## Materials:

Sponge Sample Seawater

| Part 1  | Part 2                                    | Part 3  |
|---|---|---|
| Glass Bowl Petri Dish Forceps Dissecting microscope Scalpel Depression Slide Bleach solution Compound microscope dilute hydrochloric acid Microscope slides cover slip glass stirring rod Ruler | Glass Bowl<br>Food color<br>small pipette | Scalpel Forceps Petri Dish Sharpie small beaker Dissecting needle Tissue Sieve Compound microscope Microscope slides cover slip |

Part 1 - Observation of the anatomy of a sponge

- 1. After filling your glass bowl half full of seawater obtain your sponge sample.
- 2. Observe the sponge sample remove the sponge from the glass bowl and place in a petri dish. Using your camera take a picture of the sponge and insert it in the box below and label the species. Next observe under the dissecting microscope. *Briefly record your observations in the box below*

| COMMENTS: |  |  |
|-----------|--|--|
|           |  |  |

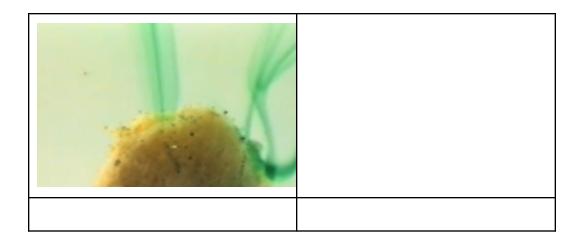
| Name of Sponge: |
|-----------------|
|-----------------|

- 3. Return the sponge sample to the glass dish. Next we will be observing the components of the body wall of the sponge. The sponge body wall is composed of an outer and inner layers of cells with an area in between composed of loose cells, spicules and/or spongin. In this part we will be observing the spicules of your sponge sample.
  - a. Using forceps obtain a very small piece of sponge (~1mm long and 1mm across)
  - b. Place your piece on a depression slide and add three drop of bleach solution and let it sit for 2 minutes to dissolve the tissue.
  - c. Grind the sponge material with the tip of a stirring rod to break up the piece.
  - d. Cover with a coverslip and observe under the compound microscope and take pictures at both 40x and 100x. Insert the pictures in the boxes below:

| Above picture take at magnification | Above picture take at magnification |
|-------------------------------------|-------------------------------------|
|                                     |                                     |
| Above picture take at magnification | Above picture take at magnification |

- e. The spicules are either made of calcium carbonate of silica. Add a few drops of dilute hydrochloric acid and observe under the microscope for signs of bubbling (calcium carbonate will bubble but not silica)
- f. Use the information below to determine what class these sponges below to
  - i. Class 1 : Calcarea ( Calcispongiae) Spicules composed principally of calcium, are monoaxon or three or four branched.
  - ii. Class 2: Hexactinellida (Hyalospongiae)Spicules composed principally of silica, are 6- rayed,
  - iii. Class 3: Denmospongiae (Siliceous) Spicules or horny fibers or both are present. If spicules of silica are present in an intertidal form, the sponge probably belongs to this class. Spicules, if present, are never six-rayed.
- g. In the box below list the class you believe your sample belongs to.

1. Place the sponge in the glass bowl. Using the small pipette place a drop of food coloring near the sponge near the ostia, observe the movement of the dye out of the osculum. Take pictures of this process and insert them in the boxes below: (Notice the example)



2. When done pour out the water and replace with fresh seawater.

## Part 3 - Reaggregation of sponge cells

- 1. Take the smaller half of the petri dish and scratch lines in the bottom of the plastic with the dissecting pin. (This will give an attachment site for the reaggregation sponge cells) Also write your group and period number on the top of the petri dish.
- 2. Fill the small beaker ¼ full of seawater.
- 3. Cut off a piece of sponge approx 2cmx2cm.
- 4. Place the sponge in the tissue sieve and place the screened end into the water in the beaker. Depress the plunger of the sieve all the way slowly and you should see dissociated sponge cells being released from the sieve.
- 5. Pour this dissociated cells into our petri dish and take several pictures with your camera and insert them in the boxes below

6. Take a drop from the petri dish and prepare wet mount slides and observe the dissociated cells. Take several photos and insert them in the box below

|        | Above picture take at magnification      | Above picture take at magnification   |
|--------|--|---------------------------------------|
|        |  |                                       |
|        | Above picture take at magnification      | Above picture take at magnification   |
|        |  |                                       |
|        |  |                                       |
| 7. Re  | eturn 24 hours later and take pictures o | of the petri dish to observe clumping |
|        |  |                                       |
| L      |  |                                       |
| 8. Re  | eturn 24 hours later and take pictures o | of the petri dish to observe clumping |
|        |  |                                       |
| 9. Re  | eturn 24 hours later and take pictures o | of the petri dish to observe clumping |
|        |  |                                       |
| 10. Re | eturn 24 hours later and take pictures o | of the petri dish to observe clumping |
|        |  |                                       |
| _      |  | <del>.</del>                          |

## Types of Sponge Spicules

