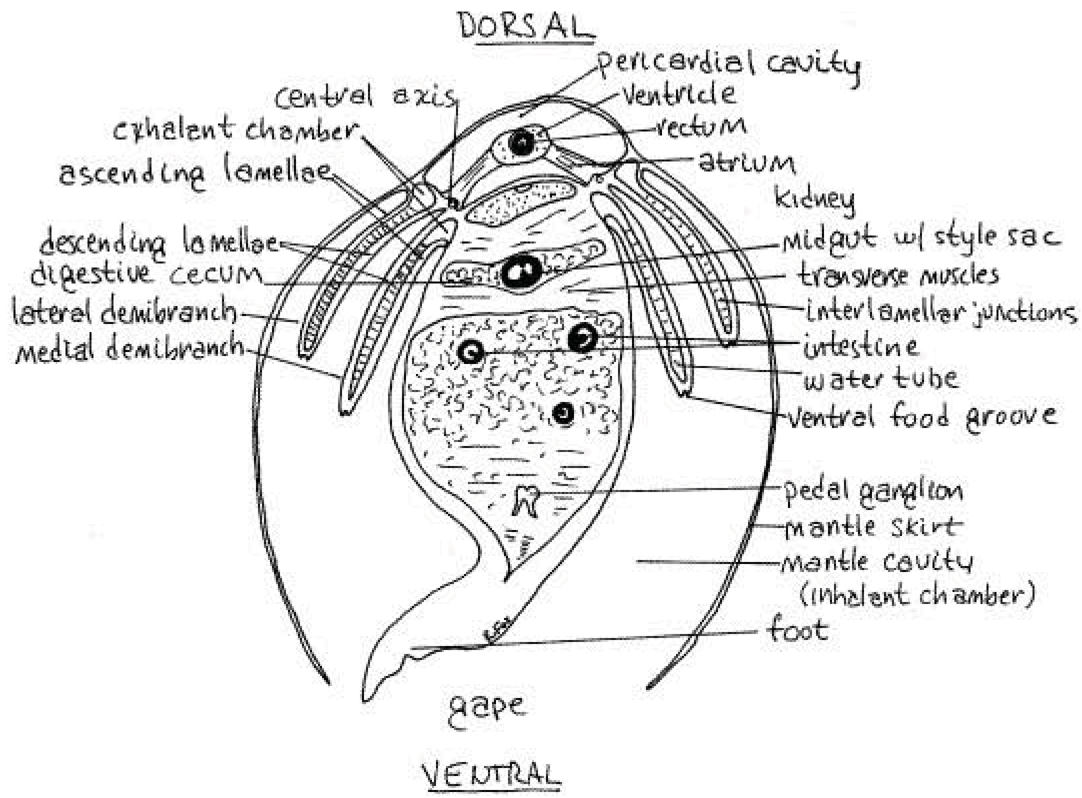
Bivalve66L.gif



The surface of the lamella is conspicuously ridged. Each ridge is a plica, running parallel to the short axis of the gill. Each plica is made up of several gill filaments and contains a water tube (Fig 5, 12-98C,D). The water tubes are part of the exhalant chamber.

At regular intervals a thicker filament is specialized to form the margins of the water tube, separate it from adjacent water tubes, and hold the opposite lamellae together. These special filaments are larger are the 'principle filaments'. The other, more numerous and smaller filaments are known as 'ordinary filaments'. Two successive principle filaments and the ordinary filaments between them form one plica, or ridge. The opposite principle filaments are held together by permanent interlamellar junctions.

Figure 5. Cross section of *Mercenaria* with the shell omitted. Bivalve67.La.gif

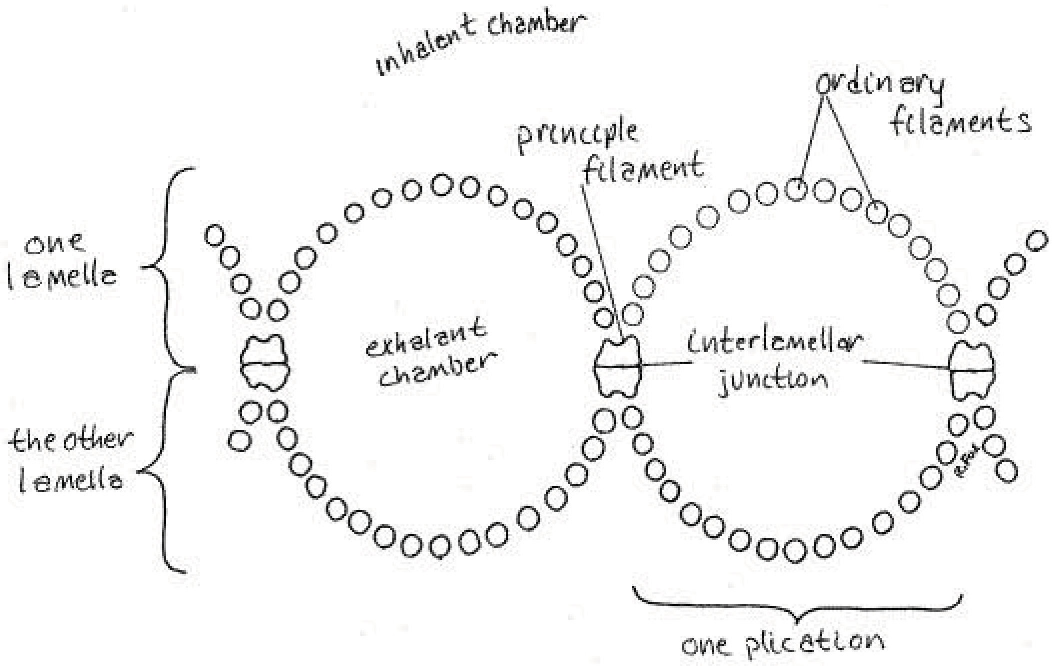


Examine one of the plicae with the highest power of your dissecting microscope and you will see faintly the long slender gill **filaments** that make up its walls. Venus clams such as *Mercenaria* and *Tapes*, have eulamellibranch gills in which adjacent filaments are permanently attached to each other by broad tissue interfilamentar junctions (Fig 12-98). The only openings between filaments are the microscopic ostia. If you are having trouble making out the details of the gill surface because of poor contrast, you may be able to improve the situation by placing a little 1% toluidine blue/seawater on the surface of the gill.

The opposite walls of each water tube are made of opposite ends of the same filaments (Fig 12-98C,D). The medial surface (lamella) of the lateral demibranch is composed of the proximal (descending) end of the filaments and the lateral surface of the distal (ascending) end of the same filaments. The connections from one lamella to the other are interlamellar junctions and they contain blood vessels.

The ciliated longitudinal groove along the ventral edge of each demibranch is a **ventral food groove** used to transport food particles anteriorly to the mouth (Fig 5). The ciliary beat in the food grooves is anterior, toward the head, labial palps, and mouth whereas that of the lamella is either dorsal or ventral.

Figure 6. Frontal section of two plications (vertical channels) of a scallop gill. Bivalve84La.gif



During feeding and respiration a water current bearing food and oxygen enters the inhalant chamber via the inhalant siphon. The water enters the ostia and passes into the water tubes of the exhalant chamber. Food particles are filtered from the current as it passes through the ostia, remain on the inhalant side where they are mixed with mucus and transported to the food grooves by cilia.

>1b. If your clam is living, remove it from its dish and place it, on a cloth towel, on the stage of your dissecting microscope. Do not get seawater or magnesium chloride on the microscope. Arrange the animal so the surface of a lamella is horizontal. Place a drop of carmine/seawater suspension on the lamella. Watch the motion of the particles along the face of the lamella and in the ventral food groove. <

>1c. With scissors, remove a small square (about 5x5 mm) from the lateral demibranch, being sure to include the ventral food groove. Make a wetmount and examine it with the compound microscope. Find the food groove along one margin and use it as a landmark. Adjust the light, focus carefully on the uppermost of the two lamellae, and study the construction of the demibranch. The surface of the lamella is composed of large parallel ridges, the **plicae**, which are themselves formed of smaller ridges, the gill **filaments**. Focus down to bring the filaments of the other lamella into view. Trace a filament to the food groove and note that it reverses direction here and extends up the opposite side of the demibranch. Look in the grooves between the filaments and you may be able to see the small oval ostia.

Labial Palps

The long narrow labial palp on each side of the mouth is an anterior extension of the gill (Figs 3, 7). Each palp consists of a **lateral** and a **medial lamella**. The lateral demibranch of the gill connects with the lateral lamella of the palp and the medial demibranch with the medial lamella. Each lamella bears a sorting **field** of ciliated ridges and grooves on *one* surface (Fig 12-100). The fields are arranged so that when the lamellae lie together, the sorting fields face each other. A ciliated oral groove lies between the tow sorting fields and leads to the mouth.

Mucus and food particles from the gills move along the food grooves to the labial palps where the sorting fields separate desirable organic matter (food) from indigestible mineral particles. Organic particles and mucus move via the ciliated oral groove to the mouth whereas mineral particles, also bound in mucus, move to the tip of the palp from which they are dropped into the inhalant chamber. This mixture of discarded mineral particles and mucus is known as pseudofeces and it is periodically expelled through the *in*halant siphon by sudden contractions of the adductor muscles.

>1d. If you have a living specimen, place carmine/seawater on the sorting field of one of the labial palps to visualize the ciliary currents. Look for particles moving parallel to the ridges and others moving across the top of the ridges but perpendicular to them. Look also for a string of particles and mucus moving along the margin to the distal tip of the lamella and still another moving along the oral groove between the lamellae to the mouth. You may be able to see the food string disappear into the mouth. Repeat with chalk dust collected from the chalk tray of a blackboard. <

Trace the lamellae of the labial palps across the midline and note that each is connected with its counterpart on the opposite side of the body. These two thin folds of tissue are the lips. The lip that connects the two lateral palps is the upper lip and the one that connects the two medial palps is the lower lip (Fig 12-100). The oral groove is between them. Exactly on the midline, between the upper and lower lips, is the mouth. Tilt the animal and find the mouth with the dissecting microscope. This area may have been damaged when the anterior adductor muscle was cut. The mouth is easiest to find if it has a string of carmine red mucus entering it.

Internal Anatomy

Hemal System

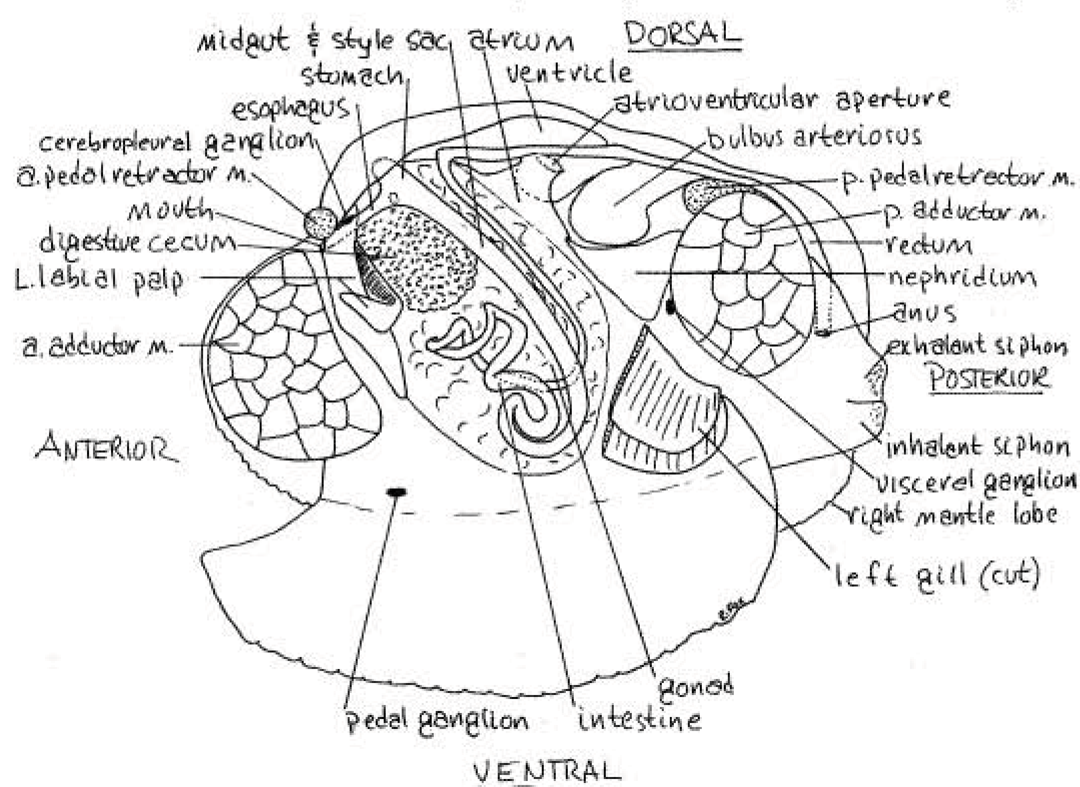
The bivalve hemal system consists of heart, arteries, blood, and an extensive hemocoel. Only the heart will be studied in this exercise.

The heart lies in the **pericardial cavity** located dorsally just below the hinge (Figs 3, 5, 7, 12-89B). It is surrounded by the thin membranous **pericardium**. The body wall in this area is very thin and the pericardium is close to the surface. The pericardial cavity is a remnant of the coelom and the pericardium is peritoneum. The dark reddish brown (pale beige in *Tapes*) **nephridium**, or kidney, can be seen on the walls of the pericardial cavity, especially posteriorly. Paler, greenish brown tissue situated farther anterior is the **digestive cecum**.

!!

With fine scissors, make a *shallow*, longitudinal, middorsal incision in the thin dorsal body wall and pericardium paralleling and extending the length of the hinge ligament. This cut opens the pericardial cavity to reveal the **heart** within. The heart consists of a single large **ventricle** into which the paired right and left atria empty (Fig 7). The ventricle lies on the midline and is a long, soft, ovoid organ. It is penetrated by the posterior intestine, or **rectum**, which runs the length of its lumen (Fig 7). The intestine can be seen faintly through the walls of the ventricle.

Figure 7. Dissection of the left side of *Mercenaria*. The left side of the visceral mass has been dissected. The left mantle skirt and most of the kidney and left gill have been removed. Bivalve68La.gif



With a blunt probe, carefully lift the edge of the ventricle and find the **left atrium** attached to its left side (Fig 7). The atrium is a thin-walled, transparent, truncated triangle extending from the ventrolateral pericardial wall to the ventrolateral wall of the ventricle. The truncated apex of the atrium attaches to the ventricle and its broad base extends along the length of the central axis of the gill. Oxygenated blood from the gills drains into the atria and from there enters the ventricle.

>1e. If your animal is alive, count the heartbeats for three minutes and calculate a pulse rate for your specimen. If your animal is in magnesium chloride it will have to be transferred to seawater before the heart will beat. **<**

Anteriorly, the ventricle narrows to become the wide, transparent anterior aorta which may be visible beside the base of the anterior pedal retractor muscle. The anterior aorta is dorsal to the intestine. The intestine emerges from the visceral mass at the end of the aorta and bends almost 90° to enter the lumen of the ventricle (Fig 7).

Posteriorly, the ventricle constricts to become the posterior aorta which then immediately expands to form the large swollen **bulbus arteriosus**. The size of the bulbus varies depending on the amount of blood it contains. The posterior aorta then narrows and continues posteriorly, ventral to the rectum.

>1f. With fine scissors, open the ventricle with a longitudinal middorsal incision. The rectum, which runs through the lumen of the ventricle, is easily seen. Use fine forceps to pull the cut edge of the ventricle toward you so you can look inside and see the inner surface of the left ventricular wall. Find the atrioventricular aperture between the atrium and ventricle and insert a probe into it to demonstrate continuity between the two chambers. Notice the bundles of muscle fibers in the lumen of the ventricle. **<**

Exhalant Chamber

The ventrolateral floor of the pericardial cavity is the roof of the **exhalant chambers** (Fig 5). Insert the blunt probe into the exhalent aperture and into the exhalant chamber. The transparent roof of the chamber extends anteriorly from the exhalent siphon but may have been cut when you severed the posterior adductor muscle. The visceral ganglion of the nervous system is in the tissue between the roof of the chamber and the adductor muscle. In living clams the ganglion is orange (neuroglobin). Be careful that you do not destroy it.

>1g. Open the exhalant chamber by tearing the ascending lamella of the lateral demibranch away from its attachment to the mantle. Look inside the chamber and find the upper ends of the **water tubes** inside the plicae. Insert a blunt probe into the exhalent siphon to demonstrate its continuity with the exhalant chamber. Above the gills the exhalant chamber is divided into two longitudinal channels, one above each demibranch. The attachment of the central axis of the gill to the dorsal mantle wall separates the two chambers. The **medial exhalant chamber** lies above the medial demibranch and the **lateral exhalant chamber** lies above the lateral demibranch. Look at the cut gills and find **water tubes** and the **interlamellar junctions** that define them. Much of the roof of the exhalant chamber is also the floor of the pericardial cavity. The dark nephridium can be seen in the roof of the chamber at the posterior end of the pericardial cavity. Another dark portion of the nephridium can be seen at the anterior end of the pericardial cavity. **<**

Excretory System

The two **nephridia** , or kidneys, are large metanephridia, one on each side on the floor and walls of the pericardial cavity at the dorsal margin of the visceral mass (Figs 5, 7, 12-89B). They are brown or reddish-brown (in life) and are easily seen without dissection although their structure is not apparent. The nephridia are elaborate ducts extending from the pericardial cavity to nephridiopores in the exhalant chamber. Urine is released from this pore into the exhalant water stream.

>1h. You may want to study the nephridium in more detail (but a freshwater mussel would be a better choice for this purpose). The nephrostome is a small opening from the antero-ventral corner of the pericardial cavity into anterior end of the nephridium. Most of the inner wall of the pericardial cavity is smooth but where it covers the region of the nephrostome it is folded minutely and the nephrostome is hidden in the folds. Sometimes it helps to put a little pressure on the adjacent nephridium to cause fluid to be extruded from the opening. The nephrostome opens into the renopericardial canal that leads into the lumen of the nephridium. The nephrostome is a ciliated funnel and the nephridium lumen is derived from coelomic space. The antero-ventral region of the nephridium, into which the canal opens, is glandular. The lumen of this region is continuous with a thin-walled postero-dorsal region that functions as a reservoir, or bladder. The right and left nephridia are connected across the body by a small duct. Each nephridium extends posteriorly from the nephrostome, mostly embedded in other tissues. The reservoir opens into the exhalant chamber via a small nephridiopore. The nephridiopore is located in a tiny dimple on the roof of the exhalant chamber. This inconspicuous pore is between the medial demibranch and the visceral mass and is at about the level of the posterior end of the ventricle. It is just posterior to the small genital papilla on which the gonoduct opens. **<**

Digestive System

The bivalve gut consists of a mouth, esophagus, stomach, digestive ceca, midgut, rectum, and anus. A complex stomach with style and style sac are present but bivalves have no buccal cavity, radula, radular sac, or odontophore. Most of the gut is within the visceral mass and cannot be seen without dissection. If you plan to study the nervous system, refer to the nervous system section and find the cerebropleural ganglia at this time as they are usually destroyed during the dissection of the anterior gut.

" Remove the left gill and left labial palp so you have unobstructed access to the left side of the visceral mass. Relocate the **mouth** on the anterior midline between the upper and lower lips of the labial palps (Fig 7, 12-89B). The mouth opens directly into the esophagus. The short esophagus passes through the greenish digestive cecum to join the stomach (Fig 7). The anterior end of the stomach, which is otherwise embedded in the visceral mass, lies very close to the surface of the antero-dorsal corner of the visceral mass and can sometimes be seen without cutting.

"

Insert a blunt probe through the mouth into the esophagus to the stomach. The probe will follow the gut lumen and you can see it (the probe) through the gut wall. The **esophagus** extends obliquely dorso-posteriorly between the bases of the two anterior pedal retractor muscles (Fig 7). The esophagus more or less parallels the edge of the dental shelf in the vicinity of the cardinal teeth. The walls of the esophagus bear fine, longitudinal folds, or rugae.

Open the **stomach** by inserting a blade of your fine scissors into the mouth and cut along the esophagus, following the probe to the stomach. Once you have entered the stomach, cut posteriorly through the lateral wall of the visceral mass and digestive cecum, opening the stomach as you go (Fig 7). Continue this cut posteriorly, paralleling the dorsal border of the visceral mass. The incision should follow and open the intestine (= midgut) which extends posteriorly from the stomach. Continue this cut, following the intestine, around the posterior curve of the visceral mass to the foot.

Periodically use a Pasteur pipet or squirt bottle to blow away the accumulating debris that would otherwise obscure your view of the gut lumen. Examine the intact right wall, floor, and roof of the stomach (Fig 12-102). Find the opening of the esophagus on the midline at the anterior end of the stomach. It lies under a fleshy overhang of tissue.

Two openings to the digestive ceca are located on the floor of the stomach just posterior to the opening of the esophagus. A branching network of tubules extends from these openings throughout the ceca (Fig 12-103B).

On the right of the esophageal opening, and extending dorsally to the left side, is a large, ciliated **sorting field** (Fig 12-102). The field is composed of numerous fine ridges and grooves. A thick shelf on the right side of the stomach marks the posterior right border of the sorting field and separates the dorsal and ventral portions of the stomach from each other.

The roof and much of the left wall of the stomach are covered by a thin, chitinous **gastric shield** but it is difficult to see (Fig 12-102). Although the shield itself is nearly invisible, the wall of the stomach under it is smooth and can thereby be distinguished from the surrounding walls.

The **intestine** extends from stomach to anus (Fig 7). Its anterior end is the midgut whereas the rectum is its posterior end. The midgut consists of a descending intestine and ascending intestine. The descending intestine exits the posterior end of the stomach and parallels the dorsal and posterior edges of the visceral mass. It is divided into two parallel tubes by two longitudinal ridges of tissue that approach each other in the middle of the midgut lumen (Fig 5, 12-103A). These two ridges are **typhlosoles**. The typhlosoles are ciliated ridges that transport particulate material.

The major typhlosole is on the lower right side of the midgut and is the larger of the two. It begins in the anterior stomach and its edge is thin and sharp. The minor typhlosole begins at the posterior end of the stomach, lies on the dorsal left wall of the midgut, and has a broad, bilobed edge.

The two side by side tubes of this region of the gut are the dorsal **style sac** and the ventral **intestine**. The style sac of your specimen may or may not contain a **crystalline style**. The style is produced only when needed and if your animal has been maintained without food, it probably will not have a style. The style is a *very* long, narrow, flexible, pellucid, gelatinous rod that occupies the length of the style sac and protrudes from the sac into the stomach lumen. It is composed of hydrolytic enzymes (amylase, cellulase, lipase) secreted by the glandular walls of the sac. It stirs the stomach contents, serves as a windlass to help pull mucus/food strings from the labial palps into the stomach, and is a source of digestive enzymes for the extracellular hydrolysis of carbohydrates and lipids in the stomach. Protein digestion occurs intracellularly in the digestive ceca.

The intestine extends posteriorly from the stomach, curves around the posterior edge of the visceral mass to the foot where it changes direction and turns anteriorly as the ascending intestine (Fig 7). It soon changes direction again and heads dorsally. Only the intestine, and not the style sac or the style, continues past this point.

The intestine loops in a characteristic pattern through the visceral mass, and emerges at the base of the anterior aorta (Fig 7). Here it makes a right angle turn to extend posteriorly through the center of the pericardial cavity and ventricle as the **rectum**. Beginning at the point where the style sac ends, the intestine is characterized by the presence of a single, large, rounded **typhlosole** that occupies most of its lumen (Fig 5). Continue tracing the intestine a little beyond the point where the typhlosole begins so you can get a good look at this part of the gut. The gut is easiest to trace by opening it and following the typhlosole. Trace the intestine as far as you wish into the visceral mass. If you have the time you may follow it all the way to the heart but the procedure is tedious and time-consuming.

Locate the **rectum** where it exits the heart and trace it posteriorly. It passes between the bases of the two posterior pedal retractor muscles and then curves over the top of the posterior adductor muscle and part of the way around its posterior margin. It ends in the exhalant chamber at the **anus** on the posterior edge of the adductor muscle. The anus is dorsal to the inner aperture of the exhalent siphon. Fecal pellets released from the anus are caught in the exhalant current and swept out the siphon. (In *Tapes*, the rectum flares trumpetlike to end at the anus.)

Nervous System

The bivalve nervous system is relatively simple and consists of four major pairs of ganglia (cerebral, pleural, pedal, and visceral), connectives and commissures between them, and the nerves radiating from them (Fig 12-119).

Despite its simplicity, the system is not easily studied. The ganglia can usually be found but tracing the connectives and nerves is difficult. In living animals the ganglia are orange or yellow with neuroglobin, making them easier to recognize. The bivalve sensory system is weakly developed. Most sensory receptors are in the middle fold of the mantle margin.

The cerebral and pleural ganglia are coalesced and the resulting two cerebropleural ganglia are close to each other atop the anterior end of the esophagus, very close to the mouth (Fig 7). They are embedded in connective tissue on the posterior dorsal edge of the anterior adductor muscle. The two cerebropleural ganglia are connected to each other via a short transverse cerebral commissure across the top of the esophagus and nerves extend from them to the labial palps, anterior adductor muscle, anterior mantle, and anterior pedal retractor muscle.

From the cerebropleural ganglia a pair of visceral nerves (= cerebrovisceral connectives) runs posteriorly through the dorsal visceral mass to the visceral ganglia. The visceral ganglia are located on the anterior face of the posterior adductor muscle between the origins of the two posterior pedal retractor muscles (Fig 7). They are fused with each other on the midline. The visceral ganglia send nerves to the siphons, posterior adductor muscle, posterior pedal retractor muscle, posterior mantle, nephridium, gill, and heart.

A pair of cerebropedal connectives extends ventrally and posteriorly from the cerebropleural ganglia to the pedal ganglia in the foot. The two pedal ganglia are fused with each other and lie on the midline in the visceral mass, very near the muscles of the foot (Figs 5, 7). The pedal ganglia innervate the musculature of the foot. Look for them on the border between the foot and the visceral mass ventral and anterior to the point at which the midgut makes its sharp turn dorsally.

Reproductive System

Like most bivalves, *Mercenaria* is gonochoric and the two **gonads**, either ovaries or testes, fill most of the space in the visceral mass (Figs 5,7). The size of the gonads depends on the degree of development. Although there are two of them, they cannot usually be distinguished from each other.

>1i. Determine the sex of your clam by making a wetmount of some of the gonadal tissue and examining it with the compound microscope. Look for gametes, either large spherical **eggs** or tiny flagellated **spermatozoa**. <

The gonads occupy remnants of the embryonic coelomic cavity but that will not be apparent in gross dissection. Each gonad empties into the exhalant chamber via a gonoduct that opens at a gonopore at the tip of a small genital papilla just anterior to the nephridiopore. The genital papilla is located at about the level of the posterior end of the ventricle in the medial exhalant chamber but is difficult to locate.

Cross Sections

>1j. If extra specimens are available, you may want to make a cross section. This works best if the tissues are firm, as are those of preserved or steamed specimens. If you have fresh or living specimens, steam them, shell and all, in a pot with a little boiling water for about ten minutes to denature and solidify the proteins. Remove the clam from the shell and, using a very sharp scalpel, razor blade, or scissors make one or more cross sections entirely through the body including the gills and mantle. Most useful is a slightly oblique section passing through the heart and anterior end of the base of the foot (Fig 5). Rinse the sections gently and then immerse them in fluid in a small dissecting pan for study with the dissecting microscope. Use Figure 5 to identify the structures already familiar to you from your conventional dissection. <

References

Brooks WK . 1890. Handbook of Invertebrate Zoology. Bradlee Whidden, Boston. 352p.

Brown FA . (ed) 1950. Selected Invertebrate *Types*. Wiley, New York. 597p.

Petrunkevitch A . 1916. Morphology of Invertebrate Types. MacMillan, New York. 263p.

Ruppert EE, Fox RS, Barnes RB. 2004. Invertebrate Zoology, A functional evolutionary approach, 7 th ed. Brooks Cole Thomson, Belmont CA. 963 pp.

Supplies

Dissecting microscope

Compound microscope
Dissecting pan or culture dish
Isotonic magnesium chloride
Living or preserved clam (*Mercenaria* or *Tapes*)
Empty, cleaned shell
Pliers
Slides and coverslips
Carmine-seawater suspension
1 % toluidine blue in seawater

If the process of opening living clams is considered to be too difficult, clams can be easily opened by streaming them a stock pot with about an inch of water for about 10 minutes or until the valves gape. This provides quick access to the soft anatomy but, of course, removes many advantages of using fresh material. Cilia will no longer be beating and textures, colors, sizes and shapes will be altered. Even so, steamed clams are preferable to preserved.

Lander University

Invertebrate Anatomy OnLine

Corbicula fluminea©

Asian Clam

13mar2006

Richard Fox

Lander University

Preface

This is one of many exercises available from ***Invertebrate Anatomy OnLine***, an Internet laboratory manual for courses in Invertebrate Zoology. Additional exercises, a glossary, and chapters on supplies and laboratory techniques are also available at this site. Terminology and phylogeny used in these exercises correspond to usage in the Invertebrate Zoology textbook by Ruppert, Fox, and Barnes (2004). Hyphenated figure callouts refer to figures in the textbook. Callouts that are not hyphenated refer to figures embedded in the exercise. The glossary includes terms from this textbook as well as the laboratory exercises.

Systematics

Mollusca P, Eumollusca, Conchifera, Ganglioneura, Ancyropoda, Bivalvia C, Metabranchia SC, Eulamellibranchia SO, Veneroida O, Corbiculoidea SF, Corbiculidae F (Fig 12 125, 12 122)

Mollusca P

Mollusca, the second largest metazoan taxon, consists of Aplacophora, Polyplacophora, Monoplacophora, Gastropoda, Cephalopoda, Bivalvia, and Scaphopoda. The typical mollusc has a calcareous shell, muscular foot, head with mouth and sense organs, and a visceral mass containing most of the gut, the heart, gonads, and kidney. Dorsally the body wall is the mantle and a fold of this body wall forms and encloses that all important molluscan chamber, the mantle cavity. The mantle cavity is filled with water or air and in it are located the gill(s), anus, nephridiopore(s) and gonopore(s). The coelom is reduced to small spaces including the pericardial cavity containing the heart and the gonocoel containing the gonad.

The well-developed hemal system consists of the heart and vessels leading to a spacious hemocoel in which most of the viscera are located. The kidneys are large metanephridia. The central nervous system is cephalized and tetraneurous. There is a tendency to concentrate ganglia in the circumenteric nerve ring from which arise four major longitudinal nerve cords.

Molluscs may be either gonochoric or hermaphroditic. Spiral cleavage produces a veliger larva in many taxa unless it is suppressed in favor of direct development or another larva. Molluscs arose in the sea and most remain there but molluscs have also colonized freshwater and terrestrial habitats.

Eumollusca

Eumollusca, the sister taxon of Aplacophora, includes all molluscs other than aplacophorans. The eumolluscan gut has digestive ceca which are lacking in aplacophorans, the gut is coiled, and a complex radular musculature is present

Conchifera

Conchifera, the sister taxon of Polyplacophora, includes all Recent molluscs other than aplacophorans and chitons. The conchiferan shell consists of an outer proteinaceous periostracum underlain by calcareous layers and is a single piece (although in some it may appear to be divided into two valves). The mantle margins are divided into three folds.

Ganglioneura

Most Recent molluscs are ganglioneurans, only the small taxa Aplacophora, Polyplacophora, and

Monoplacophora are excluded. Neuron cell bodies are localized in ganglia.

Ancyropoda

The mantle cavity, with its gills, is lateral. The calcareous portion of the shell is bivalve, with the valves opening laterally and joined dorsally by a derivative of the periostracum.

Bivalvia [C]

Bivalvia is a large, successful, and derived taxon. The body is laterally compressed and enclosed in a bivalve shell. The two valves are hinged dorsally. The the foot is large and adapted for digging in the ancestral condition. A crystalline style is usually present but never is there a radula. The mantle cavity is lateral and in most bivalves the gills are large and function in respiration and filter-feeding. The head is reduced and bears no special sense organs. The nervous system is not cephalized. The group includes scallops, clams, shipworms, coquinas, marine and freshwater mussels, oysters, cockles, zebra mussels, and many, many more.

Metabranchia [sC]

Metabranch gills are adapted for filter feeding. Water enters the mantle cavity posteriorly.

Eulamellibranchia [SO]

Eulamellibranchs have gills with tissue interfilamentar connections.

Veneroida [O]

Shell is usually equivalve and without a nacreous layer.

Laboratory Specimens

Corbicula fluminea is an Asian species that was introduced to the west coast of North America around 1925. Since that time it has spread across the continent and is present in streams, canals, lakes, and reservoirs south of 40 ° North latitude. Its range continues to expand and it can be collected locally for laboratory use in many parts of the United States. It is common in California, western Arizona, parts of Washington and Oregon, throughout the southeast north through Kentucky and sporadically in more northern states. It is absent from most of the Great Plains and Great Basin. *Corbicula* lives in sand or gravel bottoms with the posterior third of shell exposed above the substratum. It has very short siphons and consequently must live at the sediment surface.

Corbicula is often abundant and population densities can reach $130{,}000/m^2$ but are usually much less, about $10\text{-}3000/m^2$. It is used as human food in Asia. It is often common in reservoirs where its densities are greatest near the shore.

Corbicula is hermaphroditic, both simultaneous and protandric, has a benthic crawling larva known as a **pediveliger** which has made it possible for this species to spread rapidly both upstream and downstream in any drainage to which it is introduced. *Corbicula* competes with native mussel species and is thought to reduce their population densities and may be responsible for the extinction of some species.

Corbicula is recommended as for the study of bivalve anatomy as an alternative to the widely used freshwater mussels. It is a typical eulamellibranch and in many areas it is abundant and readily available, making live dissection feasible at low cost. Furthermore, it is an undesirable alien species. It is preferable to use introduced, overwhelmingly abundant exotic species for study than to sacrifice increasingly scarce native freshwater mussels, several of which are threatened or endangered. Conduct the dissection under magnification in a small dissecting pan immersed in 7% ethanol (if living) or tap water (if preserved).

External Anatomy

Study the external features of an intact clam. The soft parts are completely enclosed in the **shell**. The shell consists of two **valves** (Figs 1, 2), right and left. The two valves are held together along their dorsal margins by the **hinge**. The **umbo** is a protuberance beside the dorsal margin of each valve. The umbo is displaced slightly toward the anterior end of the valve. Knowing **dorsal** and **anterior** find **right, left, ventral,** and **posterior.**

Note the shiny brown organic **periostracum** covering the outside of the valves. Bright white calcareous layers of the shell may also be visible where the periostracum has been eroded, especially near the umbos

Living *Corbicula*, like most bivalves, are difficult to anesthetize and open. At their first detection of anything undesirable in their environment the animal “clams up” and refuses to expose itself to an anesthetic. *Corbicula* is much easier to open than are larger clams such as *Mercenaria*.

Use the following instructions to open the clam so that the adductor muscles are cut, the right valve is removed, and the clam is left cradled in its left valve with its right surface exposed for observation. Be very careful that the scalpel does not slip and cut you instead of the clam. Refer to Figure 2 or 3 to determine the location of the anterior and posterior adductor muscles. These muscles must be cut before the clam can be opened.

Carefully slip the blade of a scalpel between the valves at the anterior end of the clam. Do not push toward your hand while you do this. Cut through the anterior adductor muscle and then gently push on the scalpel handle so that the tip of the blade is against the inside surface of the ***right*** valve. Carefully work the blade around the ventral perimeter of the shell from anterior to posterior so that the blade scrapes the soft tissue away from the right valve. Cut the posterior adductor muscle. You should not cut any of the soft tissue other than the adductor muscles. To that end, be sure to keep the blade against the right valve.

When the two adductor muscles are severed or scraped away from the right valve, gently lift the right valve. Open the shell slightly so you can see inside and use the scalpel to scrape gently (not cut) all remaining soft tissue away from the right valve so it stays with the rest of the animal in the left valve. When all soft tissue is removed from the right valve, remove the valve and set it aside. The complete clam is now present in its left valve with its right surface uppermost and ready for study. The right mantle skirt may have been damaged by the scalpel but the rest of the clam should be intact.

Place the clam in a small dissecting pan of 7% non-denatured ethanol. The clam should be completely immersed in the anesthetic. The right surface of the clam should be up. Once in the anesthetic the clam will begin to relax. Until that time its muscles, including those of the mantle, will be contracted. Set the pan aside. While you wait for the clam to relax, study the anatomy of an empty shell.

Shell

Examine a cleaned **shell**. It consists of two **valves** (Fig 1, 2). The **umbo** is a protuberance beside the dorsal margin of the valve. It is often called the "beak" and is the oldest part of the valve. It makes a good landmark for orienting the clam. It is dorsal and in most bivalves is displaced toward the anterior end of the valve and/or points toward the anterior end. The plane of symmetry passes between the two valves, which are thus right and left. Place the two valves together, orient the animal, find the **plane of symmetry** and relocate the major directions; **dorsal/ventral, anterior/posterior,** and **right/left**.

The two valves of your dried shell are probably no longer connected to each other but in life they would be held together along their dorsal margins by an articulation known as the **hinge** (Fig 2, 12-92B). The umbos are situated beside the hinge and arch toward it and toward each other.

The hinge region possesses projections of the shell known as **hinge teeth** and a pad of elastic protein known as the **hinge ligament** (Fig 2, 12-92B). The teeth are readily visible on the inside of the hinge of each valve and the ligament should also be visible unless it has been broken off by handling. It is a dark brown mass of protein that becomes very brittle in dried specimens. In *Corbicula* it is external and located immediately posterior to the umbo.

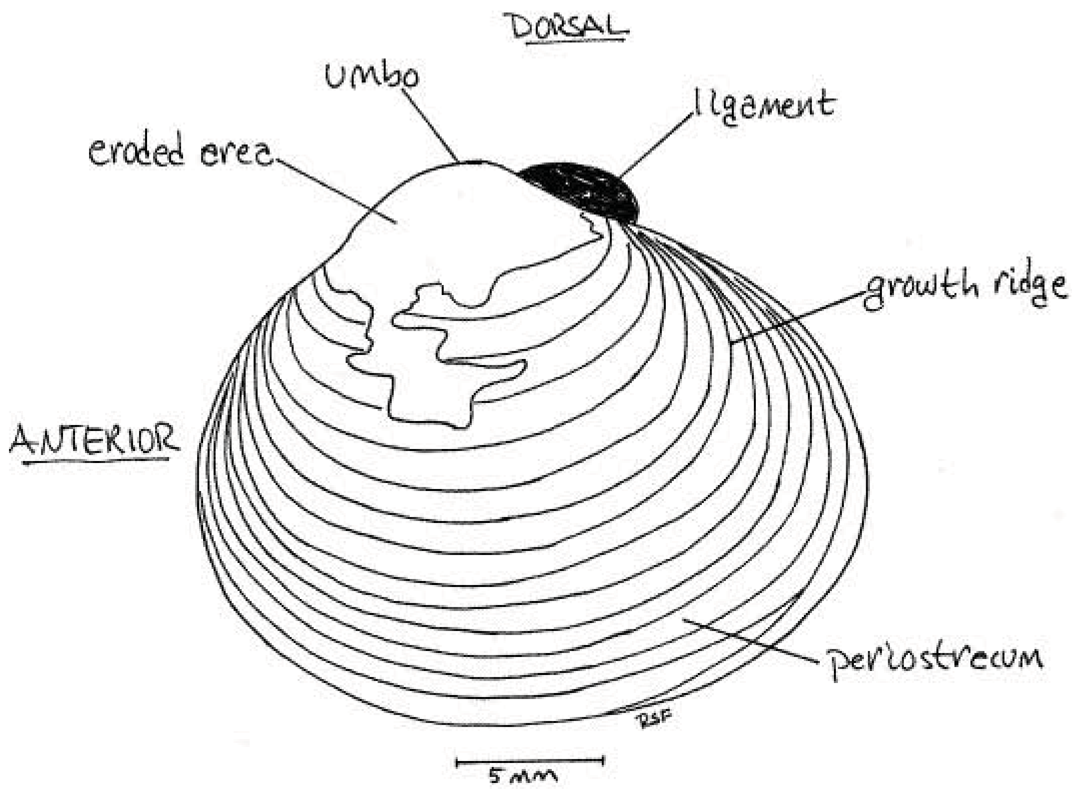
Shell Layers

The typical bivalve shell consists of three layers; the outer periostracum, middle prismatic layer (= ostracum), and the inner lamellar layer (= hypostracum) (Fig 12-91). All three layers are secreted by the mantle epidermis. *Corbicula* has a well developed and conspicuous periostracum and lamellar layers but the prismatic layer is reduced. Most of the mass of the shell is the lamellar layer.

The **periostracum**, which in *Corbicula* is dark olive brown or black, is the outermost layer. It is composed of the protein conchiolin. Inside the periostracum is a chalky white **prismatic layer** of calcium carbonate crystals deposited over an organic collagenous matrix. The periostracum, in the vicinity of the umbo especially, is often eroded in freshwater clams. Consequently, the underlying white calcareous layer is exposed and visible externally. In areas where the periostracum is missing the underlying calcareous shell is subject to erosion by acidic water so that the shell is often pitted. Innermost is the thick, **lamellar layer** , which is also calcium carbonate and an organic matrix. It can be seen covering the inside surface of the valves. It is purple and white.

In some molluscs the structure of the lamellar layer is such that its appearance is smooth and lustrous. This type of shell is known as "mother of pearl" or nacre (pronounced NAKE ur). The lamellar layer of freshwater “pearly” mussels (Unionida) is nacreous but that of *Corbicula,* and other Veneroida, is not.

Figure 1. Exterior of the left valve of the Asiatic clam, *Corbicula fluminea*. Mussel83La.gif



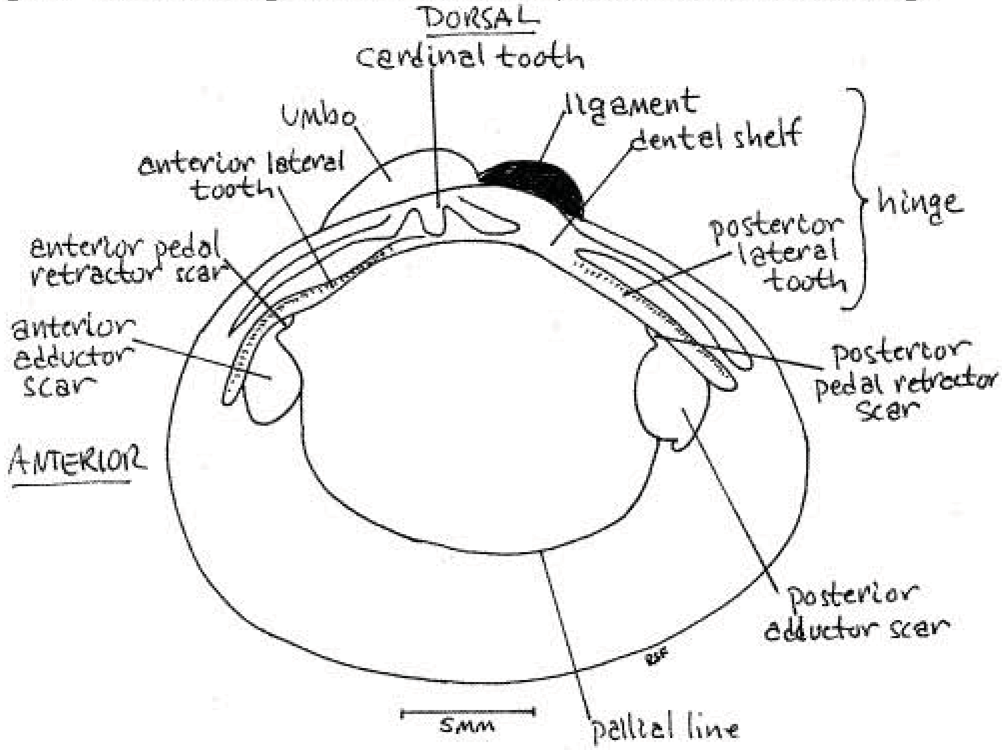
New shell material is deposited by the mantle epidermis along the margins of the valve. Periods of growth are marked by conspicuous concentric growth ridges on the outer surface of the valve (Fig 1).

Hinge

Look at the inside of one of the valves and observe the architecture of the hinge region. *Corbicula* is a good species for demonstrating the basic pattern of bivalve hinge teeth. The function of the hinge teeth is to keep the valves in alignment. The dentition on the right and left valves differ (because they must mesh with each other) but in *Corbicula* the differences are slight so it makes no difference which valve you study.

In the center of the hinge, immediately ventral to the umbo, are the cardinal teeth (Fig 2, 12-92B). (In freshwater mussels the teeth in this position are known as pseudocardinal teeth.) In *Corbicula* there are three cardinal teeth in each valve and they are easily recognized because they look like teeth.

Figure 2. Interior of the right valve of the Asiatic clam, ***Corbicula fluminea***. Mussel84La.gif



In addition to the cardinal teeth, two lateral teeth are present in the hinge of each valve but they don't look much like teeth. They are low straight ridges paralleling the dorsal edge of the valve. The **anterior lateral teeth** are anterior to the cardinal teeth and the **posterior lateral teeth** are posterior to them. The right valve has two of each, whereas the left valve has only one. Look at the two valves and see if you understand the functional reason for this asymmetry.

Fit the two valves together to demonstrate how the teeth mesh together to keep the valves in alignment when closed. Try to shear the two valves past each other while the valves are tightly closed and you will appreciate the effectiveness of the hinge teeth.

The teeth are located on a part of the hinge known as the **dental shelf**. There is typically a cavity under the dental shelf and inside the umbo known as the **beak cavity**. *Corbicula* has a deep beak cavity.

Muscle Scars

In life the two valves are pulled together by a pair of adductor muscles, one anterior and one posterior. These muscles extend transversely across the clam from one valve to the other. When contracted they pull the valves together. This action also stretches the hinge ligament, which is elastic. When the adductor muscles relax the hinge ligament returns to its original shorter length and this pulls the umbos closer together and the ventral margins of the valves move apart. The shell thus opens slightly along the ventral border. The small gap thus created between the edges of the two valves is the **gape**. It is just wide enough to allow the foot to slip out.

Look again at the inside surface of one of the valves. You will see two smooth elliptical areas, one anterior and one posterior. These are the **anterior** and **posterior adductor muscle scars**, respectively, and they are the sites of attachment of the adductor muscles.

Associated with the adductor muscle scars are scars of the pedal retractor muscles that withdraw the foot into the shell before the valves are adducted. These small scars are located on the margins of the adductor scars and often coalesced with them.

The **anterior pedal retractor muscle scar** is located at about 1:00 on the circumference of the anterior adductor scar. It is hidden by the overhang of the anterior lateral tooth. The **posterior pedal retractor muscle scar** is located at about 11:00 on the outline of the posterior adductor scar and is hidden by the posterior lateral tooth.

The **pallial line** extends from one adductor scar to the other and parallels the ventral border of the valve. This line marks the site of attachment of the mantle and its pallial muscles. The mantle will be considered in more detail later.

Soft Anatomy

Introduction

Turn your attention to the opened, live clam you set aside earlier. It should be anesthetized now and unable to contract. Place the dissecting pan on the stage of the dissecting microscope and examine the animal with low power. If the soft anatomy is intact, you will be looking at the outside surface of the right **mantle skirt** (Fig 3, 12-90). The right mantle skirt (= mantle lobe) is penetrated by the two adductor muscles. You cut these muscles in order to open the shell but they will still be in place attached to the left valve. Find the **anterior adductor muscle** ventral to the anterior lateral tooth and the posterior adductor muscle ventral to the posterior lateral tooth (Fig 3, 12-89A).

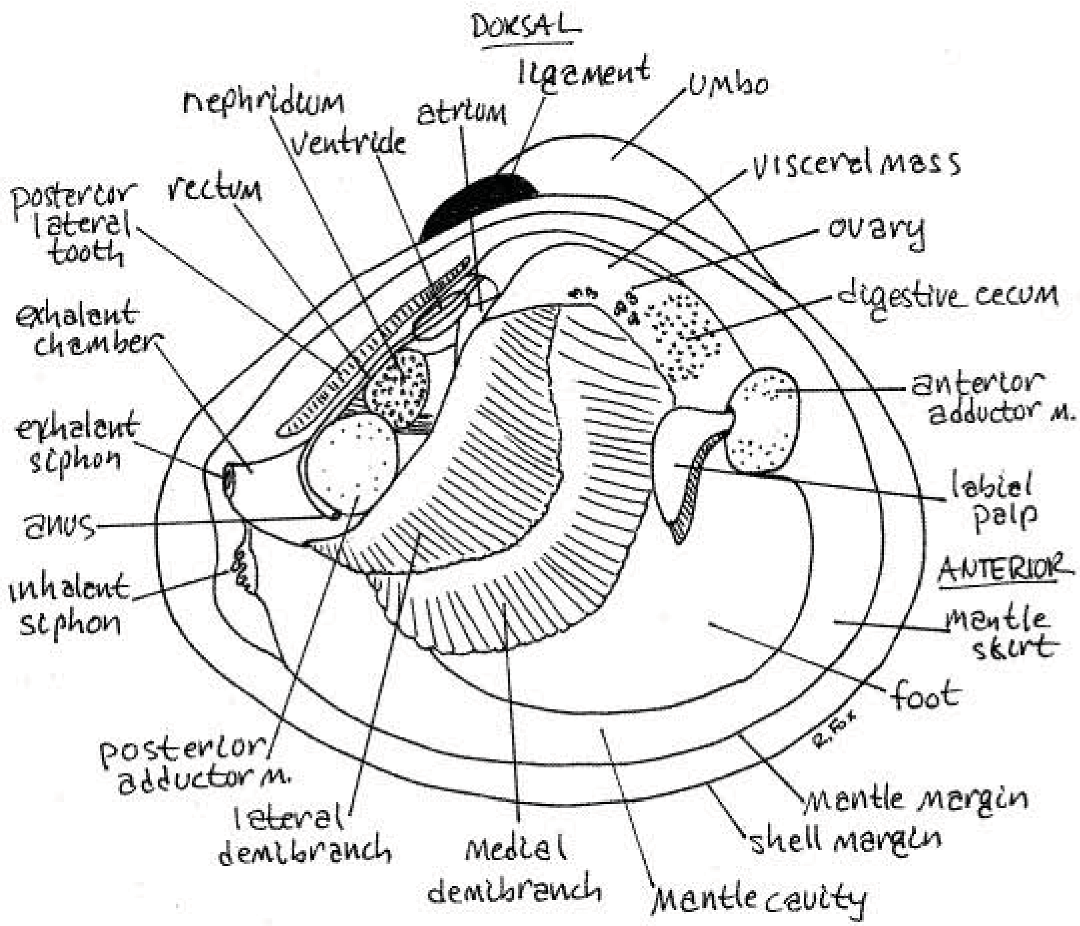
Mantle and Mantle Cavity

In life the periphery of the right mantle skirt would be attached to the right valve by a sheet of transparent, slightly yellowish **periostracum** but, since you have removed the valve, that connection has been broken. With magnification, look at the edge of the right mantle skirt to see the remnants of this sheet of periostracum. It looks like plastic wrap or cellophane. The periostracum is secreted by the margin of the mantle and the sheet you see was, before dissection, continuous with the periostracum covering the right valve. Lift the right mantle skirt and find the margin of the **left mantle skirt**. The periostracum of this skirt should still be intact and connected with the shell. Examine it with the dissecting microscope.

The space between the right and left mantle skirts is the **mantle cavity**. This space is outside the body and is not a body cavity, even though it is largely enclosed by the shell and mantle. In life it is filled with the water that is the animal's environment. The mantle cavity consists of two parts. The part you see now is its **inhalant chamber** (= branchial chamber).

Look at the posterior **edges** of the right and left mantle skirts and see that they are joined with each on the midline to form a pair of openings, the **siphons** (Fig 3, 8, 12-89). The mantle tissue is thickened in the vicinity of the siphons and is pigmented. The ventral siphon is the **inhalant siphon** and it is continuous with the inhalant chamber. It is the larger of the two and its external opening is guarded by **tentacles** of various sizes whose purpose is sensory and mechanical (to exclude large particles). Use a needle or probe to demonstrate the connection between the inhalant siphon and the inhalant chamber.

Figure 3. The right side of ***Corbicula*** with the right valve and mantle skirt removed. Mussel85La.gif

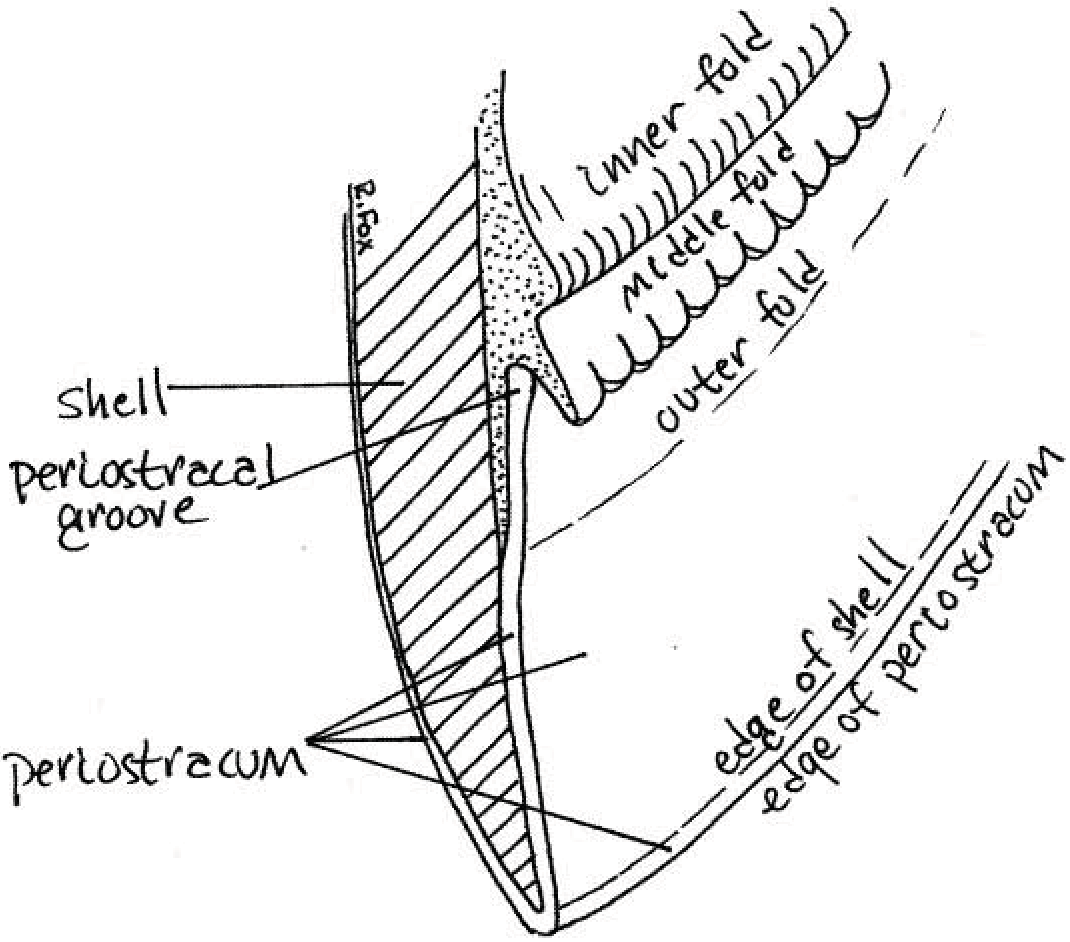


The dorsal opening is the **exhalant siphon** and it is the smaller of the two siphons (Fig 3, 8, 12-89). It does not possess the array of sensory tentacles found on the inhalant siphon. It is the outlet from the exhalant chamber of the mantle cavity dorsal to the gills. You cannot demonstrate the continuity between the exhalant siphon and the exhalant chamber at this time.

The mantle cavity is divided by the gills into a ventral **inhalant chamber** (which you have seen) and a dorsal exhalant chamber, or suprabranchial chamber, (which you cannot see yet). Water passes through the inhalant siphon to enter the inhalant chamber, flows across the gills and then into the exhalant chamber. As the water crosses the gills food particles are filtered from it and oxygen is removed. From the exhalant chamber the water flows out the exhalant siphon.

Use fine scissors to remove the right mantle skirt. Leave the siphons intact but cut around them to remove the rest of the right mantle. The margin of a typical bivalve mantle skirt has three longitudinal folds, each with a specific function (Fig 4, 12-91). The **outer fold** secretes two of the layers of the shell (periostracum and prismatic layer), the **middle fold** is sensory, and the **inner fold** is muscular. The lamellar layer of the shell is secreted by the entire outer surface of the mantle skirt, not by the folds.

Figure 4. Section and oblique view of the shell and mantle margin of *Corbicula*. Mussel86La.gif



Examine the margin of the left mantle skirt with the dissecting microscope at about 12X. You may also find it instructive to refer back to the edge of the right mantle skirt occasionally. Adjust the light and focus carefully. Find the **periostracum** emerging from near the edge of the skirt (Fig 4, 12-91). Note that it is attached to the mantle and then extends to the inner edge of the valve and wraps over this edge to continue over the outer surface of the valve. The periostracum is secreted by the inner surface of the outer fold of the mantle margin. It arises in the **periostracal groove** between the outer and middle folds.

Gills

Removal of the right mantle exposed the inhalant chamber of the mantle cavity to view and gave you access to the structures in it. You can now see the right gill, foot, visceral mass, and left mantle skirt (Fig 3, 12-89A). The left gill is hidden by the foot and visceral mass. The right gill is a double sheet of corrugated tissue lying on top of the foot and visceral mass (Fig 3). It extends obliquely across the mantle cavity.

The **foot** is a large semicircular mass of muscle occupying most of the ventral region of the mantle cavity (Fig 3). The size of the foot varies depending on its state of contraction. It is smaller in preserved specimens.

The **visceral mass** is the thick globular mass of tissue dorsal to the foot and ventral to the hinge (Fig 3, 12-89B). The foot is attached to the ventral border of the visceral mass. The left mantle skirt has already been identified. In dissected specimens contractions of its muscles may pull it away from the margin of the valve.

Look at the right gill. It is a single gill, or **holobranch**, even though it may appear to be two. It is a typical eulamellibranch gill whose dual purposes are filter feeding and gas exchange. In addition, as is typical of many other freshwater bivalves including the freshwater mussels, part of it serves as a brood chamber for the incubation of eggs.

The gill consists of two sheets of coalesced filaments folded into a "W" shape (in cross section) (Fig 12-90, 12-96C,D). It is attached to the dorsal wall of the mantle cavity by a **longitudinal central axis** coinciding with the middle point of the "W". This sheet divides the mantle cavity into the ventral inhalant chamber and the dorsal exhalant chamber. To get from the ventral chamber to the dorsal, water must pass through ostia (pores) in the gills.

The **holobranch**, or whole gill, is composed of two half gills, or **demibranchs**. Each demibranch is a sheet of fused filaments. Each demibranch corresponds to one of the two "Vs" of which our "W" model is composed. Closest to you is the **lateral demibranch** of the right gill and under it is the larger **medial demibranch** (Fig 3). The two are attached to each other and to the mantle along the **longitudinal central axis** of the gill. They are also attached to the body (either mantle or foot) along their borders. The demibranchs are hollow and the space inside them is the exhalant chamber.

The surface of each side of a demibranch is a lamella. Each demibranch has lateral and medial surfaces, and thus two lamellae. Each holobranch thus has four lamellae. Each demibranch is connected to the central axis by its **descending lamella** and to the mantle skirt or foot by its **ascending lamella** (Fig 12-90).

The lamellae are covered by a ciliated epithelium and some of these cilia (the lateral cilia) generate the feeding current that brings water in through the inhalant siphon and then through the ostia into the exhalant chamber and then out the exhalant siphon.

Bivalves feed on suspended particles too large to pass through the ostia and thus are retained on the inhalant side of the gill. Other cilia (the frontal cilia) are responsible for moving these particles, both organic and mineral, over the surfaces of the lamellae and eventually to the labial palps and mouth.

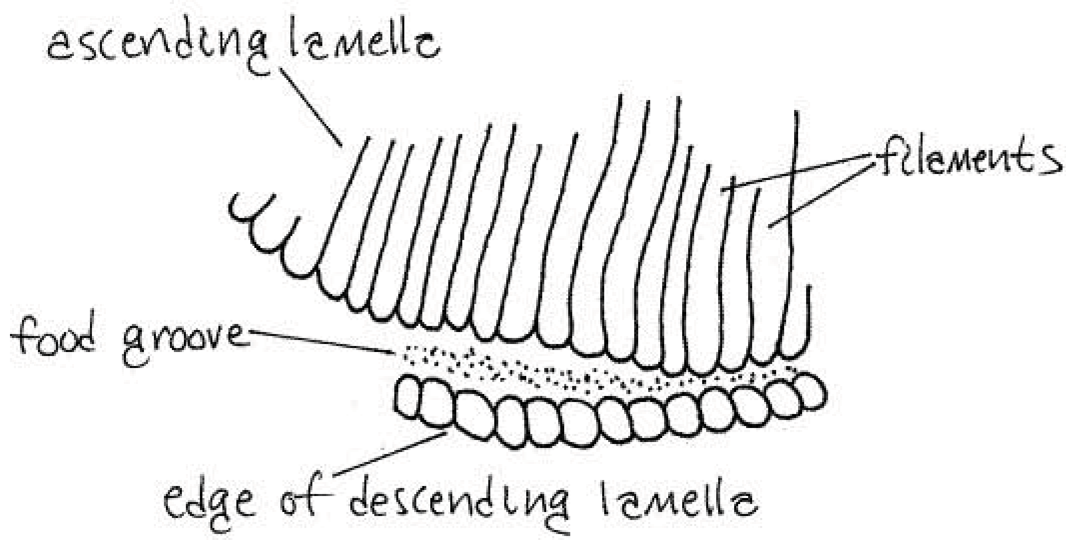
> 1a. If your specimen is alive, remove it from its pan and looking at the gills, with magnification, while they are covered by a thin film of water. Focus on areas where light is reflected from the surface and you will see it shimmer from the activity of the cilia. <

Bivalve gills are composed of numerous long, fused filaments. Look as the surface of a demibranch with about 25X magnification. At this magnification you can easily see the parallel **filaments** (Fig 5, 12-96D) of which the lamella, demibranch, and holobranch are composed.

Look at the ventral edge of one of the demibranchs with high power. Here you see the filaments of one lamella bend 180 ° and become the filaments of the opposite lamella (Fig 12-90). Along the bend, which is the free edge of the demibranch, there is a distinct ciliated **food groove** (Fig 5, 12-96B,D). The cilia in this groove create a stream of mucus and food particles that moves anteriorly to the labial palps and ultimately to the mouth.

>1b. If you have a living specimen, place it in a dish of seawater and arrange it in the dish so the flat surface of the exposed lamella is horizontal, or nearly so. Look at the surface of the gill with magnification. Place a little chalk dust or a drop of carmine-seawater suspension on the surface of the gill while watching it with the dissecting microscope. You should be able to see the particles moving rapidly over the gill to the ventral food groove. The particles, and the mucus surrounding them, are moved anteriorly by the ciliary transport mechanism of the food groove. <

Figure 5. A small portion of the food groove on the ventral margin of the medial demibranch of *Corbicula*. Mussel87La.gif



>1c. Remove about 2-3 mm of the edge of a demibranch, place it on a slide, tease the filaments apart, affix a coverslip, and examine it with the compound microscope. If your specimen is alive, the beating cilia of the filament will be easy to see. Look for the **ventral food groove** at the edge of the gill. Note that it is a deep groove with a narrowed opening formed by the ends of the filaments of the descending and ascending filaments. In living specimens, the beating cilia of the filaments are easily seen. <

In *Corbicula*, every 16 th filament of the descending lamella is connected by interlamellar junctions to the same filament on the opposite ascending lamella. The other 15 intervening filaments are not connected in this fashion and are free to bulge away from each other. Consequently, the surface of the gill appears corrugated, or plicate. Each ridge is a group of 16 filaments held together only by the 16 th filaments.

!!

With fine scissors cut along the central axis of the right gill at its posterior end, in the vicinity of the posterior adductor muscle. This will reveal the **exhalant chamber** inside the gill. Note the vertical water tubes extending into the demibranchs (Fig 12-98C,D). Water from the inhalant chamber passes through ostia in the lamella to enter the water tubes. From the water tubes it moves to the exhalant chamber and exhalant siphon. With a probe, demonstrate the connection between the exhalant chamber and the exhalant siphon.

Labial Palps

Strings of food and mucus are moved anteriorly along the ventral food grooves of the demibranchs to the labial palps at the anterior end of the mantle cavity on either side of the mouth (Fig 3). There is a labial palp on the right of the mouth and another pair on the left. Each pair consists of two triangular sheets of tissue, known as palp lamellae that resemble small gills (Fig 12-100). The outermost is the **lateral lamella** and hidden under it is the **medial**

lamella.

One surface of each lamella is a **sorting field** of ciliated ridges and grooves. The other surface is smooth. The ciliated surface of each lamella faces the opposing ciliated surface of the other lamella. More specifically, the medial surface of the lateral lamella faces the lateral surface of the medial lamella. Food and mucus from the food groove of the gill move onto the sorting fields where organic food particles are separated from mineral particles. The food moves along a ciliated oral groove to the mouth, again in a mucus string powered by cilia. The mineral particles, also mixed with mucus, are discarded into the inhalant chamber as **pseudofeces**. Occasional contractions of the adductor muscles compress the chamber and expel the pseudofeces through the *in*halant siphon. Sorting on the gills and labial palps is imperfect and final sorting occurs in the stomach.

The labial lamellae of each side are connected with their counterparts on the opposite side of the anterior visceral mass by a pair of transverse lips. The right and left lateral lamellae are connected by the **upper lip** and the right and left medial palps are connected by the **lower lip** (Fig 12-100). The upper lip passes dorsal to the mouth and the lower lip passes ventral to it. The food string travels in the oral groove between the upper and lower lips to reach the mouth.

Hold the clam with one hand so you can see the anterior surface of the visceral mass and the lips with the dissecting microscope. Use a *minuten nadel* to lift the upper lip so you can see the **mouth**. The mouth is a tiny, inconspicuous opening located on the midline between the two lips. It can be difficult to demonstrate.

>1d. Separate the two lamellae of the left labial palp as if opening a book. Place a little carmine-seawater on the ridged surfaces of the lamellae and watch it as it is transported by their cilia. Try to trace currents and watch for the development of a stream of particles in the oral groove leading into the mouth. This is probably the easiest way to find the mouth. The mouth is a small and inconspicuous pore but is easy to see if it has a string of red carmine particles entering it. <

Visceral Mass

Spend a moment or two on a superficial preliminary examination of the visceral mass before considering it in more detail. Relocate the **visceral mass** (Fig 3). It is the large, thick mass of tissue situated immediately ventral to the hinge and occupying the dorsal half of the valve.

The laterally compressed, muscular, cream-colored **foot** is attached to the ventral edge of the visceral mass. While its appearance is variable, it is likely to be more or less tongue-shaped with the tongue probably pointing anteriorly.

The visceral mass is thickest dorsally and here you may see dark lobes of the **digestive ceca** through its walls. The color of the digestive ceca depends on the color of the food and may be yellowish, brownish, green, etc.

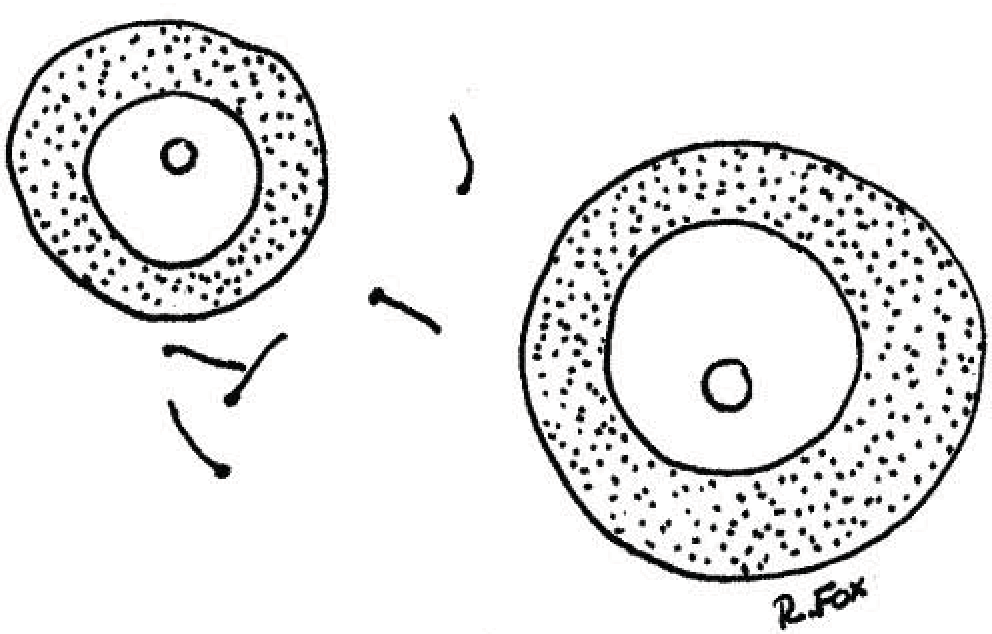
You may also see parts of the gray **nephridium,** or kidney. The thin-walled **pericardial cavity** and the heart are located on the dorsal median edge of the visceral mass immediately ventral to the middle of the posterior lateral tooth (Fig 3, 8). A lobe of the nephridium is present just posterior to the pericardial cavity. The heart is located inside the pericardium.

Although you cannot see it now, the rectum passes through the pericardial cavity, extends posteriorly just ventral to the posterior lateral tooth, between the tooth and the posterior adductor muscle, and then ends at the anus, in the exhalant chamber, on the posterior side of the posterior adductor muscle.

Gonad

Most of the interior of the visceral mass, dorsal mantle skirts, and foot are filled with the **gonads**. *Corbicula* is hermaphroditic and there may be testis, ovary, or both present in your specimen. These organs reach the surface of the visceral mass and parts of them are visible without dissection (Fig 3).

Figure 6. The eggs of *Corbicula* with sperm drawn at the same scale. Mussel88La.gif



The testis is bright white and the ovary is dark gray. The **ovary** consists of innumerable fairly large digitiform follicles which contain female sex cells in various stages of development. The follicles are easily seen on the surface of the visceral mass and in the dorsal part of the mantle skirts. Don't confuse the ovary with the nephridium, which is also gray and composed of digitiform processes. The processes of the nephridium are much smaller than those of the ovary.

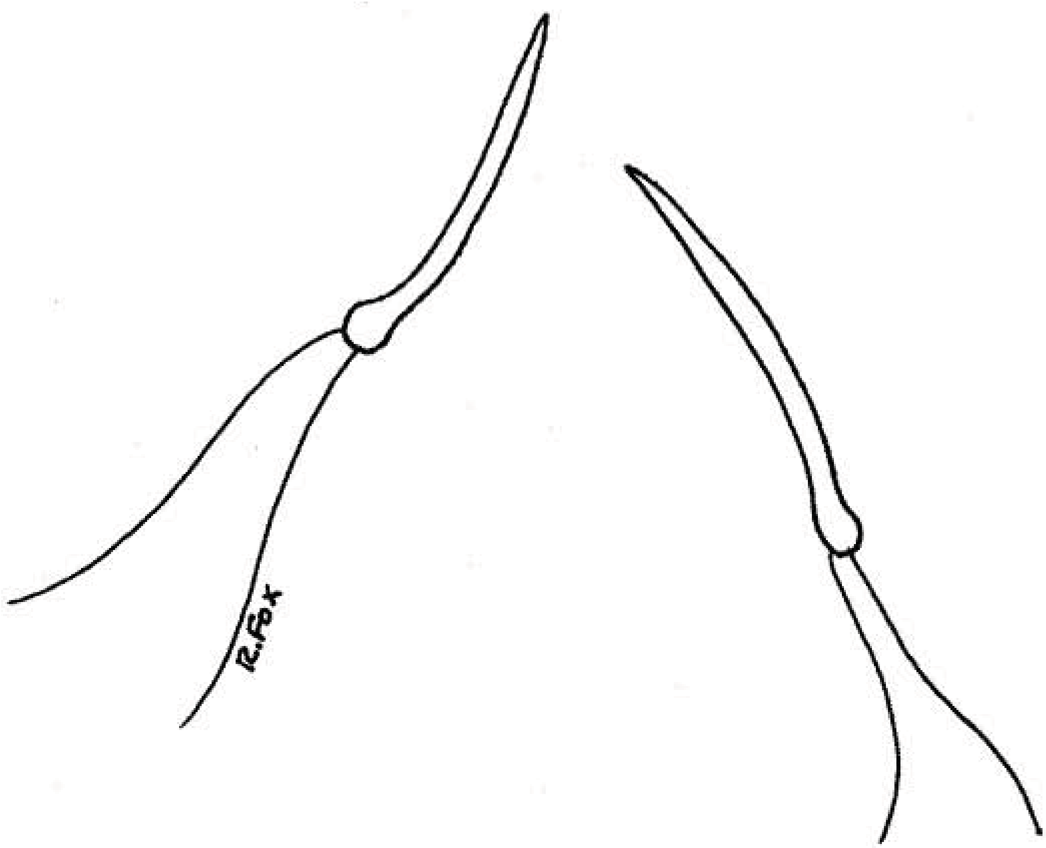
The **testis** consists of microscopic seminiferous tubules which contain male sex cells in various stages of development. You cannot see individual tubules with the dissecting microscope.

>1e. Make two wet mounts using small bits of ovary for one and testis for the other. Squash the tissue in a drop of water and add a coverslip. Examine the preparation with the compound microscope and look for gametes. Eggs are large nucleate spheres (Fig 6). Sperm are tiny, elongate and biflagellate (Fig 7). <

Sperm are released to the environment in large balls (morulae), each consisting of thousands of sperm, via the exhalant siphon. These enter the inhalant siphon of another clam where the morulae break up into individual sperm which penetrate the gills and enter the exhalant chamber where they fertilize eggs present there. The eggs are then brooded in the interior water tubes of the medial demibranchs.

The larvae (pediveligers) are crawlers, not swimmers, and thus are well adapted for life in flowing water where they can move upstream or downstream along the bottom and avoid being swept downstream as would planktonic larvae. Nor are they dependent on fishes for dispersal as are the parasitic glochidia larvae of freshwater mussels. Larvae are released twice yearly, once in spring and in fall.

Figure 7. The biflagellate sperm of *Corbicula*. Mussel89La.gif



Pericardial Cavity and Heart

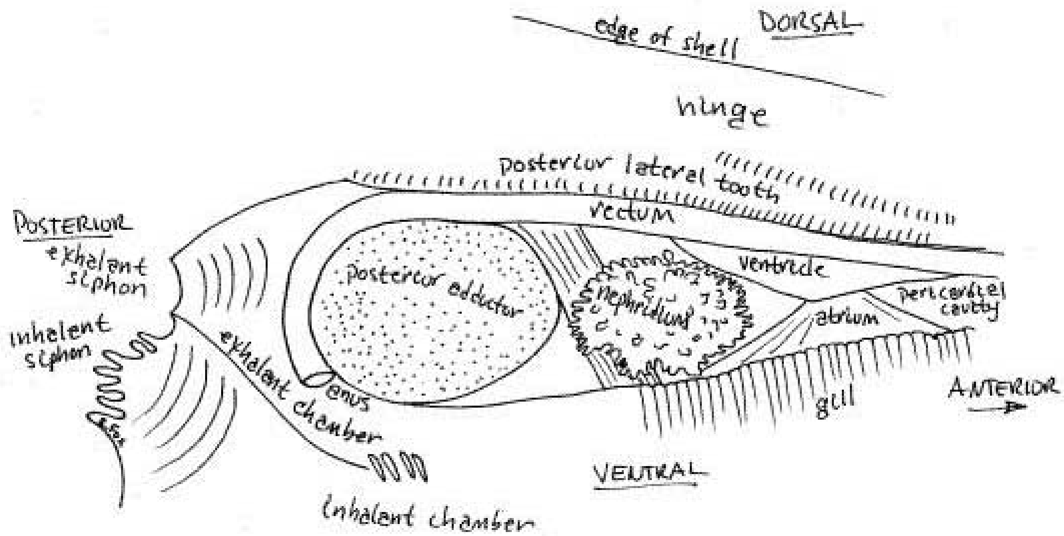
"

The heart and pericardial cavity are enclosed in a thin membranous **pericardium** ventral to the posterior lateral tooth (Fig 3, 8). Use your fine scissors to make a longitudinal incision through the pericardium to open the pericardial cavity. Extend this incision posteriorly around the dorsal edge of the posterior adductor muscle and then to the siphons. Cut away the right side of both siphons. This will expose the rectum, heart, and exhalant chamber (Fig 8).

Find the **heart** in the **pericardial cavity**. It consists of a muscular ventricle to which are connected two thin-walled atria, one from each gill. The **ventricle** has thick, opaque walls and is wrapped around the tubular **rectum** (Figs 3, 4). The triangular, thin-walled, transparent right **atrium** extends from the dorsal edge of the right gill to the right side of the ventricle. The atrium may have been damaged or destroyed when you opened the pericardium.

The **rectum** enters the pericardial cavity anteriorly, passes through the ventricle, and then exits the pericardial cavity posteriorly (Fig 8). It lies immediately ventral to the posterior lateral tooth. It crosses the **posterior pedal retractor** muscle and then passes over the dorsal surface of the posterior adductor muscle, between the muscle and the lateral tooth, and then curves around the posterior side of the muscle to end at the anus located at about 7:00 on the circumference of the muscle. The anus opens into the exhalant chamber.

Figure 8. Side view from the right of the pericardial cavity, heart, kidney, and exhalant chamber of *Corbicula*. Mussel90La.gif



References

Britton JC, Morton B. 1982. A dissection guide, field and laboratory manual for the introduced bivalve *Corbicula fluminea*. Malacologia Rev. Supp. 3:1-82.

Heard WH. 1968. Mollusca, G1-G26 in F. Parrish (ed), Keys to water quality indicative organisms (Southeastern United States). Federal Water Pollution Control Administration, Washington.

McMahon RF. 2001. Mollusca: Bivalvia, pp.331-430 in Thorp JH, Covich AP (eds.), Ecology and classification of North American freshwater invertebrates, 2nd ed. Academic Press, San Diego. 1056pp.

Pennak RW. 1989. Fresh-water invertebrates of the United States, 3rd ed. Wiley, New York.

Ruppert EE, Fox RS, Barnes RB. 2004. Invertebrate Zoology, A functional evolutionary approach, 7th ed. Brooks Cole Thomson, Belmont CA. 963 pp.

Supplies

Dissecting microscope
Compound microscope
Small dissecting pan
Empty cleaned shell
Living or preserved *Corbicula*
7% non-denatured ethanol
Carmine-seawater suspension

Corbicula can be collected, sometimes in enormous numbers, along the shallow littoral zone of lakes and reservoirs. They are especially easy to collect when exposed by deliberate water level drawdown in the winter. At other times they can be collected by wading with a shovel and sieve or from a boat or dock using an Ekman dredge or similar sampler. Once collected, specimens can be held in finger bowls, but out of water, in a refrigerator for several weeks. They should be moistened occasionally.

Corbicula can be maintained indefinitely in laboratory aquaria on strained (babyfood) spinach but they will not grow or reproduce on this diet (Britton & Morton, 1982).

Bivalve Introduction

Bivalves are Mollusks

Bivalve Anatomy

Economics & Impact

In the Classrooom

Dissection Guides:

Actinonaias ligamentina

Lander University

Invertebrate Anatomy OnLine

Actinonaias©

Freshwater Unionoid Mussels

25may2007

Copyright 2005 by

Richard Fox

Lander University

Preface

This is an exercise from ***Invertebrate Anatomy OnLine***, an Internet laboratory manual for courses in Invertebrate Zoology. Additional exercises, a glossary, and chapters on supplies and laboratory techniques are also available. Terminology and phylogeny used in these exercises correspond to usage in the Invertebrate Zoology textbook by Ruppert, Fox, and Barnes (2004). Hyphenated figure callouts refer to figures in the textbook. Callouts that are not hyphenated refer to figures embedded in the exercise. The glossary includes terms from this textbook as well as the laboratory exercises.

Systematics

Mollusca [P], Eumollusca, Conchifera, Ganglioneura, Ancyropoda, Bivalvia [C], Metabranchia [SC], Eulamellibranchia [SO], Unionoida [O], Unionoidea [SF], Unionidae [F], Ambleminae [sF] (Fig 12 125, 12 122)

Mollusca [P]

Mollusca, the second largest metazoan taxon, consists of Aplacophora, Polyplacophora, Monoplacophora, Gastropoda, Cephalopoda, Bivalvia, and Scaphopoda. The typical mollusc has a calcareous shell, muscular foot, head with mouth and sense organs, and a visceral mass containing most of the gut, the heart, gonads, and kidney. Dorsally the body wall is the mantle and a fold of this body wall forms and encloses that all important molluscan chamber, the mantle cavity. The mantle cavity is filled with water or air and in it are located the gill(s), anus, nephridiopore(s) and gonopore(s). The coelom is reduced to small spaces including the pericardial cavity containing the heart and the gonocoel containing the gonad.

The well-developed hemal system consists of the heart and vessels leading to a spacious hemocoel in which most of the viscera are located. The kidneys are large metanephridia. The central nervous system is cephalized and tetraneurous. There is a tendency to concentrate ganglia in the circumenteric nerve ring from which arise four major longitudinal nerve cords.

Molluscs may be either gonochoric or hermaphroditic. Spiral cleavage produces a veliger larva in many taxa unless it is suppressed in favor of direct development or another larva. Molluscs arose in the sea and most remain there but molluscs have also colonized freshwater and terrestrial habitats.

Eumollusca

Eumollusca, the sister taxon of Aplacophora, includes all molluscs other than aplacophorans. The eumolluscan gut has digestive ceca which are lacking in aplacophorans, the gut is coiled, and a complex radular musculature is present.

Conchifera

Conchifera, the sister taxon of Polyplacophora, includes all Recent molluscs other than aplacophorans and chitons. The conchiferan shell consists of an outer proteinaceous periostracum underlain by calcareous layers and is a single piece (although in some it may appear to be divided into two valves). The mantle margins are divided into three folds.

Ganglioneura

Most Recent molluscs are ganglioneurans, only the small taxa Aplacophora, Polyplacophora, and Monoplacophora are excluded. Neuron cell bodies are localized in ganglia.

Ancyropoda

The mantle cavity, with its gills, is lateral. The calcareous portion of the shell is bivalve, with the valves opening laterally and joined dorsally by a derivative of the periostracum.

Bivalvia ^{C}

Bivalvia is a large, successful, and derived taxon. The body is laterally compressed and enclosed in a bivalve shell. The two valves are hinged dorsally. The the foot is large and adapted for digging in the ancestral condition. A crystalline style is usually present but never is there a radula. The mantle cavity is lateral and in most bivalves the gills are large and function in respiration and filter-feeding. The head is reduced and bears no special sense organs. The nervous system is not cephalized. The group includes scallops, clams, shipworms, coquinas, marine and freshwater mussels, oysters, cockles, zebra mussels, and many, many more.

Metabranchia ^{sC}

Metabranch gills are adapted for filter feeding. Water enters the mantle cavity posteriorly.

Eulamellibranchia ^{SO}

Eulamellibranchs have gills with tissue interfilamentar connections.

Unionoida ^{O} (Paleoheterodonta)

The lamellar layer of the shell is nacreous (pearly). The apertures are poorly developed and the shells are equivalve. Modern classifications of unionoid mussels recognize two families in North America; Margaritiferidae and Unionidae. Two subfamilies of unionids, Ambleminae and Unioninae, are recognized. Most amblemines are North American where they occur exclusively (or almost so) in the Atlantic and Gulf of Mexico drainages. Approximately 75% of North American genera and 80% of species belong to Ambleminae. The North American Ambleminae belong to the tribes Amblemini, Pleurobemini, and Lampsilini.

Natural History

Native North American freshwater bivalves are either mussels in the order Unionida ^{O} (Margaritiferidae ^{F} and Unionidae ^{F}) or fingernail clams (Veneroida ^{O}: (Corbiculoidea ^{SF}: Sphaeriidae ^{F}). Introduced bivalves include the now widely dispersed Asian clam, *Corbicula fluminea*, and the rapidly dispersing zebra mussel, *Dreissena polymorpha*.

Freshwater mussels (Unionoidea ^{SF}) occur worldwide but they are most abundant in North America, with their greatest diversity in the Ohio River Drainage of the Southeastern United States. They should not be confused with the unrelated marine mussels (Filibranchia ^{SO}, Pteriomorpha ^{O}) from which they differ in many important respects.

Freshwater mussels are usually found in medium to large rivers in water less than two meters deep. A few species inhabit the quiet water of lakes but most are riverine. Small creeks and small lakes have few mussels although they may have diverse faunas of other bivalves such as the sphaeriid clams. Mussels live in soft, sedimentary bottoms with the foot and anterior end of the shell and body dug into the sediment with the posterior end exposed. Adult mussels move slowly and never very far from the site of settling after leaving the fish host. Dispersion occurs as larvae parasitic on fishes. Like other bivalves, mussels are suspension feeders using the gills as filters to remove particulate organic matter, detritus and plankton, from the water. Adults unionoids can be 4-30 cm in length.

Most freshwater mussels are gonochoric and cross fertilize without copulation. Fertilization is external and occurs in the water tubes of the female demibranchs where embryos are brooded to the larval stage. This is sometimes referred to as internal fertilization but, since the water tubes are part of the mantle cavity, and not the gonoduct, it is topologically outside the body and it is probably preferable to consider it to be external. The larva is the unique glochidium, an external parasite of fishes. Glochidia live only attached to the fish host and without the appropriate fish the life cycle cannot be completed.

Our freshwater mussels are imperiled by several human activities including habitat destruction (especially the impoundment of rivers), siltation from agricultural and construction runoff, organic and chemical pollution, competition with introduced exotics such as Asian clams and zebra mussels, extirpation of fish hosts for the larvae, and direct exploitation, originally for the button industry and now as a source of beadstock for the cultured pearl industry. Of about 300 unionoid taxa once known from North America 70% are Endangered, Threatened, or of Special Concern. Twenty one taxa are Endangered and possibly Extinct, 77 are Endangered and extant, 43 are Threatened, 72 are of Special Concern, 14 are Undetermined, and only 70 taxa, or 24%, are Currently Stable. Harvesting of mussels is not federally regulated except for species on the federal list of endangered or threatened species although many states regulate species and sizes that can be harvested.

Unionoid genera differ from each other in hinge morphology, hinge tooth morphology and development, shell thickness, the shell inflation, shell ornamentation, elaborations of the mantle margin, and use of the demibranchs for brooding. Species and genera are difficult to identify.

Laboratory Specimens

Many native North American freshwater mussels are threatened or endangered and are protected by state and federal legislation. Obviously, these species should never be used for dissection in teaching laboratories. Whenever possible alternatives to native unionoid mussels should be used for the study of bivalve anatomy. Clams (*Mercenaria mercenaria*), marine mussels (*Mytilus edulis*), and oysters (*Crassostrea virginica*) are often available alive and inexpensively from local fish markets and supermarkets, even in the interior of the continent. If locally collected living specimens of freshwater bivalves are desired, it is much better to use the abundant, widespread, invasive Asian clam, *Corbicula fluminea*. Dissection instructions for each of these species are available at ***Invertebrate Anatomy OnLine*** .

To avoid further damage to endangered and threatened populations native mussels should not be collected locally for laboratory use unless the instructor can identify the specimens to species. Federal legislations provide for substantial penalties for collection or possession of Threatened or Endangered species and some states require permits. Biological supply companies provide specimens of what we assume are species without conservation concern, classified as Currently Stable, for laboratory use. The actual identity of commercially supplied specimens varies and can only be known by identifying the specimens upon arrival at your institution. The catalogs advertise them as *Unio* or *Anodonta* but these names are used *sensu lato*, in the broad sense. Both genera, once large, have been split into several smaller genera and as the genera are currently defined, there are no species of *Unio* in the Americas.

Anodonta has thin shells without hinge teeth. Other genera, including genera such as *Actinonaias* and *Lampsilis* have thick shells and hinge teeth. Both types are marketed under the designation " *Anodonta* or *Unio*". This exercise was prepared using preserved, commercially supplied *Actinonaias* (marketed under the name " *Anodonta-Unio*") but can be used with fresh or preserved specimens of any unionoid mussel. The dissection is best conducted with the specimen covered with tap water although this may prove impracticable until both valves have been removed. A dissecting microscope should be used as needed.

Although the exercise is written to be used with preserved specimens, it can also be used with living or freshly killed animals. Living specimens are very difficult to open due to the ability of the adductor muscles to hold the valves tightly together indefinitely. Literature accounts suggest placing living mussels in warm tap water (55-60° C) for a few minutes. This is said to result in relaxation of the muscles and consequent gaping of the valves. Alternatively, mussels can be boiled or steamed briefly, until the valves gape open. Any procedure that kills the specimen is, of course, incompatible with later observation of ciliary activity on the gills, labial palps, and stomach walls or beating of the heart. Once the valves have separated, a sharp scalpel can be inserted between the valves to sever the anterior and posterior adductor muscles. Living specimens should be anesthetized in 5% non-denatured ethanol in water.

External Anatomy

Shell

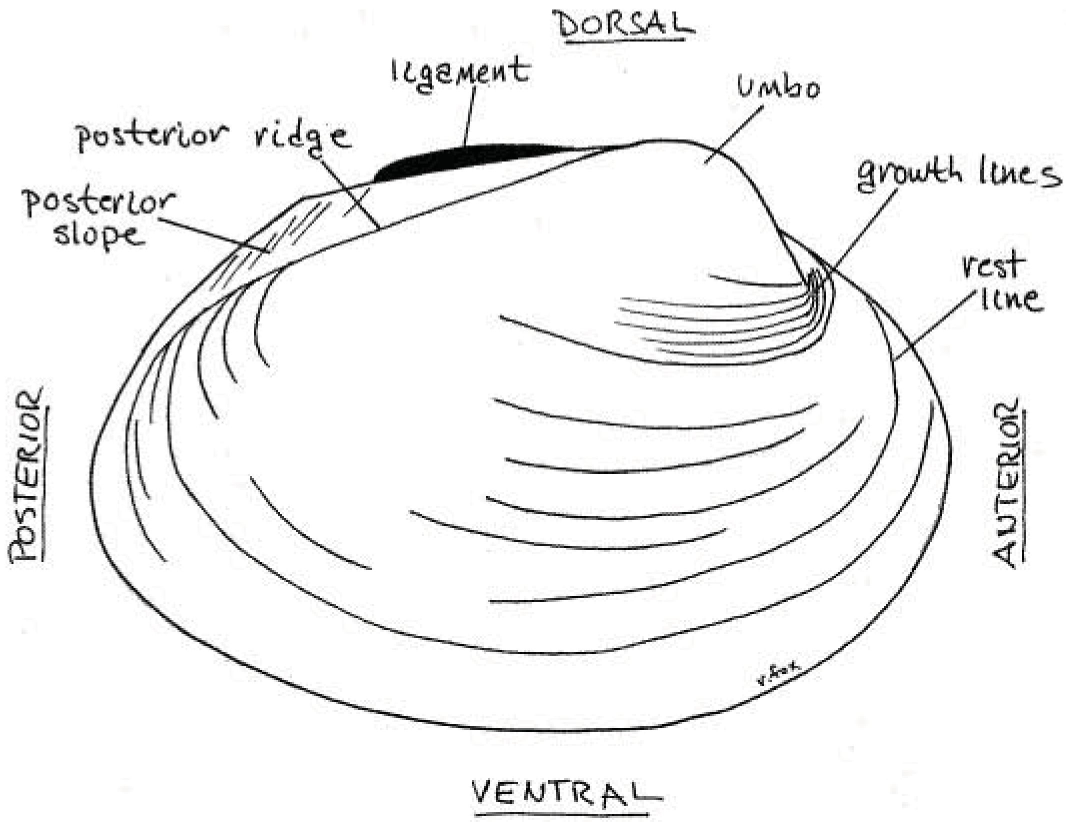
External Shell Features

Examine a cleaned dry shell from which the animal has been removed or, if such a shell is not available, an intact specimen with the animal still within the shell (Fig 1). The bivalve **shell** consists of two halves, or **valves**. The **plane of symmetry** passes between the two valves and divides the animal into right and left sides, which in most cases are more or less mirror images of each other.

The two valves articulate with each other along the dorsal midline via a **hinge** mechanism which may be simple and toothless (*Anodonta*, *Pyganodon*) or complex and toothed (most unionoids). The valves are held together along the hinge by a dark brown proteinaceous **hinge ligament** (Fig 1, 12-92B).

The valves can move laterally, away from each other, along the anterior, ventral, and posterior margins but are always in contact along the dorsal hinge. This lateral motion opens a space, known as the **gape**, along the anterior, ventral, and posterior margins and thus providing the animal with access to its environment. If you have an intact preserved specimen the gape will be present and obvious, although it will probably be larger than it would be in life. If you have a living specimen the valves will be held tightly together and there will be no gape.

Figure 1. External view of the right valve of *Actinonaias*. Mussel146L.gif



On each side of the hinge, anterior to the ligament, each valve displays a raised area known as the**umbo**, or beak (plural = umbones) (Fig 1, Fig 12-92B). The umbo is the oldest part of the shell. The two umbos almost touch across the midline. The umbo is anterior to the externally visible portion of the hinge ligament.

The anterior position of the umbo and its location with respect to the ligament can be used to recognize the anterior end of the clam without resorting to soft anatomy. Use the umbo to identify the **anterior** end of your mussel and the hinge to identify **dorsal**. Use these landmarks to find **ventral** and **posterior** and to determine which valve is the**right valve** and which is the **left valve**.

Relocate the **ligament** and note that it is outside the hinge and posterior to the umbo. In all freshwater bivalves the ligament is external. The posterior portion is obviously external and is clearly visible on the outside of the hinge even when the valves are closed. The anterior portion, although still external, is hidden when the valves are closed. The critical criterion being the position of the ligament with respect to the hinge teeth. If the ligament is outside the line of hinge teeth, it is external, if inside the line of teeth, it is considered to be internal. The ligament, being elastic, antagonizes the two adductor muscles and opens (abducts) the valves when the adductors are relaxed (Fig 12-92A). An external ligament is stretched when the valves are closed and pulls the valves apart when the adductors are relaxed. An internal ligament, on the other hand, is compressed when the valves are closed and pushes the valves apart when the adductors relax.

The two valves of a unionoid mussel are similar to each other, a condition known as **equivalve**. Some clams, such as the marine oysters and jingles, have very different right and left valves and are inequivalve. Furthermore, the anterior and posterior ends of freshwater mussels are more or less similar to each other and the valves are said to be **equilateral**. Equilateral valves are symmetrical on a dorsal ventral axis. In contrast, the two ends of some bivalves, such as the marine mussels (Fig 12-110B,E) and pen clams (Fig 12-110C) are very different, are not symmetrical, and are inequilateral although they are equivalve.

Numerous closely spaced, fine, concentric **growth lines** are visible on the outside surface of the valves. Larger, more widely spaced, concentric ridges are **rest lines**. An oblique **posterior ridge** extends from the umbo in a posteroventral direction to the posterior margin of each valve. In *Actinonaias* the ridge is inconspicuous but in some genera it is strong and well developed. The region of the valve posterior to the ridge is the **posterior slope**. Depending on species, a variety of spines, corrugations, rays, or pustules may ornament the valves externally but none of these is present in *Actinonaias*.

If you are using cleaned valves to study, go directly to "Internal Shell Features". If you have an intact specimen, skip to "Preview of Soft Anatomy".

Internal Shell Features

If you have a cleaned shell, its internal features can be studied now. If you have an intact specimen, you must wait until the shell has been opened and the animal removed before you can study the inside of the valves (Fig 2, 12-92B).

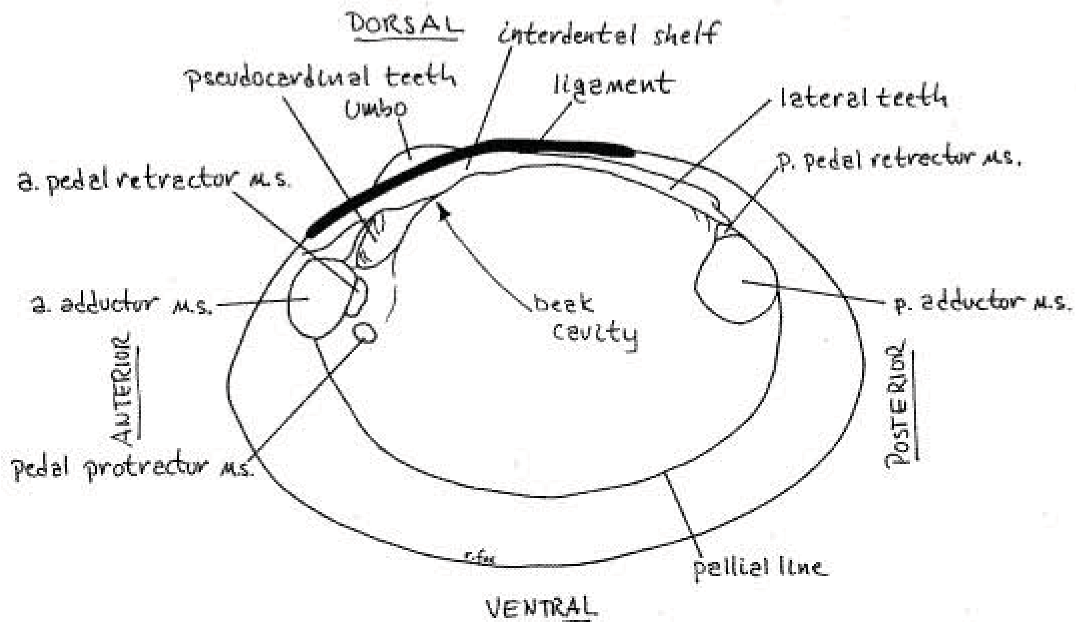
Relocate the hinge region along the dorsal margin of the valve. Find anterior and posterior, dorsal and ventral. The inside surface of each valve bears scars of the muscles that attached to it. Among these are the conspicuous **anterior and posterior adductor muscle scars** (Fig 2, 12-92B). The anterior scar is the smaller of the two. The **pallial line**, along which the pallial muscles of the mantle insert, is a conspicuous line or groove paralleling the margin of the valve from the anterior adductor muscle scar to the posterior adductor muscle scar. The mantle is attached to the valve along this line. The small **pedal protractor muscle scar** is located near the posterior ventral margin of the anterior adductor scar. Dorsal to the protractor scar are the **anterior pedal retractor muscle scars**. The **posterior pedal retractor muscle scar** is dorsal to the posterior adductor scar and may be continuous with it or slightly separated from it. These muscles originate on the shell and insert on the foot. Their action is to help extend the foot out of the gape (pedal protractors) or withdraw the foot into the mantle cavity (pedal retractors).

The **umbo** rises above the hinge and the **ligament** extends posteriorly from the umbo. Most unionoid hinges are equipped with **hinge teeth** to prevent shearing and keep the valves aligned when the powerful adductor muscles pull them together. The teeth are the pseudocardinal teeth and the lateral teeth. The teeth of the right and left valves are not symmetrical. Instead a tooth on one valve opposes, and fits snugly into, a depression on the opposite valve.

The jagged, massive **pseudocardinal teeth** are far anterior, near the anterior adductor muscle scar. *Actinonaias*, like most unionoids has one large pseudocardinal tooth in the right hinge and two in the left.

The **lateral teeth** are on the posterior end of the hinge and are long smooth ridges. The right hinge has one lateral teeth and the left hinge has two. The pesudocardinal teeth look a little like vertebrate teeth but the laterals do not. Compare the left and right valves to demonstrate to yourself the way the teeth fit together and prevent shearing.

Figure 2. Internal view of a right valve of *Actinonaias*. a. = anterior, p. = posterior, m.s. = muscle scar. Mussel147L.gif



Anterior lateral teeth are absent in mussels and some lack teeth altogether (*viz.* Anodontinae; *Anodonta*, *Pyganodon*). The Asian clam, *Corbicula*, and the sphaeriid clams (both Corbiculoidea[SF]) have true cardinal teeth preceded and followed by anterior and posterior lateral teeth, respectively. In unionoids true cardinal teeth are absent and the ancestral anterior laterals have been modified to form the pseudocardinals.

Mollusc shells are secreted in layers by the mantle epithelium. The three layers of the typical bivalve shell are present and well developed in mussels. All three layers are secreted by the mantle, each by a specific region of secretory epithelium.

The outermost layer is the proteinaceous **periostracum**. In unionoid mussels its color varies but is usually black, brown, or yellow (Fig 12-91). It may be patterned, often with rays. In older individuals the periostracum is often damaged or eroded and much of it may be absent, especially near the umbo.

The innermost layer of the shell, the one adjacent to the animal itself, is the **lamellar layer** (= hypostracum, Fig 12-91) composed of thin calcareous sheets, or lamellae, of **nacre** (pronounced NAKE ur), or mother of pearl, layered on top of each other. The shiny lustrous nacre is visible on the inside of the valve. Most of the inside surface of the valve, except for the extreme outer border, is covered by nacre. The color of the nacre varies with species.

The calcareous, white **prismatic layer** (= ostracum, Fig 12-91) lies between the periostracum and lamellar layers. It can be seen externally in areas near the umbo where the periostracum is eroded, exposing the white prismatic layer beneath it. It is composed of vertical prisms, polygonal in cross section.

The periostracum is impermeable to water and its presence protects the underlying calcium carbonate layers from dissolving and being eroded by the surrounding water. Its presence at the edge of the shell is essential for the continued secretion of new prismatic layer. It seals the margin and creates a chemically regulated extrapallial space in which new shell can be precipitated (Fig 12-91). In older areas of the shell, i.e. nearer the umbo, the protective periostracum is often broached and the prismatic layer may be exposed and subsequently eroded.

> **a.** With the dissecting microscope examine a broken shell of a mussel, or other large bivalve such as *Mercenaria*, and view the shell layers in cross section. The piece you examine should include the original edge of the shell. The **periostracum** forms a thin organic layer on the outer surface. The prismatic and lamellar layers are distinctly different in this view. The **prismatic layer**, which lies immediately below the periostracum, is composed of conspicuous, more or less vertical, closely spaced columns, or prisms, of calcium carbonate. As you know, the

prisms are vertical to the surface of the shell. The **lamellar layer** on the other hand consists of more or less horizontal sheets lying inside the prismatic layer. These lamellae are parallel to the surface of the shell. Note that the lamellar layer extends almost to the edge of the shell but that closest to the edge only the prismatic layer is present. The extreme edge is peripheral to the area where the lamellar layer is being secreted and consists entirely of recently secreted periostracum and prismatic layer with no lamellar layer yet present (Fig 12-91). <

Preview of Soft Anatomy

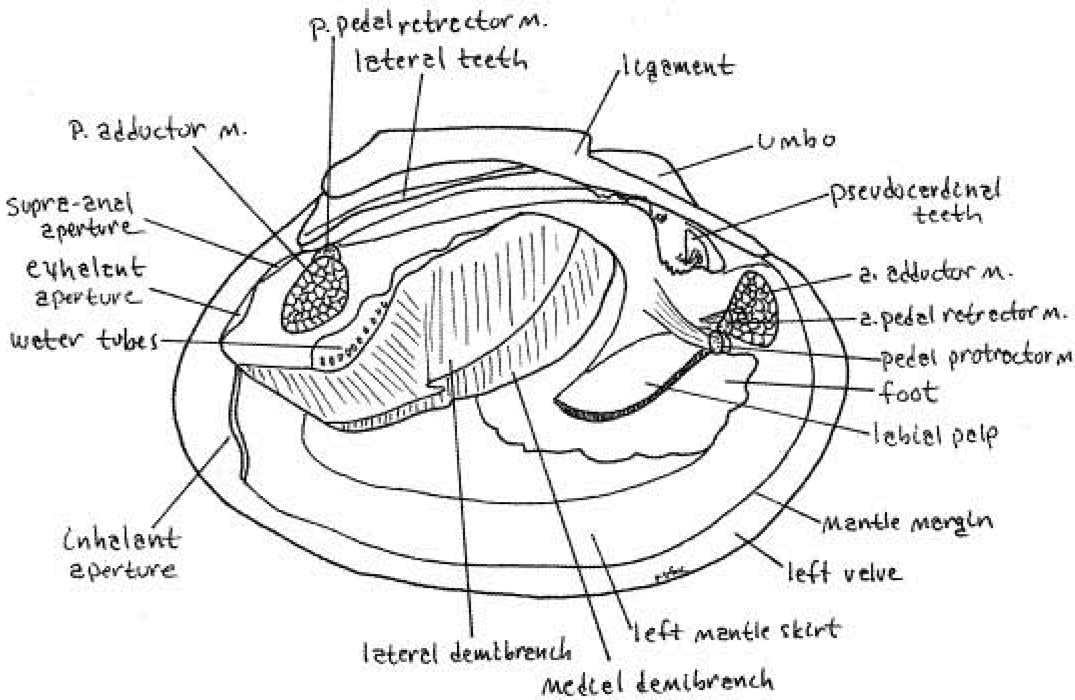
Commercially supplied preserved specimens will arrive gaping, usually with a wooden peg holding the valves apart in a wide ventral gape. The major features of the external soft anatomy can be previewed through this gape before a valve is removed. With living specimens this preview must wait until the adductor muscles have been cut. Look into the gape and note the most obvious features for use later as landmarks. You may have to force the valves a little farther apart to improve your view but, if so, be careful that you do not tear or otherwise damage the soft tissues.

The mantle, a molluscan apomorphy, is the dorsal body wall which typically exhibits modification in different ways in different classes. Under each of the two valves of a bivalve the mantle forms a thin sheet of soft tissue. These two sheets are the right and left **mantle skirts** (also known as mantle lobes or mantle folds by other authors) and they enclose the animal like a cloak (*i.e.* mantle) (Fig 12-90). The two skirts are joined to each other and to the body along the dorsal midline, under the hinge. Laterally and ventrally the space enclosed by the two skirts is the **mantle cavity**. In life it is filled with circulating water. The circulation is driven by cilia on the gills. Posteriorly the margins of the right and left mantle skirts conspire to form a pair of openings, the ventral **inhalant aperture** and the dorsal **exhalant aperture**. Species of Unionidae and Margaritiferidae have simple apertures rather than siphons. Among the Unionoida only Mycetopodidae, Iridinidae, and Hyriidae have siphons.

In the center of the mantle cavity is the laterally compressed, anteriorly directed **foot**, which in preserved or dissected living specimens will be strongly contracted with a wavy ventral edge. The large mass of tissue dorsal to the foot is the **visceral mass** in which are located most of the organ systems including gonad, kidney, heart, and gut

The bivalve **head** is weakly developed and lacks the concentration of sensory equipment typical of the heads of other molluscs. In bivalves most sensory receptors are located in the mantle margin, especially in the vicinity of the apertures.

Figure 3. **View of the external soft anatomy of the right side of an undissected specimen. The right valve and mantle skirt have been removed resulting in the partial opening of the right exhalant chamber and exposure of a few water tubes belonging to the lateral demibranch. Because this is a preserved specimen tissues are unnaturally contracted. a = anterior, m = muscle, p = posterior.** Mussel149L.gif



On each side of the visceral mass, between the mass and a mantle skirt, is a long, leaf-like gill. It may seem to you that there are two gills on each side but there is actually only one. At the anterior end of each gill there is a smaller, but also leaf-like labial palp. The palps resemble miniature gills and like them are double but there is actually only one on each side. Immediately anterior to the foot and labial palps is the **anterior adductor muscle** extending transversely from valve to valve. Hold the specimen so you can look into the posterior end of the mantle cavity. Here you will see the **posterior adductor muscle** extending across the cavity from valve to valve. The action of the two adductor muscles is to bring the two valves together (adduct the valves).

Soft External Anatomy

" After previewing the soft anatomy remove the right valve as follows. Identify the right valve and then look into the gape and find the anterior and posterior adductor muscles. With a scalpel or blunt probe scrape the two adductor muscles away from their attachment to the right valve. This will be easy to do in preserved specimens, in fact, the adductors may already be separated from their attachments. This process should also separate the protractor and retractor muscles from the valve. (The adductors of fresh specimens must be cut with a sharp scalpel. The posterior muscle should be cut immediately to the right of the rectum. Be careful that you do not cut any tissues other than the two muscles.)

Separate the margin of the right mantle skirt from the periostracum that attaches it to the right valve. Gently lift the right valve away from the left, leaving the right mantle skirt behind, with the remainder of the mussel. Use the blunt, flat handle of the scalpel as necessary to push, not cut, the mantle skirt away from the inner surface of the right valve. You will notice that the right mantle skirt is attached to the right valve, not only by the periostracum along its edge, but also by a line of pallial (pallial = mantle) muscles that parallels the valve margin. As you break the connection of the pallial muscles with the valve note that a distinct **pallial line** (Fig 2, !2-92B) marking the former attachment site is thereby revealed. All soft tissues, including the right mantle skirt, should remain cradled in the left valve. Break or cut the **ligament** and remove the right valve.

If you have not yet studied the inside of the shell go back to "Internal Shell Features" and do so using the right valve.

Muscles

Look at the body of the animal in the left valve. Return the right mantle skirt to its original position and inspect its outer surface. This is the surface that would normally lie adjacent to the inner surface of the right valve. Several muscles originate on the valves and pass through the mantle skirt to their insertion. Some are adductor muscles inserting on the other valve whereas others are pedal muscles inserting on the foot.

Find the large **anterior** and **posterior adductor muscles** (Fig 3). You have already seen their scars on the valves. These adduct the valves by bringing them together on the midline thereby closing the gape. No muscles antagonize the adductors, instead the elastic ligament opens the gape and stretches the adductors.

Unionoids also have a pair of pedal protractor muscles to assist in protruding the foot out of the gape and two pairs of pedal retractor muscles to withdraw the foot back into the mantle cavity before the adductor muscles close the gape. The protractors and retractors are antagonists and their origins and insertions must be arranged so they can have opposite actions. Both originate at scars on the inner surface of the valve (Fig 2) and insert on the lateral surface of the foot and visceral mass. Protractor origins (on the shell) are ventral to those of retractors whereas protractor insertions (on soft tissue) are dorsal to those of the retractors.

Near the postero-ventral corner of the anterior adductor find the right **pedal protractor muscle** (Fig 3). It is a small, but conspicuous, cord of muscle fibers extending obliquely posteriorly and dorsally from its scar on the right valve (Fig 2). Its origin on the valve is ventral to that of the pedal protractor muscle (Fig 2). You can see the muscle by lifting the mantle skirt to reveal the foot. It is usually easy to see and is often pale, either tan or white.

The right **anterior pedal retractor muscle** is near the pedal protractor but is a little harder to find. Its distal end (origin) is contiguous with the posterior edge of the anterior adductor muscle (Fig 3). The **posterior pedal retractor muscle** can be seen immediately dorsal to, and contiguous with, the dorsal edge of the posterior adductor muscle. It extends to the dorsal edge of the foot.

The mantle is attached to the inside of the valves by pallial muscles along the periphery of the mantle and by two small inconspicuous muscles extending from the dorsal mantle to the valve near the umbones.

Foot

The **foot** is a large, laterally compressed complex of muscles with a central hemocoel on the ventral midline of the visceral mass. It will be contracted in both preserved and fresh dissected specimens. In life it is capable of great extension, of which you see no hint in your preserved specimen. The ancestral bivalve foot was adapted for digging in soft sediments and that continues to be true of almost all North American freshwater bivalves, native and introduced, except *Dreissena* (Fig 12-105). The pedal retractor and protractor muscles originate on the shell and extend to their insertions on the foot.

Mantle Skirt

The two mantle skirts are lateral outfoldings of the mantle, or dorsal body wall of the visceral mass. They extend ventrally on either side of the mantle cavity, visceral mass, and foot and underlie the valves. The **right mantle skirt** should presently be uppermost in your preparation and should be hiding most of the remaining soft anatomy from view. Move the right mantle skirt dorsally to reveal the mantle cavity, foot, and visceral mass. This will also uncover the left mantle skirt lying intact against the left valve. Avoid disturbing the fragile (in preservative) connection between the left skirt and left valve.

Use the dissecting microscope (8X) and good illumination to examine the ventral margin of the left skirt and note that it consists of four distinct longitudinal folds or ridges extending along its entire length (Fig 4, 12-91). The parallel folds are separated by three parallel grooves. Use fine forceps and needles to demonstrate the presence of four side-by-side folds separated by three grooves. In most bivalves only three folds, inner, middle, and outer, are present but in unionoids one of the original three folds is divided into two folds to give a total of four.

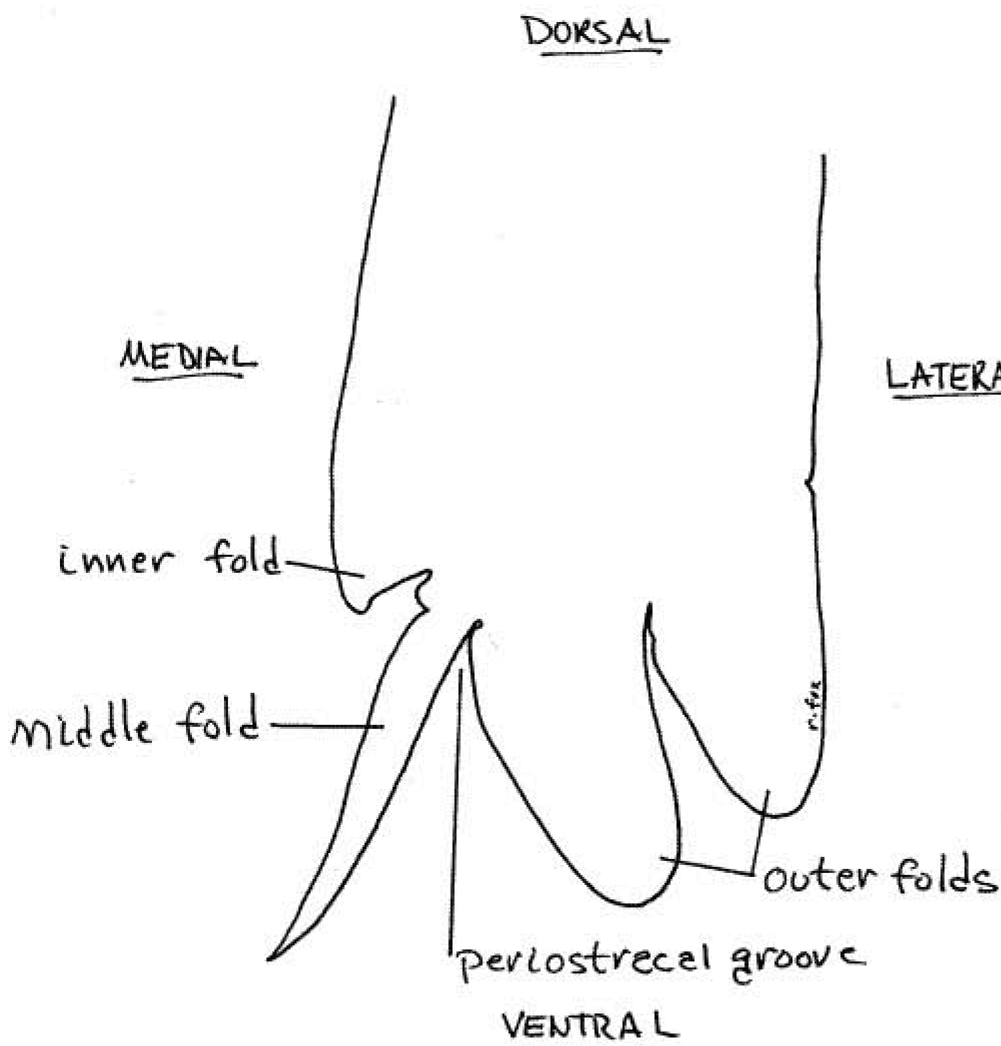
The thick, wide **inner mantle fold** can be recognized by the small papillae along its free edge. It is the muscular fold containing the radial pallial muscles that extend to the pallial line as well as longitudinal pallial muscles whose fibers parallel the mantle margin (Fig 12-91). It is not involved in secreting the shell. The groove separating this fold from the middle fold is shallow.

The next two folds are separated by the deep **periostracal groove**. The periostracum originates in this groove and in an intact specimen will still be connected here. New periostracum is secreted by the epithelium of the medial surface of the outer fold, deep in the groove. Look along the edge of the left mantle skirt for places where the skirt remains attached to the shell by a thin, transparent, yellowish-brown, cellophane-like **periostracum**. The periostracum emerges from the periostracal groove between the outer and middle folds. The attachment between periostracal groove and periostracum is very fragile, and easily destroyed in preserved specimens. It may not be intact in your specimen. The attachment is robust in fresh specimens.

With forceps tug the periostracum out of the periostracal groove without damaging the edge of the skirt. Now, with magnification, examine the edge of the left skirt in a region from which the periostracum has been removed.

The thin **middle mantle fold** lies close to the outer mantle fold (once the periostracum has been removed) and the two may appear to be a single fold. Use a microneedle to tease the two folds apart and demonstrate the deep periostracal groove between the outer and middle folds. The periostracum is secreted by secretory cells of the outer fold at the bottom of this groove. The middle mantle fold is sensory.

Figure 4. Cross section of the ventral margin of the mantle skirt. Mussel148L.gif



The thick **outer mantle fold** (the one closest to the shell) is glandular and its outer epithelium secretes the prismatic layer of the shell. In unionoids the outer fold is actually composed of two folds. The entire outer surface of the mantle skirt secretes the lamellar (nacreous) layer of the shell. The mantle folds are not involved in secretion of the lamellar layer.

At some point during the dissection the weakened (by preservative) attachments between muscles and shell in preserved specimens may fail so that the mussel falls out of the left valve. You may take advantage of this, if you wish, to view all aspects of the external anatomy without the interference of a valve. Be sure you are always able to replace the animal in its correct position and orientation in the left valve.

Visceral Mass

The large central part of the body is the **visceral mass** (Fig 3, 12-90). The foot occupies its median ventral border and the two mantle skirts arise from its dorsal margin. Most of the visceral organs, including the heart, kidney, gut and digestive ceca, and gonads, of a mollusc are contained within the visceral mass. The pigmented tissues of some these organs can be seen through the body wall at present but will be considered in more detail later.

The **pericardial cavity** occupies the extreme dorsal edge of the visceral mass between the posterior adductor muscle and the umbo. It is covered dorsally by a thin region of the body wall. This body wall is usually opaque in preserved specimens but may be translucent in fresh material. The pericardial cavity is the much reduced bivalve coelom, the chief body cavity of molluscs being the hemocoel. The heart, consisting of two atria and a ventricle, is located in the pericardial cavity, as is a portion of the rectum. (If you are dissecting a living mussel the beating heart may be visible inside the thin body wall and pericardium.) Later in the dissection the pericardium will be opened to reveal its contents.

The brown **kidney** lies close to the surface of the visceral mass lateral, anterior, and posterior to the pericardial cavity (Fig 12-89B). The greenish digestive cecum (= digestive gland) shows through the body wall of the anterior visceral mass just posterior to the anterior adductor muscle. The yellowish gonad is situated in the central region of the mass dorsal to the foot (Fig 12-89B).

Mantle Cavity

Chambers

The two mantle skirts enclose a large water space known as the **mantle cavity**. The gills form the roof of this chamber. In life position the mantle cavity is in communication with the external environment via two apertures, the inhalant and exhalant apertures (Fig 12-89A).

On each side of the visceral mass and foot, a gill divides the mantle cavity into a ventral inhalant chamber (= branchial chamber) and a dorsal exhalant chamber (= suprabranchial chamber, epibranchial chamber, cloacal chamber, anal chamber). The gills are the floor of the exhalant chamber and roof of the inhalant chamber. The **inhalant chamber** is currently visible to you as the large space into which the gills protrude (Fig 3, 12-89A). Most of the exhalant chamber, however, is currently hidden. Looking into the exhalant aperture the space you see is the posterior end of the **exhalant chamber**. Water enters the inhalant aperture and flows into the inhalant chamber. It then passes through minute ostia in the gills to enter the exhalant chamber from which it exits through the exhalant aperture. The mantle cavity is a space outside the body of the mussel even though parts of it appear to be internal. It contains a current of river water that circulates through the mussel.

Apertures

In unionoids the two mantle margins are largely independent of each other and are not fused except posteriorly where they form two apertures to channel water in and out of the mantle cavity. Together the right and left posterior mantle margins form the ventral inhalant aperture and the dorsal exhalant aperture (Fig 3, 12-106, 12-89). The right and left mantle skirts are fused together dorsal and ventral to the **exhalant aperture**. The exhalant aperture opens from the exhalant chamber of the mantle cavity to the outside.

The **inhalant aperture**, although much larger than the exhalant, is not so obvious in a gaping specimen in which the mantle edges do not touch. It is formed by thickened pads on the margins of the right and left mantle edges. When the valves are close together, so are these pads and they then form an opening, the inhalant aperture. Push the right and left posterior mantle edges together recreate the inhalant aperture. The dorsal margin of the inhalant aperture is formed by the fusion of right and left mantle skirts which also forms the ventral margin of the exhalant aperture. In contrast, there is no fusion of tissues to form the ventral margin of the inhalant aperture. The inhalant aperture is equipped with short chemosensory **papillae** but these will be contracted in preserved specimens. The exhalant aperture has no sensory papillae. The inhalant aperture opens from the outside into the inhalant chamber (= branchial chamber) of the mantle cavity.

By looking into the exhalant aperture you can see the posterior adductor muscle passing transversely across the exhalant chamber. The tubular **rectum** can be seen on the midline of the posterior adductor muscle. The rectum ends there at the **anus**, which you can also see by looking in the exhalant aperture.

Look dorsal to the posterior adductor at the junctions of the margins of the right and left mantle skirts. In most unionoids (*viz*, Ambleminae [sF] and Unioninae [sF] but not Margaritiferidae [F]) there are one or more **supra-anal apertures** between the two skirts. These openings are auxiliary exhalant apertures dorsal and anterior to the exhalant aperture. Use a blunt probe to demonstrate the continuity of the supra-anal aperture(s) with the exhalant chamber.

Gills

Observe that each gill (there is one on each side) is composed of two half-gills known as **demibranchs**. A whole gill is a **holobranch**. Each holobranch is derived from a single bipectinate gill of the ancestral bivalve and thus should still be thought of as a single gill. The mussel has two holobranchs (one right and one left) composed of two demibranchs each for a total of four demibranchs. Each holobranch consists of a **lateral demibranch** adjacent to the mantle skirt, and a **medial demibranch** adjacent to the visceral mass and foot (Fig 3, 12-90). The holobranch is attached to the roof of the mantle cavity by a longitudinal **central axis**. The central axis lies between the two demibranchs.

" Use a pair of scissors to remove the right mantle skirt with a longitudinal incision between the dorsal margin of the right lateral demibranch and the right mantle skirt. Start the incision immediately ventral to the posterior adductor muscle and avoid cutting into the region of the apertures. Extend the incision anteriorly and then dorsal to the labial palps and remove the skirt. This incision will open the exhalant chamber but the gill and labial palp will remain with the body. Modify the incision if necessary so that the exhalant chamber is open along the entire length of the gill. Do not open the pericardial cavity at this time.

Bivalve gills are composed of numerous slender gill filaments joined together to form sheets. Use 30X of the dissecting microscope to look at the surface of the lateral demibranch and you will see the very fine parallel **gill filaments** (Fig 12-96D). Each filament begins at the central axis, drops down into the inhalant chamber then reverses direction sharply and climbs back up to the roof of the chamber where it attaches beside (lateral or medial) to the central axis. Each filament is attached to the filament anterior to it and the one posterior to it. Collectively all the filaments are joined together form a sheet, or lamella. Each demibranch is composed of two lamellae (Fig 12-96D). One, the **descending lamella** is composed of the filaments that drop down from the central axis (Fig 12-90). The other, the **ascending lamella**, is composed of the same filaments on their way back up to the top of the inhalant chamber.

Look at the lateral demibranch of the right gill. It should be facing you. The surface you see is the **ascending lamella of the lateral demibranch**. Lift the demibranch and look at its other side. This is the **descending lamella of the lateral demibranch**. Look at its dorsal edge to see the **central axis**. With the lateral demibranch held up and out of the way you are looking at the **descending lamella of the medial demibranch**. It arises at the central axis. Finally, lift the medial demibranch and look at its medial surface. This heretofore hidden surface is the **ascending lamella of the medial demibranch**. Got it? Two holobranchs, two central axes, four demibranchs, eight lamellae in one mussel.

Most bivalves, unionoids included, use their gills for suspension feeding as well as gas exchange. Both functions require that water (with dissolved oxygen and suspended food particles) enter the inhalant chamber, pass through tiny openings in the gill lamellae (between the filaments) into the exhalant chamber and then out the exhalant aperture. Oxygen and food particles are removed as the water passes through the lamellae. Adjacent filaments are held together by interfilamentary junctions but these are not unbroken continuous connections (Fig 12-96D). Gaps in the junctions are known as ostia and provide a route for water to flow through the lamellae.

" With fine scissors cut a 2x2 mm square of the ascending lamella and rinse both sides of it with a vigorous stream of water from a squeeze bottle. Make a wetmount and examine it with 400X of the compound microscope. Most of what you see will be **filaments**. Tissue **interfilamentary junctions** holding the filaments together will also be visible. The clear areas between the filaments, where the junctions are interrupted are **ostia**. Focus carefully on the edges of the filaments to see the **lateral cilia** extending from the filament into the ostia (Fig 12-99B, 12-98). These are the cilia that generate the feeding/respiratory current through the system. Other cilia, known as frontal cilia, are on the inhalant surfaces of the lamellae and are responsible for moving food particles and mucus over the gill. Frontal cilia may be difficult to see in this preparation.

Look again at the surface of the ascending lamella of the lateral right demibranch. Large evenly spaced ridges extend across the demibranch parallel to the filaments. These are tissue **interlamellar junctions** responsible for holding the ascending and descending lamellae of the demibranch together. The interlamellar junctions divide the exhalant chamber into vertical **water tubes** (Fig 3, 12-98C, D) that arise in the demibranch and empty into the exhalant chamber. We have seen that the lamella appears finely corrugated because it is composed of filaments but coarser corrugations are also present and these are the water tubes. The demibranch was opened in the vicinity of the exhalant aperture earlier when you cut away the right mantle skirt. Find the openings to the water tubes inside the lateral demibranch (Fig 3). Note that the tubes open into the exhalant chamber.

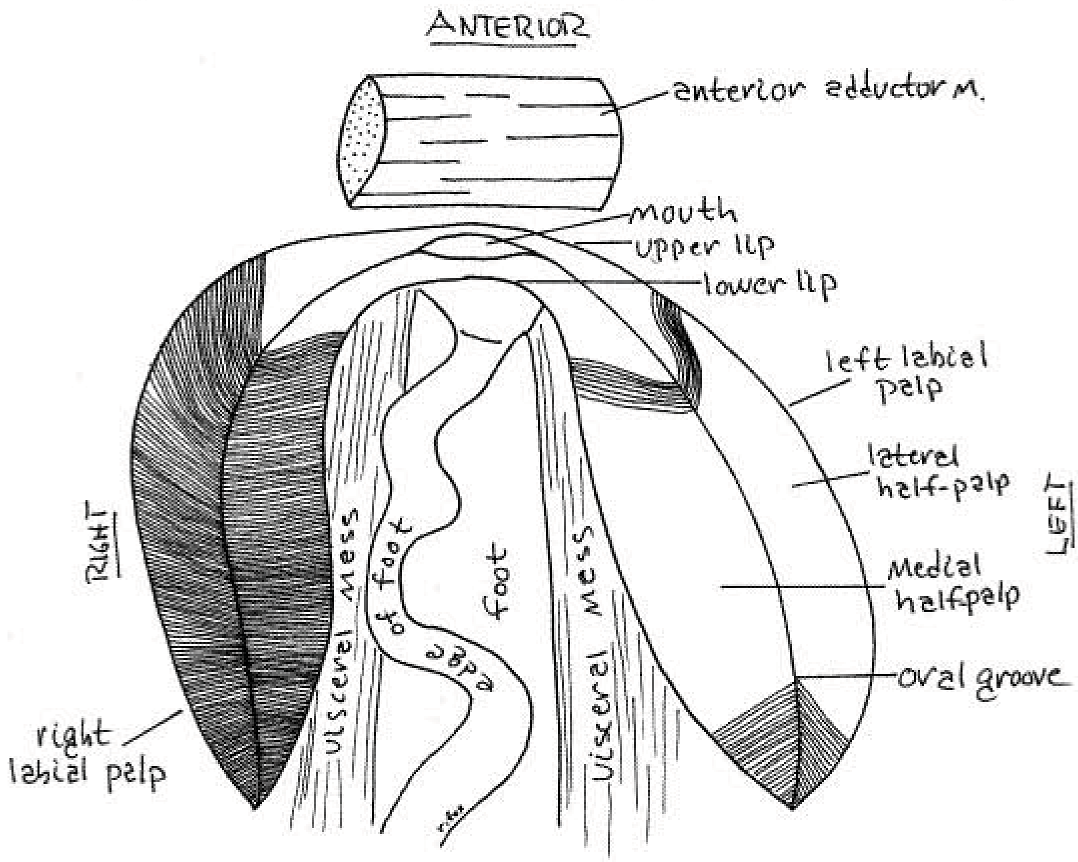
The kidneys and gonad connect via through separate ducts with openings in the anterior end of each exhalant chamber. Both are small slits of which the nephridiopore is slightly lateral to the gonopore.

Female unionoids retain their eggs and brood them in the water tubes. Note if eggs, embryos, or larvae are present and if so return later during your study of reproduction to make a wetmount of them.

Labial Palps

A large, leaflike labial palp is located on each side of the visceral mass and gill at the anterior end of the mantle cavity (Fig 3, 12-112A). Each palp resembles a small gill and like the gill is bilobed, being composed of two similar, demibranch-like half palps. The lateral half-palp is associated with the lateral demibranch of the whereas the medial half-palp corresponds with the medial demibranch.

Figure 5. Ventral view of the anterior end of the foot and visceral mass. The palps are opened to reveal the sorting fields. Most of the ciliated ridges of the left palp have been omitted. Mussel150L.gif



Find the right labial palp and lift the lateral half-palp to expose the medial half-palp (Fig 5). Particles and mucus are transported by ciliary currents from each demibranch to the associated half-palp for sorting. Examine the now-exposed surfaces of the half-palps with magnification to see that they are composed of an array of ciliated ridges and grooves. These form a ciliary sorting field across which a string of mucus and food particles from the gills passes on its way to the mouth. While crossing the palpal sorting field mineral particles are imperfectly separated from organic food particles. The mineral particles drop off the edges of the palp into the inhalant chamber as pseudofeces whereas food particles continue on to the mouth. In life the ciliated, ridged surfaces of the two halves of the palp face each other and the sorting field is not visible from the outside. In the crease where the two half-palps join is a ciliated oral groove that goes to the mouth (Fig 12-94D). Mineral particles are transported laterally, away from this groove and food particles move along it.

If the mussel is still in the left valve, remove it and set the valve aside. Hold the mussel so you can view the anterior end of the visceral mass and foot with magnification (Fig 5, 12-100). Open the right palp so the sorting fields of both half-palps are completely exposed. Manipulate the mussel so you can trace the anterior ends of each half palp anteriorly toward the midline. The anterior end of the lateral half-palp tapers to a narrow ridge of tissue that extends anteriorly to cross the midline where it joins the similar anterior extension of the left lateral half-palp. The anterior extension of the right medial half-palp similarly joins with the left medial half-palp. These two ridges cross the anterior midline of the visceral mass immediately dorsal to the foot and ventral to the anterior adductor muscle. Located between them is the large, open mouth (Fig 5, 12-89B). The ridge connecting the lateral half-palps is dorsal to the mouth and forms the upper lip. The lower lip connects the two medial half-palps below the mouth. The mouth is much easier to see in unionoids than in most other bivalves.

Internal Anatomy

Nervous System

The central nervous system is typical of bivalves and consists of paired ganglia connected by commissures and connectives (Fig 8, 12-119). In life the ganglia contain neuroglobin which, if it has not faded in preservative, imparts a rusty orange, brownish, or yellow color and makes them easier to locate.

The large visceral ganglion is easily found making it a good starting point for dissection of the CNS. The paired visceral ganglia are fused together on the midline to form what appears to be a single ganglion. It is on the ventral margin of the posterior adductor muscle and may or may not be visible through the thin epithelium covering the midline of the ventral surface of this muscle. Remove this epithelium to expose the ganglion. Three major pairs of nerves exit the ganglion. A pair of conspicuous cerebrovisceral connectives (= visceral nerves) connect it with the cerebral ganglia. The connectives can be traced anteriorly into the visceral mass for a short distance but anteriorly they are embedded in the mass and cannot be seen without careful dissection. A pair of large posterior pallial nerves from the visceral ganglia serve the posterior mantle margin (Fig 12-119, 12-91). A pair of large branchial nerves from the visceral ganglion extend to the gills. The heart is served by a pair of small nerves from the visceral ganglion.

The smaller cerebral ganglia are situated anteriorly, one on each side of the mouth (Fig 8). They are more difficult to locate than the visceral ganglia. Find the mouth and the anterior pedal protractor muscle and pedal protractor muscle where they emerge from the visceral mass. The lips of the labial palps pass over this area which is separated from the lateral corners of the mouth by a few millimeters. The ganglia are not immediately adjacent to the mouth. Carefully remove the thin body wall from the area just described to reveal the ganglion. The pleural ganglia are fused with the cerebral ganglia and cannot be distinguished from them in gross anatomy.

The two cerebral ganglia are connected by the slender cerebral commissure which arches dorsally over the mouth. A cerebropedal connective extends from each half of the cerebral ganglion to the pedal ganglion. An anterior pallial nerve extends longitudinally along the anterior mantle margin after arising from the cerebral ganglia. Each half of the cerebral ganglion sends a nerve to the anterior adductor muscle.

The two pedal ganglia are fused on the midline in the dorsal edge of the foot, embedded in the foot muscles (Fig 8). They can be exposed only through a careful dissection beyond the scope of this exercise. They connect with the cerebral ganglia by the cerebropedal connectives mentioned above. The short pedal commissure is embedded between the two contiguous pedal ganglia and cannot be seen in gross view. Together these elements (cerebral ganglia, cerebral commissure, cerebropedal connectives, pedal ganglia, and pedal commissure) form a nerve ring around the anterior gut (esophagus). Motor nerves from the pedal ganglion the muscles of the foot. A pair of spherical statocysts is located lateral to each pedal ganglion.

The peripheral nervous system consists of sensory and motor nerves extending to and from the above ganglia. The most important have been mentioned.

Hemal System

The bivalve hemal system consists of a heart, arteries, veins, and a hemocoel consisting of large, blood-filled sinuses. The blood is colorless. The heart is located in the dorsal pericardial cavity mentioned earlier, and is easily demonstrated. The remainder of the hemal system is difficult to see in these specimens.

Relocate the thin dorsal body wall on the dorsal midline just anterior to the posterior adductor muscle. This part of the body wall covers the pericardial cavity (Fig 3, 12-89B). Use fine scissors to make a longitudinal incision through this thin body wall to open the pericardial cavity. The incision should extend from the posterior adductor muscle anteriorly to near the posterior edge of the umbo. Be careful that you cut only through the thin body wall and that you do not damage the organs within the pericardial cavity.

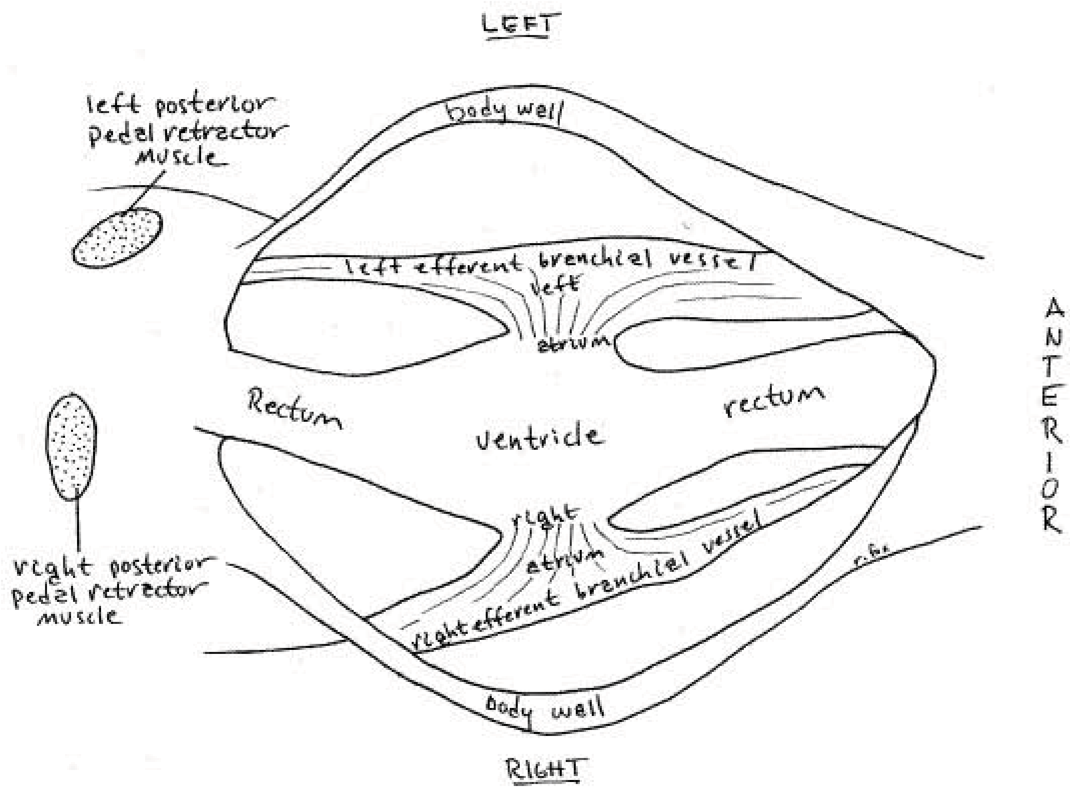
The relatively large space this incision exposes is the **pericardial cavity** lined by a peritoneum known as the pericardium. It is a coelomic space and contains the heart and rectum. A large tube, the **rectum**, enters the cavity anteriorly, extends the longitudinally for length of the cavity, and then exits posteriorly to end at the anus on the posterior margin of the posterior adductor muscle (Fig 6, 12-89B). The rectum passes through the center of the heart ventricle.

The **heart** consists of a single medial ventricle and two lateral atria (= auricles), one on either side of the ventricle. The atria and ventricle are both contractile.

The large and conspicuous **ventricle** is wrapped around the rectum and is easy to find, although its relationship with the rectum may surprise you. Blood exits the ventricle via an unpaired median anterior aorta lying on the dorsal surface of the rectum, and an unpaired median posterior aorta on the ventral surface of the rectum. The aortae are difficult to demonstrate. The anterior aorta supplies the visceral mass, anterior adductor muscle, gonad, foot, anterior mantle, and kidney. The posterior aorta supplies the posterior adductor muscle, pericardium, rectum, and the posterior mantle. The venous return consists of veins and sinuses ultimately draining into the vena cava, a large sinus ventral to the pericardial cavity and between the two kidneys. Blood from the vena cava goes to the gills via the afferent branchial vessels where. Oxygenated blood leaves the gills via the efferent branchial vessels to the atria. The atria drain into the ventricle.

Gently pull the ventricle and rectum to the side, away from the body wall while watching under magnification. This will enhance your view of a thin, triangular, membranous **atrium** connecting the side of the ventricle with the membranous efferent branchial vessel from the right (or left) gill.

Figure 6. Dorsal view of the opened pericardial cavity. Mussel151L.gif



Use fine scissors to open the right side of the ventricle with a longitudinal incision along the entire ventricle. This will open the **ventricular lumen** for study. Observe the thick muscular walls of the ventricle. Notice the conspicuous tubular **rectum** passing longitudinally through the lumen. Look on the right side of the ventricle for an **atrioventricular aperture** between the atria and ventricle. The aperture is guarded by an **atrioventricular valve** consisting of two thin, membranous, longitudinal folds of tissue arranged like lips above and below the aperture. Although large, these lips can be difficult to distinguish from the muscular ventricular walls and from each other. Use fine forceps and a microneedle to probe the tissues and demonstrate the lips. The two lips form a one-way valve that prevents the backflow of blood from the ventricle to the atria. You can see, by examining the configuration of the lips, how back pressure on the membranes would close the aperture.

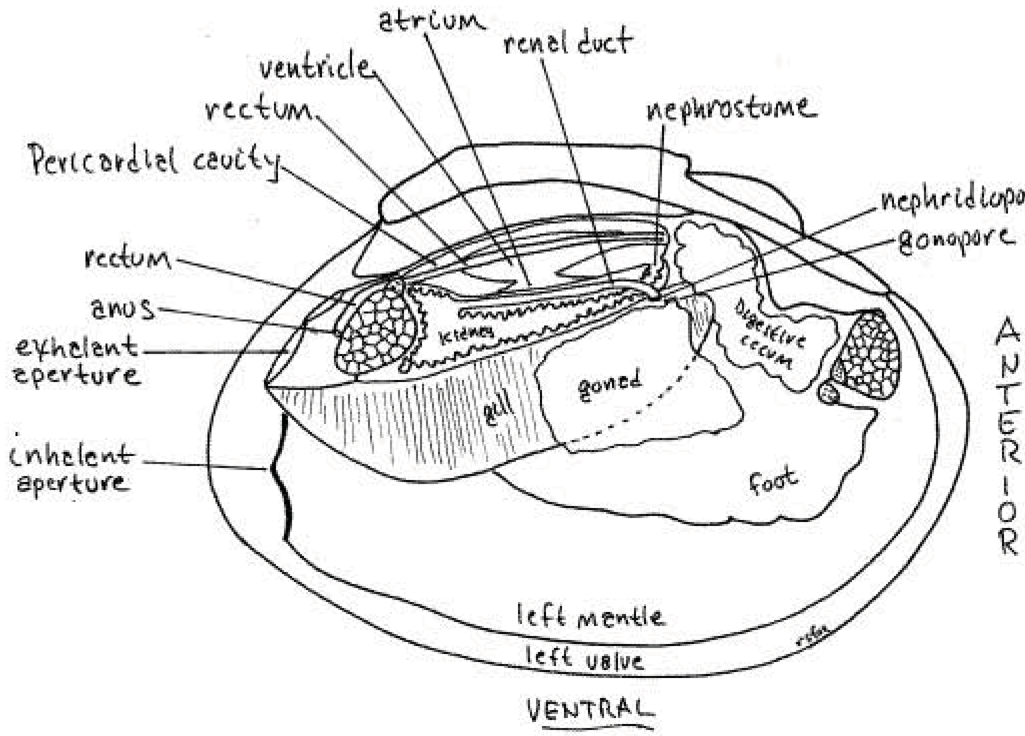
Contractions of the atria propel blood through the atrioventricular aperture into the ventricle. Subsequent contraction of the ventricle closes the atrioventricular valve and forces blood into the anterior and posterior aortae.

Excretory System

The bivalve excretory system consists of a pair or large, elaborate metanephridia, or kidneys, draining the pericardial cavity to the exhalant chamber (Fig 12-89, 12-118). Remember that the pericardial cavity is a coelom. Each kidney is an elaborate tube extending from the nephrostome, opening from the pericardial cavity, to the nephridiopore, opening into the exhalant chamber. A special elaboration of the atrial wall, equipped with podocytes, is specialized for ultrafiltering the blood into the pericardial cavity to form the primary urine. The primary urine enters a nephrostome and passes through the kidney to the nephridiopore. During passage through the kidney lumen the primary urine (ultrafiltrate) is modified, chiefly by reclamation of solutes, and the resulting final urine, mostly water, is released from the nephridiopore into the exhalant chamber. The role of the kidneys in freshwater bivalves, living as they do in a hyposmotic environment, is chiefly osmoregulatory. The kidneys constantly pump excess water out of the tissues and back into the environment. Ammonia, the chief end product of nitrogen metabolism, is lost by diffusion across the mantle and gill surfaces.

Each of the two **kidneys** (= metanephridium, organ of Bojanus, renal organ) is a large sac located ventral, lateral, and posterior to the pericardial cavity (Fig 7). With fine scissors make a longitudinal cut through the body wall along the dorsal margin of the right gill and ventral to the pericardium.

Figure 7. Dissected unionoid mussel viewed from the right side showing the pericardial cavity and kidney. The gill and visceral mass are drawn as if transparent. Mussel152L.gif



The kidney is recurved on itself and open at both ends (Fig 7, 12-120C, 12-118). Proximally it is a large spacious glandular region that opens from the anterior pericardium via the nephrostome. The distal, downstream end of the kidney is a non-glandular renal duct that opens into the anterior exhalant chamber via the nephridiopore. The ventral wall of the pericardial cavity is adjacent to the dorsal wall of the glandular portion of the kidney. If your incision was deep enough it will have opened the glandular kidney and revealed its lumen. The walls have a glandular appearance and consist of dark brownish gray tissue. The nephridiopore is a slit-like opening at about the level of the middle of the foot but it is not easy to demonstrate. The gonopore, either male or female, is nearby, slightly medial to the nephridiopore.

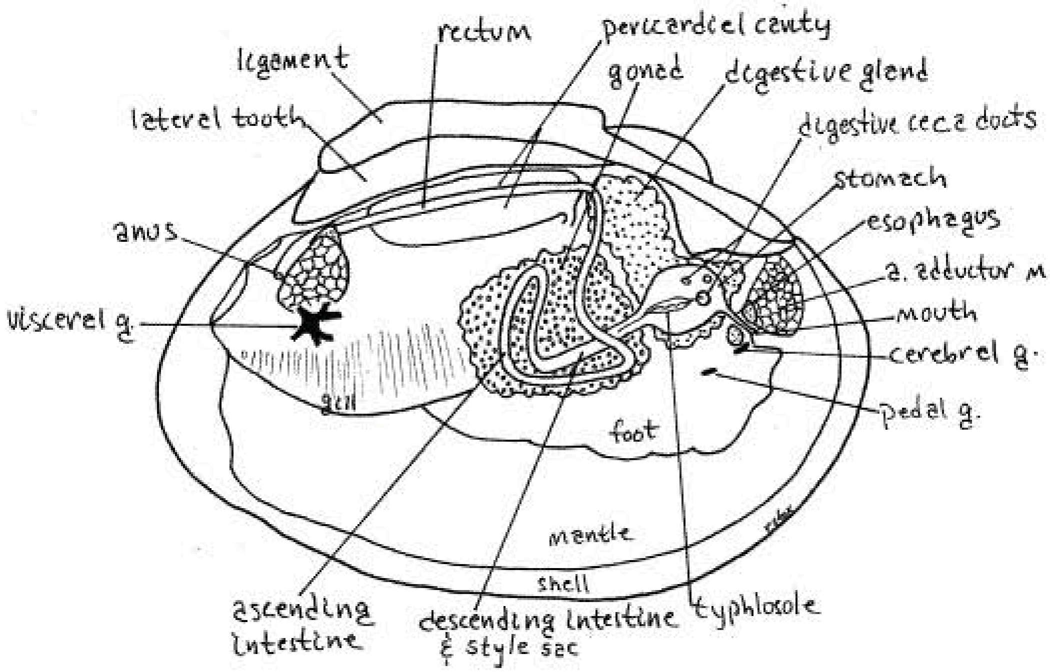
The two pericardial glands (= Keber's organs) are elaborations of the pericardial peritoneum equipped with abundant podocytes where ultrafiltration occurs (Fig 12-118). These brown organs can be seen through the body wall at the anterior end of the pericardial cavity. They receive blood from the efferent branchial vessel and ultrafilter it into the pericardial cavity to form the primary urine.

Reproductive System

Anatomy

Unionoids are gonochoristic with external cross fertilization. Some sexual dimorphism is present but it is usually not conspicuous (the shell of females of some species is more inflated than males to provide the space for brooding). The reproductive system consists of a single gonad, either ovary or testis, and two short gonoducts. The gonad, although a derivative of the coelom, is independent of the pericardial cavity. It does not share ducts with the kidney (Fig 12-120C). It arises in bivalve evolution through the fusion of an ancestral pair of gonads. The two gonoducts, right and left, emerging from a single gonad, reflect this double origin. The gonad is large and occupies the space in the visceral mass extending ventrally from the kidney to the dorsal edge of the foot (Fig 8, 12-89B). Each gonoduct empties into the anterior exhalant chamber via a gonopore.

Figure 8. Dissected *Actinonaias* showing the gonad, digestive system, and ganglia. The star-shaped visceral ganglion is shown a little larger than life size. A= anterior, g = ganglion, m = muscle. Mussel99L.gif



The shapeless gonad can be exposed by cutting away the thin body wall from the side of the ventral visceral mass at the top of the foot. It fills the space in the ventral visceral mass and is dorsal to the muscular foot.

Life Cycle

Females brood eggs and embryos to the glochidia larva stage in the water tubes (Fig 12-121C, D). Females shed eggs into exhalant chamber from which they move into the water tubes of some or all demibranchs. In some (*viz. Amblema, Quadrula, Fusconaia, Gonidea, Tritogonia, Plectomerus, Quincuncina,* and *Megalonais*) both demibranchs on each side are used as brood chambers and are visibly swollen in brooding females but in the remaining Unionidae, including *Actinonaias*, *Lampsilis*, and *Anodonta*, only the lateral demibranchs are so used.

Sperm are shed into the river (or lake) from the exhalant aperture of a male individual. If caught in the inhalant flow of a female mussel, they pass through the ostia to enter the water tubes of the demibranchs. The waiting eggs are fertilized and retained in the water tubes where development begins and continues to the glochidia larva stage.

Glochidium Larva

Females release glochidia larvae through the exhalant aperture (Fig 12-121E). Glochidia are modified veliger larvae and are parasitic, requiring a fish host before metamorphosing into a juvenile mussel capable of independent existence. Glochidia parasitize and feed on a fish host before dropping off into the sediment to metamorphose into a juvenile mussel.

> b. Inspect the water tubes for the presence of glochidia larvae. If present, make a wetmount with a wax supported coverslip and study it with the compound microscope. Glochidia are derived bivalve larvae with a pair of **valves**, **adductor muscle**, sensory bristles, and a larval thread (Fig 12-121C). Species that parasitize the skin of their fish hosts also have a conspicuous hook at the ventral border of each valve (Fig 12-121C). Species that attach to the gills lack the hooks. <

Digestive System

The digestive system consists of mouth, esophagus, stomach with digestive ceca, intestine, and anus. Remove the mussel from both valves if you have not already done so. The large mouth is on the midline at the anterior end of the visceral mass flanked by a labial palp on each side (Fig 5, 8, 12-89B). Hold the mussel with one hand and examine the anterior midline of the visceral mass to find the mouth.

The mouth opens into the short esophagus which extends posteriorly to the stomach. Insert a blunt probe into the mouth and look into the esophagus while holding the mussel on the stage of the dissecting microscope.

With the mussel in a small dissecting pan of water on the stage of the dissecting microscope use fine forceps to dissect away the thin body wall posteriorly from the right side of the mouth. You may already have done some of this to expose the right cerebral ganglion. Removal of the body wall will expose the esophagus, which is a short but wide, very flat, thin-walled tube about the width of the mouth (Fig 8). With fine scissors open the esophagus posteriorly to the stomach. The esophagus is lined by a ciliated epithelium. Further dissection of the digestive system is destructive and should not be attempted until all other organ systems have been studied.

Extend the esophageal incision posteriorly to open the anterior stomach. Initially you need cut only the thin gut wall but to open the posterior stomach you must cut through a thick layer of the wall of the visceral mass.

The stomach is surrounded by the two greenish digestive ceca which can usually be seen, without dissection, through the surface of the visceral mass posterior to the anterior adductor muscle (Fig 8). The ceca are diverticula of the stomach to which they remain connected by ducts opening from the stomach walls (Fig 12-103B).

The **stomach** is a large chamber in the anterior dorsal visceral mass (Fig 8, 12-102). With magnification examine the anterior folded walls of the stomach. Anteriorly are four **openings to the digestive ceca**. The largest opening is low on the left wall, another is to the right of it, and two others on the dorsal wall. All these openings are in the anterior half of the stomach. Insert one blade of your fine scissors one of the apertures and open the duct with which it connects. This duct will soon enter a greenish **digestive cecum**. The ceca walls are elaborated to form abundant **acini**, or pouches, lined with secretory and absorptive epithelium (Fig 12-103B). Absorption and most digestion, both intra- and extracellular takes place in the ceca. Indigestible particles are returned to the stomach. Some extracellular digestion takes place in the stomach.

Much of the wall of the stomach bears the fine but conspicuous, parallel, ciliated ridges and grooves of **sorting fields** whose function is to separate incoming particles and send organic particles to the digestive ceca and mineral particles to the intestine.

On the ventral stomach wall is the large protuberant ciliated **typhlosole** whose role is shunt mineral particles wasted from the digestive ceca to the intestine. Note that a slender ridge continuous with the larger portion of the typhlosole exits the large ventral aperture.

Posteriorly the intestine and style sac share a common bilobed aperture from the stomach. This aperture is partially divided, by two ridges, into the **intestine** on one side and the **style sac** on the other. Notice that the typhlosole enters this opening (Fig 8, 12-102). Most material entering the intestine is indigestible mineral particles and the chief function of the intestine is feces formation and storage.

The ciliated, secretory epithelium lining the style sac secretes and rotates an elongate, flexible, pellucid rod, the **crystalline style**, which extends out of the style sac into the lumen of the stomach. The distal (stomach) end of the style rubs against a chitinous plate, the gastric shield, in the wall of the stomach. The style is composed of digestive enzymes secreted by the sac epithelium and is present only in individuals that are feeding or have recently fed. It is resorbed in starved individuals. Consequently it may not be present in your specimen. It is large an unmistakable when present.

Follow the lumen of the style sac and intestine into the gonad ventral to the stomach. The style sac and intestine extend, side by side with continuous lumina, into the visceral mass where they are surrounded by the gonad. Here the style sac eventually reaches a dead end and stops but the intestine continues on making several loops until it eventually reaches the anus. The intestine is divided into three regions but its path through the visceral mass is difficult to follow. Use your scissors to trace the intestine lumen as far as you can. Beginning in the posterior stomach insert one blade of the scissors into the opening of the intestine and style sac and cut completely through the thick wall of the visceral mass. Most of this wall is **gonad**. You will cut through digestive ceca (greenish) and gonad (yellowish) as you trace the gut.

The **descending intestine** exits the posterio-ventral end of the stomach and its lumen is beside and continuous with that of the style sac for the length of the sac (Fig 8, 12-89B, 12-103A). It and the sac are a straight bilobed tube extending into the gonad in the ventral visceral mass.

Deep within the visceral mass, the style sac ends and the intestine continues as the **ascending intestine**, or middle limb of the intestine. This second of three regions of the intestine loops through the gonad and then extends dorsally to exit the gonad. The walls of the middle intestine are thin, making this limb difficult to trace in its wanderings.

Having exited the gonad, the gut extends posteriorly as the **rectum** (Fig 8, 12-89B). The rectum, which you have already seen, enters the pericardial cavity, passes through the ventricle, exits the cavity, and curves dorsally over the posterior adductor muscle to end at the **anus** on the posterior side of the muscle. Insert a teasing needle into the slit-shaped anus to demonstrate its presence.

The wall of the rectum is folded to form a large typhlosole that occupies most of the lumen. Relocate the rectum where it enters the anterior end of the pericardial cavity and trace it through the ventricle. With a longitudinal incision open the rectum and find the **typhlosole**.

References

Alderman JM, Ratcliffe JA, McDougal LA. North Carolina Freshwater Mussels; Wildlife Species and Conservation. www.ncwildlife.org/pg07_WildlifeSpeciesCon/pg7b1a.htm

Bogan A . Workbook and Key to the freshwater bivalves of North Carolina http://www.naturalsciences.org/research/inverts/bogan.html

Brooks WK. 1890. Handbook of invertebrate zoology for the laboratory and seaside work. Bradlee, Whidden Pub, Boston. 392pp.

Bullough WS. 1958. Practical invertebrate anatomy, 2 nd ed. MacMillan, London. 483 pp.

Burch JB. 1973. Freshwater unionacean clams (Mollusca: Pelecypoda) of North America. US Env. Protection Agency, Biota of Freshwater Ecosystems, Project 18050ELD, Contract 14-12-894. Govt. Printing Office stock number 5501-00488. 176pp.

Campbell DC, Serb JM, Buhay JE, Roe KJ, Minton RL, Lydeard C. 2005. Phylogeny of North American amblemines (Bivalvia, Unionoida): prodigious polyphyly proves pervasive across genera. Invertebrate Biology 124(2):131-164.

Cummings K,Bogan A,Watters GT,Mayer CA . Freshwater Mollusk Bibliography http://ellipse.inhs.uiuc.edu/mollusk/

Freeman WH, Bracegirdle B. 1971. An Atlas of invertebrate structure. Heinemann Educational Books, London. 129 pp.

Johnson RI . 1970. The systematics and zoogeography of the Unionidae (Mollusca: Bivalvia) of the southern Atlantic Slope Region. Harvard Univ, Bull. Mus. Comparative Zoology 140(6):263-447.

McMahon RF. 2001. Mollusca: Bivalvia, pp.331-430 in Thorp JH, Covich AP (eds.), Ecology and classification of North American freshwater invertebrates, 2 nd ed. Academic Press, San Diego. 1056pp.

Morse MP and Zardus JD. 1997. Bivalvia, pp. 7-118 in Harrison FW, Kohn AJ, Microscopic anatomy of invertebrates 6A, Mollusca II. Wiley-Liss, New York. 414 pp.

Parmalee PW, Bogan AE. 1998. The freshwater mussels of Tennessee. Univ. Tennessee Press, Knoxville. 328 pp

Pennak RW. 1989. Fresh-water invertebrates of the United States, 3 rd ed. Wiley, New York.

Pierce ME. 1950. Fresh-water clams or mussels. Pp. 334-336 (with figure on p.332) in Brown FA. Selected invertebrate types. Wiley, New York. 597 pp.

Ruppert EE, Fox RS, Barnes RB. 2004. Invertebrate Zoology, A functional evolutionary approach, 7 th ed. Brooks Cole Thomson, Belmont CA. 963 pp.

Simpson GB . 1884. Anatomy and physiology of *Anodonta fluviatilis.* Rept. New York State Museum 35:169-191, pls 3-13. Detailed and well illustrated.

Strayer DL, Jirka KJ . 1997. The pearly mussels of New York State. New York State Museum Mem. 26:1-113, pls 1-26.

Williams JD, Neves RJ. Freshwater mussels: A neglected and declining aquatic resource.
http://biology.usgs.gov/s+t/noframe/f076.htm.

Williams JD, Warren MI, Cummings KS, Harris JL, Neves RJ . 1993. Conservation status of the freshwater mussels of the United States and Canada. Fisheries 18(9):6-22.

Supplies

Preserved (or fresh) unionoid (pearly) freshwater mussel
Empty shell consisting of right and left valves
Dissecting pan
Dissecting microscope
Dissecting set
Broken piece of the ventral edge of a valve

Lander University

Invertebrate Anatomy OnLine

Mytilus edulus©

Blue mussel

4jul2006

Richard Fox

Lander University

Preface

This is one of many exercises available from ***Invertebrate Anatomy OnLine*** , an Internet laboratory manual for courses in Invertebrate Zoology. Additional exercises can be accessed by clicking on the links to the left. A glossary and chapters on supplies and laboratory techniques are also available. Terminology and phylogeny used in these exercises correspond to usage in the Invertebrate Zoology textbook by Ruppert, Fox, and Barnes (2004). Hyphenated figure callouts refer to figures in the textbook. Callouts that are not hyphenated refer to figures embedded in the exercise. The glossary includes terms from this textbook as well as the laboratory exercises.

Systematics

MOLLUSCA ^{P}, Bivalvia ^{C}, Pteriomorphia ^{sC}, Isofilibranchia ^{SO}, Mytiloida ^{O}, Mytiloidea ^{SF}, Mytilidae ^{F} (Fig 12-122, 12-125)

Mollusca ^{P}

Mollusca, the second largest metazoan taxon, consists of Aplacophora, Polyplacophora, Monoplacophora, Gastropoda, Cephalopoda, Bivalvia, and Scaphopoda. The typical mollusc has a calcareous shell, muscular foot, head with mouth and sense organs, and a visceral mass containing most of the gut, the heart, gonads, and kidney. Dorsally the body wall is the mantle and a fold of this body wall forms and encloses that all important molluscan chamber, the mantle cavity. The mantle cavity is filled with water or air and in it are located the gill(s), anus, nephridiopore(s) and gonopore(s). The coelom is reduced to small spaces including the pericardial cavity containing the heart and the gonocoel containing the gonad.

The well-developed hemal system consists of the heart and vessels leading to a spacious hemocoel in which most of the viscera are located. The kidneys are large metanephridia. The central nervous system is cephalized and tetraneurous. There is a tendency to concentrate ganglia in the circumenteric nerve ring from which arise four major longitudinal nerve cords.

Molluscs may be either gonochoric or hermaphroditic. Spiral cleavage produces a veliger larva in many taxa unless it is suppressed in favor of direct development or another larva. Molluscs arose in the sea and most remain there but molluscs have also colonized freshwater and terrestrial habitats.

Eumollusca

Eumollusca, the sister taxon of Aplacophora, includes all molluscs other than aplacophorans. The eumolluscan gut has digestive ceca which are lacking in aplacophorans, the gut is coiled, and a complex radular musculature is present.

Conchifera

Conchifera, the sister taxon of Polyplacophora, includes all Recent molluscs other than aplacophorans and chitons. The conchiferan shell consists of an outer proteinaceous periostracum underlain by calcareous layers and is a single piece (although in some it may appear to be divided into two valves). The mantle margins are divided into three folds.

Ganglioneura

Most Recent molluscs are ganglioneurans, only the small taxa Aplacophora, Polyplacophora, and

Monoplacophora are excluded. Neuron cell bodies are localized in ganglia.

Ancyropoda

The mantle cavity, with its gills, is lateral. The calcareous portion of the shell is bivalve, with the valves opening laterally and joined dorsally by a derivative of the periostracum.

Bivalvia C

Bivalvia is a large, successful, and derived taxon. The body is laterally compressed and enclosed in a bivalve shell. The two valves are hinged dorsally. The the foot is large and adapted for digging in the ancestral condition. A crystalline style is usually present but never is there a radula. The mantle cavity is lateral and in most bivalves the gills are large and function in respiration and filter-feeding. The head is reduced and bears no special sense organs. The nervous system is not cephalized. The group includes scallops, clams, shipworms, coquinas, marine and freshwater mussels, oysters, cockles, zebra mussels, and many, many more.

Metabranchia sC

Most bivalves are metabranchs. The gills are adapted for filter feeding and water enters the mantle cavity posteriorly.

Filibranchia SO

Filibranchs are suspension-feeding bivalves with filibranch gills.

Pteriomorpha O

Pteriomorph bivalves are epibenthic and live on, rather than in, the bottom. They may be attached or unattached, may have a byssus or not, and may cement one valve to the substratum or not. The foot is reduced and the mantle margins are not fused. The gills are large and used for filter feeding. There is a tendency to reduce or loose the anterior adductor muscle. Siphons are absent or reduced. This taxon includes the well-known arcs, mussels, scallops, pen clams, and oysters.

Laboratory Specimens

Marine mussels are metabranch bivalves with filibranch gills. Almost any marine mussel will serve for this exercise as most are similar in the basic features of their anatomy. The exercise is written specifically for *Mytilus edulis*, the blue mussel (Fig 12-110A), with parenthetical comments on differences from *Geukensia demissa*, the ribbed mussel (Fig 12-110E). From the standpoint of general anatomy the differences between these two species are minor. Both are common but *Mytilus* is more widespread and has the added advantage of being available alive in inland fish and supermarkets. Freshwater mussels are unrelated and are not described by this exercise.

Mytilus edulis is found, often in high population densities, in Europe, on both coasts of North America from the Arctic to the Middle Atlantic States on the east coast and south to California on the west. It forms dense and extensive mats on hard substrata. It is a valued seafood and supports a commercial fishery where it is abundant. It reaches about 8 cm in length. Large specimens are often overgrown by other organisms.

Geukensia demissa occurs along the east coast of North America from Nova Scotia to Florida and is common in salt marshes and among oysters in the southeastern United States. It is an intertidal species reaching its largest size (about 10 cm) in salt marshes where it lives in the sediment attached to saltmarsh cord grass, *Spartina alterniflora* (Fig 12-110E).

Living specimens should be relaxed in isotonic magnesium chloride and dissected under magnification, immersed in magnesium chloride in a small dissecting pan.

Shell

Obtain an empty **shell** for study. Like that of all bivalves, it is composed of two similar **valves**. Each valve is elongate and the exterior is relatively smooth but has concentric **growth rings**. (The valves of *Geukensia* are sculptured with strong radial ribs). The anterior end is pointed and the posterior is broadly rounded (Fig 1). The dorsal margin is convex whereas the ventral is weakly concave. There is a slight permanent gape, where the valve margins do not meet, near the middle of the ventral midline between the two valves. This is the **byssal gape** which accommodates the byssus.

The two valves are held together in life by the long, straight, developed **hinge** occupying the anterior end of the dorsal margin (Fig 1). Note the narrow, straight, proteinaceous **ligament** extending for the length of the hinge. Hinge teeth are weakly developed in mussels. The inconspicuous **umbos** are located at the anterior end in *Mytilus* (but are a little posterior to the end in *Geukensia*). The valve is covered by the conspicuous, dark yellowish brown or black, proteinaceous **periostracum**. This outermost layer of the shell can be seen folded over the ventral edge of the valve in fresh specimens. The three shell layers are, from outside in, the organic periostracum, calcareous prismatic layer (= ostracum), and calcareous, nacreous, lamellar layer (= hypostracum) (Fig 12-91). The valves are often eroded so that the chalky white calcareous prismatic layer shows through the dark periostracum

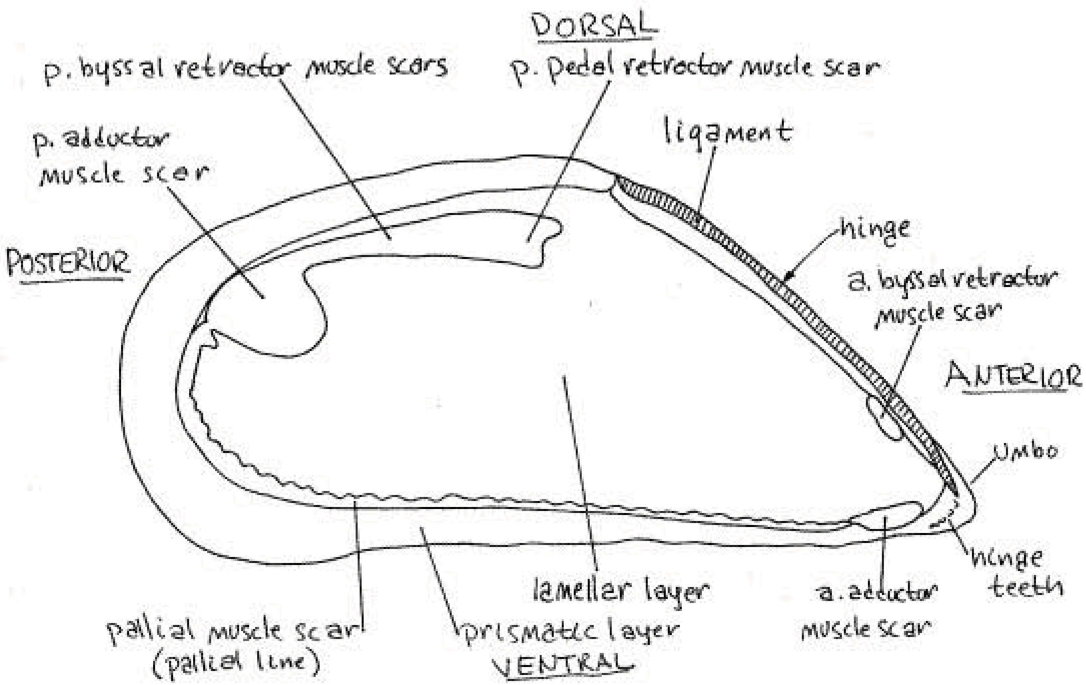
Use your knowledge of the antero-posterior and dorso-ventral axes to determine which valve is **right** and which **left**. Mussels are **equivalve** (right and left valves are nearly identical and symmetrical). The opposite is inequivalve. Look at the outside of one of the valves. Each valve is strongly asymmetrical, a condition referred to as **inequilateral**. The anterior end of the valve does not resemble the posterior (Fig 1). The opposite is equilateral.

Most of the interior of the valve is pale but the margins are dark. The line separating the two is the **pallial line** (Fig 1). It is the line of attachment of the mantle to the valve.

A small **anterior adductor muscle scar** can be seen at the anterior end of the valve (Fig 1). It lies on the pallial line on the ventral edge of the valve. The much larger **posterior adductor muscle scar** is located at the posterior end, displaced to the dorsal side. These scars mark the sites of attachment of the adductor muscles to the valves. Remember their location.

The scar of the **anterior pedal-byssal retractor muscle** is a small, pale, slender, elongate depression under the overhang of the anterior edge of the valve below the ligament (Fig 1). The scar of the **posterior pedal-byssal retractor muscle** is a large, lobed, narrow, dark area extending anteriorly from the dorsal edge of the posterior adductor scar.

Figure 1. Interior of the left valve of ***Mytilus edulis*** (redrawn from White, 1937). Bivalve70La.gif



External Anatomy

Living (or preserved) *Mytilus* are easily opened, unlike *Mercenaria*). Hold the mussel with its right valve uppermost. Insert the tip of a screwdriver or the blunt end of a forceps into the byssal gape and twist it to force the valves to gape all around, especially posteriorly. Refer to an empty valve to help you remember the position of the posterior adductor muscle. This muscle is located near the dorsal margin just posterior to the hinge.

Slip a long, sharp scalpel blade into the posterior gape and cut the posterior adductor muscle. The muscle is easily recognized by feel because it is the only firm, resisting structure in the vicinity. You can also look into the gape and see it. Try to avoid cutting any other tissues around the muscle. It will, however, be necessary to cut the black tissue connecting the right and left mantle skirts at the siphons. The rectum and anus are also in this vicinity and you will want them intact later.

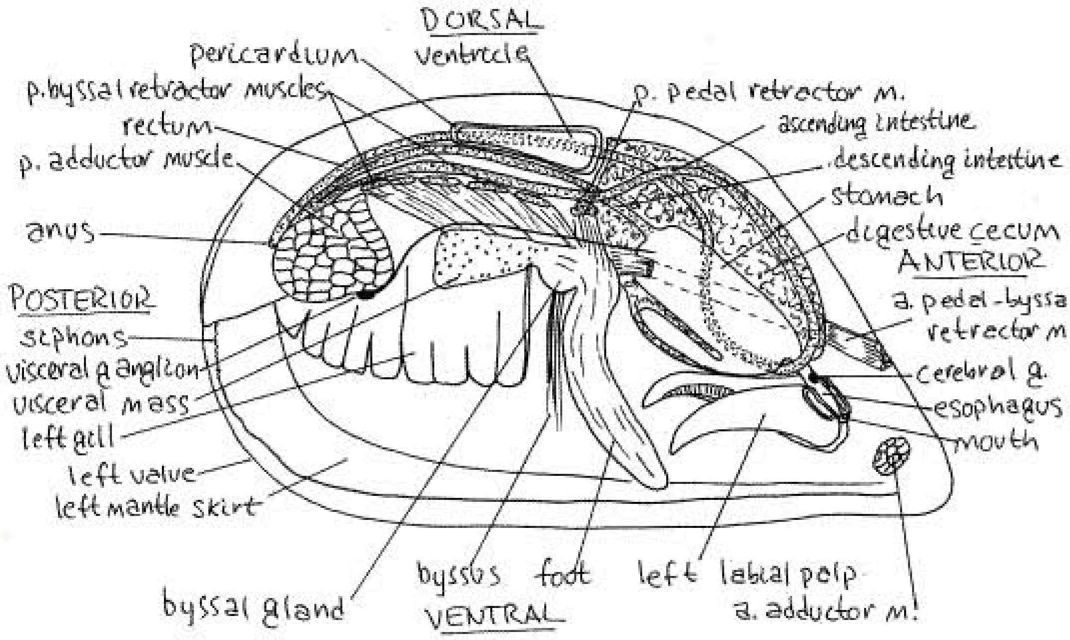
Cut the small anterior adductor muscle. The anterior adductor is on the ventral margin just posterior to the anterior tip of the shell.

Lift the right valve slightly and separate it from the soft tissue (right mantle skirt) adhering to its inside surface. Use a scalpel to scrape the right posterior pedal-byssal retractor muscle and the right anterior pedal/byssal retractor muscle away from the right valve.

Remove the right valve. The well developed proteinaceous ligament that extends along most of the length of the hinge (Fig 1, 3) and must be cut or torn to remove the valve. Place the left valve, with the animal contained in its concavity, in a 12 cm culture dish or small dissecting pan of isotonic magnesium chloride (use tapwater for preserved specimens).

Later in the exercise, after the animal has relaxed, replace the magnesium chloride with seawater so the heart will continue beating, or resume beating if it has stopped. The animal must be immersed in fluid during the dissection and most of the work should be done on the stage of the dissecting microscope.

Figure 2. Lateral view of a dissected *Mytilus edulis* from the right side (redrawn from White, 1937). The right valve, mantle skirt, gill, and labial palps have been removed. Part of the right gill has been removed. The visceral mass has been opened and much of it has been removed. Bivalve71La.gif

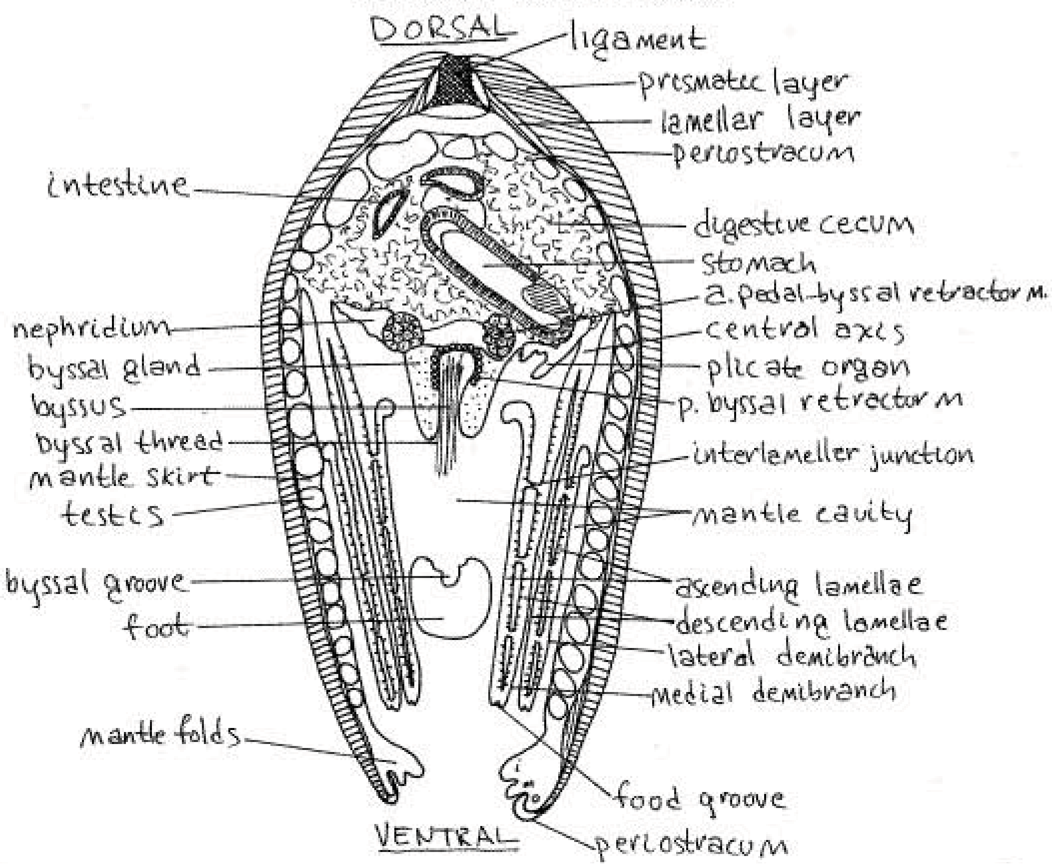


Mantle

The animal is enclosed by the large right and left mantle skirts (lobes) which line the inner surfaces of the two valves (Fig 2). The space between the two mantle skirts is the inhalant chamber of the mantle cavity (Fig 3). In life it is filled with seawater. The two skirts are connected dorsally to each other and are attached to the valves along the pallial line.

The mantle skirts are much thicker than is usual in bivalves because they contain the gonads (Fig 3). When ripe, the male mantle is creamy beige whereas that of females is reddish.

Figure 3. Cross section of *Mytilus edulis* (redrawn from White, 1937). The section is at a level immediately posterior to the foot. A posterior curve of the distal foot resulted in its inclusion in this section. o = outer, m = middle, i = inner. Bivalve72La.gif



Find the *left* mantle skirt. It should still be attached to its valve. The mantle margins of mussels provide a good example of the basic tripartite condition characteristic of most bivalves. Look at the ventral margin of the mantle skirt. This margin should still be attached to the margin of the left valve. It consists of three folds; outer, middle, and inner (Fig 3). The muscular **inner fold** is medial and is the largest. It forms a conspicuous bulging ridge running along the edge of the mantle margin and it bears a thin papillate crest extending along its length.

Lateral to the inner fold is the much smaller, sensory **middle fold**. The **periostracal groove** separates the middle fold from the outer fold. The periostracum is secreted by the inner (medial) surface of the **outer fold** and thus originates in the periostracal groove. The outer fold is secretory but cannot be seen clearly because it is covered by the periostracum, which it secreted.

The newly secreted **periostracum** remains attached to the bottom of the periostracal groove (Fig 3) and extends outward from the groove, over the outer fold, and around the edge of the valve. Grasp the newly formed periostracum with a fine forceps and tug it. It is very tough and pliable. Tear a bit of it away so you can see the outer mantle fold. The outer surface of the outer fold secretes the prismatic (middle) layer of the shell. The entire outer surface of the mantle skirt (not fold) secretes the lamellar (inner) layer and is in broad contact with it.

"

Cut away the newly secreted periostracum on the edge of the *left* valve along a 2 cm length of the valve margin. Use fine scissors or a scalpel to do this. Lift the freed mantle margin and look beneath it at the shell. You will see that the mantle is attached to the shell a short distance from its margin. This attachment is accomplished by the pallial muscles in the muscular fold (inner fold) of the mantle margin. This line of muscle attachment parallels the margin of the valve and is the **pallial line**. Posteriorly, the right and left mantle skirts together form the obscure ventral **inhalant** and dorsal **exhalant siphons** (Fig 2). The skirts are connected by a small, transverse, dark brown **branchial membrane** between the two siphons. The epithelium in the vicinity of the siphons is darkly pigmented. A vertical, median membrane extends ventrally from the branchial membrane. Neither of the two siphons is distinct. They are weakly modified areas of the mantle margin and are not complete tubes as are the siphons of many bivalves. They are short and do not extend from the shell. Being epifaunal, *Mytilus* does not need the tubular siphons characteristic of infaunal burrowers.

Adductor Muscles

Examine the two adductor muscles which you cut in order to open the animal (Fig 2). The **anterior adductor muscle** is reduced and is much smaller than the **posterior adductor muscle** as it is in all mussels. This disparity in the size of the two adductor muscles is referred to as the heteromyarian condition and it is associated with the presence of a proteinaceous holdfast called the byssus, which will be discussed later. The heteromyarian condition is derived from the more primitive, and more common, dimyarian condition in which the two adductor muscles are of similar sizes.

Gills

"

Use scissors to remove the *right* skirt of the mantle. This will expose the large right **gill** extending the length of the mantle cavity on the right side of the visceral mass (Figs 2, 3, 12-110B). A gill is formed of the combined filaments attached to the central axis. The entire gill is a **holobranch** (=whole gill) and includes the filaments on both sides of the axis. There is only one gill on the right and one on the left even though it may look to you as if there are two on each side.

Each **holobranch** consists of two **demibranchs**, or half gills, one medial and one lateral (Fig 3, 12-96). Do not mistake demibranchs for complete gills. Find the **lateral** and **medial demibranchs** of the right gill.

Each of the two surfaces of a demibranch is a **lamella**. Each demibranch thus has two lamellae (Fig 3, 12-100). Each holobranch, since it is composed of two demibranchs, has four lamellae.

The two demibranchs are attached to each other along the **central axis** (Fig 3, 12-96). This longitudinal axis extends the length of the dorsal wall of the mantle cavity between the visceral mass and the mantle skirt. The demibranchs hang freely into the mantle cavity and are attached to the body wall at the central axis.

In cross section each holobranch can be likened to a capital W (\/\/) or, more accurately, a "double V" (Fig 3, 2-96C,D). The central axis by which the gill is attached to the body is represented by the middle point of the **W**. Each **V** of the \/\/ is a demibranch. Each demibranch **(V)** is composed of two lines, \ and /, which represent the lamellae. The four lamellae of the holobranch are the four straight lines of which the **W** is composed (i.e. \ / \ /).

The two lamellae that drop from the central axis down into the mantle cavity are the **descending lamellae** (Fig 3, 12-100). Each holobranch has two, each demibranch has one. The descending lamellae of adjacent demibranchs face each other. The lamellae that rise back up into the mantle cavity are the **ascending lamellae**. The descending lamellae of a holobranch are the inner two straight lines of our W (/\) and the ascending lamellae are the outer two (\ /).

In mussels and scallops, the upper ends of the ascending lamellae are not attached to the body wall and are entirely free (Fig 3, 12-100). The ascending lamella of the lateral demibranch of the right gill is facing you now (if you have been following instructions). Put your new knowledge to use by naming the remaining three lamellae of the right gill.

Look at the surface of a lamella under magnification and see the numerous very narrow **gill filaments** of which it is composed. They are not grouped into ridges (plicae) as are those of most bivalves, and the surface of the demibranch is flat, except for the minute ridges of the individual filaments.

> 1a. If your animal is alive, you can see cilia beating on the surface of the gills. This is best accomplished by removing the animal from its dish and looking at the gills, with magnification, while they are covered by a thin film of water. Look at places where light is reflected from the surface and you will see it shimmer from the activity of the cilia. **<**

In mussels. most ciliarv currents on the lamella beat ventrallv toward the **ventral food arooves** on the ventral to edges of the demibranchs (Fig 3, 12-99A). In mussels the dorsally directed currents are weak. The currents in the ventral food grooves are longitudinal, toward the labial palps at the anterior end. Look carefully at the ventral edge of a demibranch and find its food groove. A weak anterior longitudinal current exists along the dorsal free edge of the ascending lamellae but not along the central axis of the holobranch.

>1b. If you have a living mussel, place it in a dish of seawater and arrange it in the dish so the flat surface of the exposed lamella is horizontal, or nearly so. Look at the surface of the gill with magnification. Place a little volcanic ash, chalk dust, or a drop of carmine-seawater suspension on the surface of the gill while watching it with the dissecting microscope. You should be able to see the particles being moved quickly over the gill to the longitudinal food groove on its ventral margin. The particles, and the mucus surrounding them, are moved anteriorly by the ciliary transport mechanism of the food groove. Leave the mussel in seawater so its heart will recover and resume beating. **<**

Mussels and scallops have **filibranch gills**. Such primitive and are presumed to be the original condition of the lamellibranch gill. In a filibranch gill, adjacent filaments are held together only by ciliary interfilamentar junctions and are easily pulled apart (Fig 12-98A,B). The eulamellibranch gills of most other bivalves, such as *Mercenaria* and *Corbicula*, are held together by solid, vascularized tissue junctions (Fig 12-98C,D). The filaments of eulamellibranch gills have, in fact, grown together to form a continuous sheet perforated by small pores.

Each filament bears frontal cilia on its outer edge and lateral cilia on the flat surfaces facing adjacent filaments (Fig 12-97B, 12-98B). The lateral cilia generate the feeding/respiratory current whereas the frontal cilia move food particles along the surface of the gill to the food grooves.

>1c. If you have a living specimen, you can demonstrate the roles of the frontal and lateral cilia. Locate the free dorsal edge of the ascending lamella of the lateral right demibranch. That is the lamella facing you. While watching under magnification, place a drop of dye/seawater solution on the surface of the lamella. Some of the dye will be propelled by the frontal cilia toward the ventral food groove but some of it will be moved through the gill and into the exhalant chamber by the lateral cilia. Because the dorsal edge of the lamella is unattached, you can see the interior of the exhalant chamber and can see the current of dye appear suddenly in this space. **<**

The gills divide the mantle cavity into a ventral inhalant chamber and a dorsal exhalant chamber. Water enters the inhalant chamber from the ventral inhalant siphon (Fig 12-89A). It then passes between the gill filaments to enter the exhalant chamber. It flows back into the sea through the dorsal exhalant siphon. The large **inhalant chamber** is readily visible between the two mantle skirts whereas the exhalant chamber can be seen only by removing or opening a demibranch.

Use a *minuten nadel* to separate two adjacent gill filaments from each other and then look inside the demibranch. Note that there are no tissue connections between adjacent filaments and thus they are easily separated. The space you now see within the demibranch is the **exhalant chamber**. The chamber is not divided into water tubes or vertical channels as it is in *Mercenaria* and *Argopecten*. Note that adjacent filaments are held together loosely by cilia and not by permanent tissue junctions. This is the defining characteristic of filibranch gills.

>1d. Remove about 2-3 mm of the edge of a demibranch, place it on a slide, tease the filaments apart, affix a coverslip, and examine it with the compound microscope. If your specimen is alive, the beating cilia of the filament will be easy to see. Look for the **ventral food groove** at the edge of the gill. Note that it is a deep groove with a narrowed opening formed by the ends of the filaments of the descending and ascending filaments. In living specimens, the beating cilia of the filaments are easily seen. See if you can distinguish the lateral from the frontal cilia. **<**

Adjacent lamellae (not filaments) are held together by widely spaced **interlamellar junctions** (Fig 3, 12-96). These are small "spot welds" and are not continuous and do not divide the exhalant chamber into water tubes. Pull the dorsal edge of the ascending lamella of the lateral demibranch toward you so you can see into the exhalant chamber. There you will see numerous interlamellar junctions holding the ascending and descending lamellae together.

Any kind of junction between opposing lamellae is an interlamellar junction. Any junction between adjacent filaments is an interfilamentar junction. The interfilamentar junctions of mussels are ciliary whereas the interlamellar junctions are small tissue connections between the descending and ascending lamellae of a demibranch.

Labial Palps

At the anterior end of the visceral mass is a pair of elongate, triangular, flat **labial palps**, one right and one left (Fig 2, 12-89A, 12-100). The palps are ciliated and are used to transfer food from the gills to the mouth. Each palp consists of a **lateral** and a **medial lamella** associated with the lateral and medial demibranchs respectively. There is one lamella for each demibranch. One surface of each lamella is covered by ciliated ridges and grooves. The ridged surfaces of the two lamellae of a palp face each other. The ridges are perpendicular to the long axis of the palp. A longitudinal ciliated **oral groove** extends along the junction between the two lamellae.

Each lamella, medial and lateral, is connected physically with its counterpart on the opposite side. These transverse connections form a pair of **lips** above and below the mouth (Fig 12-100). Thus the right and left lateral palps are connected with each other by the **dorsal lip** above the mouth and the right and left medial palps are connected by the ventral **lip** below the mouth.

The **mouth** is a small opening located on the anterior midline of the visceral mass between the dorsal and ventral to lips. The oral groove runs between the upper and lower lips to enter the mouth.

The ciliated ridges and grooves form a sorting field to partially separate the mineral and organic particles collected on the gill surfaces. Ciliary currents in the grooves move mineral particles to rejection currents along the free margins of the lamella. Ciliary currents on the crests of the ridges and in the oral groove move organic particles toward the mouth. The oral groove transports these particles, between the lips, to the mouth. Final sorting will occur in the stomach.

The rejection current runs along the free edge of the lamella to the pointed tip of the palp. Rejected sediment, trapped in mucus and known as **pseudofeces**, drops into the inhalant chamber of the mantle cavity. Posteriorly directed currents on the ventral mantle margin and posteroventral currents on the mantle surface move the pseudofeces posteriorly to the *inhalant* siphon. The particles are ejected from this siphon when periodic contractions of the adductor muscles force spurts of water out of the inhalant chamber.

>1d. Place a little carmine-seawater on the opposing surfaces of the two left palps and watch it as it is transported by their cilia. Try to trace currents and watch for the development of a stream of particles in the oral groove leading into the mouth. This is probably the easiest way to find the mouth. The mouth is a small and inconspicuous pore but is easy to see if it has a string of red carmine particles entering it. **<**

Respiratory Surfaces

The gills are not the only, or even the most important, respiratory organs of mussels. The inner surfaces of the mantle skirts are also responsible for gas exchange but the chief respiratory surfaces are the **plicate organs** (Fig 3). Each of the two plicate organs is a longitudinal row of transverse folds of epithelium between the gill and the visceral mass. They are heavily vascularized, more so than the gills.

" Carefully remove the right gill to expose the right plicate organ. Look deep in the crease between the central axis of the gill and the visceral mass. In living specimens the folds are white and show up clearly against the golden brown kidney or dark brown digestive cecum behind them.

Visceral Mass

Removal of the right gill exposes the visceral mass and foot. You should now be looking at the exposed right side of the **visceral mass** (Fig 2). It is the largest part of the body and contains most of the organs. It lies on the median plane in the center of the mantle cavity. To the left of the visceral mass are the left gill, left mantle skirt, and left valve in that order. Point the anterior end of the animal to your right.

Look at the **visceral mass** lying between you and the left gill. It is elongated along its antero-posterior axis and is compressed from side to side. Look through the body wall for the large, dark brown **digestive cecum**, which occupies most of the space in the anterior visceral mass. It is located immediately posterior to the mouth and can be seen through the body wall. The esophagus, stomach, and much of the intestine are embedded in the digestive cecum and visceral mass but will not be seen at this time. If the right gill has been removed, the digestive cecum will be easy to see.

The nephridium is visible in the dorsal mantle cavity under the surface of the visceral mass beside the gill. It is yellow brown. The plicate organ is on its surface.

Foot and Byssus

The small wormlike **foot** is located in about the middle of the ventral margin of the visceral mass (Fig 2, 12-110B). In mussels the foot is not used for digging and is not a typical bivalve foot. Its function is the formation and manipulation of byssal threads.

The foot is muscular is composed of an outer layer of circular muscles around an inner core of longitudinal muscles. There are also some oblique fibers. The pedal hemocoel inside the foot is a hydrostatic skeleton that allows these muscles to antagonize each other. Contraction of the circular muscles extends the foot whereas contraction of the longitudinals withdraws it. Partial and selective contraction of the longitudinal muscles can bend the extended foot.

The foot has a longitudinal **byssal groove** along its posterior margin (Fig 3, 12-110B). The **byssal gland** is located at the base of the foot and byssal groove (Figs 2, 3, 12-110B). You may be able to see **byssal threads** emerging from it. The byssal groove originates at the gland, which secretes liquid protein that is formed into threads by the byssal groove. The threads are attached to the substratum by the foot and then allowed to harden.

Collectively the resulting cluster of protein threads is the **byssus (**Fig 2, 12-110B). It may or may not still be present in your animal. It is a holdfast extending from the visceral mass to the substratum. The proximal ends of the threads enter the visceral mass and are attached to numerous muscles, the byssal retractor muscles that run through the mass to insert on various parts of the shell (Fig 2). These white muscles can be seen easily through the integument of the visceral mass of *Mytilus*. (They are less easily seen in *Geukensia*.)

Pedal and Byssal Muscles

The foot and byssus are moved with respect to the body by a set of extrinsic muscles that originate on the shell and insert on the foot or byssal gland. Among these is a pair of **anterior pedal-byssal retractor muscles** of the foot and byssus (Fig 2). These are white, cordlike muscles extending from their origin at the base of the foot along the anterior edge of the visceral mass. They diverge slightly to their separate insertions on the shell under the anterior end of the ligament. You found their scars earlier and may want to refer once again to the empty valve to confirm their position.

Several pairs of **posterior byssal retractor muscles** originate at the base of the foot and fan out dorso-posteriorly to their insertions on the shell in a line anterior to the posterior adductor muscle. These are also white, cordlike paired muscles. The retractor muscles can retract the foot but their primary function is to pull the animal to the substratum to which the byssus is attached.

The anteriormost of this series of muscles is sometimes known as the **posterior pedal retractor muscle** (Fig 2).

Internal Anatomy

Hemal System

The **pericardial cavity** is located dorsally at the very top of the visceral mass, just ventral to the middle of the dorsal margin of the shell. In *Geukensia* it is ventral to the posterior third of the hinge and in *Mytilus* it is posterior to the hinge. It is a coelomic space and is lined with peritoneum. The walls of the cavity are relatively thick and opaque in *Geukensia* but thin and translucent in *Mytilus*.

" Open the pericardial cavity carefully with a shallow, dorsal, longitudinal cut made with fine scissors. It may already have been opened accidentally. The posterior intestine, which is the **rectum,** extends longitudinally through the cavity but is enclosed for most of its length by the ventricle of the heart.

The **heart** is associated with the rectum in the anterior part of the pericardial cavity. In *Mytilus* the single **ventricle** is a pale yellowish mass that surrounds the tubular rectum. At its anterior end the ventricle swells to form the **aortic bulb** at the base of the aorta. The rectum passes through the center of the bulb.

" With fine scissors open the left wall of the aortic bulb and look for the openings of the major arteries. The **aorta** is median and unpaired and exits the bulb on its dorsal anterior wall, dorsal to the rectum. It supplies the anterior mantle and dorsal esophagus with blood. The paired **posterior pallial arteries** arise from the lateral floor of the aortic bulb. They supply the posterior mantle. A very large, unpaired **coeliac artery** exits the floor of the aortic bulb on its midline. It immediately branches into several important arteries to the stomach, intestine, and digestive ceca. (In *Geukensia*, a **posterior aorta** exits the ventricle posteriorly and runs ventral to the rectum. There is no posterior aorta in *Mytilus*).

On each side the ventricle connects with a membranous **atrium**. Hold the mussel erect, with its dorsal edge up and focus on the ventricle. Pull the ventricle to one side and you will see the atrium being stretched on the opposite side by its connection with the ventricle. The atria extend ventrolaterally on the sides and floor of the pericardial cavity. Each of the two atria occupies most of the length of the cavity and is attached posteriorly to the other atrium. Their lumina are not continuous, however. The atria receive oxygenated blood from the efferent branchial vessels draining the gills.

The lobulated brown **pericardial glands** are associated with the atria. They are a part of the excretory system and are involved in the production of the primary urine in the pericardial cavity.

Reproductive System

Mussels are gonochoric. The gonads extend throughout most parts of the body except the gills, muscles, and foot (Fig 3). Most of the gonad is in the mantle skirts, thus accounting for the unusual thickness of the mantle in these bivalves. Some of the gonad is in the visceral mass (Fig 3). Ovaries are reddish in *Mytilus*, purplish in *Geukensia* whereas testes are cream in *Mytilus* and yellow in *Geukensia*.

>1e. Make a smear preparation of a bit of the mantle of a mature specimen and examine it with the compound microscope for eggs or sperm. If your specimen is living, make the slide with seawater and look for motile sperm. **<**

On each side of the body the many lobes of the gonad connect by a converging system of ducts leading to the gonopore on the tip of the **genital papilla**. This papilla is located on the roof of the mantle cavity where the medial demibranch attaches to the visceral mass. This places it in the exhalant chamber dorsal to the medial demibranch but you can see it without dissection because the gill is not attached to the foot

Excretory System

The two **nephridia**, or kidneys, are complex, elongate, brown organs lying in the roof of the mantle cavity at the base of the gills (Fig 3, 12-118). They extend from the labial palps to the posterior adductor muscle. In your dissection the right kidney should be easy to see under the cut edge of the left gill and plicate organ. Anteriorly it may lie on the surface of the digestive cecum, which is also brown, but darker, so the two are readily distinguishable.

Each nephridium connects to the pericardial cavity via a renopericardial canal. Each of the two renopericardial canals opens from the pericardial cavity via a small pore in its anterior floor. Each nephridium empties into the exhalant chamber via a nephridiopore located atop the tiny urinary papilla on the base of the posterior side of the larger genital papilla.

Pericardial glands are also associated with the excretory system. The pericardial glands invest the outer walls of the atria and give that part of the heart its characteristic brown color.

Nervous System

The nervous system consists of the usual bivalve ganglia, connectives, and nerves. The major features of the system are superficial and can be revealed simply by removing the epidermis covering them. An extensive dissection is not necessary. Care should be taken to avoid damage to the digestive system, which you have not yet studied.

The paired cerebral ganglia are situated beside the esophagus (Fig 2). They are beside the posterior margin of the lower lip touching the medial edge of the anterior pedal-byssal retractor muscle. In life, the ganglia usually contain orange or reddish neuroglobin and, when they do, are easily seen through the thin integument, without dissection or magnification. If they lack this pigment (as preserved specimens always do) they will be harder to find.

" Carefully remove the integument covering the area described above. Look for the right **cerebral ganglion** in the corner between the lower lip and the anterior pedal-byssal retractor muscle. You may want to insert a blunt probe into the mouth to help you find the esophagus. The thick **cerebral commissure** arches over the esophagus to connect the right and left cerebral ganglia.

A large common trunk of the cerebropleural and cerebrovisceral connectives exits each cerebral ganglion and extends posteriorly beginning on the ventral border of the anterior pedal-byssal retractor muscle. The trunk curves around the lateral border of the retractor and ends on the dorsal surface of the posterior end of that muscle. On the way around the lateral edge of the muscle it bifurcates into the cerebropedal and cerebrovisceral connectives.

The cerebropedal connective extends posteroventrally along the anterior pedal-byssal retractor muscle to the pedal ganglion. The connectives and the ganglion can be seen by cutting the epithelium covering the groove between the pedal-byssal retractor and the visceral mass medial to it. The ganglion is usually yellowish or orange. Nerves to the foot, byssal gland, and both pedal-byssal retractor muscles arise from it. The two pedal ganglia touch each other on the median plane and their neurons are connected by the short transverse pedal commissure.

The cerebral ganglia, cerebral commissure, cerebropedal connectives, pedal ganglia, and pedal commissure make up a circumesophageal nerve ring that loosely encircles the esophagus. A small nerve arises at the point where the common trunk bifurcates. It runs to a statocyst located near the surface of the body in the angle between the cerebropedal and cerebrovisceral connectives.

The cerebrovisceral connnective on each side extends posteriorly across the outer surface of the digestive cecum in the visceral mass. It lies just inside the integument and crosses the lateral surfaces of the dorsal ends of the posterior pedal-byssal retractor muscle. It parallels and is medial to the line of attachment of the gill to the body. In this area it can be seen as a white band through the transparent epithelium. The cerebrovisceral connective has branches to the digestive cecum, intestine, gonad, and nephridia. The areas surrounding the retractor and adductor muscles may have been damaged when you cut the muscles to open the shell. If that is the case it may be necessary to trace the cerebrovisceral connective on the other side.

The cerebrovisceral connective extends posteriorly to the visceral ganglion (Fig 2) located on the ventral, slightly anterior, surface of the posterior adductor muscle. The right and left visceral ganglia are close together and are connected by the transverse visceral commissure. Nerves to the mantle, siphons, nephridia, foot, and posterior adductor muscle arise from the visceral ganglia. It is possible that the visceral ganglia were destroyed when you cut the posterior adductor muscle.

Digestive System

" Relocate the tiny slit-shaped **mouth** on the median plane between the upper and lower lips (Fig 2). It opens into a short, straight **esophagus**. Insert a blunt probe into the mouth to reveal the location of the esophagus. Leave the probe in the esophagus and with fine scissors cut along the probe. This will open the esophagus with a lateral longitudinal incision along the right side of the visceral mass. The esophagus runs straight posteriorly, angling slightly dorsally, to open into the anterior end of the stomach (Fig 2). The esophagus and stomach are embedded in the digestive cecum. Extend the incision posteriorly into the stomach.

" The esophagus widens suddenly in the anterior dorsal visceral mass to become the **stomach** located ventral to the posterior part of the hinge (Fig 2). Continue the incision along the left wall of the stomach. Rinse the inside of the stomach with squirts of water from a pipet and look at its lumen and walls.

The **sorting field** is a complex of ciliated ridges and grooves on the stomach wall whose function is to separate digestible organic particles from indigestible mineral particles (Fig 12-102). The organics are sent to the digestive cecum for digestion and the minerals directly to the intestine to become feces.

A thin chitinous plate, the **gastric shield**, is located on the anterior wall of the stomach. The **crystalline style** is a transparent, jelly-like rod that protrudes into the stomach from the style sac and rests against the gastric shield. It is composed of digestive enzymes, especially carbohydrases and lipases, but there are no proteases in it. The style is very long and extends far back into the **style sac,** which is combined side by side with the **intestine** (Fig 12-103A), to the level of the posterior adductor muscle. This portion of the intestine is known as the descending intestine (= direct intestine). You will follow it soon but in the meantime do not pull the crystalline style out of the style sac. The enzymes of the style are secreted by the style sac epithelium and the style can be reabsorbed when it is not needed. It is often absent in individuals that have not fed recently.

The style sac and descending intestine exit the posterior end of the stomach. They are joined side by side and their lumina are continuous. A pair of opposing typhlosoles, or ridges, separate the lumen of the tube into two parallel and contiguous channels, one of which houses the style and is the style sac. The other is the descending intestine. The style sac continues a distance posteriorly and then ends blindly.

Cilia in the walls of the style sac rotate the style causing its anterior end to rub against the gastric shield. This abrades its anterior end and releases enzymes into the stomach lumen. Rotation of the style also helps pull a string of mucus and food particles into the stomach from the mouth. This string originated on the gills and labial palps. Several ducts from the digestive cecum open into the stomach. Their number and position vary from individual to individual. In some individuals there is a ventral diverticulum of the stomach (Fig 2).

The **intestine** leaves the posterior end of the stomach and loops through the visceral mass before eventually reaching the anus. It is composed of three regions of approximately equal length (Fig 2), of which the last is the rectum. The **rectum** (Fig 2), which is the posterior third of the intestine, can be seen without any additional dissection as it passes through the pericardial cavity. The rectum ends at the anus located in the exhalant chamber above the posterior adductor muscle (Fig 2). The posterior rectum is often damaged when the posterior adductor muscle is cut to open the shell early in the dissection.

Although it is impractical to trace the intestine posterior to the style sac. The descending intestine extends posteriorly from the stomach and lies beside the style sac as has been discussed. The descending intestine and style sac continue side by side posteriorly to a position dorsal to the posterior adductor muscle. Here the intestine makes a sharp bend dorsally, to the right, and then turns and runs back anteriorly as the ascending intestine (= recurrent intestine) (Fig 2).

The posteriormost region of the intestine is the rectum. Upon reaching the vicinity of the esophagus the ascending intestine reverses direction and turns posteriorly to become the rectum. The rectum extends posteriorly and enters the pericardial cavity. The rectum leaves the pericardial cavity posteriorly and runs over the dorsal curve of the posterior adductor muscle to terminate at the anus in the exhalant chamber.

References

Field IA. 1922. Biology and economic value of the sea mussel, *Mytilus edulis*. Bull Bur. Fisheries 38:127-259.

Ruppert EE, Fox RS, Barnes RB. 2004. Invertebrate Zoology, A functional evolutionary approach, 7th ed. Brooks Cole Thomson, Belmont CA. 963 pp.

White KM. 1937. *Mytilus*. Liverpool Marine Biol. Committee. 31:1-117, pls 1-30.

Supplies

Dissecting microscope
Compound microscope
Slides and coverslips
Living or preserved marine mussel
Empty shell
Screwdriver
Small dissecting pan or culture dish
Volcanic ash, chalk dust, or carmine-seawater suspension
Dye/seawater solution

Lander University

Invertebrate Anatomy OnLine

Crassostrea virginica©

American oyster

25may2007

Richard Fox

Lander University

Preface

This is one of many exercises available from ***Invertebrate Anatomy OnLine*** , an Internet laboratory manual for courses in Invertebrate Zoology. Additional exercises, a glossary, and chapters on supplies and laboratory techniques are also available at this site. Terminology and phylogeny used in these exercises correspond to usage in the Invertebrate Zoology textbook by Ruppert, Fox, and Barnes (2004). Hyphenated figure callouts refer to figures in the textbook. Callouts that are not hyphenated refer to figures embedded in the exercise. The glossary includes terms from this textbook as well as the laboratory exercises.

Systematics

Mollusca ^{P}, Eumollusca, Conchifera, Ganglioneura, Ancyropoda, Bivalvia ^{C}, Metabranchia ^{SC}, Filibranchia ^{SO}, Pteriomorpha ^{O}, Ostreoidea ^{SF}, Ostreidae ^{F} (Fig 12-125, 12-122)

Mollusca ^{P}

Mollusca, the second largest metazoan taxon, consists of Aplacophora, Polyplacophora, Monoplacophora, Gastropoda, Cephalopoda, Bivalvia, and Scaphopoda. The typical mollusc has a calcareous shell, muscular foot, head with mouth and sense organs, and a visceral mass containing most of the gut, the heart, gonads, and kidney. Dorsally the body wall is the mantle and a fold of this body wall forms and encloses that all important molluscan chamber, the mantle cavity. The mantle cavity is filled with water or air and in it are located the gill(s), anus, nephridiopore(s) and gonopore(s). The coelom is reduced to small spaces including the pericardial cavity containing the heart and the gonocoel containing the gonad.

The well-developed hemal system consists of the heart and vessels leading to a spacious hemocoel in which most of the viscera are located. The kidneys are large metanephridia. The central nervous system is cephalized and tetraneurous. There is a tendency to concentrate ganglia in the circumenteric nerve ring from which arise four major longitudinal nerve cords.

Molluscs may be either gonochoric or hermaphroditic. Spiral cleavage produces a veliger larva in many taxa unless it is suppressed in favor of direct development or another larva. Molluscs arose in the sea and most remain there but molluscs have also colonized freshwater and terrestrial habitats.

Eumollusca

Eumollusca, the sister taxon of Aplacophora, includes all molluscs other than aplacophorans. The eumolluscan gut has digestive ceca which are lacking in aplacophorans, the gut is coiled, and a complex radular musculature is present.

Conchifera

Conchifera, the sister taxon of Polyplacophora, includes all Recent molluscs other than aplacophorans and chitons. The conchiferan shell consists of an outer proteinaceous periostracum underlain by calcareous layers and is a single piece (although in some it may appear to be divided into two valves). The mantle margins are divided into three folds.

Ganglioneura

Most Recent molluscs are ganglioneurans, only the small taxa Aplacophora, Polyplacophora, and Monoplacophora are excluded. Neuron cell bodies are localized in ganglia.

Ancyropoda

The mantle cavity, with its gills, is lateral. The calcareous portion of the shell is bivalve, with the valves opening laterally and joined dorsally by a derivative of the periostracum.

Bivalvia C

Bivalvia is a large, successful, and derived taxon. The body is laterally compressed and enclosed in a bivalve shell. The two valves are hinged dorsally. The foot is large and adapted for digging in the ancestral condition. A crystalline style is usually present but never is there a radula. The mantle cavity is lateral and in most bivalves the gills are large and function in respiration and filter-feeding. The head is reduced and bears no special sense organs. The nervous system is not cephalized. The group includes scallops, clams, shipworms, coquinas, marine and freshwater mussels, oysters, cockles, zebra mussels, and many, many more.

Metabranchia sC

Most bivalves are metabranchs. The gills are adapted for filter feeding and water enters the mantle cavity posteriorly.

Filibranchia sO

Filibranchs are suspension-feeding bivalves with filibranch gills.

Pteriomorpha O

Pteriomorph bivalves are benthic and live on the bottomof ther sea where they may be epifauna. They may be attached or unattached, may have a byssus or not, and may cement one valve to the substratum or not. The foot is reduced and the mantle margins are not fused. The gills are large and used for filter feeding. There is a tendency to reduce or loose the anterior adductor muscle. Siphons are absent or reduced. Pteriomorpha includes the well-known arcs, mussels, scallops, pen clams, jingles, and oysters.

Laboratory Specimens

This exercise is written specifically for the American oyster, *Crassostrea virginica* but it applies equally well to other species of *Crassostrea* and with a little modification, to *Ostrea*. *Crassostrea* has a large promyal chamber on the right side (Fig 6) that is lacking in *Ostrea*. *Crassostrea* is oviparous, whereas *Ostrea* eggs are fertilized in the exhalant mantle cavity and gestated there. *Crassostrea* is gonochoric, although sometimes they change sex after a breeding season, whereas *Ostrea* is a simultaneous hermaphrodite.

Oysters are not particularly good examples of metabranch bivalves for they differ from the "typical" condition in several respects. They have only one adductor muscle, have no foot and no siphons and have the enigmatic promyal chamber.

Oysters can be collected along the east coast of the United States from protected, estuarine habitats. They are most abundant on soft bottoms in the intertidal zone where they form reefs composed of countless individuals cemented together to form large clumps exposed at low tide. Live oysters can also be purchased at many fish markets or supermarkets at inland locations.

If possible lean specimens that have just finished spawning and thus have no gametes or glycogen reserves to obscure your view of other anatomical features. Such specimens are available in September, October, and November. Reproductive oysters with ripe gonads have thick mantles with a creamy–yellowish color. Individuals with abundant glycogen reserves also have thick mantle skirts but they are white. Starved individuals that have exhausted their glycogen reserves have thin transparent mantles and watery tissues through which organs are unobscured and readily studied. These are best suited for study. If lean individuals are not available, fat ones can be used and are satisfactory for studying the features described in this exercise.

Living specimens should be anesthetized and relaxed in isotonic magnesium chloride. Use a dissecting microscope as needed.

The edges of oyster shells are very sharp and can easily inflict cuts when handled carelessly. Wear gloves whenever it is necessary to grasp the oyster firmly. Injury from a slipping oyster knife is also possible. Exercise care when opening the shell.

Natural History

Oysters are attached epifaunal bivalves that live with the left valve cemented to the substratum. They have abandoned the ancestral bivalve biotope of burrowing in soft sediments. Consequently the have lost the foot and siphons. They have also lost the anterior adductor muscle, retaining only the posterior adductor. Lacking a foot, they also lack the pedal protractor and retractor muscles.

Oysters typically grow tightly packed in clumps, or reefs, in which they use each other as substrata for the attachment of the left valve. Because of this crowding the shape of the shell is highly variable.

Crassostrea virginica is the commercially harvested oyster of the east coast of North America. It occurs from the Gulf of St. Lawrence south to the Caribbean Sea. Sexually mature individuals vary from 5-35 cm in length.

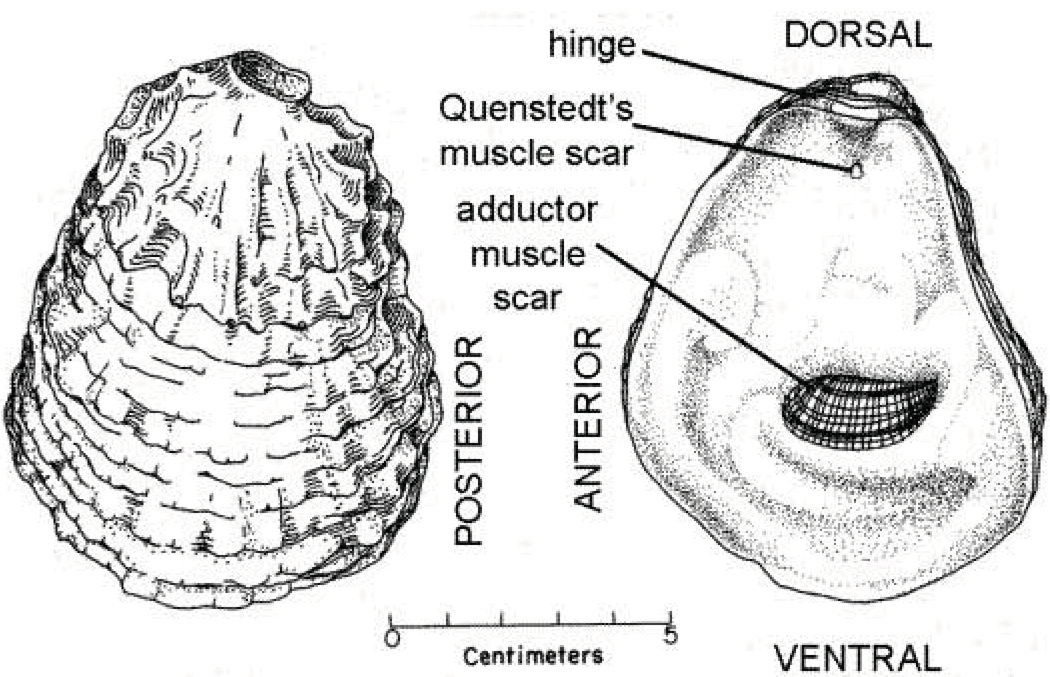
The small, commensal oyster pea crab, *Pinnotheres ostreum*, lives in the mantle cavity where it may cause deformation of the gills. Oysters are attacked by several predators including the starfish, *Asterias forbesi*, and the oyster drill, *Urosalpinx cinerea*,. The boring sponge, *Cliona celata*, inhabits the shell and weakens it. Predators and commensals are minimized in intertidal, brackish water habitats and it is here that oysters reach their highest population densities.

External Anatomy

Shell

Make a study of the shell. The exercise is written assuming you will use the shell of your intact specimen but, if available it is better to use a cleaned dry shell from which the animal has been removed. If necessary use a toothbrush to clean sediment and debris from the shell. Rinse the scrubbed shell with tapwater.

Figure 1. ***Crassostrea virginica***. Outside of left (lower) valve and inner view of right (upper) valve (from Galtsoff, 1964). Bivalve127a.gif



External Shell Features

Study the external features of the shell. One end of the shell is narrow and the other broad. The narrow end is **dorsal** and the broad end is **ventral** (Fig 1). The shell is composed of two parts, or **valves**. Oysters are **inequivalve** bivalves with a shell composed of two unequal valves. With the exception of the unequal valves oysters, like other bivalves, are **bilaterally symmetrical**. The plane of symmetry passes between the two valves, one being the right valve and the other the left. The **left valve** is the larger of the two and is the valve that is cemented to the substratum. It is deeper than the right and forms a cup that cradles the mollusc. The right **valve** is smaller and flatter, forming a cover that fits over the cup of the left valve (Fig 2). Knowing dorsal-ventral and right-left, determine **anterior** and **posterior** (Fig 1, 5). Confirm your determination by referring to the curvature of the two long margins. In most specimens the anterior margin is a relatively smooth convex curve whereas the posterior margin is concave and often irregular (Fig 1). Unfortunately, this is not true of all shells.

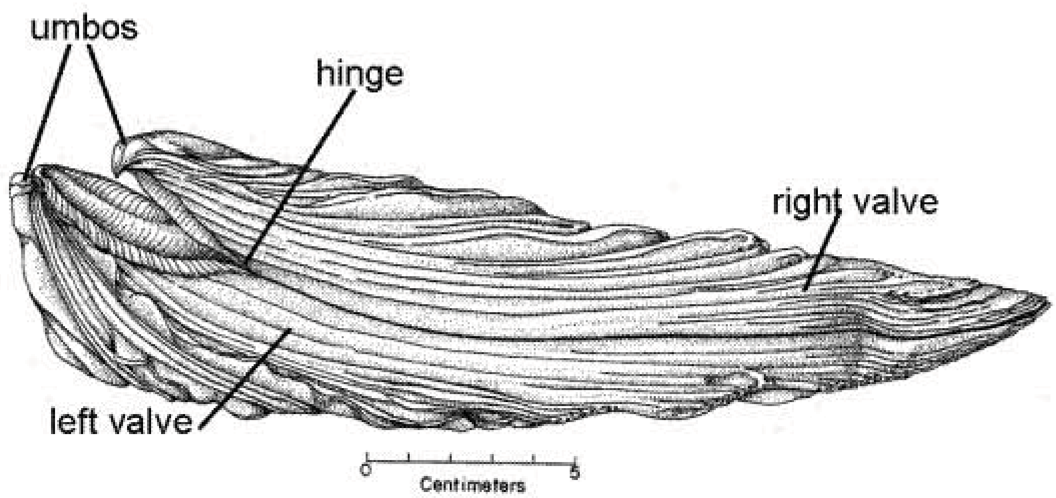
Oysters are elongated along the dorso-ventral axis in contrast with the anterior-posterior elongation of most bivalves. This can cause initial (and persistent) orientation confusion for students accustomed to typical bivalves.

The two valves are joined to each other by a **hinge** at the dorsal end (Fig 2). An internal protein hinge **ligament** in the hinge holds the two valves together. The pointed umbo, or beak, of each valve extends dorsally beyond the hinge. The tip of the umbo is the oldest part of the shell (Fig 2). The valves **gape** along the anterior, ventral, and posterior margins to allow the animal to feed and respire.

Internal Shell Features

" With a cotton work glove protecting your left hand (if right handed) remove the right valve from an oyster. The right valve is the smaller of the two valves. Hold the oyster, right valve uppermost, in your gloved left hand (unless you are left-handed, in which case reverse the instructions). Slip an oyster knife or screwdriver between the two umbos (Fig 2) at the narrow end of the oyster and pry the two valves apart. Do not force the knife toward your hand as it can easily slip and gouge your hand badly. Use the pressure to break the hinge ligament. You may have to try several positions of the knife before you meet with success.

Figure 2. Side view of a large, old specimen (from Galtsoff, 1964). The angle between the umbos determines the maximum movement of the right valve. Bivalve129a.gif



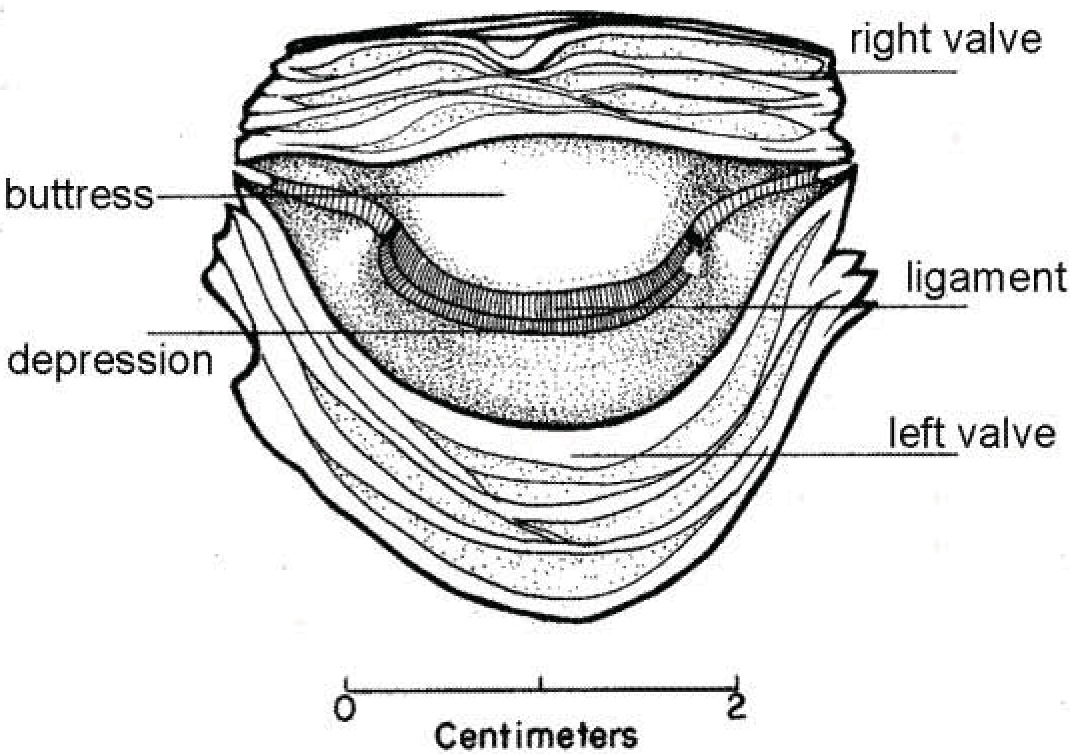
Breaking the hinge allows the valves to gape so you can slip the blade of a sharp scalpel between the two valves at the posterior edge of the shell (Fig 1, 5). Insert the blade until it encounters a rubbery resilient adductor muscle close to the middle of the posterior edge of the valve (Fig 5). Be careful you do not damage the soft tissue in the vicinity of the muscle. The rubbery texture of the muscle is unlike that of any other tissues and can be recognized by feel. You may also be able to look through the gape and see the muscle. When you find it, cut the adductor muscle with the scalpel. Without further cutting, use the scalpel to push all soft tissue away from the right valve as you gently lift the right valve away from the left. Detach the right valve and set it aside. All soft tissues should remain in the left valve. Place the left valve in a dish of isotonic magnesium chloride. Be sure all soft tissues are immersed in the anesthetic. Set the dish aside to allow the animal to relax while you study the interior surface of the right valve.

The oyster shell consists of three layers. Outermost is the organic **periostracum** composed of a thin layer of the protein conchiolin. In *Crassostrea* this layer is weakly developed but can be seen as a thin, transparent, glistening, yellowish film adhering to the outer surface of the valve. It is inconspicuous in young specimens and often absent in old. The **prismatic layer**, composed of calcium carbonate on a protein matrix, is the middle layer. Because the periostracum is thin or absent, it is the prismatic layer that is seen when viewing the outside of an oyster shell (through the periostracum). The inner layer, also composed of calcium carbonate on a protein matrix, is the **lamellar layer**. This is the layer you see when viewing the inside surface of the shell.

A large purple **adductor muscle scar** is a conspicuous feature of the inner surface of both valves (Fig 1). The scar is much closer to the posterior margin than the anterior and is ventral of the middle of the valve. The scar marks the sites of attachment of the powerful posterior adductor muscle whose action is to pull the two valves together and close the gape. It is antagonized by the hinge ligament There is no anterior adductor muscle. **Quenstedt's muscle scar** is a tiny indistinct spot just ventral to the hinge (Fig 1). Sometimes it is obvious, sometimes it cannot be found.

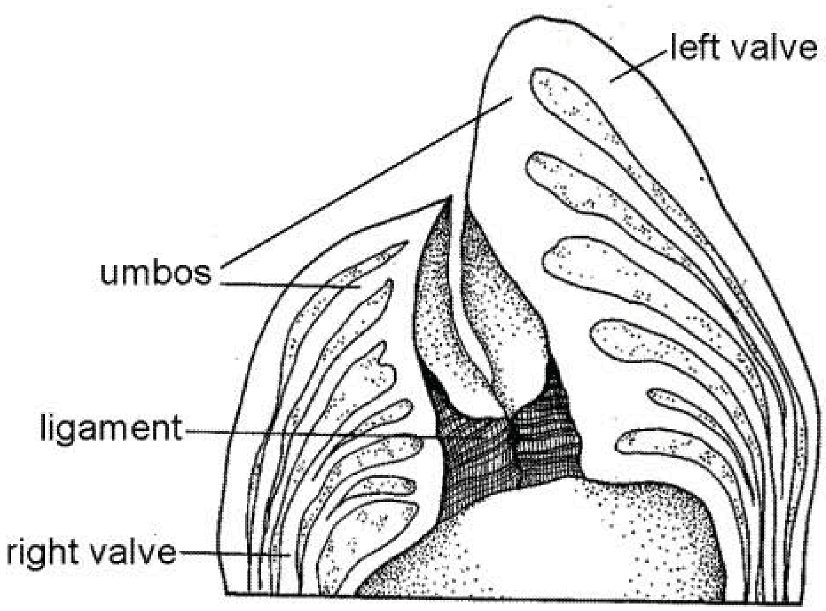
Find the **hinge** at the dorsal (narrow) end of the valve. That of the right valve includes a slight elevation, known as the buttress, of the shell margin. The buttress fits into a corresponding depression in the hinge of the left valve (Fig 3). With magnification inspect the hinge region and note that other than the buttress, it is similar to the remainder of the valve margin. Oysters completely lack any hinge teeth but the irregular curvature of the edges of the valves prevents the adducted valves from shearing past each other. If possible, compare the hinge of your oyster with one from another bivalve such as the quahog, *Mercenaria*, which has well developed hinge teeth.

Figure 3. Cross section below the hinge. The buttress of the right valve fits into the depression of the left valve (from Galtsoff, 1964). Bivalve128a.gif



The two valves are held together by a dark, proteinaceous hinge ligament, usually greenish brown in life. The ligament lies between the buttress and depression of the two valves. It is located inside the hinge and is thus an inner ligament, or resilium. (In bivalves in general, the more common position of the ligament is outside the hinge where it is known as a tensilium, or outer ligament.) The ligament will necessarily have been torn when you broke the right valve away from the left but you should be able to find its remains in the hinge at the dorsal end of the valve. The ligament is composed of the protein conchiolin (as is the periostracum) and is elastic. It, along with the adductor muscle, holds the two valves together. Contraction of the adductor muscle adducts the valves and simultaneously compresses the elastic ligament. When the muscle relaxes, elastic recoil of the compressed ligament abducts the valves, opens the gape, and stretches the adductor muscle.

Figure 4. Cross section through the hinge (from Galtsoff, 1964). Bivalve131a.gif



>a. If shells of the quahog, *Mercenaria mercenaria*, are available, study them using the description at the OnLineLab Web page for ***Mercenaria*** . Compare the *Mercenaria* shell with that of *Crassostrea*.

Soft Anatomy

Look at the oyster you set aside in magnesium chloride. Support the left valve so the surface of the soft tissue is horizontal. Touch the oyster gently with a teasing needle. If it is relaxed, it will not respond when touched. External anatomy can be studied before complete relaxation occurs. Be careful that magnesium chloride does not spill on the stage of the microscope. In reproductive individuals the diffuse hypertrophied gonads will cover most of the visceral mass and mantle in a thick layer that will obscure the kidney which could otherwise be seen through the body wall.

Preview

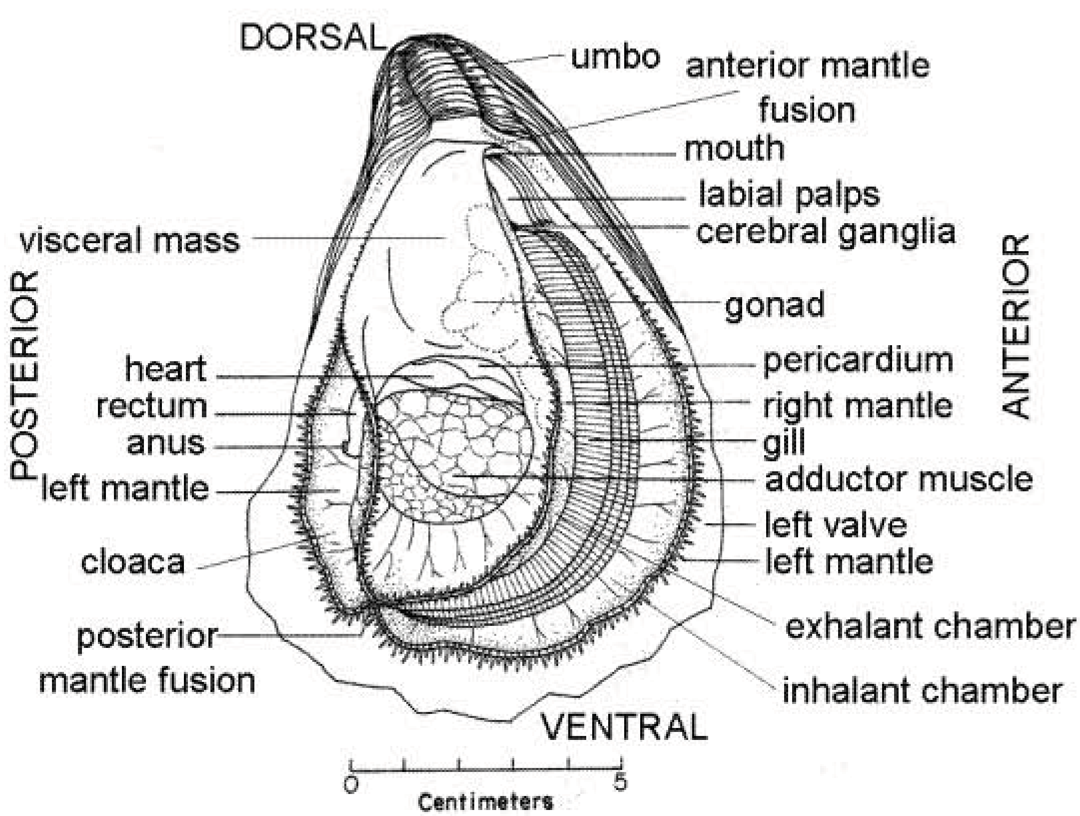
Removal of the right valve exposed the right side of the oyster to view. Arrange the oyster in the dish with the right side up and the dorsal end away from you, as in Figure 5. The oyster body consists chiefly of a large visceral mass, two mantle skirts, a mantle cavity, a large adductor muscle, a pair of gills, and a pair of labial palps (Fig 5). There is no foot, no anterior adductor muscle, and no siphons. The head is so weakly developed as to be indistinguishable from the visceral mass.

The uppermost layer of tissue arrayed before you is the right mantle skirt. It may have been damaged a little when you removed the right valve. It will probably be thick and white with glycogen reserves and gametes in the gonad. Its border is marked by a dark brown or gray line. The **adductor muscle** can be seen penetrating the mantle skirt in the posterior ventral region of the valve. It is white and does not contrast strongly with the mantle so it may not be immediately apparent. The **visceral mass**, mostly covered by the right mantle skirt, occupies the space between the hinge and the adductor muscle. The open space under the right mantle skirt is the **mantle cavity**. If you lift the anterior edge of the right mantle skirt, the conspicuous gills will be revealed. The **pericardial cavity** and heart lie on the dorsal edge of the adductor muscle.

Adductor Muscle

The large **adductor muscle** can be seen in the posterior ventral region. Its fibers run transversely from valve to valve so you are seeing it now in cross section. It may appear a bit ragged since it was cut by the scalpel. The muscle may be easier to see on the right valve where the mantle has been removed. The function of the muscle is, of course, to pull the two valves tightly together, thus closing the gape and isolating the oyster from external threats. The muscle is divided into a large, pale grey, dorsal **quick muscle** and a smaller, bright white, ventral and posterior **catch muscle** whose fibers differ in structure and function (Fig 8). The quick muscle is composed of cross-striated fibers capable of rapid response but incapable of prolonged contraction. Catch muscle, on the other hand, is composed of smooth fibers that react more slowly but that can remain contracted for long periods.

Figure 5. An oyster viewed from the right side with the right valve removed but with the right mantle skirt still in place (from Galtsoff, 1964). Bivalve132a.gif



Visceral Mass

The **visceral mass** fills most of the space between the hinge and the adductor muscle (Fig 5). A large, thin fold of the dorsal body wall extends laterally from each side of the visceral mass. These are the right and left mantle skirts and together they enclose a large water space, the mantle cavity, ventral and anterior to the visceral mass. The dark greenish brown **digestive ceca** can be seen at the surface the visceral mass posterior to the gills. Sometimes the **kidneys**, which are paler yellowish brown, may also be visible through the body wall. A pair of large crescent-shaped gills protrude from the anterior and ventral border of the visceral mass. In reproductive individuals the **gonads** fill much of the space in the visceral mass between the stomach and digestive ceca.

The rectum and anus are located in the cloaca, which is part of the exhalant chamber of the mantle cavity. Lift the right mantle skirt between the adductor muscle and the edge posterior edge of the valve. This will expose the **cloaca** (Fig 5). The **rectum** can be seen as a thick tube curving around the posterior border of the adductor muscle. It ends at the anus, whose border is flared outward.

The pointed, conical, ventral tip of the visceral mass extends on the anterior side of the adductor muscle beside the exhalant chamber. It is known as the **pyloric process** (Fig 11). The common openings of the kidneys and gonads are on the pyloric process. The heart is in the pericardial cavityon the dorsal margin of the adductor muscle.

Mantle and Mantle Cavity

As you now view the specimen the tissue closest to you is the right **mantle skirt** (Fig 5). It is penetrated by the adductor muscle. The skirt is thin and transparent and its pigmented border bears abundant **sensory tentacles**. The skirt is immediately inside the right valve and the valve was secreted the skirt. The edge of an identical **left mantle skirt** can be seen lying against the left valve (Fig 5, 7) but most of this skirt is now hidden by the visceral mass and gills. **Blood vessels** can be seen in the right mantle skirt. The two mantle skirts enclose a water space, the **mantle cavity** (Fig 6). In life the mantle cavity is filled with seawater and is divided into inhalant and exhalant chambers by the gills (Fig 5). The feeding and respiratory current enters the inhalant chamber and then passes through pores, or ostia, in the walls of the gills. Once through the ostia, the water is in the exhalant chambers above the right and left gills. These chambers coalesce in the vicinity of the anus to form the cloaca, which empties to the exterior.

Each mantle skirt is a fold of the dorsal body wall and arises from the dorsal part of the visceral mass. Dorsally the right and left skirts are joined to each other and to the visceral mass. Trace the edge of the right skirt dorsally along the anterior border of the valve until it joins the edge of the left skirt. This broad junction is the **anterior fusion** (Fig 5). Follow the edge of the right skirt ventrally and posteriorly until you find the delicate **posterior fusion** where it joins with the left mantle skirt (Fig 5). The posterior fusion of the mantle skirts marks the line of separation of the inhalant chamber of the mantle cavity from the cloaca, which is part of the exhalant chamber. Lift the right mantle skirt anterior to the posterior fusion to reveal the spacious **inhalant chamber**. Lift the right mantle skirt posterior to the posterior fusion to reveal the **cloaca**. The rectum and anus can be seen in the cloaca.

Continue following the right mantle skirt dorsally along the posterior margin until you find where it arises from the visceral mass. The origin of the mantle skirt anteriorly is hidden from view.

The edges of the mantle skirts are lobed to form three longitudinal ridges, or folds, which can be seen with magnification. With the dissecting microscope study the edge of the left mantle skirt and find the three folds. The **outer fold** secretes the shell, the **middle fold** is sensory and bears numerous **tentacles**, and the **inner fold** is muscular, but also has tentacles (Fig 12-91). The outer surface of the outer fold secretes the prismatic layer. The outer surface of the entire mantle skirt secretes the lamellar layer. The groove between the outer and middle folds is the **periostracal groove** from which the periostracum is secreted. Find a region of the left valve with the left mantle still intact and adhering to the valve. Study the mantle edge with magnification to find the outer fold and the periostracal groove. The outer fold, being the same color as the shell and covered by the middle fold, is difficult to see. Adjust the light (reduce it), lift the middle fold with a microneedle, and focus on the outer fold. Look for the thin, glistening, newly secreted sheet of periostracum emerging from the groove and extending over the outer surface of the left valve. New periostracum is difficult to demonstrate in oysters as it is thin and weak and tears free of the valve.

Gills

" Use fine scissors to remove the right mantle skirt by cutting along the line where the skirt joins the right gill for the entire distance between the anterior and posterior fusion points. Set the mantle aside. Two large crescent-shaped **gills** protrude from the anterior and ventral edge of the visceral mass into the mantle cavity (Fig 5). Each gill is known as a whole gill, or **holobranch**, and is composed of two half gills, or **demibranchs**, one of which is lateral and one medial. The four demibranchs give the impression that four gills are present but there are actually only two (Fig 5, 7).

The four conspicuously ridged demibranchs are stacked on top of each other in the inhalant chamber of the mantle cavity (Fig 5). Find the **lateral and medial demibranchs** of the **right holobranch** and the **lateral and medial demibranchs** of the **left holobranch**. In oysters there is no foot to divide the mantle cavity into right and left sides and separate the two gills.

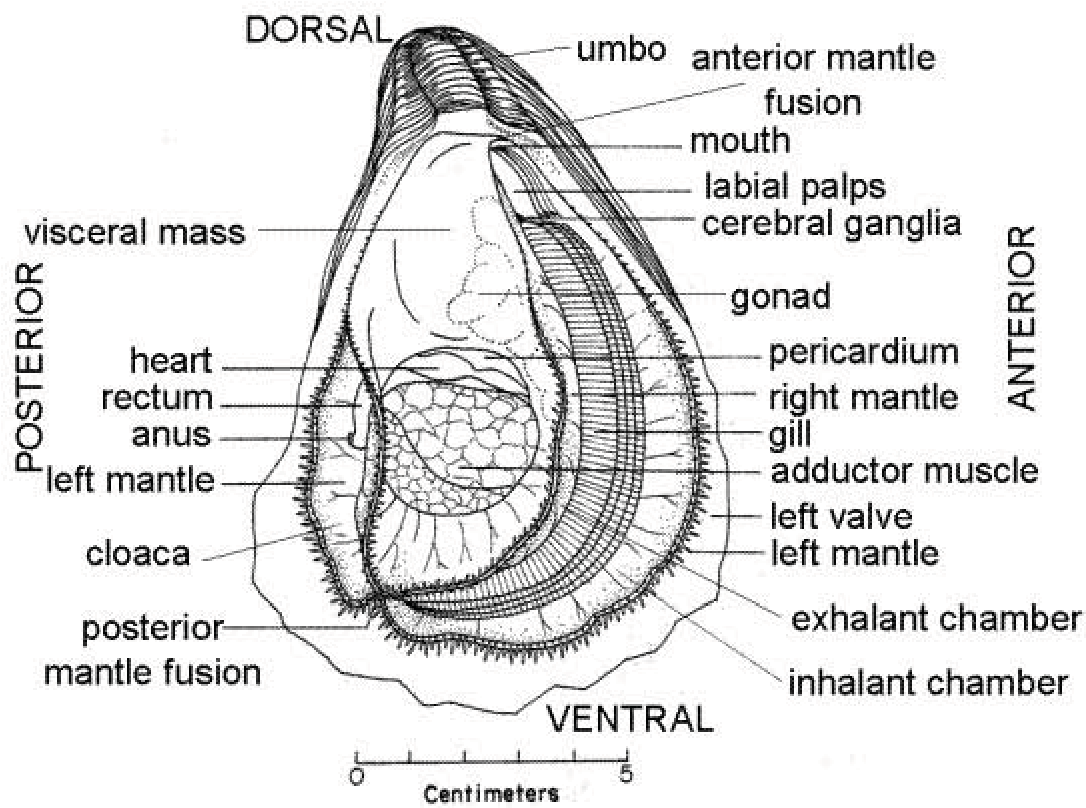
Each demibranch is composed of long narrow gill filaments which cannot be seen without magnification and then only by careful observation and sharp focus. The conspicuous ridges oriented perpendicular to the long axis of the gill are **plicae**, or plications, and each is composed of numerous filaments.

Lift the lateral demibranch of the right gill so you can see the line along which it joins the medial demibranch. This line is the **central axis** of the gill (Fig 6) and is the attachment of the gill to the body. You can see the plicae arise from the central axis and then extend down into the mantle cavity to form one side of the demibranch. At the free edge of the demibranch the filaments are bent almost 180 ° so they extend up again to form the other side of the demibranch before they end at the body. The resulting two-sided sheet is the demibranch. Each side of a demibranch is known as a **lamella** and is composed of side-by-side plicae (made of filaments). The lamella joined to the central axis is the **descending lamella** (= descending limb) whereas the opposite side of the demibranch is the **ascending lamella** (Fig 6, 12-96). The spaces inside the demibranchs, between the lamellae, are water tubes, which are part of the exhalant chamber.

The free edge of each demibranch bears a conspicuous longitudinal **food groove** (Fig 6). You may need to use fine forceps to bend the free edge of the demibranch toward you to reveal the groove. Cilia in this groove generate a current that moves a mucous string with trapped food particles anteriorly to the labial palps for sorting.

The gill filaments of which the plicae are composed have lateral cilia between the filaments to generate the ventilating and feeding current and frontal cilia on the outer surface to move particles off the lamellae. As the ventilating current moves through the lamella from the inhalant to the exhalant side, particles too large for the ostia are caught on the surface of the lamella. Ciliary currents move them ventrally to the food groove along the free margin of the demibranch. The food groove extends dorsally to the labial palps to which it transports food.

Figure 6. A cross section made just ventral to the labial palps. (from Galtsoff, 1964). Bivalve134a.gif



>b. Place a little carmine-seawater or a sprinkle of finely divided carmine powder or chalk dust on the surface of the ascending lamella of the lateral demibranch and watch as it is transported anteriorly by the frontal cilia. Watch for particles to arrive at the food groove and then change directions 90 ° and move dorsally on the way to the mouth. <

>c. With the highest power of the dissecting microscope (40X) study the surface of the ascending lamella. The unrelentingly white tissue of most bivalves provides little contrast and makes it difficult to resolve objects such as gill filaments. Slip a small square of black paper under the demibranch to provide a contrasting background . Use fine forceps as necessary to manipulate the demibranch and tug gently on the plicae as necessary. Confirm that the plicae are composed of numerous gill **filaments**, which are *much* smaller than the plicae. They are almost too small to resolve at 40X. Adjacent filaments are held permanently together by tissue **interfilamentar junctions** that cannot be broken without tearing the tissue. The interfilamentar junctions are penetrated periodically by small pores, or ostia. It is through the ostia that the feeding current passes through the lamella on its way from the inhalant to the exhalant chamber. Between the plicae the ascending lamella is joined to the descending lamella on the other side of the demibranch by tissue interlamellar junctions (Fig 6, 12-98D). These junctions divide the demibranch into vertical **water tubes** that extend up into the exhalant chamber. The interior of each plica is a water tube. <

>d. Use fine scissors to remove a 3x3 mm square of the lateral demibranch including a section of the food groove. Make a wet mount with the square being careful that it is not folded on itself. Examine it with 400X of the compound microscope with the light carefully adjusted. Focus on the **food groove** and look for beating cilia in the groove. Find the parallel gill **filaments**, which also have cilia that may still be beating.

Find **ostia** between the filaments. <

Use fine scissors to cut longitudinally along the line of fusion of the lateral demibranch with the body. Extend the incision for the entire length of the gill. This will open the right **exhalant chamber** and reveal the **water tubes** and **interlamellar junctions** in the interior of the demibranch. Trace the chamber ventrally and posteriorly to the **cloaca** just posterior to the adductor muscle. Find the anus and **rectum** on the circumference of the muscle if you have not already done so. Find the opening from the cloaca into the **left exhalant chamber**. It will be to the left of the **visceral mass**. Confirm that the cloaca is formed by the junction of the right and left exhalant chambers.

In *Crassostrea*, the dorsal end of the right exhalant chamber is expanded to form a large promyal chamber (Fig 6). There is no such modification of the left exhalant chamber nor is it present in *Ostrea*.

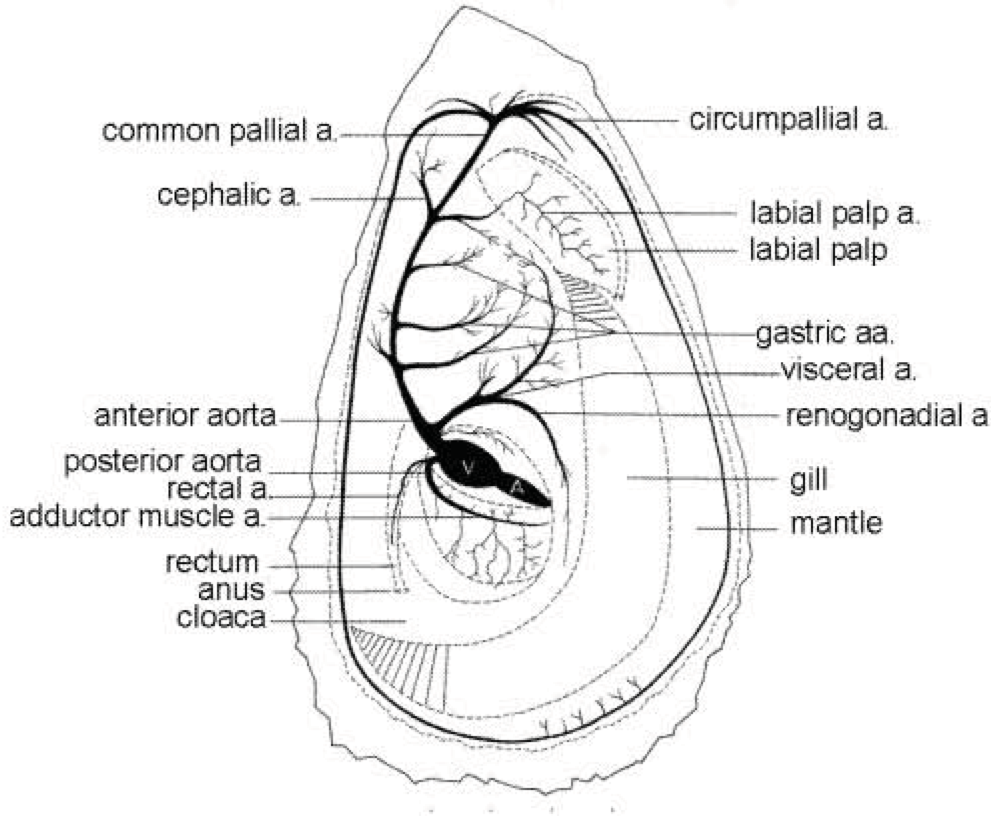
Heart and Pericardial Cavity

If it is still present, remove the right mantle skirt from the region immediately dorsal to the adductor muscle. This will reveal the thin walled **pericardial cavity** on the dorsal border of the muscle (Fig 5, 7). The wall of the cavity is the thin, translucent **pericardium**. With your finest scissors cut through the pericardium to open the cavity. The pericardial cavity is all that remains of the mollusc coelom. Inside it is the **heart** consisting of a ventricle and two atria. The **ventricle** is a large, white or gray, ovoid chamber in the posterodorsal quadrant of the cavity. The atria are smaller than the ventricle and are in the anteroventral region of the cavity. The atria drain oxygenated from the gills via the efferent branchial vessels and send it to the ventricle to be pumped to the various hemocoels via a system of arteries (Fig 7). The brownish right atrium is closer to you and the only one you can get a good look at without further dissection. Watch the ventricle carefully for a minute or so and you will probably see it contract, even if your specimen has been in magnesium chloride for an hour or two. If it does not contract give the heart a gentle squeeze with forceps and watch for a contraction in response.

The conspicuous **posterior aorta** exits the dorsoposterior corner of the ventricle and curves ventrally, still within the pericardium, to supply the adductor muscle (Fig 7). The less obvious, but larger, **anterior aorta** exits the same end of the ventricle dorsal to the posterior aorta. The anterior aorta disappears immediately into the pericardial wall but can be followed a short distance by cutting into the wall with fine scissors. It quickly gives off a large visceral aorta before itself extending anteriorly towards the head. The visceral aorta runs anteriorly and dorsally to the gonad and visceral mass. Notice that the rectum does not pass through the ventricle, or even enter the pericardium as it does in many bivalves.

>e. If you wish to observe the heartbeat, replace the magnesium chloride with seawater and let the oyster sit for about 15-30 minutes. Stimulate the ventricle with a gentle pinch and watch the resulting contractions. Eventually (about 30 minutes) the heart will resume beating spontaneously. When finished with your observations, replace the water with magnesium chloride. <

Figure 7. The arterial system of *Crassostrea* (from Galtsoff, 1964). a = artery. Bivalve140a.gif



The **accessory heart** is a conspicuous Y-shaped tube on the left wall of the cloaca ventral to the adductor muscle. Handling it with dissecting tools may cause it to collapse, in which case it cannot be seen. The accessory heart is muscular and beats, although not as rapidly as the heart itself. Its function apparently is to assist in the movement of oxygenated blood from the mantle to the efferent vessels draining the gills.

Labial Palps

" Use the scalpel to separate the left end of the adductor muscle from the left valve. Carefully remove the oyster from the left valve and transfer it to a small dissecting pan of isotonic magnesium chloride. Place the right side uppermost, facing you, as it was while in the shell. Examine your oyster and find landmarks to orient yourself so you still know dorsal, ventral, anterior, posterior, right, and left.

The two labial palps are located in the mantle cavity at the anterior end of the gills. Each gill is associated with a labial palp. Like the gills, the labial palps are bilobed giving the appearance of two palps on each side (Fig 5, 12-113). Each lobe, known as a lamella, is flat and leaflike. The two lamellae of each palp face each other. The palps are ciliated and are used to transfer food from the gills to the mouth.

Find the **right labial palp** at the dorsal end of the right gill. Find its two lamellae. The one closest to you is the **lateral lamella** of the right palp and the other is the **medial lamella**. Each demibranch is associated with a palpal lamella; the lateral demibranch with the lateral lamella and the medial demibranch with the medial lamella.

Determine that the lateral surface of the lateral lamella is smooth as is the medial surface of the medial lamella. In contrast, the medial surface of the lateral lamella and the lateral surface of the medial lamella bear conspicuous ridges and grooves. In other words, the surfaces of the lamellae that face each other are ridged and grooved and the outside surfaces are not. The ridges and grooves are ciliated and are presumed to function as a **sorting field** to separate food and mineral particles although that may not be the case for *Crassostrea*.

Observe the relationship between the lamellae of the right palp and the demibranchs of the right gill. The food grooves on the edges of the demibranchs end at the crease at the junction of the right and left lamellae of the palp. This crease is the ciliated **oral groove** and it leads to the mouth. Its cilia generate a current in the direction of the mouth. The food string of mucus and food particles from the food groove passes along the oral groove into the mouth.

Look deep under the right palp to find the **left labial palp**. Each lamella of the right palp, medial and lateral, is connected physically with its counterpart on the left palp. Trace the connections from right to left from right lateral lamella to left lateral lamella and from right medial lamella to left medial lamella. These transverse connections form a pair of **lips** above and below the mouth (Fig 12-100). Thus the right and left lateral palps are connected with each other by the **dorsal lip** above the mouth and the right and left medial palps are connected by the ventral **lip** below the mouth.

Grasp the oyster with thumb and forefinger of one hand and hold it above the dissecting pan with the dorsal end up, so you can focus on the head. Use a blunt probe to explore the region at the dorsal end of the visceral mass and locate again the upper and lower lips. With the probe push the two lips apart and find the **mouth.** It is a fairly large horizontal slit. It opens into a short esophagus which itself opens into the stomach. If you press the visceral mass gently with your fingers, stomach contents may be regurgitated out of the mouth, thus confirming its presence. The stomach contents will probably be brownish green. You should squirt this material out of your field of vision with jets of magnesium chloride from a plastic Pasteur pipet.

>f. Reflect the lateral right lamella to the side and arrange the oyster so the ridged surface of the medial lamella of the right palp is horizontal. Place a little carmine-seawater or a sprinkle of carmine powder on the surface of the palp and watch as the particles are transported by the cilia. **<**

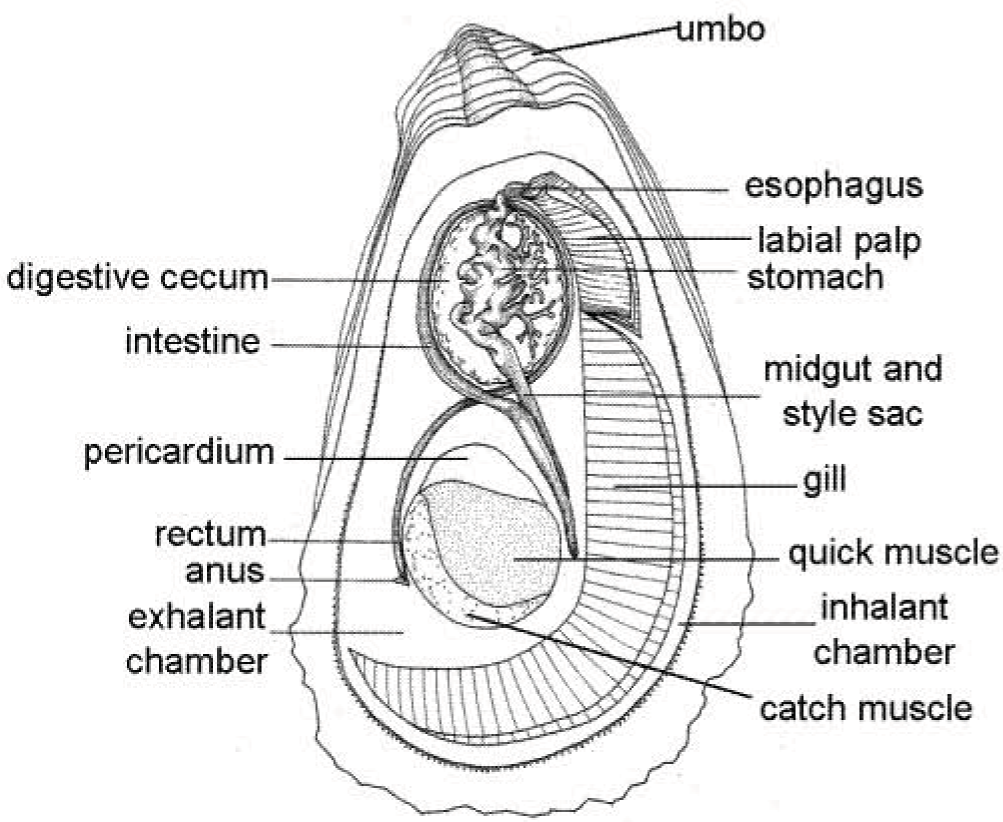
Internal Anatomy
Digestive System

The digestive system consists of mouth, esophagus, stomach, midgut and style sac, descending intestine, ascending intestine, rectum, and anus.

"

Hold the oyster with thumb and forefinger of one hand so you can focus on the region of the mouth and lips. Insert one blade of your fine scissors into the right side of the mouth and cut along the right side of the visceral mass back to the pericardial cavity. The incision should be deep enough to open the lumen of the gut, which in this region will be the stomach. Use a pipet to blow away debris and cloudy fluids. The incision will open the short esophagus and the large, irregularly lobed **stomach** (Fig 8). Extracellular digestion, using enzymes released from a rotating crystalline style, occurs in the stomach.

Figure 8. The digestive system of an oyster as revealed by latex injection (from Galtsoff, 1964). Bivalve139a.gif



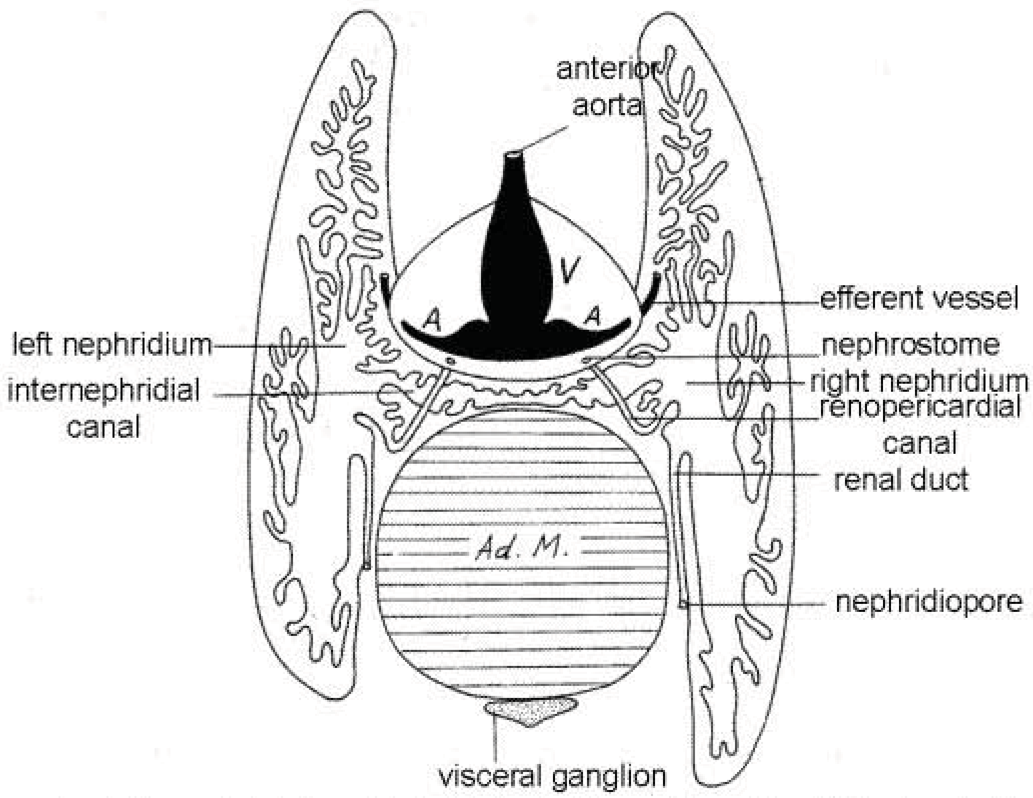
The **crystalline style** is a long, soft, gelatinous, protein rod whose distal end extends into the stomach lumen (Fig 12-102, 12-89B). It is probably homologous to the protostyle of the ancestral molluscs. The style is composed of digestive enzymes that are released into the stomach lumen as the style rotates. The style originates in a deep diverticulum of the ventral stomach wall known as the style say. The glandular epithelium of the sac secretes the enzymes and it s cilia cause the style to rotate. The free end of the style protrudes from the sac into the stomach lumen. The style is reabsorbed when the oyster has not fed recently. The style will probably not be present in your specimen.

The stomach is embedded in, and surrounded by, the greenish-brown **digestive ceca** (Fig 8, 12-89B). The ceca are diverticula of the stomach and are the site of enzyme synthesis, intracellular digestion, and absorption. Part of the digestive cecum is usually visible on the surface of the visceral mass, even in reproductive individuals. On the surface the digestive cecum appears as an irregular, dark greenish-black spot. The kidney, on the other hand, when visible on the surface is a pale yellowish brown.

" Trace the remainder of the gut as far as you can. This is best accomplished by using fine scissors to open its lumen as you did the esophagus and stomach. Use the Pasteur pipet freely to wash away debris that interferes with your view of the lumen. Tracing the gut is destructive of the remaining soft anatomy.

Figure 9. Cross section of the excretory system of *Crassostrea virginica* (from Galtsoff, 1964).

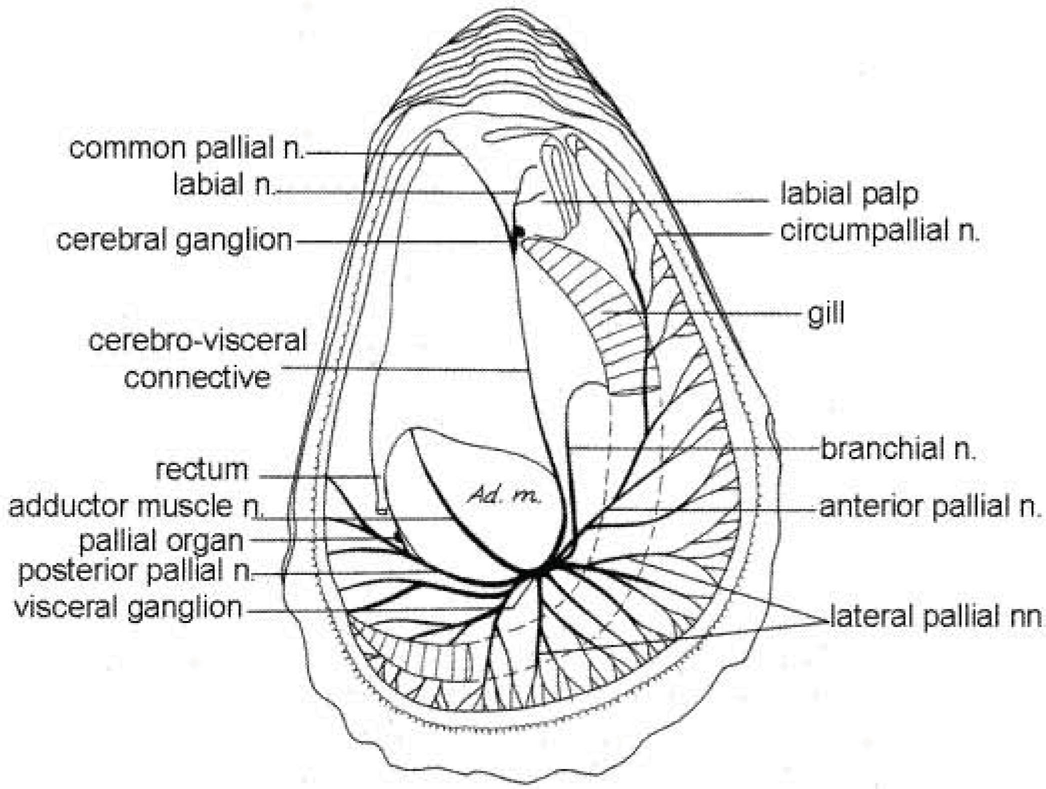
Bivalve142a.gif



Locate the opening into the combined midgut and **style sac** at the ventral end of the stomach. Use the scissors to open the midgut for its entire length. It extends ventrally to the level of the adductor muscle. The style sac and midgut are coalesced side by side and share a common lumen partially partitioned by a pair of opposing ridges (Fig 12-103A). In freshly caught, recently fed individuals a crystalline style may be present in the style sac. Together the two extend ventrally a position near the pyloric process (Fig 11) on the anterior edge of the adductor muscle.

The style sac ends here, near the adductor muscle, but the gut continues on as the intestine. Posterior to the end of the style sac the midgut becomes the **descending intestine** which reverses direction and heads dorsally again, back toward the stomach. It reaches the posterior edge of the digestive ceca, loops dorsally then anteriorly around the stomach and ceca. Having completed this loop the intestine turns ventrally again, now as the **ascending intestine**, and extends to the level of the pericardium where it becomes the **rectum** before ending at the **anus** in the cloaca.

Figure 10. The nervous system of *Crassostrea* viewed from the right (from Galtsoff, 1964). n = nerve, Ad. M = adductor muscle. Bivalve143a.gif



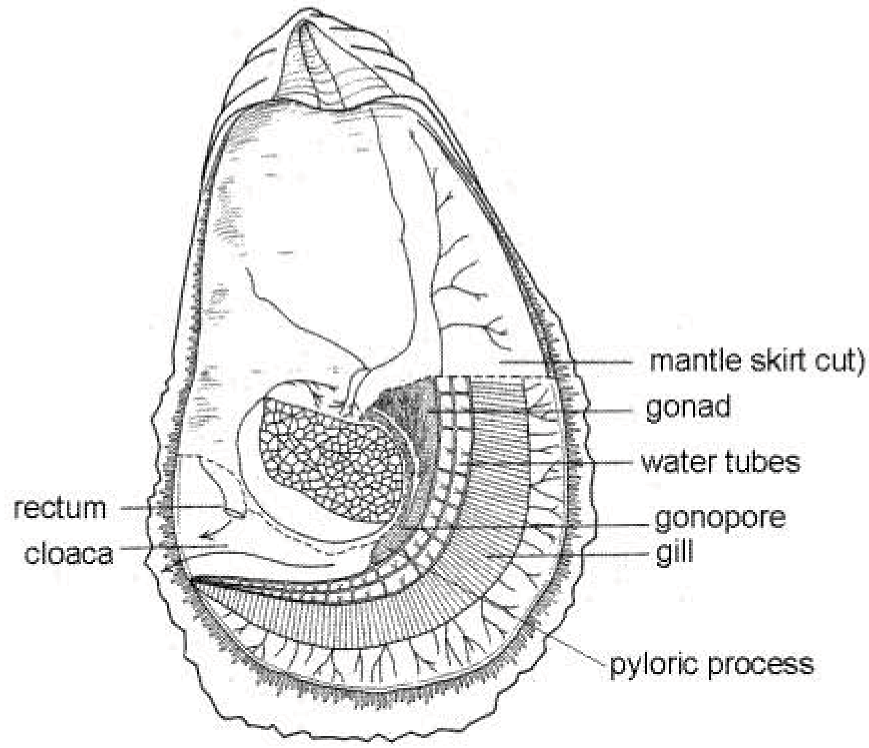
Trace the gut as far downstream as you can by opening the gut lumen with fine scissors. When you can follow it no farther, redirect your attention to the anus and rectum. Insert one blade of the scissors in the anus and open the lumen of the rectum. Cut as far dorsally as you can, opening the ascending intestine as you go. The ascending intestine will eventually reach the level of the digestive ceca and pass anteriorly to the left of the cecum and stomach. It will cross the descending intestine, which you should watch for (Fig 8). After crossing the descending intestine ventral to the digestive cecum it passes along the anterior border of the cecum until it joins the descending intestine dorsal to the cecum (Fig 8).

Excretory System

Although parts of the excretory system can be seen on the surface of the visceral mass, especially in the vicinity of the pericardium, this system will not be studied in this exercise. Exposed parts of the nephridia are pale yellowish brown.

The excretory system consists of a pair of large metanephridia, or kidneys extending from nephrostomes in the wall of the pericardium to nephridiopores in the atrium (shared with the gonad) leading to the cloaca (Fig 9). The nephrostome opens into the renopericardial duct, which connects with the lumen of the nephridium. The nephridia lie immediately below the surface of the visceral mass where they are visible without dissection in lean oysters without well-developed gonads. In fat oysters with hypertrophied gonads the kidneys will be hidden deep under a thick layer of gonad.

Figure 11. *Crassostrea* viewed from the right with part of the right mantle skirt removed to reveal the pyloric process and gonad (from Galtsoff, 1964). Bivalve144a.gif



A large, thick-walled metanephridium lies on each side of the visceral mass. The right and left metanephridia are connected across the midline by an internephridial canal between the pericardial cavity and the adductor muscle to form a H-shaped complex (Fig 9, 12-118). One upright of the H lies to the right of the pericardium and adductor muscle and the other to the left. The nephridia extend dorspoventrally from anterior to the heart to posterior to the adductor muscle.

Nervous System

The nervous system, although simple in *Crassostrea*, is difficult to demonstrate in gross dissection and will not be studied in this exercise (Fig 10, 12-119). It includes a pair of cerebral ganglia, with one ganglion on each side of the esophagus. The two are connected by the cerebral commissure that arches up over the top of the esophagus. The coalesced visceral ganglia are at the ventral end of the visceral mass on the anteroventral border of the adductor muscle. The cerebro-visceral connectives run from the cerebral ganglia to the visceral. There being no foot, there are likewise no pedal ganglia. Pleural ganglia are also absent.

In the absence of cephalic sense organs the cerebral ganglia are weakly developed and small. The visceral ganglia are much larger than the cerebral and nerves from it innervate the mantle, gills, and adductor muscle. In addition to their usual autonomic functions, the visceral ganglia also receive sensory input from the sensory tentacles of the mantle. The tentacles are photoreceptive, mechanoreceptive, and chemoreceptive.

The large white visceral ganglion can be revealed by opening the exhalant chamber and cloaca and looking between the pyloric process and the adductor muscle. The small cerebral ganglia are beside the esophagus at the bases of the labial palps. They are near the surface of the visceral mass but are usually obscured by gonad.

Reproductive System

Crassostrea is gonochoric and at any given time is either male or female (although *Ostrea* is simultaneously hermaphroditic). In reproductive individuals the gonad is large and occupies most of the space in the visceral mass between the digestive ceca and the surface. It may be several millimeters thick but is an indistinct organ without definite walls. The two gonads of the ancestral bivalves are coalesced to form a single organ with two gonoducts, right and left. Gametes exit the gonad via the gonoduct, which joins the kidney tubule to form a common chamber, the atrium, which opens into the cloaca via a pore on the pyloric process (Fig 11, 12-120B).

>e. Cut a small piece of gonad from the visceral mass beside the digestive cecum and make a wetmount using seawater. Examine the slide with the compound microscope looking for gametes. Eggs are large and irregularly shaped with conspicuous pronuclei, sperm are tiny and monoflagellated. Be sure you see oysters and gamete wetmounts of both sexes. <

References

Galtsoff PS. 1964. The American oyster, *Crassostrea virginica* Gmelin. US Fish. Wildlife Ser, Fish. Bull 64:1-480.

Ruppert EE, Fox RS, Barnes RB. 2004. Invertebrate Zoology, A functional evolutionary approach, 7 th ed. Brooks Cole Thomson, Belmont CA. 963 pp.

Supplies

Dissecting microscope
American oysters, *Crassostrea virginica*, 10-12 cm in length
Empty complete shell (If available)
Isotonic magnesium chloride
Cotton work gloves
Screw driver or oyster knife
Dissecting set with iridectomy scissors and microdissecting forceps
Carmine particles
12 cm culture dish
Canned smoked oysters
black construction paper
Plastic Pasteur pipet

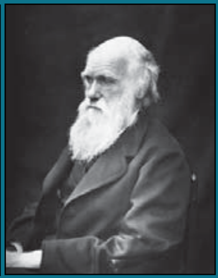
The Teacher-Friendly Guide to Evolution

Using Bivalves as a Model Organism

By Paula M. Mikkelsen & Robin Henne

PALEONTOLOGICAL
RESEARCH INSTITUTION



A professional development tool for K-12 teachers, provided by the Bivalve Tree of Life project and supported by the U.S. National Science Foundation.

ISBN 978-0-87710-495-7
PRI Special Publication no. 40

Production of the *Teacher-Friendly Guide™ to Evolution Using Bivalves as a Model Organism* was funded by the U.S. National Science Foundation under grant award DEB-0732860, "Assembling the Bivalve Tree of Life."

The interactive online version of this *Guide* (including downloadable pdfs) can be found at http://teacherfriendlyguide.org/bivalves.

1259 Trumansburg Road
Ithaca, New York 14850 U.S.A.
www.museumoftheearth.org