**GEODES - Gene Expression in Oligotrophic, Eutrophic, and Dystrophic Systems Sampling Procedures**

**Sample Timing and Location**

Lake sampling will occur at the epilimnion of Trout Bog, Sparkling Lake and Lake Mendota once during July of 2016. An integrated sample of the top 2 meters of epilimnion will be collected from each lake. The sampling locations will be the centers of Trout Bog and Sparkling Lake (marked by LTER buoys) and University Bay in Lake Mendota.

Before each run, check the weather. If there is a storm warning or high winds, consult Alex before going out on the boat. If not, record the wind speed and air temp.

**Water Column Profiles.** Half an hour before each timepoint, send the datalogger program to the sonde via the phone and lower the sonde into the water\*. Measure all parameters at every meter. Hold the sonde at each depth for 1 minute and record the readings at the end of each minute on the metadata sheets. Hold onto the safety line at all times.

\*I still need to figure out what the sonde program will be

**Photosynthetically active radiation (PAR).** Designates the spectral range of solar radiation from 400 to 700 nanometers that photosynthetic organisms are able to use in the process of photosynthesis. Lower the PAR meter through the water column, recording it every meter, until no light is detected. This is not necessary to collect at the 1AM timepoint.

**Secchi Depth.** Lower the Secchi disk on the shaded side of the boat. Remove any sunglasses. Record the depth at which you can no longer see the disk. Lower the disk another half meter, then pull up, recording the depth when you first see the disk. Average the two readings. Repeat with each person on the boat. This only needs to be performed once per lake.

**Water Samples**

Water samples are taken from the integrated top 2 meters of epilimnion, using an integrated water column sampler. Wash the sampler and the carboy on the side of the boat you will not be collecting samples on. Take 3 water columns, then place in carboy and mix well. Aliquot or filter subsets of this sample for various analyses, as described below.

**RNA/DNA Samples.** Filtration volume size will depend on the particulate load of the water. Because of this, you should filter each sample for 3 minutes. Generally 400 - 500 ml of sample water is required, but up to a liter may be required during clear water phase. Filtering and storing should be performed in less than 5 min to avoid errors resulting from changes in gene expression of the bacterial populations in the lake. Measure the volume of water filtered for the first replicate. **Store the filtered water from the second two replicates into two separate bottles for nutrient analyses.** DNA will be collected once for each lake.

**Preservation** - Sample filters should be stored in screw cap tubes and frozen in liquid nitrogen in the dark until extraction. Cryobaby labels and cryotubes should be used so that labels and caps do not come off. Place all screw cap tubes from each timepoint in a 50 mL Falcon tube, then drop into the liquid nitrogen dewer.

Whether on board or in the lab, all apparatus should be clean. Assemble the filtration apparatus by gently resting a sterile 0.22 um pore-diameter nitrocellulose filter on the clean 47 mm filter holder. Assemble a 14-16 size silicone tube in the portable peristaltic pump and attach the filter holder to one extreme of the tube. Attach cheesecloth in the other extreme with a zip tie to avoid bigger particles to pass. Filtration should not exceed ¾ of the pump speed in order to avoid high filtration pressure.

**Whole water samples.** Using the peristaltic pump with no filter attached, fill two nutrient bottles with 250 mL of whole water from the integrated sample. Fill one 15 mL Falcon tube with whole water for bacterial production assays, place in Ziploc bag, and put in the thermos (fill an additional Falcon tube for SAGs if needed). Fill the thermos with a surface grab of water – this water will not be analyzed, as it will provided the incubation conditions for the bacterial production assays. Fill one container with 250 mL of whole water for phytoplankton counts. Fill another with 250 mL of whole water for metabolomics. Pour 1L of whole water into the dark chlorophyll sampling bottle. Pour any remaining water over the side of the boat.

**Post-field processing**

**Chlorophyll.** Filter three replicates from the dark chlorophyll bottle through glass fiber filters. Filter 250 mL for each replicate. Place each filter in a film canister and store at -20C.

**Nutrients.** Put both filtered and unfiltered nutrient samples at -20C.

**Phytoplankton.** Add three drops of glutaraldehyde to the phytoplankton container. Store at 4C.

**Metabolomics.** Wrap bottle in tin foil and store at 4C.

**SAGs**. Add 1 mL of water from Falcon tube to each prepared SAG tube with GlyTE buffer in the fridge. Store at -20 C. This only needs to occur once per lake.

**Equipment maintenance.** Plug in the peristaltic pump and make sure it is set to “charge.” Every other timepoint, switch out the sonde batteries and charge the one not in use. Unplug the power cord from the datalogger before removing the sonde battery. Change the cheesecloth on the end of the peristaltic pump hose after every timepoint.

**Supplement - Sampling Protocol**

Initially, depth profile including temperature, pH, dissolved oxygen, and conductivity data must be collected at a maximum of 1-meter intervals. A thermocline exists if greater than 1°C change occurs within a depth change of 1 meter or less. Determination of the existence of a thermocline is essential for downstream analysis.

**Timing**

Samples will be taken at 5AM, 9AM, 1PM, 5PM, 9PM, 1AM during a period of 48 hrs.

**Sampling**

**Water quality components**

Using filtered water:

* Nutrients (Nitrogen, Phosphorus, Ammonia, Urea and Polyamides)

Using unfiltered water:

* Nutrients (Total Nitrogen and Phosphorus)
* Chlorophyll
* Biomass Production
* Phytoplankton
* Metabolomics

**Physical parameters**

* Temperature
* pH
* Conductivity
* Dissolved Oxygen
* Secchi Disk Transparency
* Pressure
* Chlorophyll
* Phycocyanin