04/11/13

Oly Relaxation Experiment Protocol/Notes

1. Wash the 6 quart shoebox containers and allow to dry.
2. Put lab tape on the bottom of the containers in order to make a 5x2 grid.
3. Weigh out the correct amount of magnesium sulfate (Epsom salts) for 2 liters of water.
   1. We will have this prepared ahead of time (for at least the first test)
   2. For Example, for the first round of treatments:

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment | Volume Freshwater | Volume Saltwater | Mass MgSO4 |
| Control | 0 L | 2 L | 0 g |
| 75 g/L | 1 L | 1 L | 150 g |
| 85 g/L | 1 L | 1 L | 170 g |
| 100 g/L | 1 L | 1 L | 200 g |

* 1. There will be 3 repetitions of each treatment

1. Put the correct mixture of water (see above) into each shoebox.
2. Add the weighed out MgSO4 to the appropriate container, label, and stir until dissolved.
3. Record the temperature of the water in each shoebox as well as the oysters’ current location.
4. Obtain clean seawater for recovery.
5. Add oysters one by one to the containers. Make sure only one is in each grid box. Place the cup side down.
6. Take initial notes of openness/responsiveness
7. Every 15 minutes, check each oyster and take the following notes
   1. If the oyster is open.
   2. If open, check for response to touch. Gently tap on the shell ~3 times and note response/speed of response.
   3. If there is no response to touch, take the oyster out of the water, if it stays open remove it from the treatment and put into clean water. Record the time.
      1. If it closes, observe and record the speed of response. (Slow, normal, etc.)
      2. If the oyster stays open for ~15 seconds or does not completely close, this counts as anesthetized and should be removed.
8. Each treatment and replicate should have a unique bag in which to put the oysters after treatment. This way we can track mortality later.
9. After two hours, remove all of the oysters from treatment, put them into their treatment’s recovery bag. If, during the course of the experiment, any oysters have not opened at all they should get a separate recovery bag.
   1. For example, during preliminary testing, in one treatment three oysters never opened at all. We put them into a separate recovery bag from the rest of their treatment because they were never exposed to the treatment. I don’t feel that their mortality should be considered part of the test group.

Time point: Notes: Time point: Notes:

Treatment:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Repetition:\_\_\_\_\_\_\_\_\_\_ Notes:

1. 2.

3. 4.

5. 6.

7. 8.

9. 10.

~ 420 Oysters Total

The 20 control can be reused.

No algae present

Need 80 oysters

Algae present

Need 80 oysters

Food

Range of 3 concentrations, possible temperature change, 2 Reps of each

Decide which temperature range to continue with

Temperature

Range of 3 conc., 2 reps for each treatment

Temperature decrease

Ex. 15-11 degrees

Need 80 oysters

Temperature increase

Ex. 11-15 degrees

Need 80 oysters

No temperature change

Need 80 oysters

Decide which concentration range to continue with

High Concentration

0, 75, 85, 100 g/L

Need 120 oysters

Mid-Concentration

0, 25, 50, 75 g/L

Done on 4/11

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