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

LAB I. Ph. Chordata, Subph. UROCHORDATA; Ph. PHORONIDA, Ph. BRACHIOPODA, and Ph. BRYOZOA (The "Lophophorates")

I. Ph. CHORDATA

A. general characteristics and taxonomy

Chordates are the sister group to [hemichordates+echinoderms], which together complete the deuterostomes. The phylum Chordata includes three subphyla: vertebrates, cephalochordates (= amphioxus, sister group to vertebrates), and urochordates (= tunicates, sister group to the other two). Chordates share a derived character, the **notochord**—a stiff rod composed of gel-filled cells wrapped in a fibrous sheath—at some stage of development. Chordates also have **pharyngeal gill slits** (also shared with hemichordates, and also perhaps with extinct echinoderms), a **dorsal, hollow nerve cord** (DHNC), and a **post-anal** tail.

Subph. Urochordata (= Tunicata)—Tunicates are all **marine filter feeders**. They are all enclosed within an outer covering, the **tunic**, a living exoskeleton that is composed of water, a protein matrix, the polysaccharide **tunicin** (which closely resembles the plant polysaccharide cellulose), and sometimes $CaCO_3$ spicules. The tunic is a "living exoskeleton" that is pervaded by blood vessels, and can "grow" as the animal grows larger in size.

Cl. Ascidiacea—Adults are all **benthic** and **sessile**, with well-developed tunics. A large internal **pharynx** with abundant ciliated **pharyngeal slits** is used to drive water motion. Food is collected on a large **mucous sheet** produced by the **endostyle** that is moved by pharyngeal cilia from ventral to dorsal, and then collected into a mucus string that is fed continuously into the gut. Adults lack a DHNC and a notochord, though these characteristics are present in the **tadpole** larval stage. Ascidians may be **solitary** or **colonial**. The ascidian tunic can store high levels of certain heavy metals, including vanadium, gold, and iron, which are concentrated from seawater and delivered by the blood. Ascidians are also known to store ammonium ions in the tunic.

Cl. Larvacea—Adults are all **pelagic**, tadpole-like animals that secrete and live within a large gelatinous **house**. The house has internal channels to direct locomotory and feeding currents produced by the beating of the muscular tail. Food is captured on a **mucous web** secreted by the endostyle (as in ascidians), but the web is held outside the body in the path of feeding currents. The house is abandoned when it becomes clogged with debris or feces.

Cl. Thaliacea—Adults are all **pelagic** filter-feeders that are somewhat similar in form to benthic ascidians, but with the oral siphon anterior and the atrial siphon posterior. The tunic tends to be clear, making these animals largely transparent (as is common in pelagic organisms). The pharynx is complete in **O. Pyrosoma**; is a sheet that separates anterior from posterior in **O. Doliolida**; and is reduced to a simple **pharyngeal bar** without stigmata in **O. Salpida**. Note how this series of reductions in the pharynx is correlated with changes in both locomotion and feeding. (a) Locomotion is achieved by ciliary currents in pyrosomes, by a mixture of ciliary currents and muscular contractions in doliolids, and entirely by muscles in salps. The muscular contractions, involving large bands of muscle in the body wall, force water out the atrial siphon as the oral siphon is closed by sphincter muscles. (b) Feeding is accomplished by ciliary currents in both pyrosomes and doliolids, which have stigmata, but by muscular contractions in salps, which lack stigmata and instead use muscular contractions to force water through the mucus net suspended between the pharyngeal bar and the endostyle. Various forms of **colonial growth** are seen in the adult form of pyrosomes and in the complex life histories of doliolids and salps.

***Subph. Cephalochordata*—**The 23 species of lancelets, or amphioxus, are small, fish-like animals that live partially buried in sand in shallow water where they filter tiny food particles out of the water using mucus and gill slits on the enlarged pharynx. They have a well-developed axial musculature used for swimming or escaping predators.

Subph. Vertebrata*—Not covered in lab, for purely anthropocentric, historical reasons.*

B. EXERCISES

Cl. Ascideacea

- <u>External morphology and "behavior"</u>. The solitary ascidians *Molgula manhattensis* (the "sea grape") and *Styela plicata* are common members of the "fouling community" on local piers and floating docks. Pick up (with your hands!) a live specimen of each species, and gently squeeze to get a feeling for the texture and firmness of the tunic. Take these specimens to your lab bench in a bowl of clean sea water. Allow the animal, undisturbed, to open the **oral** (or buccal) and **atrial siphons**, which might have closed off using sphincter muscles when the animal was disturbed. While you wait for your specimens to relax and open the siphons, examine the slide in the next exercise.

- <u>Internal anatomy</u>. *Ecteinascidia* sp. is a colonial ascidian, with colony members connected by stolons. Examine a prepared slide of a single zooid using high magnification on your **dissecting** microscope. Although the slide contains a single zooid, colony members are connected by stolons and are physiologically integrated by **blood vessels** that run between zooids. Carefully draw the features as you observe them.

Orientation to body axes (A-P and D-V) is made easier by the clear tunic and staining of the internal organs. The end of the zooid with the two siphons is **anterior** (the so-called "brain" is located between the two), and the atrial siphon is positioned **dorsal** to the oral siphon. These two features will allow you to identify the **posterior** and **ventral** sides as well. As you sketch your specimen, you should label the A, P, D, and V sides. Now that you are oriented, are you looking at the **right** or **left** side of your animal? (Note that internal structures may be more visible from the left side.)

The zooid is surrounded by the transparent **tunic**. Look for $CaCO_3$ spicules embedded in the tunic near the siphons. Locate the **buccal (oral) siphon** at the center of the anterior end and the **atrial siphon** dorsal to it. The **mouth**, just inside the oral siphon, is encircled by a ring of **buccal tentacles** (one feature that differentiates the two siphons). The mouth opens into the very large **pharynx**, which is perforated throughout by elongate oval **gill slits (stigmata)**. Surrounding the pharynx is a large open space, **the atrium**. Although the atrium looks like it is dorsal to the pharynx, it actually wraps around the left and right sides as well to create a "jacket" that water enters after leaving the gill slits. The walls of the atrium attach to the pharynx along the ventral side.

Review how water is drawn into the pharynx and through the stigmata by the action of **lateral cilia** on the edges of the stigmata. The pharynx therefore serves as the principle organ for gas exchange in addition to feeding. Food particles are filtered by a **mucous sheet** secreted by the darkly-stained **endostyle** along the ventral side of the pharynx. A different set of cilia, the **frontal cilia**, move the continuously secreted sheet around the inside surface of the pharynx from ventral to dorsal, where the mucus is collected into a string and transported by cilia into the gut.

At the bottom of the pharynx is a short curved **esophagus,** leading to the larger pouch-like **stomach** posterior to the pharynx. On its ventral end the stomach narrows to the **intestine**, which leads to the dorsal and anterior-running **rectum**. The **anus** empties into the atrium, where digestive wastes are carried out of the atrial siphon by respiratory currents.

TQ: How does material in the gut change form from esophagus to anus? Describe the feeding process that leads this “food” to have the appearance of a “string” in the early part of the gut.

In the loop between the esophagus and intestine are the **gonads**. The **testis** is a darkly-stained horseshoe, with a dark **sperm duct** leading to the atrium near the anus. The smaller **ovary** is a cluster of large eggs in the center of the horseshoe, with an oviduct that opens posterior to the anus. (The oviduct is difficult to see unless it contains embryos being gestated.)

Now transfer the slide to the **compound** microscope to examine the pharynx in detail at 100X or higher. Because you are looking through the pharynx, the gill slits on the two sides of the pharynx are superimposed. Try to focus carefully on one plane to see the regularity of the slits in the pharynx wall--about 30 rows with 60 slits per row. Also examine the **endostyle** to see that it is a groove flanked by two thick ridges of tissue.

- <u>Live specimens</u>. Now return to your live specimens of *Styela* and *Molgula*, trying not to disturb them greatly. Note differences in the texture of the tunic—*Styela* has a thick leathery, bumpy surface, whereas the surface of *Molgula* is smooth, white, and translucent (if *Molgula’s* tunic is clean you may be able to see the loop of the intestine through the left body wall.

Although the principle mechanism of water movement is ciliary, sudden muscular contractions of the body wall can provide a protective mechanism for **backflushing** the pharynx if sediment or other material enters the mouth.

TQ: Would you expect the buccal siphon to be longer and narrower than the atrial siphon, or vice versa? Explain your reasoning, and relate your explanation to design features in other organisms. With the animal quite relaxed, view these features and test your hypothesis.

There should be one *Styela* and one *Molgula* cut open to reveal (a) the thickness of the tunic, (b) the complexity of the pharynx surface, and (c) the form and layout of stigmata.

✂ With fine scissors, you can try removing a small, single-layered piece of pharynx, and make a wet mount, being sure the tissue is not folded over itself. Compare the layout and shape of the stigmata at 100X, then 400X. Find the conspicuous **lateral cilia** on the margins of the gill slits, which creates water flow through the stigmata. Look also for **longitudinal** and/or **transverse blood vessels** that run through the pharynx, carrying exchanged gases from this major respiratory organ. Note that all of these features may be m ore visible in the

- *Compound ascidians.* We may have two very different examples of **compound** ascidians, in which the zooids are enclosed by a common tunic. Begin as before by touching the two types and noting differences in the texture of the tunic. Take samples to your dissecting microscope to look at external details of the fine structure of the colony.

  (a) An unidentified compound ascidian is currently growing as a thin, flexible film over much of the other biomass on floating docks near Folly Beach. Note how these animals depend on the substrate provided by other animals, and could potentially be detrimental in their effects. Find the **siphons** on the surface of the colony, and try to determine (using carmine particles if necessary) where the oral and atrial openings are located. See if you can determine using high magnification through the semi-transparent tunic how the internal organs are distributed.

  (b) Examine a small chunk of the thick, gelatinous colonies of "sea liver" (*Aplidium stellatum*), a rubbery colonial tunicate. Zooids are arranged in circular groups under the common tunic, although it can be difficult to see the internal structure of these colonies.

Species	Zooid size (mm)	Tunic thickness (mm)	Form (Solitary, clonal, colonial, compound)	Distinct external features, characteristics of the pharynx and stigmata

- *Tadpole larva.* Examine a prepared slide of the **tadpole larva** of a compound ascidian, *Amaroucium pellucidum*. Compound ascidians typically brood their young inside the colony to an advanced stage of development, with many of the internal organs that will be functional in the adult stage already present (though non-functional) in the larva. Note the long **tail** coming off of the ovoid **larval body**. You should be able to see a thick, transparent, almost invisible **tunic** surrounding the body. The **notochord**, visible down the center of the tail, is used to antagonize longitudinal muscles that contract to cause the tail to move side to side. At the anterior end locate two or three **adhesive papillae**, used to attach just prior to metamorphosis. Just dorsal and posterior to the adhesive papillae is the **cerebral vesicle** (= sensory vesicle) that contains a **statocyst** for gravity detection and an **eyespot** for photoreception. Posterior to the vesicle, and at the center of the body, is a mass that is the non-functional **pharynx** and **atrium**, with the siphons enclosed within the tunic. Although you may not see details of these structures, if you focus carefully you should be able to see the few large **protostigmata** in the developing pharynx.

Cl. Larvacea

- *Larvaceans*. Examine the preserved specimens, a far cry from the pleasure of seeing a live animal moving in its secreted house. Note similarities with the larval ascidian—the long, **muscular tail** (with a notochord, in the adult stage) used to drive swimming and feeding currents in the house. These preserved animals are valuable, so try to just get a sense of their general structure and similarity to ascidian larvae on the dissecting microscope.

Cl. Thaliacea

- *Salps*. The pelagic salps have a tunic and body wall that is remarkably clear. Salps can be distinguished from doliolids, in part, by the prominence of **muscle bands**, the presence of the slim **pharyngeal bar** in place of the pharynx, and the corresponding **absence of stigmata**. The mucous sheet used in food collection is simply suspended between the **endostyle** and **pharyngeal bar**, separating the oral (anterior) and atrial (posterior) chambers. Currents used for both locomotion and feeding are created by contractions of muscle bands to force water out the **posterior atrial siphon**, with the **anterior oral siphon** closed off. Locate these structures, along with the digestive system, in the preserved specimens.

Salps are also noteworthy for the unhappy fate they suffer from certain **hyperiid amphipods**, which *take over* the tunic and spend their lives swimming through the water, using their jointed appendages to propopel water out the atrial siphon! Hyperiid amphipods may also be on display. (Note that these animals are rumored to be the model for the aliens in the Aliens movies.)

II. The "LOPHOPHORATE PHYLA" and Ph. KAMPTOZOA

Three phyla, the Bryozoans, Brachiopods, and Phoronids—which form a clade within the Lophotrochozoa—share a derived feature, the lophophore: a crown of ciliated tentacles that surrounds the mouth. The lophophore has its own fluid-filled coelom separate from the body coelom. The complete gut (when present—the anus has been lost in the articulate brachiopods) is U-shaped, with the mouth located **inside** and the anus **outside** the ring of tentacles. A fourth phylum, the Kamptozoa, also have a tentacled crown, but this crown lacks a coelom and may not be homologous to a lophophore, and the anus is located **inside** the crown. Relationships between kamptozoans and the other three phyla are uncertain.

A. TAXONOMY

The "lophophorate" phyla

Ph. Phoronida—The 15 or so species of phoronids are all long, worm-shaped tube dwellers. Some burrow in soft and others in hard substrata, though all secrete a chitinous tube. These animals are unusual (though not unique among invertebrates) in having oxygen-binding pigments (hemoglobin) within blood cells.

Ph. Brachiopoda (“arm-foot”)—Adults superficially resemble bivalve molluscs in having the viscera enclosed between two calcitic shells. However, brachiopods have dorsal and ventral valves (unlike the right and left valves of bivalves), and most are permanently attached to substrates by a **pedicle,** a tough, flexible stalk that comes out the posterior end of the shell. Also unlike in bivalve molluscs, the shell valves are operated by opposing sets muscles: adductor muscles (for closing) and diductor muscles (for opening), without an energy-storing ligament. The viscera of some brachiopods contain toxins that render them unpalatable.

Cl. Articulata—Shell valves (always $CaCO_3$) have an articulating hinge with interlocking teeth that prevent sliding of the valves relative to one another. Some members of this class have a calcitic internal support, the **brachidium**, which supports the large lophophore. The **incomplete** gut ends blindly.

Cl. Inarticulata—The shell valves (typically $CaPO_4$) have no articulating hinge or brachidium. The gut is **complete** and the anus opens just outside the tentacle ring.

Ph. Bryozoa (“moss animal”)—All species (mostly marine, some freshwater) are colonies of genetically-uniform **zooids**. Individual zooids live within secreted gelatinous, chitinous, or calcitic box-shaped **houses**, and the lophophore is protruded from the box under various mechanisms that take advantage of increased internal pressure. Zooids are interconnected and can share nutrients. A number of specialized terms are necessary to appreciate the anatomy: the soft parts of each zooid (lophophore, gut, and other organs) constitute the **polypide**; the house around the polypide consists of the **cystid** (the cellular body wall that secretes the house) and the **zooecium** (the secreted, non-living portion). Zooid polymorphism is common. Non-feeding colony members may be specialized for defending (**avicularia**) and cleaning (**vibracula**) the colony, for attachment to the substrate, or for exchange of coelomic fluids between adjacent zooids, or for brooding young (**ooecia)**.

Ph. Kamptozoa—The kamptozoans, or “entoprocts,” have a crown of tentacles similar to the lophophorate phyla, but numerous dissimilarities suggest that they are not part of the lophophorate clade. The tentacles have fluid-filled cores that are part of a hemocoel rather than a true coelom and the digestive system, although U-shaped, exits with the anus located within the crown (distinguishing the “entoprocts,” or anus within”, from the “ectoproct” bryozoans). They are nevertheless typically introduced with the lophophorate phyla given the striking similarities and a lack of certainty about their phylogenetic position.

EXERCISES

Ph. Phoronida

- Phoronids live in **chitinous** (what does this mean?) but **non-calcified** (what does that mean?) **tubes**, which are impregnated with sand grains. The sand grains give an indication of the kind of sediment these animals live burrowed into, with the lophophore tentacles protruding from the sediment surface. Unfortunately our specimens were recently damaged and are not available for inspection.

TQ: Making reference to both the internal structure of the lophophore and the habitat where these animals live, *describe* three possible functions served by the lophophore of phoronids.

Ph. Bryozoa

- *Zooid form*. Examine a prepared slide of an individual zooid of the freshwater bryozoan *Pectinatella*. The most conspicuous features are the **zooecium**, **lophophore**, and **gut**:

>>> The **zooecium** is underlain by the **epidermis**, a layer of **circular muscle**, a layer of **longitudinal muscle**, and the **peritoneum** that lines the large body **coelom**. The body wall (all of these structures together) is known as the **cystid**, as distinct from the rest of the zooid, the **polypide** (the "movable" portion). The polypide includes **lophophore**, **gut**, **gonads**, and **funiculus** (a thin coelomic connection that moves nutrients among colony members).

>>> The U-shaped **gut** is partitioned into **pharynx**, **stomach**, and **rectum**, all separated by distinct sphincters. Can you see material in the gut? Where is the anus located?

>>> The **lophophore** and the remainder of the anterior end of the polypide can be extended and retracted. A pair of lophophore **retractor muscles**, extending from the base of the lophophore, can quickly pull the introvert back into the zooecium. Feeding requires that the lophophore be extended, which is accomplished by contracting the **circular muscles** in the body wall. This pressurizes the coelomic fluid to pop the lophophore out of the **orifice**.

- *Colony form*. Bryozoans all live in physiologically-integrated *colonies*. We may have live examples of three different colony forms (**branching, encrusting, and gelatinous**). You should examine samples of all three under your dissecting scope to see (1) the behavior of individual zooids, (2) the distribution of zooids in colonies, and (3) the composition of the **zooecium**, the outer wall of the colony. Patiently watch to see the lophophores emerge from zooecia and begin feeding. With backlighting, carefully observe natural particles in the water as they are drawn down into the tentacled crown. You may also see the tentacles flicker occasionally as particles are encountered. These flickers are accomplished, of course, by longitudinal muscles in the tentacle walls.

[Note how the species with **branching** morphologies can resemble branching hydroid colonies. On microscopic inspection the differences should be clearer: bryozoan **zooids** are each contained within individual **zooecia**, their lophophores are **retractible**, and the body contains a number of organs not found in the simple hydroids.]

Species name	Colony form	Zooecium composition	Zooid distribution	Sketches

TQ: Watching *Bugula*, notice the direction of water flow created by the lophophore. Given the position of the anus, would the direction of water flow hinder or contribute to the removal of digestive wastes from where the mouth is located? How might the efficiency of waste removal differ depending on different colony growth forms?

TQ: Without disturbing them, watch lophophores both extend and retract. Based on your observations, how do you think the coordination of tentacle retraction among zooids might be accomplished? Now, gently disturb a single zooid with a probe, and then more vigorously disturb the zooid. Is the coordinated retraction local or global for each type of disturbance? How might it achieve more coordinated retraction?

Ph. Brachiopoda

- *Bivavled shell*. Examine an empty shell of the articulate brachiopod *Terebratalia transversa*. The convergence between shells of brachiopods and bivalve molluscs is absolutely remarkable. For example, the shell is composed of three layers, with an outermost **periostracum** composed of **protein**, and two inner layers composed mainly of calcium carbonate (in articulate brachipods) or calcium phosphate (in inarticulates). Also, the shell is secreted by the underlying epidermis, known as the mantle, in a fashion similar to the bivalve molluscs.

However, differences between brachipods and bivalve molluscs are substantial. Brachiopod valves are D&V whereas those of bivalves are L&R. The plane of bilateral symmetry therefore divides each brachiopod valve into more or less equal right and left sides. The **dorsal valve** is the smaller, and often the flatter, of the two and it acts as a lid. The **ventral valve** is the larger and is usually the deeper of the two, and contains most of the body. The ventral valve contains a large posterior opening, the **aperture**, through which protrudes the **pedicle**, a tough fleshy stalk. Find the aperture and use it to determine which end is posterior. The valves separate from each other at the **gape**, or opening, along their anterior margins.

>>> Use the landmarks above to find **anterior, posterior, dorsal, ventral, right,** and **left.** Find the **plane of symmetry** of these bilaterally symmetrical animals. Make sure you understand the orientation of the animal and can contrast this orientation to that of a bivalve mollusc.

A complex **hinge** with **hinge teeth** are present at the posterior end where the valves **articulate** with one another. The hinge teeth maintain the proper alignment between the valves as they open and close. Put the two valves together so the hinge teeth articulate and note the closeness of the fit. The ventral valve, of course, bears the aperture for the pedicle.

- *Articulate brachiopod*. Examine the opened, preserved articulate brachiopod specimen. Note the large horseshoe-shaped **lophophore**, the **mantle tissue** pressed against the valves of the shell, and **coelomic channels** in the mantle tissue. **Gametes** may give a brown or yellow color to these coelomic channels. Also note the prominent **muscles** (adductor and diductor) used to open and close the shell valves. Use the illustration in your book to orient yourself to these features, and the illustration given in lecture to understand the shell opening and closing mechanism.

- *Inarticulate brachiopod*. The genus *Lingula* is known, virtually unchanged, from fossils extending back at least 400 million years, making it the *oldest known animal genus*. While examining *Lingula*, note that in inarticulates, the **valves** are almost equal in size (the dorsal valve is *slightly* longer) and the **pedicle** protrudes from between them. The long pedicle of inarticulates is used to anchor the animal into sediment, whereas the shorter pedicle of articulates usually attaches them to a firm substrate. The valves are held together with **muscles** and have no hinge or articulation. The shell of inarticulates has a high protein content, making it more flexible than an articulate shell. Test this idea by bending gently with forceps. In addition, the major mineral composing the shell is calcium phosphate ($CaPO_4$) rather than calcium carbonate.

Examine the **lophophore** shape with right and left arms (brachia). Note the diversity of muscles, including the central pair of **anterior adductor muscles** and the single **posterior adductor muscle**. The **gonads** occupy much of the space in the posterior part of the body. You may also see parts of the digestive system, especially the large greenish **digestive cecum** posterior to the anterior adductor muscles.

- *Fossils*. Examine any fossil brachiopods that are available. Brachiopods, in particular articulated brachiopods, have an extensive fossil record. However, the diversity of articulated brachiopods has declined considerably since its peak about 350 million years ago.

TQ: Scientists have observed that the decline in articulate brachiopod diversity coincided with the rise of diversity of bivalve molluscs. What are two similarities about their lifestyles and use of resources that would lead them to compete? State a hypothesis for why brachiopods might have lost out in competition with bivalve molluscs.