3 August 2011

Dear colleagues:

Thanks for your interest in participating in our large-scale collaboration to enhance our existing database aimed at forecasting toxic cyanobacterial blooms in freshwater habitats (lake, reservoirs, ponds, large rivers) throughout the southeastern U.S. This project is funded for three years (2011-2014) through a USGS National Institutes for Water Resources grant (2011AL121G**)**.

Project homepage - http://wilsonlab.com/bloom_network/

Thus far, we have collected water samples from hundreds of sites in Alabama, Florida, Georgia, Tennessee, and Kentucky and found some really interesting patterns between algal abundance and important nutrients, such as phosphorus and nitrogen. As algal blooms continue to threaten aquatic communities, pets, and humans, I think this project will have important implications for the entire Southeast and beyond.

Briefly, we are requesting that our collaborators help us collect epilimnetic water samples for the analyses of cyanobacterial toxins (microcystin, cylindrospermopsin, and saxitoxin), phycocyanin, and off-flavors, as well as phytoplankton enumeration from your routine sampling sites during July or August 2012 to 2014. We will provide the sampling supplies and request that the samples are returned to us each September once sampling is completed. We will forward any data we collect from the samples back to our colleagues and request that other available water quality data collected for each sample site (nutrients, chlorophyll, temperature, etc.) are returned to us. Please find the timeline and standard operating procedures associated with these efforts attached. If you have any questions about these items, please let me know.

Again, thanks for interest in this project. Please let me know if you have any questions, and I look forward to working with you.

Sincerely,

Dr. Alan Wilson
Auburn University, Fisheries and Allied Aquacultures
203 Swingle Hall, Auburn, AL 36849
Phone: 334-246-1120, Email: wilson@auburn.edu, Website: www.wilsonlab.com

TIMELINE

1. **28 February-1 March 2013** (Orlando, FL) and **14-15 March 2013** (Auburn, AL) – water quality workshops. Stay tuned for announcements about registration. Space is limited for each workshop.
2. By **1 April 2013**, using a preformatted excel spreadsheet (see **Protocols** page of project website - http://wilsonlab.com/bloom_network/protocols.html), please send Alan a list of sample sites that will be sampled in July or August (2012) including a return address where sampling supplies (filters, bottles, etc.) can be sent prior to sampling. Given the large number of collaborators, we are only interested in receiving ONE SAMPLE PER WATERBODY DURING THE SUMMER (July-August) – i.e., we do not need samples from multiple sites per waterbody or the same site in July and August. Also, let me know if you need a filter size different from 47mm or do not have a filter tower (I might be able to find one for you to borrow this summer). If you will not be able to analyze samples for chlorophyll, TSS, nutrients, and Secchi, let me know so that I can send additional supplies. This information will be used to determine the quantity of sampling supplies that will be sent to each collaborator.
3. By **1 May 2013,** Alan will ship sampling supplies (i.e., filters, film canisters to store filters, glass vials, parafilm, plastic centrifuge tube, and glass bottles with 1% Lugol's preservative) to all collaborators.
4. During routine lake monitoring in July and August (2012), collaborators will collect additional integrated (surface to sampling depth) epilimnetic seston samples for toxins (2x), phycocyanin (2x), extra filter (2x), off-flavor (1x), phytoplankton (1x), and other toxins (1x) at each site, if possible. One surface whole water sample will also be collected (1x). We are happy to receive fewer samples per site, if the full sampling suite is too time consuming. The standard operating procedures are below.
5. By **1 October 2013**, collaborators will send all samples to Alan Wilson (Auburn University, Fisheries, 203 Swingle Hall, Auburn, AL 36849, phone: 334-246-1120) for analyses.
6. ASAP, associated water quality data for all sample sites will be returned to Alan so that the data can be included in the database and model verification. Please use preformatted excel spreadsheet (see **Protocols** page of project website - http://wilsonlab.com/bloom_network/protocols.html)
7. ASAP, Alan will return all algal toxin, phycocyanin, off-flavor, and phytoplankton data to the collaborators.

STANDARD OPERATION PROCEDURES

1. Samples are requested from routine sampling sites (freshwater lakes, reservoirs, large rivers, ponds) visited in July or August (2012). Only one sample per waterbody per summer is necessary. We are not targeting sites with cyanobacterial blooms but hope to have samples from waterbodies across the productivity gradient. We are only interested in getting samples from freshwater sites at this time. We are not currently requesting samples from streams or brackish waterbodies.
2. At each sample site, collect depth profile data for temperature, conductivity, pH, dissolved oxygen, and light (if available). Measurements taken at every 0.5 to 1.0 meters are fine, but should be relevant for lake depth. Measure Secchi depth (meters). Record GPS coordinates. If you are unable to get GPS coordinates in the field, please use WHAT'S HERE? feature of google maps for GPS coordinates. To get coordinates, find site on http://maps.google.com/ and then right click on sampling location. GPS coordinates will be listed in search box. Cut and paste into excel file.
3. Determine edge of epilimnion from temperature and/or dissolved oxygen data.
4. Using a tube sampler (we use a clear vinyl, weighted tube or a clear PVC pipe for this sampling; pictures here http://wilsonlab.com/bloom_network/gear.html), collect an integrated water sample from the surface to near the edge of the epilimnion. Samples from multiple tube hauls (2 to 4 depending on depth and volume needed) are poured into a clean bucket, mixed well before pouring into a clean, acid-washed and DI-water rinsed, plastic bottle or cubitainer (large collapsible plastic container), which will be stored on ice until being returned to the lab for processing. If a tube sampler is unavailable, a discrete water sample collected **below** the surface (a depth of 1 meter or arms length) is OK. Ideally, we are looking for a representative water sample from the mixed layer where phytoplankton will be most numerous, but not the surface. Sample preparation (described below) can be completed in the field, if desired. Note that low-productivity systems will require larger volume samples (10L or more). Please plan accordingly.
5. **Integrated epilimnetic samples** - We are interested in getting at least four types of samples from each water sample. For each sample, please include the following information on the sample label: **collaborator name, sample type, site, date, depth sampled (meters), volume filtered (milliliters) –** see labels here- http://wilsonlab.com/bloom_network/publications/sample_labels.xlsx
    a. Seston on filter (for toxins and phycocyanin analyses and extra filters, 2 samples per sample type per site = 6 filters per site) - filter mixed water sample onto 47mm, A/E filter, more volume is better (1000 ml to 200 ml, depending on productivity; record volume on label), after filtration, fold filter twice, and store one filter per film canister, store film canisters in freezer (-20 °C). Be sure that all water added to filter tower is filtered. If the filter clogs quickly, use a new filter and add less volume. It is not critical to filter in the dark, but do keep in mind that chlorophyll is light sensitive. So, avoid prolonged exposure to sunlight if possible. If you do not have a filter tower assembly or have a tower which uses filters of different size than 47mm, please let me know so I can order the size filter you need. It does not matter what side of the filter (rough or smooth) you use. Be sure to mix sample well before filtering.
    b. Raw water (for off-flavor analyses, 1 sample per site), fill glass vial (no air bubbles), close cap tightly and parafilm cap to prevent gas release, store in fridge (4 °C). Be sure to mix sample well before filling vial.

c. Preserved water (for phytoplankton enumeration, 1 sample per site) - pour roughly 100ml of mixed water sample into 125ml glass bottle with Lugol's preservative (leave small air bubble), store at room temperature. Be sure to mix sample well before filling bottle.
d. Raw water (for other toxins, 1 sample per site), fill 50ml plastic centrifuge tube to 30ml line and freeze (-20 °C). Be sure to mix sample well before filling tube.

6. **Surface sample** – Given that human health recreational toxin exposure is related to surface conditions, we will collect one surface toxin sample in addition to our epilimnetic samples.
    a. Raw water from SURFACE (for toxins, 1 sample per site), fill 50ml plastic centrifuge tube to 30ml line and freeze (-20 °C).
7. Shipping information
    a. Return all samples at the conclusion of your sampling for the entire sampling season (e.g., September) for analyses.
    b. Group samples by sample type (toxins, phycocyanin, etc.) in large ziplock or trash bags to prevent water from entering sample containers.
    c. Ship all samples and extra, unused supplies and gear at the conclusion of your sampling season.
    d. Plan to have sampled shipped on Monday or Tuesday to prevent lost samples from sitting in a warehouse over a weekend.
    e. Plan to have samples picked up on AFTERNOON to prevent the samples from sitting in a hot warehouse.
    f. Ship cold samples (frozen filters, frozen plastic tubes, and refrigerated glass vials) in one **WELL-INSULATED COOLER** on **REGULAR ICE** using **STANDARD OVERNIGHT DELIVERY** service. Use a lot of ice volume relative to sample volume to prevent warming. Be sure to package items well to prevent box from warming or leaking water. Also, be sure to use a good quality cooler to keep samples cold. Coolers will be returned to sender.
    g. Phytoplankton bottles and extra supplies/gear should be returned in another box using **GROUND** service. Be sure to package glass bottles well with bubble wrap after caps have been sealed to prevent breakage.
    h. Shipping address:
    Alan Wilson
    Fisheries - Auburn University
    203 Swingle Hall
    Auburn, AL 36849
    phone: 334-246-1120
    email: wilson@auburn.edu
    i. Please send tracking information when items are on their way so we can be on lookout for them
    j. Contact Alan (wilson@auburn.edu) for Fedex account info.
    k. You can provide a chain of custody form for the samples, if needed.
8. Once in-house analyses are complete, please send other water quality data to Alan Wilson using associated Excel spreadsheet - http://wilsonlab.com/bloom_network/protocols.html. Minimum data needs include; collaborator name, state, site type, site name, site ID (if applicable), GPS coordinates, sample date, sample depth (meters), surface temperature (°C), surface conductivity (µS/cm), surface pH, surface dissolved oxygen (mg/L), Secchi depth (meters), chlorophyll a (µg/L), total phosphorus (µg/L), total nitrogen (µg/L), and

total suspended solids (mg/L). If you are unable to provide these data, please let Alan know. Other data (SRP, NH_3, NO_3, NO_2) are welcome, if available. Please do not alter primary columns in spreadsheet to help data integration.

9. Please document any changes to the SOP, where appropriate, and any issues during sampling or sample processing.

IF ANY OF THESE NOTES ARE UNCLEAR, PLEASE LET ME KNOW.