GEODES Sampling Protocol

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# 1 hour before you go out on the boat

* Check the weather. If there is a storm warning or high winds, check with Alex before going out on the boat.
* If the weather poses no threat, record the local temperature and wind speed on the metadata sheet.
  + Mendota weather: <http://metobs.ssec.wisc.edu/buoy/>
* Pack the boat, check that equipment is charged and ready to use, and prepare datasheets and sampling bottles. Fill out the sampling checklist as you go.
* Change the cheesecloth on the end of the filtering tubing
* Set sail 45 minutes before the official timepoint

# 30 min before your timepoint

* Arrive at the sampling location
* Measure water column profiles using the sonde and the PAR meter
* Collect the water sample within 10 minutes of the official timepoint

# At your timepoint

* Collect an integrated sample of the epilimnion using the tubing
* Begin filtering for RNA and collecting metdata samples as you go

# Back on shore

* Place samples in the fridge or freezer, as necessary
* Filter for chlorophyll
* Charge the pump and the sonde phone
* Change the sonde battery every other timepoint
* Record which battery and pump you used so the next crew knows which ones to take
* Deliver Falcon tube of water Robin or Alex

# How to use the sonde

* BE VERY CAFEFUL WITH THE SONDE. IT PROBABLY COSTS MORE THAN YOUR CAR.
* Is everything charged?
  + Phone should be plugged into the wall between timepoints.
  + The battery should be switched with the one charging every other timepoint.
    - With the cables attached to the battery NOT plugged into the control panel, remove the terminal ends from the battery and lift battery out of its case. Clip charger onto the used battery and insert the charged battery in the exact reverse of what you just did (do not touch cables when they are plugged into the control panel)
  + GENTLY remove the water-filled cap on the sonde sensors and replace it with the weighted guard. Have someone else hold the sonde while you do this.
* Inside the cooler, you will find the battery (in another box) and the control panel.
  + THESE ELECTRONICS SHOULD NEVER GET WET. DO NOT OPEN THE COOLER WHILE ON THE WATER.
  + To turn the sonde on and off, plug the battery cables into the control panel before you leave the dock.
  + It will beep and shine a blue light when it is on
* Controlling the sonde
  + Open the pink phone and click on the “LoggerLink” app. Make sure the phone’s Bluetooth and Wi-Fi is on (drag down from top of screen and click the icons – if on, they will be green)
  + A picture of the control panel with the name “CR1000” will be on the Loggerlink screen. Click on this to connect.
  + Once the sonde is connected, you should see real time measurements from the sensor
  + While holding on to the safety line to the boat, lower the sonde into the water and hold it steady at every meter for 1 minute, starting at the surface. Manually record sensor data from the first LoggerLink tab at the end of each minute, including the exact time of the measurement.
  + You will measure the top 10 meters of the water column
  + Re-coil the cable and safety line nicely as you bring the sonde back to the surface.
  + Before disconnecting the sonde, save the data tables by going to the “Collect” tab in LoggerLink, selecting “All Data” then “Collect” on the top right, clicking the eye sample to the right of the sonde table when it is finished collecting, then click “Share” icon on the top right, and select “Save to Dropbox.” The phone will automatically sync with my computer’s Dropbox folder once you are back at the CFL
* Back on shore
  + Turn off the sonde by unplugging the power source cables from the control panel.
  + Remove the sensor guard and re-attach the water filled cup, filled with fresh tap water.

# How to use the PAR meter

* Turn on the PAR meter and lower it on the sunny side of the boat
* Starting at the surface, record the PAR measurement every 1 meter until you reach a depth of 6 meters.
* This step is not necessary at the 1AM timepoint

# How to collect the integrated epilimnion sample

* Dip the bottle in surface water and shake it to wash
* Lower the weighted end of the tubing until the other end is nearly at the water surface. Hold on to the safety line while you do this!
* Pull the tubing back up to the boat. Right before the bottom of the tubing reaches the surface, reach into the water and insert the stopper at the bottom end of the tubing.
* Hold either end over the 4L sampling bottle and remove its stopper to release the water
* Shake the bottle to integrate the sample

# Filtering for RNA

* The person handling the filters should wear gloves. Make sure you have fresh cheesecloth on the end of the filtering tubing for each timepoint.
* Run 50 mL water sample through the tubing and filter holder with no filter.
* Place a 0.22 micron Supor filter grid-side facing the direction the water is coming from in the filter holder. Place the O-ring on top of the filter using the tweezers.
* Turn the pump on at ¾ speed
* Filter for a specific amount of time set that day based on the filter clogging time on that lake
* When the time is up, open the filter holder and carefully fold the filter like a hot dog 3x. Squish this rolled up filter into a 2 mL cryogenic tube labelled with the appropriate number.
* Record the time filtered and the timepoint on the sheet corresponding to that tube number
* Make sure the tube is well-sealed, then drop into liquid nitrogen.
* Repeat this process 4 times. Discard the flow through of the first filter, then keep the flowthrough of the second filter for nutrient testing. Discard the flowthrough from the remaining filters.

# Collecting filtered water samples

* While you are filtering for RNA, you can collect the flow-through for nutrient analysis.
* Wash the inside of the bottles by shaking with a small amount of filtered water inside, then dumping this water
* Collect two replicate bottles of 60 mL each of filtered water

# Collecting unfiltered water samples

* Collect small volumes of unfiltered water by running the pump with no filter in the filter holder. Wash all bottles with unfiltered water before filling.
  + 10 mL in a 15 mL Falcon tube for bacterial production assays. This is stored in the blue thermos filled with surface water.
  + Two replicate bottles of 60 mL each for nutrients
  + These samples include the cheesecloth pre-filtration
* Collect larger volumes by pouring water directly from the sampling bottle. Wash all bottles with unfiltered water before filling, and use the graduated cylinder to measure volumes below 250 mL.
  + 150 mL for phytoplankton analysis
  + 150 mL for cyanotoxin analysis
  + 1 L in a dark bottle for chlorophyll analysis
  + This does not include a pre-filtration step

# Filtering for chlorophyll

* Collect unfiltered water in a tin-foil wrapped 1L bottle
* Use the same yellow pump to filter
* Put a Whatman filter in the filter holder grid side up using tweezers
* Filter 250 mL of water from the chlorophyll bottle through the glass fiber filter
* Do NOT filter through a cheesecloth
* Fold the filter up with tweezers and place in a well-labelled 2 mL cryogenic tube.
* Make sure the tube is well-sealed, and drop it into the liquid nitrogen
* Take three replicate filters for each timepoint
* Discard the remaining water in the dark bottle
* When finished, flip the switch on the pump to “charge.” IF THE CHARGE LIGHT IS NOT ON, THE PUMP IS NOT CHARGING.

# What gets stored where?

* You should have 4 RNA filters and 3 chlorophyll filters for each timepoint in the liquid nitrogen dewar.
* Phytoplankton samples go in the fridge after 2 mL of Lugol’s solution is added
* Nutrient samples and cyanotoxin samples go in the freezer
* The 15 mL Falcon tube should be given to the person running bacterial production assays immediately upon return.