**Field methods**

**Updated 7/2/20**

Following the packing list to fill the coolers with supplies for the field (do this the Friday before our Monday scheduled trips). Each station sampled will get its own bag of supplies. Large coolers get ice packs. Small cooler gets dry ice. (Dry ice is on first floor) If there is no dry ice, Toft’s sells dry ice, so does Kroger and Meijer. Liquid nitrogen is also needed each week. Fill the liquid nitrogen container on Sunday so it is cold enough to hold liquid nitrogen when you fill it again Monday morning.

In the field, at each site visited, write down in the log book the time arrived at the station and fill in appropriate parameters measured by the handheld YSI sonde. Take measurements of light fields, every 0.5 m using the Li-Cor light meter until 0. Repeat. Write these down in the log book. No need to take light field measurements at the Bridge site due to shading by the bridge deck (too dark to get good data)

Collecting water. Use the Van Dorn bottle to collect water samples at a depth of one meter. Use three separate casts to fill 3 separate 1 L amber bottles (for chlorophylls mostly). This is 4 casts/4 bottles at ODNR 4 and Bridge and 5 casts/5 bottles at ODNR 1 and EC 1163. When you fill a bottle, rinse the bottles by adding a small volume of water, capping the bottle, shaking it and pouring the contents overboard. Repeat two more times, then fill bottle completely. Store the bottles as threesomes (or foursomes) in bags.

At ODNR1, ODNR4, EC1163 and the Bridge, two additional sterivex samples per site must come from the water just above the sediment (or as deep as the sampler goes in the case of 1163). There is a spare bottle in the coolers for temporary storage of the water, which can be rinsed in between stations.

Water for nutrient analysis. Take one (250 mL) nutrient bottle and fill the ‘Total Nutrients’ bottle with water from one of the bottle filled above, fill with 200mL to allow for expansion when freezing. (Rinse bottle as above) For ‘Dissolved Nutrients,’ fit a 0.2 um Sterivex cartridge filter on the end of a 60 mL syringe filled with water from the same bottle the Total Nutrient sample was taken (Rinse bottle with small amount of filtered water). Push 50 mL of the water into the 60 mL Dissolved Nutrients bottle. Two dissolved nutrient bottles per site. Place both nutrient bottles in the cooler (wet ice is OK for this).

Water for toxin analysis. For ‘Total Toxin’ pour a small volume of whole water from one of the chlorophyll bottles above so that the glass vial is **half** full, cap the vial and place in the cooler. (Over filling can cause the frozen bottles to break) For dissolved toxin, use a Sterivex filter and syringe to push through a small volume into the Dissolved Toxin vial. Fill halfway, cap and put in the cooler. Keep samples in the same Ziploc bag as the nutrient samples.

Water for MBio toxin analysis: Seth will be doing this but 20mL of well-mixed whole water will be extracted and analyzed using the manufacturer’s protocol (final document is currently being developed).

Sterivex filters for DNA and RNA. At each station, prepare a series of filters with water filtered through them using the 60 mL syringes until resistance is built up due to biomass. Some stations only need two filters, others need 6 or more. Label each with the date, station and volume filtered (very important!). Volume will vary depending on turbidity of water. (Some may only be able to push 30mL through) Remove as much water from filter as possible by pushing air through the Sterivex. Fill with RNALater for 3 of the sterivex until they are about 3/4 full. Let them sit for 2-3 min. When the filter is prepared, cap the Luer lock end with the white screw cap and plug the nipple end with clay. IMMEDIATELY toss DNA filters into the dry ice cooler and the RNA filters into the liquid nitrogen container. Given the time and tedium it takes to do this filtering, you might dedicate one person at each station to do this, and rotate at the next station. Tough luck for the guy who gets the 10 filter station. Write down volume filtered in field book.

**If time is short, DNA Sterivex can be done in lab.**

Muddy Creek – **2 total:** 2 surface DNA sterivex

ODNR6 – **2 total:** 2 surface DNA sterivex

ODNR4 – **9 total:** 2 above sediment DNA sterivex, 4 surface DNA sterivex, 3 surface RNA sterivex

Bridge – **9 total:** 2 above sediment DNA sterivex, 4 surface DNA sterivex, 3 surface RNA sterivex

ODNR2 – **2 total:** 2 surface DNA sterivex

Buoy2 – **2 total:** 2 surface DNA sterivex

ODNR1 – **9 total:** 2 above sediment DNA sterivex, 4 surface DNA sterivex, 3 surface RNA sterivex

EC1163 – **9 total:** 2 above sediment DNA sterivex, 4 surface DNA sterivex, 3 surface RNA sterivex

Bells – **2 total:** 2 surface DNA sterivex

EPA sampling. Bottles will be filled according to EPA protocols and forms filled out accordingly. See protocol below.

Back in the lab. After the stop at Toft’s for ice cream and the 1 hour drive back to the lab, store samples **immediately** upon arrival. First, the Sterivex filters are put in labeled boxes in the -80 freezer. Nutrient samples and toxin samples are put in large bag marked by date and put in a -20 freezer. Next filter for chlorophylls – depending on the ‘greenness’ of the water, filter 5 or 10 mL volumes, using pipettes, onto 25mm 0.2 um polycarbonate filters, and freeze individually in test tubes labeled with station, date and **volume filtered** (very important). Record time of processing.

Water for qPCR. Triplicate samples per site will be collected. Depending on the ‘greenness’ of the water, filter 25 or 50 mL volumes, using a graduated cylinder, onto 25mm 1.2 um polycarbonate filters, fold the filter in half with the biomass on the inside place into cryovials labeled (using a cryopen) with station, date sample type (qPCR) and **volume filtered** (very important). Store in a cardboard or plastic box in the -80 freezer in 513.

Water for fluoroprobe: From each station take a 25mL aliquot of water and analyze it on the Fluoroprobe in 513. Store all data on the associated laptop next to the instrument in a folder labeled Sandusky Bay Fluoroprobe 2020.

Lugol’s samples. For microscopy of phytoplankton, preserve 10 mL volumes from each site in Lugol’s solution. Fill a 15 mL Falcon tube (same tubes as the chlorophyll filters) with a water sample, and add Lugol’s iodine so the water is yellow/orange in color (urine color is fine). Store at room temperature away from direct strong light.

Other field-related duties may emerge as the summer develops, but these are the major activities on sampling days.

**Replicate equals one cast of the VanDorn. Each bottle should represent one replicate.**

**Protocol for Ohio EPA sampling in support of the Sandusky Bay Impairment Designation**

The Ohio 2020 Integrated Water Quality Monitoring and Assessment Report provides a plan for sampling Sandusky Bay to determine whether it can be listed as impaired under Section 303d of the Clean Water Act. In 2019, Lake Erie scientists developed a plan dependent on total (particulate and dissolved) microcystin concentrations as the impairment criterion (see below).

For Microcystin analysis, the protocol is simpler than in previous years. It requires sampling of 7 individual sites listed below, mixing equal volumes from each site into single container, and freezing the composite sample for analysis by Ohio EPA staff. Each week we also prepared a duplicate sample (an identical sample split from the original composite), a replicate (a composite sample prepared from an additional cast from the 7 sampling sites), and a field blank (distilled water). Chlorophyll procedure is the same from previous years, filtering 250 mL from ODNR 1, 2, 4, 6 as well as a field blank (distilled water) and a duplicate and replicate randomly picked from the four sites each week and recorded. The specific details are provided below:

1) Sample each of the seven sites with two separate casts, one for the analytical sample and one for the replicate. Fill bottles mindful of the volumes needed (250 mL volumes for EPA chlorophyll, 100 mL to be mixed into the composite sample, 100 mL for the duplicate from the analytical sample, plus any additional volumes for our work – dissolved and total nutrients, etc.).

2) Back in the lab, mix the samples from the analytical casts by inverting each bottle 5 times, then add 50 mL from each bottle into a clean acid-washed 1 L bottle. Invert 5 times, then decant the mixture into two - 125 mL bottles provided by EPA for the analytical sample. One is the original, the other is the duplicate. The replicate bottle can be 50 mL from each bottle into a clean acid-washed 1 L bottle.

3) Repeat the process for the replicate cast, filling one 125 mL bottle with a mixed sample from the seven sites.

4) Fill a 125 mL bottle with milliQ water for the field blank.

5) The end product for each week should be four bottles – the analytical composite sample, a duplicate of the analytical sample, a replicate sample from an additional cast at each site, and the field blank.

6) 250 mL from each ODNR site is filtered onto GF/C filters as has been done in the past with a duplicate and replicate randomly chosen/recorded each week.

Fluoroprobe data will be obtained from the composite water sample and samples from individual sites for our records.