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

LAB B. Ph. PORIFERA and Ph. CNIDARIA (Cl. Hydrozoa)

I. Phylum PORIFERA (*porus* = hole; *ferre* = to bear; L)

Although once classified as plants—or even mere organic secretions deposited by other animals—sponges are true multicellular animals. They are composed of aggregated, specialized cells, but they lack true epithelial tissue layers. (For this reason, sponges are sometimes referred to as "Parazoa," the sister group to the "Eumetazoa" or animals with "true" epithelia.) Sponge cells are organized to the extent that they produce complex internal and external morphologies that relate to environmental conditions, but they are not organized around a regular body axis (which lends to their morphological flexibility). Internally, cells form channels and chambers; externally, the body may be organized to form branches or encrusting mounds. And despite popular belief, sponges are internally dynamic, constantly reworking their morphologies and moving along surfaces. The clade that includes modern sponges originated in the Precambrian, over 545 mya (million years ago).

A. Grades and taxonomy

It is important to distinguish two ways that sponges are categorized. First, a sponge's **"grade of construction"** refers to its structure, regardless of its evolutionary relationship to other sponges. Although all sponges have a simple anatomy when compared with other multicellular animals, we recognize at least three grades that vary in complexity of the layout of internal channels and chambers (see lecture notes for illustration):

Grade	Description
Asconoid (A)	The body wall is not folded to form channels or chambers. The choanocytes line only the central spongocoel, and there is typically only a single osculum.
Syconoid (S)	Infolding of the body wall creates choanocyte-lined channels that increase surface area, allowing for a greater number of choanocytes per body volume. The narrower spongocoel, like the outer surface, is lined by pinacocytes.
Leuconoid (L)	The thick body contains numerous tiny chambers, each lined by a greater number of choanocytes, connected by a complex series of channels.

Second, sponge species belong to different phylogenetic **clades** (3 taxonomic classes). Ideally, taxonomy should reflect evolutionary history, but it might not reflect grade of construction (that is, a sponge's grade does not necessarily indicate its clade). To determine which class a sponge belongs to, the most important characteristics to note are the shapes and material content of the spicules:

Taxonomic class	Description
Calcarea	**All sponges that have spicules made of calcium carbonate ($CaCO_3$).** All are marine, generally from shallow water. All three grades of construction (*A, S, L*) are found. Spicules may be 2-, 3-, or 4-pointed.
Hexactinellida	**All sponges that have silicon dioxide (SiO_2) spicules with six points.** All are marine and are generally large and from deep water. Syconoid-like construction, with much of the body formed from a syncytium (layer of multinucleate cells lacking cell membranes).
Demospongiae	**All sponges that have spongin fibers <u>or</u> SiO_2 spicules with 2, 3, or 4 points <u>or</u> both.** Freshwater or marine, all *L*, 95% of all known species.

Please let me know in the margins if anything should be fixed or clarified about the lab.

TQ: Given these descriptions of clades and grades, in which particular case is the *grade of construction* sufficient to tell which *clade* a sponge belongs to? In which case is knowing the *clade* sufficient to say which *grade* it will be?

B. Exercises

1) Grades of construction

The sponge body consists of cells held together by a connective tissue layer known as **mesohyl**. In some species the mesohyl can include inorganic **spicules (**of $CaCO_3$ or SiO_2), long fibers of the collagen protein **spongin**, or both. These materials that make up the **endoskeleton** are secreted by amebocytes.

Take back to your bench a single bowl that contains relatively small-bodied representatives of each of the major sponge grades: asconoid, syconoid, and leuconoid. Based on what you know, can you guess which is which? The main goal is to note differences in size and body wall thickness, which relate to differences in internal complexity and the distribution of choanocyte-lined surfaces.

- ***Asconoid grade***. Examine your specimen of *Leucoselenia* on high power with transmitted light. Note the very small size (typical of this grade), the distinct terminal **osculum** for each tube, and the protruding spicules that appear to create a fuzzy covering of the body. Make a simple cartoon sketch of the tube showing the positions where you expect to find **choanocytes**, **pinacocytes**, and **ostia**. Indicate the path you would expect water flow to follow, and *be sure to deduce and record the taxonomic class with your drawing.*

- ***Syconoid grade***. Examine the preserved specimen of *Sycon (Scypha* or *Grantia* are the old names). Unlike in asconoid sponges the spongocoel is not lined by choanocytes, which instead occur in channels that run radially from the center to the outer edge. The choanocyte channels are contained in the finger-like projections that cover the surface of the body.

To better visualize syconoid construction, get and examine a prepared slide of *Sycon* (be sure to look at the cross-section). ✍In a simple sketch label the **spongocoel**, the channels that are lined by **choanocytes** (the darker-stained, oval cells), and the inhalant canals (next to the choanocyte canals, but not lined by choanocytes) where water first enters. Indicate on your diagram the path of water flow generated by beating flagella, including the numerous microscopic **ostia** that would be found on the walls of the choanocyte channels. Also, label where you would expect to find the microscopic flat cells (**pinacocytes**) that line both the exterior of the sponge *and* the spongocoel.

Reproduction. With no gonads (i.e., organs), **where do gametes come from**? In sponges, well-nourished choanocytes sink down into the mesohyl and *de-differentiate* into cells that undergo meiosis to produce gametes. Reproduction occurs when sponges in their female stage"capture" sperm into vacuoles (as they would a food particle!) and transfer the sperm to an egg (again, as they would transfer food to an amebocyte).

In your prepared slide of the syconoid sponge, find the large dark **embryos**. They are

normally found embedded in the mesohyl beneath the choanocyte layer. Those with a single nucleus are unfertilized eggs. Those that have a large hollow center are multiple-celled blastulae.

- ***Leuconoid grade***. Examine the small leuconoid sponge in your bowl. Leuconoid is by far the most common grade. Thousands to millions of microscopic chambers (lined by choanocytes) are connected by water canals (lined by flat pinacocytes) that bring water from the outside ostia and remove water through one or many oscula. Leuconoid sponges are typically much larger in size than asconoid or syconoid sponges. Note the thickness of your specimen, and the loss of prominence of the spongocoel (though you should still be able to find the osculum).

To better visualize leuconoid construction, get and examine a prepared slide of *Rhabdodermella* in cross-section. This small sponge, chosen for sectioning because it can fit on a slide, has a *relatively simple* leuconoid structure. ✍In a simple sketch, note the narrower central **spongocoel** and the numerous, small flagellated **chambers**, each one lined by choanocytes. Eggs or other developmental stages might be embedded in the mesohyl.

TQ: Why would the syconoid geometry (simple channels lined by choanocytes leading from ostia to the spongocoel) be less effective at increasingly larger sizes shown by leuconoids? Briefly give the scaling argument for why internal complexity must increase as the size of the sponge increases.

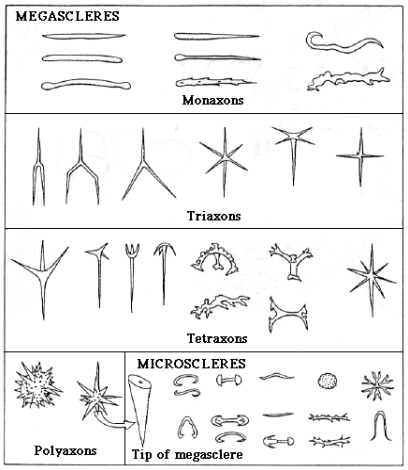
2) <u>General morphology</u>. Intact sponges are hard to describe or draw because they don't have many external features or symmetry. Use the live, dried, or preserved specimens to gain an appreciation of diversity in morphology, noting the following:

(a) Variation in **body size** and **shape**.
(b) Despite a general **lack of symmetry**, whether the body shows any type of regular organization.
(c) The position of **oscula** where water exits. In most cases, the **ostia** where it enters are too minute and numerous to see.
(d) **Smell** (if live) to get a sense of how chemical defenses may play a role in defense.
(e) For **live species**, cut a cross-section of the sponge with a razor blade and examine the **thickness of the body wall** relative to the size of the **spongocoel**.
(f) For each **dried skeleton**, squeeze to get an idea of how compliant or stiff each one is, and deduce whether or not the skeleton has a heavy component of **spicules vs. spongin**.

3) <u>CSI (Class of Sponge Investigation)</u>. In this exercise you will try to deduce the taxonomic class to which two specimens belong. To save time, we have set up samples of two sponges in bleach to digest away the organic tissue, leaving behind spicules that might be present in the tissue. You will examine the spicules and then test the material properties of any you find. Share your observations with other groups.

► We have placed very small (1 mm^3) pieces of two mystery sponge species in their own labeled microcentrifuge tubes (A and B), added a drop of dilute bleach, and heated for >60 min. The bleach will oxidize the organic material to CO2 and water, but will leave behind inorganic spicules, which do not dissolve with bleach.

► Pipette the fluid at the bottom of the tube and place it as two separated drops on a slide, covering one drop with a cover slip and leaving the other uncovered to dry. Start on low magnification and go to higher magnification to examine the spicules under the cover slip. **Be sure to rinse pipettes in order not to cross-contaminate them.**



A "taxonomy" of spicules

Sketch any materials you see in the spaces below, including spicules. Can you identify any of the spicule forms as represented in the figure? Compare with another group if you do not see spicules.

► Now, add a drop of 8% hydrochloric acid (HCl) to the uncovered drop on your slide. Acid will not react with <u>siliceous</u> (SiO2) spicules, but will react with <u>calcareous</u> (CaCO3) spicules as follows:

$$2\,HCl + CaCO_3 \leftrightarrow CaCl_2 + H_2O + CO_2$$

liberating the carbon dioxide as gas bubbles. You may notice a slight fizzing under low magnification, or the bubbles will collect at the top of the water drop. Spicules made of SiO_2 should not react with acid (unless some organic material remains). Can you deduce to which taxonomic class each prepared specimen belongs? What are the possible grades of construction?

Tube	Spicule types (draw)	Class? (if deducible)	Possible Grade(s)?	Notes

TQ: What could you conclude about the clade and grade of the sponge if the tissue digestion by Chlorox resulted in no spicules at all?

4) <u>Honorable mention</u>

(a) **Glass sponges** (class Hexactinellida) have typically long siliceous (SiO_2) spicules with six ("hex") points. In addition to providing support to the body wall, spicules can also be used to attach

the sponge to sediment. The hexactinellid sponge *Euplectella* ("Venus' flower basket") is not uncommon on deep ocean bottoms, where they may be "rooted" by the long tuft of spicules. Note how the six-pointed spicules are fused into a beautiful lattice- like skeleton. Sponges may not show symmetry, but there can certainly be regular patterns created by the cells that make up the body.

(b) **Boring sponges**, as the name implies, bore into the calcium carbonate structures produced by other kinds of invertebrates. A local yellow sponge, *Cliona*, typically lives within tunnels that they excavate within oyster shells. The ostia and oscula are present on the tips of "knobs" sticking through holes in the bored shell, but most of the sponge body is in passages below the shell surface. **View** a colony under magnification to see the position and density of ostia and oscula on the knobs.

If boring sponges are not available, examine shells they once inhabited. Note the abundant small openings penetrating the surface. This process, known as **bioerosion**, is accomplished by special archeocytes, the etching cells, that chemically remove tiny chips of calcium carbonate and release them into the excurrent canals. Boring sponges are one reason that mollusc shells do not accumulate, and why coral skeletons have not taken over tropical seas.

TQ. Based *just* on what you know about its lifestyle, to which taxonomic class can you be fairly certain *Cliona* does <u>not</u> belong? Why?

II. Ph. CNIDARIA

Cnidarians are diploblastic, non-coelomate animals with true epithelia. Some distinguishing features of the phylum and their significance:

- a single body axis, which creates radial symmetry (cf. poriferans, which lack body symmetry)
- two distinct tissue layers: an outer epithelial cell layer (**epidermis**) that derives from embryonic ectoderm, and an inner epithelial cell layer (**gastrodermis**) that derives from embryonic endoderm; in anthozoans, the pharynx leading down from the mouth is epidermal
- a jelly-like extracellular matrix (**mesoglea**) sandwiched between the two tissue layers. In some classes (e.g. anthozoans but not hydrozoans) the mesoglea can contain **mesenchyme** cells (and the mesoglea is sometimes confusingly referred to as mesenchyme); otherwise the mesoglea is acellular. The amount and composition of the mesoglea affects the mechanical properties of the cnidarian body; sedentary sea anemones and other polyps have relatively little mesoglea, while active medusae have a relatively large amount and use the springiness to restore the shape of the bell after a contraction
- an oral surface surrounded by a ring of tentacles. Tentacles, and sometimes other parts of the body, are covered with cells called **cnidocytes** that contain stinging or adhesive nematocysts.
- an "**alternation of generations**" between a polyp phase and a medusa phase. Both phases are diploid. When both phases are present, the medusa phase is *typically* pelagic and reproduces sexually while the polyp phase is typically benthic and reproduces asexually.
- good fossilization of some groups that produce hard parts (generally $CaCO_3$). Cnidarian fossils are found from the Late Proterozoic (900-600 mya).

A. Taxonomy and distinguishing features (Ph. Cnidaria, Cl. Hydrozoa only)

As we have discussed, different classes and orders within classes emphasize the polyp and medusa phases of the life cycle to different degrees, with various examples of the evolution of polypmorphism. The following classification is incomplete, but contains common orders:

Cl. Hydrozoa – Life cycle sometimes includes asexually reproducing polyp and sexually reproducing medusa. Hydrozoan medusae (=hydromedusae) have a **velum**, unlike scyphomedusae. Hydrozoan polyps have no internal **septae**, unlike anthozoan polyps. The mesoglea of both polyps and medusae is entirely acellular.

O. Hydroida – In hydroids the polyp stage is typically *colonial, benthic, polymorphic*, and covered by a chitinous **perisarc**. A medusa may be present but is more often absent. Members of the **Subo. Thecata** have a hydrotheca (ex. *Obelia*), while members of the **Subo. Athecata** lack one (ex. *Tubularia*).

O. Siphonophora and O. Chondrophora– These two groups are pelagic, highly polymorphic colonies of polyps. Zooids (polyps) have specialized functions: flotation, locomotion, feeding, reproduction, or defense (e.g., the Portuguese Man-of-War, *Physalia physalis*). "By the wind sailor" (*Valella valella*) is the representative chondrophore.

[Cl. Anthozoa, Cl. Scyphozoa and **Cl. Cubozoa**—taxonomy continued next week.]

B. Exercises

CL. HYDROZOA, O. HYDROIDA

1) Freshwater *Hydra*. The well-known genus *Hydra* has the most common life cycle for fresh-water hydroids: a *solitary* polyp that *reproduces both asexually and sexually* and does *not include a medusa*. In contrast, marine hydroids are more often *polymorphic colonies* that sometimes (not always) include a *distinct sexual medusa phase*.

Feeding. Examine a close-up of the tentacles on a prepared slide of *Hydra*. Note the batteries of cnidocytes, which include various sizes of nematocysts. The largest nematocysts clearly show the capsule inside the cell, including the **cnidocil** which acts as a trigger for the firing of the capsule.

Reproduction. Examine the prepared slide of *Hydra* in reproductive condition, showing "gonads"—either ovaries or spermaries. Since true gonads are organs, the appearance of gonads in an animal with a "cellular" rather than "organ" grade of construction may be surprising. Also potentially surprising is that the sexual phase is a polyp rather than the typical medusa.

So, what explains the appearance of gonads in these solitary polyps? What you are looking at is an extreme example of an evolutionary trend in hydroids: retention of the medusa phase by the polyp. In *Hydra*, the medusa has become so unrecognizable that all that remains is a bump on the surface of the polyp stalk that acts like a "gonad." This evolutionary trend has proceeded to different degrees in marine hydroid colonies that you will examine below.

Diploblasty. Appreciate both the simplicity of this animal (with a body wall two cells thick) and the increase in complexity over sponges (which lack true epithelia). ✍Use a simple sketch of a cross-section of Hydra to label the two distinct epithelia, **epidermis** and **gastrodermis**, the *very thin*, dark layer of **mesoglea** between them, and the large, central **gastrovascular cavity** (GVC). The gastrodermis may contain the darkly stained **gland cells** that secrete mucus or digestive enzymes and other cells that complete food digestion inside large **food vacuoles**.

TQ: Cnidarians begin digestion extracellularly but, like sponges, complete digestion intracellularly. Give two main advantages of beginning digestion outside of cells, providing a scaling argument in each case.

2) Marine hydroids. Most marine hydroids exist as colonies of polyps attached to seaweed, rocks, or dock pilings. Polyps within a colony can be specialized (e.g., for feeding, defense, reproduction), in this sense acting like specialized "organs" within the colony. Some species include a free-living, sexually reproducing medusa phase in the life cycle. More often the medusa is retained in some degenerate form, so that the *colony* appears to reproduce sexually.

Preserved colonies. As a good example of a **polymorphic** hydroid colony, look at a prepared slide of a portion of *Obelia*. ✍Carefully sketch and label a section of the colony that includes both the feeding **gastrozooids** and the reproductive **gonozooids** (which bud medusae asexually); the living tissue surrounded by the thin, protective, chitinous **perisarc** (including the **hydrotheca** that surrounds the base of each gastrozooid in these members of the Subo. Thecata); branches that include the common **GVC** running from polyp to polyp; medusa buds (= **gonophores**) budding off of the gonozooid; and batteries of **cnidocytes** on the tentacles.

As an example of a monomorphic colony, examine a prepared slide of *Pennaria*. Note single polyp type. Medusae bud off the feeding polyp close to the base of the tentacles. Note the presence of a **perisarc** (outer covering) but the absence of a **hydrotheca** (sheath around the base of the tentacles)—indicating this species is in the Suborder Athecata.

You may also have access to preserved colonies. One highly unusual species is *Hydractinia symbiolongicarpa*, a polymorphic species that grows only on the surface of mollusc shells that have become the residence of hermit crabs. See the shell on display and, to understand the detail of what you are seeing, consult the diagram in your textbook.

Live colonies. The species *Tubularia crocea* is a common fouling organism on floating docks in the winter, but it dies back by spring. Examine the unusual morphology of the colony. Each pink polyp sits at the end of a long stalk, which connects the polyp to others in the colony through gastrovascular connections. In addition to sketching the colony, examine a small clump of polyps under a dissecting microscope, and answer the following questions by labeling a sketch of the morphology:

Q: How are **structures** on an individual polyp arranged? Can you find feeding tentacles, reproductive structures, and a manubrium (short stalk leading to the mouth)?

Q: Is a hydrotheca present (that is, is the colony **thecate or athecate**)?

Q: Is *Tubularia* **monomorphic or polymorphic**? That is, do all zooids appear the same? Do reproductive structures appear separately from feeding structures?

Also to look for in living specimens:

(a) Commensals: watch for other animals that take advantage of the three-dimensional habitat created by hydroid colonies. Let your instructor know if you see anything worth showing to other students. You are especially likely to see amphipods (a shrimp-like crustacean) including the unusual elongate caprellid amphipods ("skeleton shrimp").

(b) Tentacle movement. Watch for extension, retraction, bending, flicking behavior.

TQ: The "medusoids" (= gonophores) of *Tubularia* are retained by the colony, from where they release gametes. Why do you think these species produce these specialized medusa-like structures if they never actually release them?

3) Hydromedusae. Some (but not all) hydroids have a free living (small) medusa phase. Briefly examine the medusa phase of *Pennaria* in the prepared slide. See if you can find some of the key features of a hydromedusa: **oral** and **aboral** sides, **tentacles**, **manubrium**, **stomach**, **radial canals**, and **velum** (a thin membrane attached to the margin of the bell that reduces the size of the opening).

O. SIPHONOPHORA

Siphonophores are polymorphic, pelagic colonies of polyps—they are not jellyfish (medusae). In fact, there is is no free-swimming medusa stage in their life cycle.

Briefly examine *Physalia*, the "Portuguese Man-o'-War." The large gas-filled structure (**pneumatophore**) is actually a single highly specialized polyp! The pneumatophore is the first polyp to develop (make sense?). Some of the polyps ingest food (**gastrozooids**), while others serve for prey capture and defense (long **dactylozooids**) or reproduction (**gonozooids**). A good illustration can be found in your textbook (though remember to draw what you see in the specimen, not what you see in a picture or other drawing).

O. CHONDROPHORA

Members of this order are also pelagic colonies of polyps, though there is a small, free-living medusa phase as well. The well-known species on display, *Velella velella* ("by-the-wind sailor"), has a special **pneumatophore**. Picture this animal floating at the water surface. What would happen if a strong breeze blew across the surface of the water and contacted the surface of the pneumatophore? This structure acts as a sail, causing these animals to "tack" in the wind. This polymorphic colony includes **dactylozooids** and **gonozoooids** but only a single **gastrozooid** at its center. Locate these in the specimen (see figure in your textbook) and understand their function.

TQ: Why have these organisms evolved the ability to sail? Under what conditions might a sail be adaptive?