Biology 337 *NAME:* ________________________ **Lab worksheet**
Biology of Invertebrates *LAB TIME:* ____________________ **Dr. Bob Podolsky**

LAB F. Ph. MOLLUSCA (Cl. Bivalvia & Cl. Cephalopoda)

I. Cl. BIVALVIA ("two shells") [= Cl. PELECYPODA ("hatchet-foot")]

The bivalve version of the mollusc body plan is compressed between mirror image "left" and "right" valves (shells). Unlike in gastropods, the anterior end of the body lacks a well defined "head" region and does not emerge from the shell. The mouth is anterior, but as in polyplacophorans, sensory structures are often found elsewhere (e.g. an osphradium in the mantle cavity, "eyes" and sensory tentacles on the edge of the mantle in scallops). As in gastropods, the stomach usually contains a **crystalline style**—a large rod composed of digestive enzymes and polysaccharides that is used to grind food against the **gastric plate** like a mortar and pestle—as well as a large liver-like digestive gland. Feeding in the three subclasses is accomplished by either (a) deposit feeding, (b) suspension feeding, or (c) suctorial predation in ways that relate to the structure and function of **ctenidia** (gills). Bivalves are the only mollusc class to have **lost a radula**, and the degree of **cephalization** is also highly reduced.

A. TAXONOMY

Classification has traditionally been based on ctenidium (gill) characteristics. Whether or not these three groupings are clades is currently uncertain, but we will use the traditional subclass names (see optional, newer names in brackets) in order to focus on functional differences associated with gill structure:

Subcl. Protobranchia (= "first gill")**.** All members are marine and live in or on soft substrates. The small pair of ctenidia function primarily in gas exchange, which is thought to be the primitive condition, though in some animals they can also function in limited food collection. Most feeding is accomplished by a pair of **palp proboscides** (thin, extensible projections of the mantle near the mouth) that sweep the substrate outside the shell and transfer sediment to the **labial palps**, where it is sorted before transport to the mouth in mucous strands. *[More recently classified in the subclass Palaeotaxodonta]*

Subcl. Lamellibranchia (= "plate gill"). The majority of bivalves, mostly marine but includes *all freshwater species*. The greatly enlarged and highly folded pair of ctenidia are used for filter-feeding (where suspended food particles are trapped with mucus on a filter), with feeding currents created by **frontal cilia** on the ctenidia. Trapped particles are transported by **lateral cilia** to **food grooves** on the ctenidia and then to the mouth in a mucus strand by **food groove cilia**. Many lamellibranches burrow into sediments, rock, or wood; some (mussels, scallops) attach to hard substrates using proteinaceous **byssal threads**; others sit, unanchored, on top of sediment; and some (e.g. scallops) can swim briefly by clapping the valves together and forcing water out the dorsal edge of the mantle near the hinge. *[More recently classified in two subclasses, the filibranch Pteriomorpha and the eulamellibranch Heterodonta; filibranchs have gill filaments connected by interlocking ciliary tufts, whereas eulamellibranchs have true tissue bridges between filaments]*

Subcl. Septibranchia (= "fence gill"). All are marine carnivores that feed by sucking small crustaceans (or sometimes sediment) into the mantle cavity through the use of a perforated tissue (the modified ctenidium) that separates the upper and lower chambers of the mantle cavity and can be closed and suddenly lifted to create negative pressure in the lower chamber.

Prey are ground against the chitinous covering lining the stomach, though they lack a crystalline style. *[Currently referred to as subclass Anomalodesmata]*

Note that <u>all</u> the species you will see in lab are **lamellibranchs**, by far the largest group of bivalves and the group that is most easily collected locally.

B. EXTERNAL FEATURES. A number of shells are on display for the following exercises.

<u>Shell composition and structure</u>. Last week you looked at a cross section of a clam shell. Keep in mind as you look at these shells the material properties (inorganic **calcium carbonate** and organic **conchiolin**) and the structural properties (3-layer construction: **periostracum** composed exclusively of conchiolin, thin **prismatic layer,** and thicker inner **nacreous layer**).

<u>Body orientation and growth of valves</u>. Note the pattern of growth rings on the empty shells of *Mercenaria*. Identify (a) the **umbo** (the location of the juvenile shell), (b) the **hinge**, (c) the most recently-deposited parts of the shell, and (d) the **anterior**, **posterior**, **dorsal**, and **ventral** edges of the shell. On the inside of the shell, identify separately the **anterior** and **posterior adductor muscle** scars as well as the **pallial scar** close to the ventral edge. The pallial scar shows where the mantle tissue attached to the shell near its edge. Try to locate the same features on any other shells that are available.

Mollusc Shell Growth TQ Part 2:
Last week you pondered the question of how gastropods grow and how they can make simple changes to just a few parameters in order to grow their shells differently. The same kinds of rules and constraints on growth exist for bivalves. Consider the small collection on display.

TQ1: Starting from the umbo, explain where material must be added for a bivalve to grow larger.

TQ2: Note that a valve cannot simply grow in a straight cone, like a limpet (shell #1 from last week). Rather it must curve as it grows in order that the two valve apertures stay in contact. Explain how shell must be added for the shell to curve. Then, compare two shells—the Atlantic giant cockle *Dinocardium robustus* and the disk shell *Dosinia discus*—and discuss the difference in how they would add shell to achieve their different shell shapes.

<u>Shell opening and closing</u>. In *Mercenaria*, two **adductor muscles** insert on each valve and contract to close the shell. Adductor muscles of bivalves have a special **catch** mechanism that locks contractile fibers in a contracted position without the continuous use of ATP. On the

inside of the scallop shell, note the presence of only a single large adductor muscle scar—this muscle is the part of the animal that is consumed commercially.

Closure of the shell is resisted by the **tensilium**, a protein ligament that creates the dorsal hinge and stores the energy of muscle contraction *under tension (*what does this mean?). If available, pick up shells that have been kept moist in an aquarium (the ligament becomes brittle and easily cracked when dry). Note the resting position of the shell with the animal removed. Gently aqueeze the valves together to get a sense of the resistance provided by the ligament.

If an intact scallop shell is present, look for the presence of an additional ligament (the **resilium**) that stores energy *under compression* (what does this mean?) at the hinge when the two valves are closed.

<u>Shell symmetry</u>. The two valves of every bivalve enclose the right and left sides of the body. In an **equivalve shell**, the two valves are mirror images of one another and reflect the bilateral symmetry of the animal they enclose. In an **inequivalve shell**, the shells do not look like mirror images and do not appear to reflect the bilateral symmetry of the left and right sides of the animal they enclose. Finally, in some species *each valve* appears to have its *own* internal bilateral symmetry, a condition known as an **equilateral valve.**

TQ: Among the shells provided, identify at least one species with each of the following:

1) Equivalve shell:
2) Inequivalve shell:
3) Equilateral valve:

TQ: Do two simple sketches showing the planes of mirror-image symmetry visible in *equivalve shells* and in *equilateral valves*. For each plane, explain which body regions (anterior-posterior, dorsal-ventral, left-right) are reflected in the mirror?

TQ: Why does the one species exhibited with an equilateral valve have both this additional plane of symmetry <u>and</u> an additional ligament? (*Hint: they both relate to how the movement of this animal differs from those of most other bivalves)*

EPIFAUNAL BIVALVES (live attached to hard surfaces)

<u>Attachment</u>. Some epifaunal species, like mussels and scallops, attach using a **byssus**, which is a strand of protein **byssal threads** that are glued to hard surfaces. The mussel *Brachiodontes* on

display demonstrates how mussels are often networked in clumps when they attach to the substrate as well as to one another, anchoring them into a mussel bed. Another local species, *Geukensia demissa*, the ribbed mussel, lives in soft sediment but still attaches byssal threads to hard surfaces below the sediment.

If live specimens are available, remove a bowl already containing *Geukensia* or *Brachiodontes* to your microscope for close observation. Tug gently on a mussel to see whether it is attached to the glass bowl or to another mussel. Apply slight force to take account of the strength of attachment while watching the byssal threads stretch under the microscope. Does the strength of attachment seem to be related to the production of fatter threads or more threads?

Now examine the group of live oysters, *Crassostrea virginica*. Compare this aggregation to the group of mussels. How are they attached, as compared with mussels? Carefully tug on the oysters (do not cut yourself) and see whether it is easier or harder to dislodge the oyster from its attachment.

TQ: Why do many bivalves live like this in dense groups rather than living singly? List a major advantage and a major disadvantage.

BORING BIVALVES (penetrate harder surfaces)

One example of a boring bivalve is the so called "shipworm" (*Teredo navalis*). These highly-specialized burrowers cause major economic loss by working holes into the wood of ships and pilings. In an interesting form of symbiosis seen in other wood-harvesting organisms like termites, their nutritional needs appear to be met mostly by symbionts, in this case bacteria in the gut that digest the lignin ingested by the bivalve. When <u>viewed on your dissecting microscope</u>, note the boring shells with cutting teeth, and the prominent **calcareous pallets,** surrounding the inhalant and exhalant siphons, that are used to seal off the hole when the animal withdraws.

A live or preserved specimen of one of the angel wing species may also be present. These animals bore into compacted peat (sediment and decaying plant material) by rocking the shell back and forth. Note the ridges on the shell that are used for cutting into the hard surface.

TQ: Why is the fate of a boring bivalve similar to that of the bivalve-in-a-bottle?

BURROWING BIVALVES (penetrate soft sediments)

<u>Burrowing arena</u>. Many bivalves burrow by projecting the foot into soft sediment, forming an anchor, contracting muscles to pull the shell into the sediment, and repeating until the animal is buried, in many cases several body lengths below the surface. If live specimens of the quahog

Mercenaria mercenaria or the cockle *Dinocardium robustum* are available, examine their location and body position to see if they have progressed to different depths in the sediment.

C. EXTERNAL ANATOMY OF THE BODY, INSIDE THE SHELL

Examine the opened specimen of *Mercenaria* to identify and sketch several important and prominent features of bivalve anatomy. Remember that the exposed space you are examining is *external* mantle cavity—these structures would be in contact with seawater inside the shell.

Muscles. Find the **anterior and posterior adductor muscles**, which match up with locations of scars you found in the empty shells**.** Under the microscope, try to see the *two distinct regions* of each muscle. **Catch muscle** is the "outer" crescent (anterior part of anterior muscle, posterior part of posterior muscle), composed of smooth fibers capable of sustained, slow contraction. The remainder is **fast muscle**, composed of striated fibers with abundant supplies of myoglobin (hence the red color) and mitochondria.

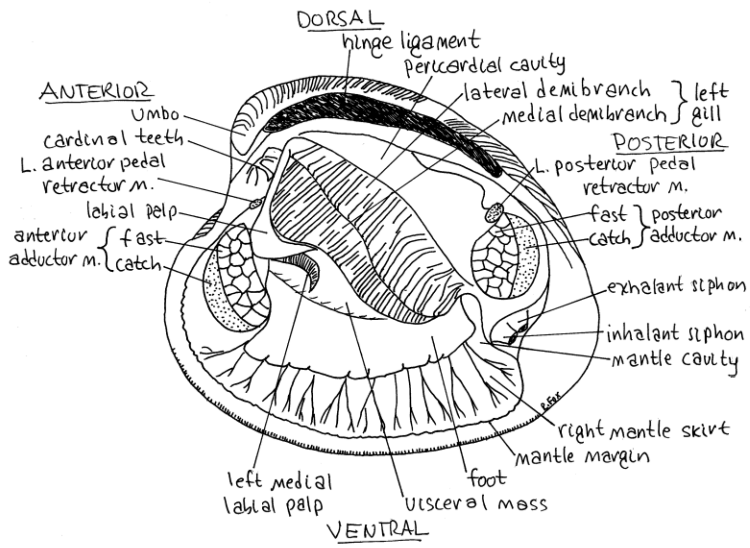

Fig. 1. The left side of *Mercenaria* (how do you know?) with the left valve and left mantle skirt removed.

Mantle cavity. In this opened specimen, the **mantle skirts** that would attach at the shell margin on the **pallial line** are detached and floating free. The body organs you are looking at sit within the mantle skirt in the **mantle cavity**, which is divided in two by the leaflike **ctenidium.** The large space you see now is the spacious **inhalant chamber**. The smaller **exhalant chamber**, which is dorsal to the ctenidium, cannot be seen, but its position can be visualized by finding the two short, tubular **siphons** at the posterior end—water enters the inhalant chamber through the lower (ventral) siphon and exits the exhalant chamber through the upper (dorsal) siphon.

Between the two ctenidia is the large bulging **visceral mass** with a thinner, muscular, hatched-like **foot** below. As you look at the exposed side of the animal, you should also identify the **labial palps**, extensions of the gill used in sorting food as it is brought to the **mouth**. On your sketch of the animal, show the pathway that a food particle would follow as it is brought into the inhalant chamber, caught by mucus on the gill, transported to a food groove and across the labial palps, and brought to the mouth for ingestion.

Ctenidium. *Mercenaria* has **eulamellibranch gills** that attach to both the body and to the mantle skirt laterally to form a complete partition between the ventral (inhalant) chamber and the dorsal (exhalant) chamber. Find these tissue connections to the body and mantle skirt. Permanent tissue bridges between adjacent gill filaments create a fine filter for suspended particles such that water passes only through microscopic **ostia** in the gill filaments.

II. Cl. CEPHALOPODA (= "head foot")

Cephalopods—including squid, octopus, cuttlefish, and chambered nautilus—are among the largest, most mobile, and most active invertebrates. All are carnivores that capture prey using arms and tentacles that are frequently covered with suckers or sharp hooks. Cephalopods have a highly developed nervous system and a camera eye that can focus images; the eye shows several features that distinguish it from the camera eye of vertebrates, a striking example of convergent evolution (and a striking example of imperfect design in vertebrate eyes). The skin contains specialized cells, **chromatophores**, that are under muscular control; they change the coloration of the skin and are used for camouflage and behavioral displays. Many cephalopod females are "semelparous"—they reproduce once, and die soon after tending and hatching their brood.

A. Taxonomy

The squid, octopus, cuttlefish, chambered nautilus, and extinct ammonites are separated into three subclasses and several orders, some of which you should become familiar with:

Subclass Ammonoidea (extinct)—Ammonites were large animals with external shells that went extinct at the end Cretaceous (with dinosaurs). Closest living relative is the nautilus.

Subclass Nautiloidea—Six known species of *Nautilus* all inhabit the Indo-Pacific. The external shell has an outermost large **body chamber** and a series of smaller chambers closed off by **septa** that are penetrated only by the **siphuncle**, a strand of highly vascularized tissue. By day individuals are as deep as 600 meters, but night migrate up to feed at coral reefs. These extraordinary migrations are made possible by the close regulation of shell buoyancy. Changes in the salinity of the blood in the siphuncle cause water to diffuse to or from the chambers of the shell. The chambers are then passively filled by gases (O_2, CO_2, and N_2) from blood. Nautiloids have pinhole eyes with no lens, and up to 90 tentacles with no suckers.

Subclass Coleoidea

Order Octopoda—Octopods live in diverse habitats, from deep ocean trenches to tide pools, feeding on crabs, clams, and shrimp using a powerful beak. One pelagic species, *Tremoctopus*, feeds on the Portuguese man-of-war and attaches pieces of the stinging tentacles to its arms, possibly as a defense. The 8 arms are all covered with suckers. During reproductive seasons, the end of one arm of males can differentiate into a specialized structure, the **heterocotylus**, that is used for introducing spermatophores to the female.

Order Sepioda—Cuttlefish and sepiolids are epibenthic animals that either bury themselves in sediment or hide on reefs, but some are nektonic (pelagic, swimming animals). The cuttlebone, an internal, modified shell, has chambers that are used to regulate buoyancy by changing the gas-to-liquid ratio in a way similar to nautilus. They have 8 arms and 2 longer tentacles with hooks used in prey capture.

Order Teuthoida—Adult squids are **nektonic**, yet they have no shell-derived buoyancy device. Some, including the giant squid *Architeuthis*, regulate buoyancy by filling their tissues with ions, typically ammonium, that are less dense than seawater. These so called "ammoniacal" squid are poor food for humans (imagine the taste!) but are an important component of the diets of many fish and marine mammals (especially sperm whales). They have 8 arms, as well as 2 tentacles that are highly specialized for prey capture.

B. EXERCISES

1. Squid dissection. Set aside at least 1.5 h for efficiently dissecting a frozen squid. Answer these questions when you come to the appropriate place on the dissection worksheet.

Table. Describe three specialized features that distinguish the cephalopods from other molluscs:

Circulatory system:	
Contractile structures of the circulatory system:	
How the mantle cavity is ventilated:	

TQ: What changes in the lifestyles of cephalopods have selected for these features?

2. Reproduction. Examine on your dissecting microscope the gelatinous "squid fingers" containing various stages of embryos (similar to those of octopuses). Note the very large yolk sac hanging from the embryo's mouth. Juvenile squid look like miniature versions of mature adults. Cephalopods are unique among molluscs for having "semelparous" reproduction, where the entire reproductive effort is put into one bout before death.

3. Buoyancy. Examine a sectioned *Nautilus* shell and compare it with figures in your text to see how the animal is positioned relative to its shell. Note the chamber and the holes through the septa dividing these chambers, through which passes the **siphuncle**, a vascularized cord of gas-releasing tissue. This tissue regulates the mixture of gas and low-density liquid present in these chambers, which are otherwise sealed off from seawater. Be able to explain precisely how the gas alters the buoyancy of the shell.

TQ: The shell of nautilus is *planispiral*—the whorls of the coiling shell remain in a single plane. The shell of the planorbid gastropod you looked at last week was also planispiral. How is the plane of spiraling different in the two? Imagine the three body axes (anterior/posterior, dorsal/ventral, or left/right). Around which axis does the shell coil in each case? In which of the two cases is bilateral symmetry of the animal also a feature of the shell?

The external shells of the extinct Subclass Ammonoidea were similar in design and function to those of the nautiloids. However, they are considered to be more closely related to coleoids (octopus, squid, and cuttlefish) than to nautiloids. They went extinct around 66 Mya along with (most of the) dinosaurs. We have an ammonoid fossil on display.

4. Internalized, coiled shells. These small shells belong to *Spirula spirula*, which comprises *its own separate order* (the Spirulida) within the coleioids, so is more closely related to octopus, cuttlefish, and squids than to nautilus**.** Like nautilus, *Spirula* is a deep-sea, highly specialized animal with a coiled shell that functions in a similar way to the nautilus shell to regulate buoyancy, except that the shell is *internal*. Note the small hole in each septum that leads to a tube containing the **siphuncle** in the live animal. Because they live deep in the ocean they are rarely seen alive, but their shells wash up frequently on beaches.

5. Internalized cuttlebones. Cuttlebones are the internalized shells of cuttlefish. They also contain chambers that allow for the control of gases and help these animals to maintain buoyancy at depth, and also play a key role in body shape. Press gently on the preserved specimen of the cuttlefish *Sepia officianalis* to feel the position of the cuttlebone inside.
   Cuttlebones are provided to pet birds as a source of calcium and as a scratching material for sharpening their beaks. These cuttlebones were more likely to have been collected from a beach, where they wash up in great numbers, than from a live animal.

6. Examine any octopuses (*not* octopi—ask me why!) on demonstration. Note that the body entirely lacks a shell or any remnant of shell. Make sure you understand the body orientation relative to other cephalopods and, more generally, other molluscs. The large "head", for example, is really the globose visceral mass. Octopuses (as the name implies) are the only group of coleioids that have only eight arms, with unstalked suckers that lack chitinous teeth.

   Note that the smallest octopus on display, the blue-ringed octopus *Hapalochlaena maculosa*, is also likely the most dangerous! To humans, blue rings are the most lethal octopus in the world (what do you predict are its most common prey?). Its venom includes tetrodotoxin (TTX), a component found in just a few other animals (pufferfish, newts, naticid snails) and actually produced by bacterial symbionts. Two ducts pass right through the brain, bringing venom down to the mouth from a pair of salivary glands. Each animal contains enough venom to inflict paralysis and eventual agonizing death on 2-3 dozen people.