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

LAB H. Ph. ECHINODERMATA (= "spiny skin"; Gr.)

Echinoderms are **deuterostomes**, a major clade that split from protostomes early in the history of coelomate divergence. The common ancestry of deuterostomes is reflected in a suite of early developmental traits involving cleavage patterns, the origin of mesoderm, the origin of coeloms, the origin of the mouth, and cell ciliation (as described and diagramed earlier in the course). The echinoderms are unusual among deuterostomes in that adults have **radial symmetry**, even though their ancestors were certainly bilaterally symmetric. The adult radial symmetry is **pentamerous** with body parts usually occurring in fives or multiples of five. Larvae of echinoderms (which retain the ancestral bilateral symmetry) have three compartments that develop into three distinct coelomic spaces in the adult.

Echinoderms have several unusual shared derived characteristics. The **water vascular system** (WVS), a coelomic compartment, functions not only as a hydraulic system to drive the movement of locomotory tube feet but also in gas exchange, excretion, and feeding. The body wall includes an *endo*skeleton (completely below the epidermis) that is composed of both **calcareous ossicles** and a thick, **mutable connective tissue** dermis. The consistency of the connective tissue can change from solid to fluid through ionic changes that are under nervous control. The calcareous plates have a special meshwork structure called **stereom,** with open spaces that allow connective tissue to grow within the hard plates. Ossicles are most prominent in echinoids, where they form the **test**, and least prominent in holothuroids, where they are only small bits embedded in the more substantial dermis. Most echinoderms have ossicles in the shape of **spines** that protrude from the body wall, giving rise to the phylum name.

Adults lack nephridia; **excretion** involves simple diffusion of ammonia through thin, permeable regions of body wall. Similarly, **gas exchange** occurs across different thin-walled structures in different classes, including **tube feet**, **papulae**, **bursae,** and **respiratory trees**. The **hemal system** (the **axial sinus** and **perihemal canals**) is surrounded by its own coelomic compartment, but it is non-contractile and its role in nutrient transport is not well understood. Instead, the chief transport system is the circulating fluid of the **perivisceral coelom**. The **nervous system** includes two epidermal nerve rings around the esophagus from which arise **radial nerves** to the periphery; there is no centralized nervous system (brain or ganglia).

All echinoderms are marine and benthic as adults. Because of the presence of hard calcareous parts the phylum has left abundant fossils; in addition to over 6000 described extant species, at least twice that number have been described as extinct species in the fossil record.

A. TAXONOMY

Cl. Crinoidea (= lily-like; Gr): The **feather stars** (unstalked and relatively mobile) and **sea lilies** (stalked and sessile). Crinoids have an extensive fossil record, extending back at least 600 million years. Although only 500 extant species are known, crinoids were dominant echinoderms in past faunas.

[NB: Crinoids form a sister group to a clade (the Subph. Eleutherozoa) composed of the remaining living classes. Because the crinoids do not have a conventional madreporite, it appears that the madreporite evolved on the branch leading to the Eleutherozoa. Crinoids are also unusual in that the oral surface is oriented upward.]

SUBPH. ELEUTHEROZOA

Cl. Asteroidea (=”star-like”; Gr). **Seastars** use tube feet for locomotion and are typically predatory. In many starfish, the arms—locked into place with mutable collagen—are used in conjunction with tube feet to pry apart the valves of clams and mussels.

Cl. Ophiuroidea (=”snake-like”; Gr). **Brittlestars** and **basketstars**. The class is named for snake-like movements of the flexible arms, which are slender and distinct from the disk. Tube feet are not used for locomotion but may be modified for feeding or gas exchange.

NB: Asteroids and ophiuroids are sometimes considered subclasses within the ***Cl. Stelleroidea****—animals with arms arrayed in a star-like pattern around a central disk..*

Cl. Echinoidea (=”spine-like”; Gr). The regular echinoids (**sea urchins**), and the irregular echinoids (**sea biscuits, heart urchins and sand dollars**). Echinoids have an internal **test** and are covered in moveable spines. In different species, the spines can function in locomotion, burrowing, food capture, and/or defense. Echinoids are typically herbivorous or omnivorous and feed using a complicated structure composed of ossicles and muscles called “Aristotle’s lantern.”

Cl. Holothuroidea (=”polyp-like”; Gr.) **Sea cucumbers** have a large perivisceral coelom, a thick connective tissue dermis, and a reduced ossicle content in the body wall. The buccal tube feet are elaborated into bushy tentacles for either suspension or deposit feeding.

B. EXERCISES

(Note: for any live specimens that are available, you must use a dissecting microscope to see the actions of spines, tube feet, and pedicillarie, which differ among the classes.)

Cl. Asteroidea

- Live specimen. *If a living specimen is available,* watch the animal move, entirely through the gliding action of tube feet. Pick up an animal and note its rigidity; flip it over in the water to examine the action of tube feet as the animal tries to right itself, partly by softening the connective tissue in the body wall to change body shape. **[Move on to the dissection, but return afterward.]**

- Dissection. Dissect a preserved specimen of the sea star *Asterias forbesi*. Instructions are given on a separate handout. Be efficient with your time! To help with your dissection, collect prepared slides of (a) a cross section of a seastar arm and (b) pedicillariae. *[You do not need to draw the arm cross section in detail*, but it may help to see some structures that are not obvious in the dissection. You can instead add notes to the diagram on your dissection handout.] In particular, examine the following structures from aboral to oral along the midline: the **WVS radial canal** (a faintly visible oval ring), the larger **hyperneural radial canal** containing the **hemal strand,** and the **radial nerve**, a thickening on the aboral midline (Fig. 3).

TQ: As you can see in the arm cross section, echinoderms tend to have a spacious body coelom. On the left, list as many functions of a coelom that you can think of, considering other animal phyla that you’ve examined in the course. On the right, name the structure in asteroids used for the same function, indicating where the coelom plays a role.

DTQ: The porous construction of stereom is also thought to help guard against cracking of ossicles. How might you explain this apparent paradox?

DTQ: What are the two epithelia that make up the thin papula wall? What components of the body wall are absent from this spot? Make a cartoon sketch of a body wall section including a papula. Label the epithelia of the papulae, the fluid spaces on either side of the wall, the location of cilia, and components of the body wall to either side of the papula.

DTQ: How do the positions of sensory structures in cnidarians and asteroids relate to similarities and differences in body symmetry and movement?

DTQ: Now draw (#1) the orientation of the two tissues below, spanning a field with two ampullae, using cilia to denote the apical sides of cells. Then, in the same way, draw (#2) the orientations of the tube foot peritoneum and the epithelium that covers each tube foot as it emerges on the other side of the ambulacral ossicles (spanning two tube feet):

Drawing #1 *Drawing #2*

Cl. Ophiuroidea

Brittlestars. Examine the preserved specimens of brittlestars from the genus *Ophiolepis*. Note that (1) the entire surface is covered with **calcareous ossicles** and a thin layer of epidermis, and (2) the slender arms come off of a distinct **central disk**, and (3) the disk contains **all of the body organs**. All three features, and others mentioned below, distinguish ophiuroids from asteroids.

- Aboral surface. The **disk** is clearly composed of a set of adjoining ossicles. Note that two key features found in asteroids (and echinoids) on the oral surface are **no longer there**: (1) the **madreporite**, which has migrated down to the oral surface, and can be detected as an anomalous plate around the mouth (try to find it!), and (2) the *anus*, which is altogether absent—ophiuroids have lost this second opening.

Under a dissecting microscope note how the arms are built from a series of **articles**, analogous to arthropod legs. Each "article" is composed of a thick **ossicle** that occupies most of the internal space of the arm (very little coelom remains), which are connected to one another with flexible **mutable collagen** and that have **muscles** running between them, forming an articulating appendage. The mutable collagen connecting the arm ossicles can be liquefied at particular joints, allowing the animal to drop the arm if disturbed. Because the arm does not contain organs, the loss is minor, and brittlestars can **regenerate** arms with remarkable speed.

In *Ophiolepis* the arms are smooth, but in many brittlestars **spines** are part of the arm plate ossicles. These spines can aid in locomotion by making contact with the substrate as muscular arms push or pull the animal using a sinusoidal wave.

TQ: Which taxon in a past lab is most analogous in terms of (1) building locomotory appendages from a series of articles? (2) Using spine-like projections from the body to make additional contact with the substrate while performing a similar movement during locomotion?

- Oral surface. Observe the central mouth and the complex set of ossicles that form **teeth** around the mouth. See if you can find the **madreporite**, which has migrated to become one of the plates surrounding the mouth. Also notice the apparent **lack of ambulacral grooves**, further distinguishing ophiuroids from asteroids. The grooves have been obliterated by the large ossicles sunk into the arm to form the articulating skeleton. If visible in some specimens, the tentacle-like **tube feet** (used for food-collection, not movement) extend through individual holes in the wall of the arms. Compare the design of the tube feet with those you saw in the asteroids.

- Bursae. On either side of the base of each arm is a **bursal slit**. The **bursae** (pouch-like cavities) inside these slits are the **main site for gas exchange** in ophiuroids. Water is circulated through the bursal slits by cilia and muscular contractions of the disk to aid gas exchange along the inner surface of the bursae. In addition, many brittlestars either **release gametes or even brood** developing embryos in these bursal pouches.

TQ: What areas in (1) molluscs, (2) decapod crustaceans, and (3) paxillosid seastars are most analogous to the bursal pouches of brittlestars (in their respiratory function)?

- Locomotion. *If live specimens are available*, note how these animals move relative to asteroids--muscular arms push against the substrate and tube feet are not involved. Can you see any evidence of on-going arm regeneration in any of the live specimens?

>>> Arm ossicles in some species act as **miniature lenses** that direct light onto photoreceptors in the arm. How would you test whether this species is sensitive to light?

>>> Gently flip a specimen over and watch its **righting behavior**. Is this accomplished in a way similar to or different from asteroids? Is the process faster or slower?

If a live specimen of *Hemopholis elongata* is available, note the bright red pigment (hemoglobin) circulating in the fluid of the water vascular system—not a traditional location of blood pigments. Not all brittlestars have such respiratory pigments.

Basketstars. The other major order of ophiuroids, the **basketstars**, have intricately branching arms that resemble a basket, which is used to greatly increase the surface area used for suspension feeding. Compare the morphology to that of brittlestars. Determine why basketstars are considered to retain the pentamerous radial symmetry characteristic of the phylum.

Cl. Echinoidea

Regular echinoids: all of the pentaradially-symmetric, tomato-shaped sea urchins.

- Test. Examine the bare sea urchin tests (carefully, as they may be fragile), composed of ossicles fused together to form a rigid structure. At the oral end is the opening for the **mouth**, which in a living specimen is surrounded by soft tissue called the **peristomial** ("around the mouth") **membrane**. At the **aboral end** is an opening for the **anus**, which in the living specimen is surrounded by soft tissue called the **periproctal** ("around the anus") **membrane**.

On the aboral end, note the five **genital plates** surrounding the periproct, each of which has a large **gonopore** that corresponds to each of the five pairs of internal gonads. The largest and most porous of these five plates is the **madreporite** (be sure you can distinguish it)—note the asymmetry created by this one structure that interrupts what is otherwise nearly perfect **pentaradial symmetry**.

Most of the surface of a sea urchin is actually homologous to the oral surface of sea stars, as evidenced by the location of **ambulacrae**. Hold a piece of the test up to the light. Look for the tiny perforations, which are the holes through which the tube feet project. By finding these perforations, you should be able to distinguish between the **ambulacral plates** (homologous to the plates that make up the ambulacrae in asteroids) and the **interambulacral plates** (which have come to fill in the spaces between ambulacral plates).

TQ: Locate two adjacent ossicles--one ambulacral and one interambulacral. Do a simple sketch of features found on the two ossicles. Label your sketch with the names and the functions of these features.

TQ: Carefully compare tests of different size under a dissecting microscopoe. What evidence can you find for whether or not sea urchins grow (1) by adding new ossicles, and/or (2) by adding more material to each ossicle?

- Spines. Examine a couple of detached spines, preferably of different sizes. Find the indented **socket** at the base of the spine. Fit this socket onto the appropriate bump (**tubercle**) on the test. Notice that larger spines have larger sockets that fit onto larger tubercles. Be sure you can describe how muscles would be used to make the spines rotate around the tubercle.

Compare pointy spines with the blunt spines of the "pencil" urchin *Heterocentrotus*. Think about what role thick, blunt spines could play in the lives of pencil urchins.

- Live *Arbacia punctulata*. One of the best visual displays in a course on invertebrates is the surface of a live sea urchin under a dissecting scope. Put yourself in the frame of mind of zoologists from the 18th century who thought some of the surface structures were actually commensal organisms growing on the surface of the urchins! Examine a live specimen using both the dissecting microscope and your unaided eye.

>>> Marvel at the long, slender **tube feet** connected to a surface such as the bowl. Try gently tugging on the animal and notice its strength of attachment. How does this animal locomote, compared to the previous two classes? What evolutionary modifications were necessary for this movement?

>>> Carefully use a probe to touch the surface of the animal and watch under the dissecting scope the action of **spines** and **pedicellariae** near the point of contact.

>>> Use the dissecting scope to examine these features on the aboral surface: **spines** of different sizes, **pedicellariae** of different sizes and shapes [in some sea urchins, the globe-shaped pedicellariae contain venom], and **tube feet**. Examine the flaps over the anus—what is unusual about their symmetry? Flip the animal over to observe the oral side features, including the **mouth** and **peristomial membrane**, the **peristomial gills**, and the **teeth of Aristotle's lantern** (the white jaws you might be able to see protruding out of the mouth—see next station for an internal view).

TQ: Most echinoids are herbivores, so they don't catch prey with their pedicellariae the way some fish-eating asteroids do. What are other possible functions of the pedicellariae?

>>> "*Aristotle's Lantern*." Examine the sea urchin that has been opened to reveal this feeding structure. This complex of ossicles creates a scaffold that allows the animal to work the set of teeth that protrude from the mouth, used mostly to cut up algae but may also scrape away at animal prey. Note the large number of ligaments that are used like guy-lines to hold the lantern in place and are also used to return the teeth to their resting position when muscles are relaxed.

TQ: Describe how and where muscles and ligaments must be attached to the structure to operate the teeth and why constraints on muscles (i.e., they only contract) make it necessary to create such a towering and complicated structure.

Irregular echinoids: sand dollars, heart urchins, and sea biscuits all show deviations from pentaradial symmetry—in fact, they have re-evolved bilateral symmetry in several features.

- <u>Oral surface</u>. Examine any preserved or dried specimens of sand dollars or sea biscuits. The oral side includes the **mouth** and **ambulacral grooves**, with **tube feet** used for feeding. As these animals are deposit feeders, the tube feet are used to pass sediment particles to the mouth.

- <u>Aboral surface</u>. The **petalloids** are small perforations in the test arranged in five petal shaped patterns. These features are homologous to ambulacrae, with specialized **respiratory tube feet** projecting from them. (Recall that in all echinoids, most of the body surface is "oral" in origin, as shown by the location of ambulacrae.) Locate three features normally associated with the aboral surface: (1) the **gonopores**, which occur in a similar position as in regular echinoids (how many can you find? ________), (2) the **madreporite**, which is the entire plate between the gonopores, and (3) the **anus** (which in fact is no longer located on the aboral surface—where is it?)

Shake the sand dollar or sea biscuit to notice the rattling of the highly modified teeth inside of the test, which are found in the bowl located near each test. These teeth are the remnants of **Aristotle's lantern**, which is more pronounced in regular echinoids.

TQ: Note how the near perfect radial symmetry of regular echinoids deviates into a tendency toward a secondary bilateral symmetry in irregular echinoids. Recall also that the madreporite breaks the perfect radial symmetry of regular echinoids (and asteroids), but in irregular echinoids, other structures determine the plane of bilateral symmetry, and the madreporite at the center does not. Locate the plane that divides “left” and “right” halves of the body of an irregular echinoid. (1) Which structures that are not consistent with pentaradial symmetry help to define this plane? (2) What structures are reflected in their bilateral symmetry?

- Spines. All irregular echinoids burrow into soft sediments, using highly modified, short spines. Examine the spines on the preserved or dried specimens, and note their size and distribution when compared with those in regular urchins. Also, examine the bare tests of these animals. Find the **tubercles** on the sand dollar and sea biscuit. Can you picture how the tiny spines would articulate on them?

Cl. Holothuroidea

- Body orientation. Examine any live or preserved sea cucumbers on display. Note the soft, elongate body. The **ambulacrae,** with tube feet used in locomotion or respiration, are homologous to those in other classes. Locate the homologous **anus** and the **mouth**, noting the change in position and body orientation. Around the mouth are a set of **bushy tentacles** (retracted in preserved specimens). These tentacles, which are highly branched and modified **podia**, are used to gather food from suspension (for **suspension feeding**) or from the substratum (for **deposit feeding**). Find the bases of these tentacles to confirm that they follow five-part symmetry, with a pair of podia per ambulacrum (as you observed in your asteroid dissection).

- Ossicles. Examine a prepared slide of *Leptosynapta* body wall. Note how the individual ossicles are reduced relative to those in other classes, and are embedded in the thick dermal wall. As with sponge spicules, the ossicles have elaborate and characteristic shapes that are sometimes useful in identifying taxonomic relationships.

Cl. Crinoidea

- Feather stars. Examine the specimen of *Antedon* encased in lucite. This shallow-water feather star shows the **calyx** (small finger-like structures at the base), the **branching arms**, and the **pinnules**. Take a preserved specimen to your desk, and examine structures in greater detail. Some of the **pinnules** may be swollen due to the presence of gametes. Examine the **tube feet**. Note that they are **tentacle-like**, as in ophiuroids, and are used solely for feeding, not for locomotion. Feather stars use the **calyx** for crawling movements (with the branches of the calyx functioning like the muscular arms of ophiuroids) and some species can also flap the arms to create swimming motions for longer-distance transport.

- Sea lilies. If a stalked "**sea lily**" is on demonstration (or a fossil of one) note the long **stalk** that extended from the body of the animal to the substrate. Note that the stalk has very large, **vertebra-like ossicles**, which permit effective bending of the stalk in currents. These animals were once quite common but are now found mostly in deep-sea environments.