General overview: We will visit five littoral sites on Thunderbird weekly during the bloom season. Some of these will be in conjunction with OWRB visits. We will also visit five sites on each of five lakes during mid-bloom with OWRB.

Proposed sites on Lake Thunderbird include: Fisherman’s Point, Sailboat, Hog Creek, Turkey Pass, South Dam

Packing List

* Secchi disk
* PVC integrated sampler
* Bucket
* Clear plastic pitcher for collecting water from integrated sampler
* 1 L bottles
  + Littoral only: 6 acid-washed 1 L Nalgene bottles (includes 1 extra)
  + Littoral + Pelagic: 12 acid-washed 1 L Nalgene botles
* Hydrolab sonde with PCY probe (NOT the one with turbidity)
* Rope and cord for Hydrolab sonde
* Both surveyors
* 8 backup C batteries for Hydrolab sonde
* White plastic sampling cage for hydrolab sonde
* cooler with ice (x 2 for littoral + pelagic)
* Pencils, sharpie markers for labeling bottles
* data sheets
* 63 µm zooplankton net + rope + spare binder clips (for pelagic, bring a second net)
* Zoop bottles
  + Littoral only: 11 x 250 ml zooplankton bottles with ethanol added for preserving tows, marked with max fill line (includes 1 extra)
  + Littoral + Pelagic: 22 x 250 ml zooplankton bottles with ethanol added
* DI squirt bottle
* Kestrel
* Printed cryo-labels for 2 ml tubes
* 1 L Nalgene filled with DI water (spare water for rinsing zoop nets)- bring extra for pelagic

Before going out

1. Check zoop net for snags in netting, detritus, or cracked latex tubing
2. Calibrate Hydrolab sonde and charge surveyors
3. Acid wash sample bottles
   1. For littoral only: 6 x 1 L Nalgene bottles
   2. For littoral + pelagic: 12 x 1 L Nalgene
4. Collect ice in coolers
   1. For littoral only: One cooler for 6 x 1 L nalgenes + 11 zoop bottles
   2. For littoral + pelagic: Two coolers that each fit 6 x 1 L Nalgenes + 11 zoop bottles
5. Mark 250 ml zooplankton bottles with 125 ml max fill line, measure out 90 ml 95% ethanol and 1.25 ml glycerin into each bottle (10 total). Pre-label bottles and caps with sampling site and date.
   1. For littoral only: 11 bottles
   2. For littoral + pelagic: 22 bottles
6. Pre-label 1L nalgene bottles and caps with sampling site and date
7. Autoclave 40 Pall A/E filters for DNA samples and dry overnight in drying oven.

In the field

1. Measure Secchi depth.
2. Collect 2 liters of integrated sample in photic zone (2x Secchi depth).
3. Rinse bucket, Nalgene bottles, and integrated sampler (tube or hose) with lake water.
4. Fill 1 1-L Nalgene bottle with integrated sample, mark with site and date, store on ice.
5. Take hydrolab profile at 0.5 m intervals, recording temp, DO, pH, conductivity, Chl-a (units & volts), PCY (units & volts).
6. Record max depth with hydrolab.
7. Zooplankton tows
   1. Littoral sites: Using 63 µm net, take two samples (three tows each)- one for morphological identification and one for meta-barcording. For each sample, take three vertical tows the full depth of the water column and rinse down into a 250 ml sample bottle with DI water.
   2. Pelagic sites: for each of two samples, take one vertical tow across the entire water column using 63 µm net and rinse down into a 250 ml sample bottle with DI water. Mark bottle with site, date, and water column depth.
8. Collect meteorological measurements using Kestrel, as indicated on sample sheet (air temp, max and average wind speed over 1-minute period).

After return to lab (must be same day)

1. Filter water and freeze filters for each site:
   1. 1 GF/F for chlorophyll (store in labeled foil packet)
   2. 1 GF/F for phycocyanin (store in labeled foil packet)
   3. 1 GF/F Syringe filter + 40 ml filtrate + 40 ml whole water frozen for toxin analysis - water should be stored in sample-triple-rinsed polypropylene tubes at -20˚C – will be sent to Loftin lab for toxin analysis (LC/MS). VWR 50-ml centrifuge tubes are correct plastic type.
   4. 1 GF/F filter + 40 ml filtrate for toxin analysis - water should be stored in sample-triple-rinsed 50-ml polypropylene tubes at -20˚C – will be kept in PELL lab for toxin analysis via ELISA
   5. Blanks for high-res mass spec: run DI water through GF/F syringe filter + 40 ml filtrate + 40 ml whole water frozen for analysis in the same way as toxins above (we do not need replicates- just one sample for each day of sampling)
   6. 0.2 µm filters for 16s, metagenomics, and qPCR – 4 filters per site. Store in 2-ml screw top centrifuge tubes. Label with pre-printed cryolabels.
2. Preserve phytoplankton
   1. Lugol’s for microscopy
      1. 10 ml whole water in 15 ml opaque tube with 100 µl lugol’s solution added
   2. Glutaraldehyde-pluronic followed by slow freezing in the -80 for flow cytometer:
      1. To a 15 ml opaque centrifuge tube, add:
         1. 50 µl 25% glutaraldehyde (final concentration 0.25%)
         2. 5 µl 10% pluronic F-68 (final concentration 0.01%)
      2. Add 4.95 ml sample (lake water or culture)
      3. Gently mix sample by inverting three times
      4. Slow freeze by putting tubes inside a Styrofoam cooler inside the -80˚C freezer for 90 minutes.
      5. Remove tubes from the Styrofoam cooler and put back into the -80˚C freezer for long term storage.
3. Freeze water for nutrients (2 x 40 ml in a labeled 50 ml tube). This water will also serve as whole water sample for our in-house toxin analysis.
4. Read water on Turner fluorometer and record on fluorometry record sheet.
   1. Turbidity
   2. Phycocyanin
   3. Chl-a (in vivo)
5. Preserve zooplankton samples for meta-barcoding.
   1. For one of the two replicate zooplankton samples from each site, filter the zooplankton sample to remove as much liquid as possible and re-preserve in 95% ethanol. The aim here is to reduce the water content of the sample, as water can lead to degradation of DNA quality. Use 95% ethanol to rinse zooplankton sample into glass scintillation vials and add 0.2 ml glycerol (1% concentration). Record site, date, tow depth and number of tows, and net size on a small piece of card stock in pencil and add to the scintillation vial. Label vial top with site and date.

After return to lab (not same day)

1. Chl-a extraction
2. PCY extraction
3. Phytoplankton flow cytometry on preserved samples
4. Zooplankton enumeration
5. Nutrient analysis on frozen water
6. DNA extraction of Pall A/E filters