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

LAB C. Ph. CNIDARIA (Cl. Anthozoa, Scyphozoa, Cubozoa), Ph. CTENOPHORA & Ph. PLATYHELMINTHES (Cl. Turbellaria)

I. Ph. CNIDARIA (Cl. Anthozoa, Scyphozoa and Cubozoa)

A. Taxonomy (cont. from last week)

Cl. Anthozoa – Medusa stage is never present. Unlike in hydrozoans, polyps have a GVC divided by mesenteries that are lined with nematocysts. Reproduction may be sexual (gametes are produced by gastrodermal cells on the mesenteries) or asexual (longitudinal fission, budding, or pedal laceration).

Subcl. Hexacorallia -- Polyps have simple tentacles, frequently in multiples of six, although numbers can vary from several to hundreds.

O. Actiniaria – The “true” sea anemones are non-colonial (though they may be clonal), lack a calcareous skeleton and have paired septa (e.g., the sea anemone *Metridium*).

O. Scleractinia – The stony corals produce a calcareous exoskeleton secreted by the epidermis. Polyps are colonial but monomorphic and share a common gastrovascular system (e.g. the staghorn coral *Acropora* and the brain coral *Diploria).*

Subcl. Octocorallia -- Polyps with eight pinnate tentacles, most colonial.

Gorgonians have an endoskeleton with a central protein rod surrounded by calcareous spicules (e.g. sea pens, the sea whip *Leptogorgia*).

Sea pansies and sea pens are fleshy, polymorphic colonies (e.g. the sea pansy *Renilla* and the sea pen *Stylatula*) with calcareous spicules embedded in soft tissue. The colony is inflated by a hydrostatic skeleton generated by the primary polyp, which forms a central axis on which lateral polyps are arranged.

Cl. Scyphozoa –Life cycle is always dominated by a large, pelagic medusa and usually includes a small benthic polyp (the **scyphistoma**). Many young medusae (called **ephyrae**) are formed by transverse sectioning (**strobilation**) of the scyphistoma. Three of the four orders have a free-swimming medusa (e.g., *Aurelia, Cassiopeia).* The odd exception is Stauromedusae (now sometimes in own class Staurozoa), with a benthic, stalked medusa that remains attached to surfaces and is never free-swimming (*e.g. Haliclystis*).

Cl. Cubozoa –As in Scyphozoa, the life cycle is dominated by a large medusa. The scyphistoma can bud to form new polyps, but a given scyphistoma gives rise to a single ephyra and does not strobilate. Cubozoans have sophisticated eyes that include a retina and lens that may be capable of forming images. They inject highly toxic venom into their prey (usually fish) via nematocysts; the neurotoxins can also be harmful or fatal to humans.

B. Exercises

CL. ANTHOZOA

SUBCL. HEXACORALLIA, O. ACTINIARIA (Sea Anemones)

- Body form. On display is the sea anemone *Aiptasia* sp., a clonal species that grows and divides prolifically on local docks and in aquaria if you are unlucky enough to introduce them. The solitary anemone *Bunodosoma cavernata* might also be on display. Try to minimize disturbance of the anemones unless the exercise calls for it. Using the dissecting microscope, do a simple sketch of the **oral disk**, including the position and variable sizes of **tentacles** and the shape and size of the **mouth**. Locate the opening to the **siphonoglyphs**, the heavily ciliated grooves that run from the mouth down the inside walls at two ends of the **pharynx**. If available, you can try to gently introduce some carmine particles close to a siphonoglyph to see, under your dissecting scope, the action of cilia sweeping water down into the GVC.

TQ: List at least three functions of a GVC in sea anemones. Which benefits most, and which is compromised most, by filling it with seawater? Why?

- Musculo-skeletal system. Sea anemones have no hard parts to support their soft tissue. How do they erect the body to move the tentacles away from the substrate and into faster current? Recall that the body is a fluid-filled cylinder wrapped by two epithelia with mesoglea between. As in many soft-bodied animals, the contractile filaments (here within the **myoepithelial cells**) are arranged in an *orthogonal* (perpendicular) array: **longitudinal** filaments run oral to aboral, and their contraction shortens the body, whereas **circumferential** (= circular) filaments run around the circumference of the body, and their contraction squeezes and lengthens the body. These two muscles are *antagonized* (their contraction is opposed) by the incompressible fluid that fills the GVC, creating a **hydrostatic skeleton**. Another set of filaments create a circular **sphincter** that controls the opening of the mouth. By contracting particular sets of filaments an anemone can contract, extend, and bend the stalk as well as the individual tentacles.

TQ: Gently disturb an individual of *Aiptasia pallida* so that it begins to withdraw. If you were to disturb the animal enough, it would shrink down to a broad, flat disk of tissue. (a) Which of the **three** sets of fibers would be contracted (C), and which relaxed (R), to achieve this shape change? (b) Which of the **three** sets of fibers would be contracted or relaxed to re-extend the stalk to put the tentacles up into the current?

	longitudinal	***circumferential***	***sphincter***
To shorten flat:	C R	C R	C R
To re-extend tall:	C R	C R	C R

TQ: Imagine two anemones of different size but exactly the same internal and external shape (that is, the smaller one would look identical to the larger if its picture were enlarged). If all parts work the same, should the smaller or larger anemone (1) pump more water per unit time? (2) take longer to fill completely? (*Hint: compare how the mechanism of filling and the volume being filled scale with size. Use a mathematical scaling argument to support your answer.)*

- Sensory and feeding behavior. Before carrying out the exercise below, first make your predictions based on the following question: *what kinds of sensory stimuli (e.g., light, touch, chemical) would you expect anemones to be sensitive to?* You will attempt four ways to elicit a behavioral response (tentacle movement, tentacle contraction, complete retraction of the oral disk, etc.). So, first predict what will happen when…

Stimulus	Prediction	Outcome, Conclusions
…the anemone is suddenly lit or shaded? (i.e., are there advantages to anemones to see light?)		
…a piece of shell drops on a tentacle? (i.e., will anemones respond to a mechanical stimulus?)		
…the piece of shell is first soaked with food before it is dropped? (i.e., is a chemical stimulus required?)		
…a piece of food is dropped on the tentacle?		

TQ: If any objects were first attached to the tentacle but then dropped, by what exact mechanism did the animal detach the object from the tentacle?

- Symbiosis. With your forceps, gently pull off tentacles of a brown ("symbiotic") *Aiptasia* and examine it in a small drop of seawater under a cover slip on a dissecting microscope. The dark brown gastrodermal cells on the inner side of the tentacle contain abundant spherical **zooxanthellae**, which are dinoflagellates that have lost their flagella after entering the symbiosis. When held under dark conditions or at depth, *Aiptasia* will bleach and exist without symbionts ("asymbiotic" individuals are white).

TQ: State 2 hypotheses for why an anemone like *Aiptaisia*, which does not need to navigate through 3-dimensional space like a medusa, would benefit from being able to detect light.

- Defense. Some anemone species have threadlike tentacles called **acontia** that are used in feeding and defense. These structures originate inside the GVC along the edges of mesentaries (partitions of the GVC) near the aboral end. Although they are inside the GVC, they can be rapidly forced out of the body when the animal is sufficiently disturbed.
    - Place a finger bowl with an individual of *Aiptasia* under a dissecting microscope. Disturb it sufficiently to watch the acontia fire out the **mouth**, and through blister-like pores in the body wall that are called **cinclides**.
    - Using forceps and scissors, collect some acontia and place them on a glass slide with seawater under a cover slip. Using 400X magnification, observe on a compound microscope with the light carefully adjusted. Be careful to wick away just enough fluid using a kimwipe to slightly compress the acontia. You may be able to see two sizes of elongated **nematocysts**, probably the most elaborate cell organelle known, with inverted tubules coiled inside. You will probably see free capsules that have already discharged their short, barbed filaments.
    - **Ask me** to set up a demonstration on the HD screen showing the firing of nematocysts exposed to acetic acid, which osmotically forces the capsules to open.

SUBCL. HEXACORALLIA, O. SCLERACTINIA (Stony corals)

“True” corals deposit a massive calcium carbonate base and form small “cups” called **corallites** that the soft tissue of each polyp can retract into. For each specimen of coral skeleton (on the back table), try to locate the corallites, imagining the array of polyps that make up the colony, all connected by a common GVC. Notice how polyps are sized and arranged differently among species as reflected by the size and position of corallites. Also, visualize how the whole colony would occupy space—some are massive heads, others flat plates, others highly branched trees.

TQ: Given your understanding of plasticity in sponges, under what environmental conditions might a *branching* species have advantages over a *flat* species*?*

SUBCL. OCTOCORALLIA

Octocorals show a number of different ways to arrange polyps in a colony. As examples, we may have live or preserved specimens of the *monomorphic* gorgonian sea whip *Leptogorgia* and the *polymorphic* sea pansy *Renilla* and sea pen *Ptilosarcus.*

- Skeleton. Examine the dried skeleton of the branching sea whip *Leptogorgia* as well as the more interconnected sea fan. Each branch has a central rod of secreted **gorgonin** (recalling spongin, what type of material is gorgonin?) encased within a rod of fused **calcareous spicules**. This internal skeleton is surrounded by mesoglea in which spicules are also embedded. The colony as a whole relies on this hard but semi-flexible structure for body support (although the polyps are still supported by a hydrostatic skeleton).

  If *Renilla* or *Ptilosarcus* is available, notice the fleshy stalk for holding in sediment and the thicker body. Both are supported by a hydrostatic skeleton and by calcareous spicules that help to stiffen the soft tissue. There is no central skeleton as in gorgonians.

- Colony form. All octocorals have polyps with exactly eight tentacles that branch in *pinnate* fashion (what does this mean?--botanists among you should know). If available, closely examine a live branch of *Leptogorgia* under a dissecting microscope to observe the small, *monomorphic* polyps. Sketch the **pinnate tentacles** and try to see the internal **pharynx** and **mesentaries** that partition the GVC, which may be visible through the transparent body wall. (The polyps normally feed at night, so they are often retracted in light).

If live *Renilla* is also available, notice that *polymorphic* construction of the colony. The primary polyp forms the central axis on which lateral polyps are arranged. Lateral polyps are specialized for feeding (**gastrozooids**) or for moving water (**siphonozooids**) through the primary polyp to keep it "inflated" with water, creating a hydrostatic skeleton.

CL. SCYPHOZOA

Unlike the classes Hydrozoa and Anthozoa, the Scyphozoa and Cubozoa are dominated by the *medusa* phase of the life cycle (but typically include a small polyp phase). These two classes differ somewhat in morphology and in the method of medusa formation, as discussed below.

- <u>Medusa body form</u>. Examine a preserved adult of *Aurelia*, the moon jelly, in water under a dissecting scope (oral side up). The **bell**, shaped like a large umbrella, has an **aboral** (umbrellar) and **oral** (subumbrellar) side. Imagine a short line that extends from the center of the aboral surface through the center of the oral surface. This **oral-aboral axis** is the axis of radial symmetry: at any given distance along the axis, similar body parts are found along any radius.

The **epidermis** covering the entire outside surface, as well as the **gastrodermis** lining the GVC, are **true epithelia**. They are transparent and hard to distinguish from the thick **mesoglea** (= mesenchyme) between them, which makes up most of the volume. The mesoglea is a connective tissue with several components:cross-linked protein fibers, a gel matrix, water, and some mobile amoeboid cells.

TQ: The consistency of mesoglea varies from firm to soft. Which component (see above) do you think varies from being highly resistant to contractions of the bell to allowing greater flexibility of body shape? (*Hint: what are the components of Jell-o™, and how does it firm up?)*

Oral surface. Orient the specimen in the dish with its <u>oral surface</u> facing you. In the center are four long **oral arms**. Where the oral arms meet they form a short tube, the **manubrium**, that leads to the **mouth**. In many scyphozoans, the oral arms are more prominent than the tentacles and provide the major surface for food capture. Under the dissecting microscope, observe that each arm bears two frilly, ribbon-like ridges that enclose a deep, ciliated groove. Food is transported by these cilia to the mouth. The edges of each groove bear small **brachial** ("arm") **tentacles** with many cnidocytes.

The *digestive* role of the GVC involves the **stomach** (just beyond the mouth) and the **gastric pouches**, where digestive enzymes are secreted and **extracellular digestion** takes place. To find the gastric pouch, look for the opaque, horseshoe-shaped "**gonad"** that sits on the floor of each pouch. (Remember that the gonads of cnidarians are actually enlarged gastrodermal cells inside of which the gametes mature.) The edge of the gastric pouch may be a faint line around the outside margins of the gonad. A number of short, threadlike **gastric filaments**, which secrete the digestive enzymes, are also located on the floor of each pouch.

TQ: What is likely the main benefit of positioning the gonads close to the gastric pouches?

The *vascular* role of the GVC involves narrow, ciliated channels that transport the partially digested food to more peripheral sites, where smaller particles are taken up for **intracellular digestion**. The GVC is lined by cilia throughout, which aids in transport and circulation. Find the 16 **radial canals** that extend from the mouth to the **ring canal** at the margin of the bell. Numerous short blind canals arise from the ring canal and extend into the marginal **tentacles** and sense organs, so that nutrition can reach every epithelium in the body.

Aboral side. Now flip your animal over to view its <u>aboral</u> side. With your finger push gently on the aboral surface to note the springy consistency of the **mesoglea**, which is essential to how the bell returns to its resting state after a contraction. On the margin of the bell are numerous, short **marginal tentacles** that bear cnidocytes. (The tentacles appear shortened by preservation, but are longer in live animals). The bell is shallowly indented at eight positions to create a slight scalloping. Complexes of sensory structures, the **rhopalia**, are located in the indentations between scallops, enclosed within folds of tissue called **sensory lappets**. Find a **statolith** (unpaired, spherical, central) inside a statocyst that is part of one of the rhopalia.

- <u>Life cycle</u>. To visualize the life cycle of a typical scyphozoan such as *Aurelia*, examine and sketch the prepared slides of (1) a **planula larva**, (2) a **scyphistoma** (the solitary polyp generation that results from the planula), (3) a **strobila** (the polyp as it undergoes transverse sectioning, or strobilation, to produce medusae), and (4) an **ephyra** (the immature medusa). On the ephyra it should be possible to identify the developing **rhopalia**, **oral arms**, **manubrium**, **stomach**, and **canals** of the GVC.

CL. CUBOZOA

"Box jellies" (some called "sea wasps") were placed within the class Scyphozoa until the 1980s. Differences in the life cycles, as well as differences in the form of nematocysts (which, like sponge spicules, are useful for taxonomy) indicate that scyphozoans and cubozoans are closely related but distinct clades, and they are now put into different classes.

- Examine the preserved specimens. Note the characteristic tetra-tentaculate anatomy (tentacles hanging from 4 points of a box-like bell) that is similar to some hydromedusae. Also similar to hydromedusae, cubomedusae sometimes have a velum-like structure that narrows the opening where water is forced out and facilitates rapid swimming.

These **fish-feeding** animals are of particular concern to humans. One species has been responsible for many human deaths in Australian waters, and swimming is banned during certain parts of the year. *Chiropsalmus quadrumanus*, the one species local to S. Carolina coast, has been blamed for the death of at least one small child in North America.

TQ: Propose two distinct hypotheses for why the venom of cubozoans might tend to be more harmful to humans than the venom of plankton-feeding scyphozoans.
Hyp 1:

Hyp 2:

II. Ph. CTENOPHORA ("comb-bearer")

Ctenophores are gelatinous, planktonic carnivores. They swim using 8 longitudinal rows of specialized, compound cilia fused into flat plates called **ctenes**. These jellies differ from cnidarians in many ways: (1) movement is typically with the **oral end** *forward*, (2) ctenophores have muscle cells below the epithelium in the mesoglea, and (3) most are simultaneous hermaphrodites. The tentacles (if present) and some other body parts are coated with **colloblasts**, adhesive cells that aid in capturing prey. Although similar in function, colloblasts are clearly not homologous to cnidocytes of cnidarians for several reasons (what are they?).

The ctenophore digestive system includes a mouth that leads to a stomach and then to a set of digestive canals. All but two of these canals end blindly; the remaining two, which open to the outside on the aboral end through **anal pores**, dispose of only very small undigested wastes—larger undigested particles still exit from the mouth. Therefore, the digestive system is not quite "complete," with one-way flow of material, as in phyla to be covered later.

A. Taxonomy

The phylum has two classes, distinguished by body form and mode of prey capture:

Class Tentaculata – Members of this class have two or more tentacles at some point in the life cycle. Typically, the tentacles (or the body lobes) are used for capture of plankton (e.g. the sea walnut *Mnemiopsis*).

Class Nuda -- As the name implies, members of this class have no tentacles (and no colloblasts). These animals eat other members of the plankton, opening their mouths wide and tearing prey items apart with big compound ciliary teeth (e.g. *Beroe*, which specializes on eating other ctenophores).

B. Exercises

- If a live ctenophore like *Mnemiopsis leidyi* is available, watch it swim. Note the walnut shape, maintained by a large volume of mesoglea. This shape clearly shows a departure from radial symmetry, with at least one additional plane of symmetry—in this case most clearly defined by the body lobes that come down around the mouth—creating a unique form of symmetry known as **biradial symmetry**.

Direct the light to shine directly on the animal. The **ctenes**, composed of compound cilia in a common membrane, will refract light like a prism. Locate the **ctene rows** running between the **oral** and **aboral** ends, paying careful attention to two aspects of ctene movement: (1) the direction that an *individual ctene* beats, which you can deduce from the direction of body movement, and (2) the direction that *the wave of beating* progresses along the row of ctenes as they beat in succession. This regular pattern, known as a **metachronal wave**, is created by the fact that ctenes on a row don't all beat simultaneously—they beat in succession. In which direction does "the wave" pass? Record your observations:

Ctene movements	**Direction (is it oral-to-aboral *or* aboral-to-oral)?**
Beat of individual ctene	
Metachronal wave	

TQ: State two distinct hypotheses for why metachronal waves pass in the direction you observed. (tricky!--be sure to discuss with your lab partner and instructor. To start your thinking, consider external forces that affect ctene efficiency and internal mechanisms that control them.)
Hyp 1:

Hyp 2:

You might also observe sudden, rapid movements that cannot be explained by the action of cilia. They are due to the contraction of muscles within the mesoglea to create a propulsive movement more like that of a cnidarian medusa.

TQ: What might be an advantage to ctenophores of generally relying more on the slow and gentle movements of ciliary propulsion, which doesn't move them very far or fast? (That is, if they have muscles, why don't they use them for typical locomotion?)

~~~Only if there is time!~~~

III. Ph. PLATYHELMINTHES ("flat worm")

Flatworms are classified as "triploblasts," with tissues derived from three embryonic tissue layers: an external epithelium derived from **ectoderm**; a midgut epithelium derived from **endoderm**; and an internal set of tissues derived from **mesoderm**. In flatworms, this third tissue consists largely of loosely packed cells called **parenchyma**, which fills most of the body, as well as distinct **muscle bands**. Because flatworms do not have a fluid-filled, enclosed body cavity, they are considered to be "acoelomate." They also lack specialized respiratory structures and a circulatory system, so the exchange of gases and metabolic wastes between cells and the outside world occurs by diffusion across the body wall. Like cnidarians, they have an "incomplete" digestive tract with a mouth that serves for both feeding and defecation.

A. Taxonomy . Today you will consider only the (mostly) free-living **Cl. Turbellaria**, one of the four classes recognized by Pechenik. Among the Turbellaria, large, marine flatworms (**O. Polycladida**) are common but difficult to find in local waters. Instead, we will look at prepared (and possibly live) specimens of freshwater planarians, members of the **O. Tricladida**.

B. Exercises

- Examine a prepared w.m. slide of the **triclad** *Planaria*. First, visualize how the **bilateral symmetry** of flatworms is formally defined by two body axes (as opposed to the single oral-aboral axis of cnidarians): a dorso-ventral (D-V) axis and an anterior-posterior (A-P) axis. Together these axes form a plane, which separates the mirror-image right and left sides of a bilaterally-symmetrical body. As their name implies, flatworms tend to be D-V compressed.

Make a very simple sketch of the anatomy of the digestive system. Food enters through the lightly-stained, mid-ventral **protrusible pharynx** with the mouth at its tip. Focus up and down to see the muscular walls of the pharynx, the **lumen** at its center, and the mouth at its posterior end. The light space around the pharynx is the **pharyngeal cavity**, into which the pharynx is withdrawn when not protruded. The dark stained area that pervades the body is the **incomplete gut** and its three main branches, which (as in cnidarians) serve as a network for distributing resources as they are digested extracellularly. Also as in cnidarians, digestion is completed in food vacuoles inside of cells. Note also the position of the **ocelli** (= eyespots), light-sensitive cells (photoreceptors) backed by opaque dark pigments.

TQ: What is the role of opaque pigments in an eye? Based on this role, which way would you point the two ocelli to optimize the way they provide information about the direction light is coming from? (Consider how radially symmetric medusae integrate such information.) Include a sketch with your explanation.

- Internal. Examine a prepared slide of *Planaria* that includes three cross-sections. *Focus only on the middle section*, which goes through the pharynx (other sections are anterior and posterior to the pharynx—visualize what this means relative to the whole-mount slide). Identify the muscular **pharynx** lying within the **pharyngeal cavity**.

As you draw a simple sketch of the ectodermal and mesodermal derivatives associated with the pharynx and its cavity, remember that (1) body walls in contact with a lumen all have the same structure: **epithelium** (epidermis) external to **circumferential muscle** external to **longitudinal muscle** external to **parenchyma**, (2) recall that the pharynx is an *invagination* of the outside body wall, so that the inside is lined by epidermis, not gastrodermis and (3) considering the area of the pharynx and its cavity, you will need to identify several epidermal layers: the lining on each of the two sides of the cavity lumen and on each of the two sides of the pharynx lumen. You might not be able to see distinct separations between each of these various layers but you should try to label where they are. Somewhat darker-stained **gland cells** visible in the pharynx epidermis secrete sticky mucous that can aid in immobilizing prey.

Note the other large spaces to the sides of the pharynx visible in this middle section**.** These are branches of the three lobed **digestive cavity** (lined by gastrodermis). These tissues contain dark-stained **gland cells** that secrete digestive enzymes. Thin bands of **D-V muscles** may be visible, but most of the body is filled by spongy **parenchyma**, also derived from **mesoderm**. The epidermis, which is ciliated on the ventral side (focus closely to see cilia), is derived from **ectoderm**.

Can you see any evidence of the pair of **ventral nerve cords**? Note that the nervous system is no longer strictly epidermal (confined to a nerve network within the epithelium), but is partly sunken in tracts below the epidermal surface.

- Locomotion. In addition to **cilia** on the ventral surface, flatworm bodies of course contain **muscles** in several orientations: circumferential (circular), longitudinal, and dorso-ventral. If a small live animal is available, put it on a slide with as little water as possible and flip the slide upside down, then place it across the top of an open bowl of smaller diameter than the slide so that you can observe its movements from “below” on a dissecting microscope.

TQ: What evidence can you see that indicates that the animal is using cilia, muscles, or a combination of the two? Which type of locomotion do you propose would be more effective at this size? At much larger body sizes? Why?