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We are still developing and optimizing this protocol

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Phytoplankton Storm Simulation

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ABSTRACT

Lab processing guide for phytoplankton culture experiments

Pre-Experimental Prep

1 Prior to the experiment you should prepare all treatment container. Be sure they are acid washed and you have plenty of nutrient

Culture Sample Collection

2 Bring all 25 culture bottles to the OL pier along with two extra sampling bottle for HPLC analysis & nutrient. You will also need a bucket, siphon and a 153micron mesh in addition to the normal mesh size.

3 Prior to culture collection, take the standard samples with a net-tow for planktoscope imaging. Take two whole water samples for HPLC and nutrient analyses as well. Take the YSI for salinity and temperature measurements as well.

4 Collect a surface water sample with a bucket. Using a siphon, you should fill water form the bucket into each container. Fill the containers according to their labels for each treatment

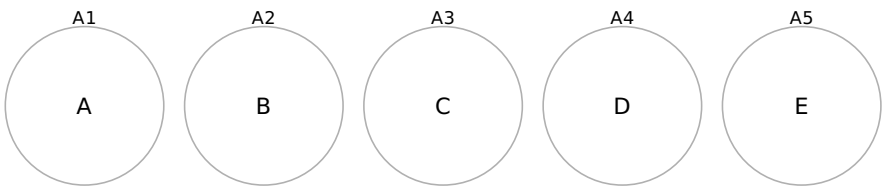
4.1 Control - 1000mL
Low DI/Swamp - 750mL
High DI/Swamp - 500mL

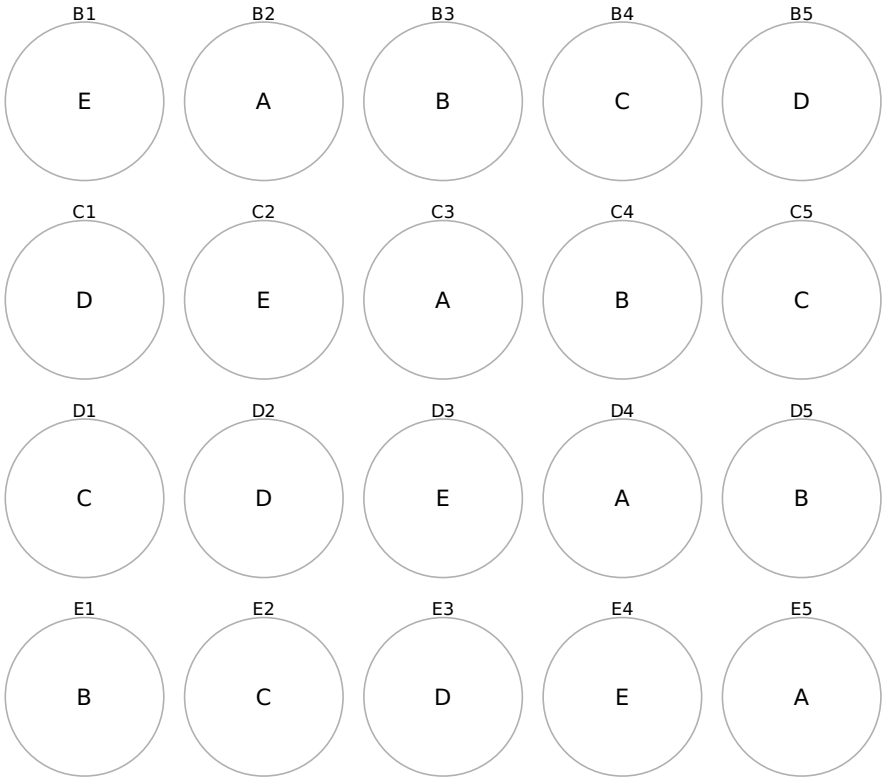
5 Collect swamp water from the nearby forest drainage area. Also get about a bucket worth of DI water. Rinse the bucket a few times prior to adding DI water. Bring DI and swamp water to the flow through seawater table.

5.1 Treatments should be added accordingly and set into the experimental layout grid.

Experimental Layout

6 Treatments should be assigned to a Latin Square design in a 5x5 grid





Treatments:
A - Control
B - Low Storm (50% salinity reduction)
C - High Storm (25% salinity reduction)
D - Low DI
E - High DI

7 Once the treatment has started, record the exact start time and let incubations occur for 48hrs.

Experimental Breakdown & Processing

8 To break down the experiment follow the steps for each bottle. Remove all bottles from the table and bring into the lab space. To reduce growth and plankton activity as you work through each bottle, place all bottles into the walk in refrigerator.

9 For each bottle, 375mL should be set aside for HPLC, 125mL for scope counts, and 500mL for planktoscope/flowcam imaging.

9.1 First, take 100mL from the sample and immediately preserve it for later settling and scope

counts

9.2 Then, take 500mL for planktoscope/flowcam imaging. To prepare this sample, filter onto a 20-micron sieve. Then, rinse the filter contents into a beaker. Aim for around a 25mL sample - this corresponds to a 20x concentration factor. It should be no more than 50mL. The more concentrated the better.

9.3 Planktoscope imaging will be done with the live sample. Generally, you can follow the planktoscope use guide. However, it is important to preserve the sample after imaging because it is so little. Thus be sure to collect the waste and not contaminate it. DI rinse between samples but don't bother with ethanol until the final clean-up. For each sample, preserve with Lugol's and store

9.4 HPLC processing. Follow the standard water filtration method from the EEL lab. Be sure to record the final filtered volume for each sample. Freeze filters immediately in the -80 storage area.

Post-Experiment Clean up

10 Once complete with the experiment, be sure to clean up and acid wash all necessary items (culture bottles). Metadata entry should also occur