CHOWAN EDENTON ENVIRONMENTAL GROUP CITIZEN SCIENTIST WATER AND AIR QUALITY MONITORING MANUAL

CHOWAN-ALBEMARLE RESEARCH SUMMER 2020

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TABLE OF CONTENTS

I. INTRODUCTION

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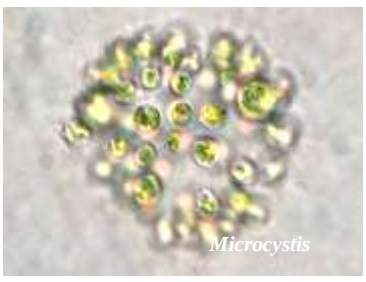
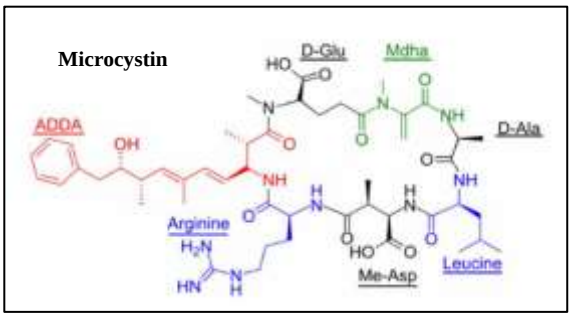
Your role in this research. Thank you for volunteering to help monitor water and air quality in your area. We couldn't perform such a thorough study without your time, generosity, and commitment to protecting our water resources. Citizen scientist efforts are incredibly important, and help foster a sense of environmental stewardship in the greater community. The data you help us collect is incredibly valuable and necessary. As a citizen scientist volunteer, you will be filtering water samples and ensuring the security of our aerosol sampling equipment at your respective location along the Chowan River-Albemarle Sound (CR-AS). The suite of air and water quality parameters we are analyzing your samples for are explained later in this manual.

What is a harmful cyanobacterial bloom (or CyanoHAB)? Cyanobacteria are ancient, photosynthetic algae that can thrive in freshwater, estuarine, and marine ecosystems on every continent (even Antarctica!). During the summer, when waters are warm and there is plenty of sunlight, cyanobacteria can bloom into dense, surface scums (Paerl & Otten, 2013a, 2013b). Nutrient-loading from fertilizers and/or untreated sewage in runoff can promote the growth of cyanobacterial blooms (Bullerjahn et al., 2016; Paerl et al., 2011). Harmful cyanobacterial blooms (CyanoHABs) can produce toxic metabolites called cyanotoxins—these toxins are hypothesized to be a result of oxidative stress on the cell. There are several

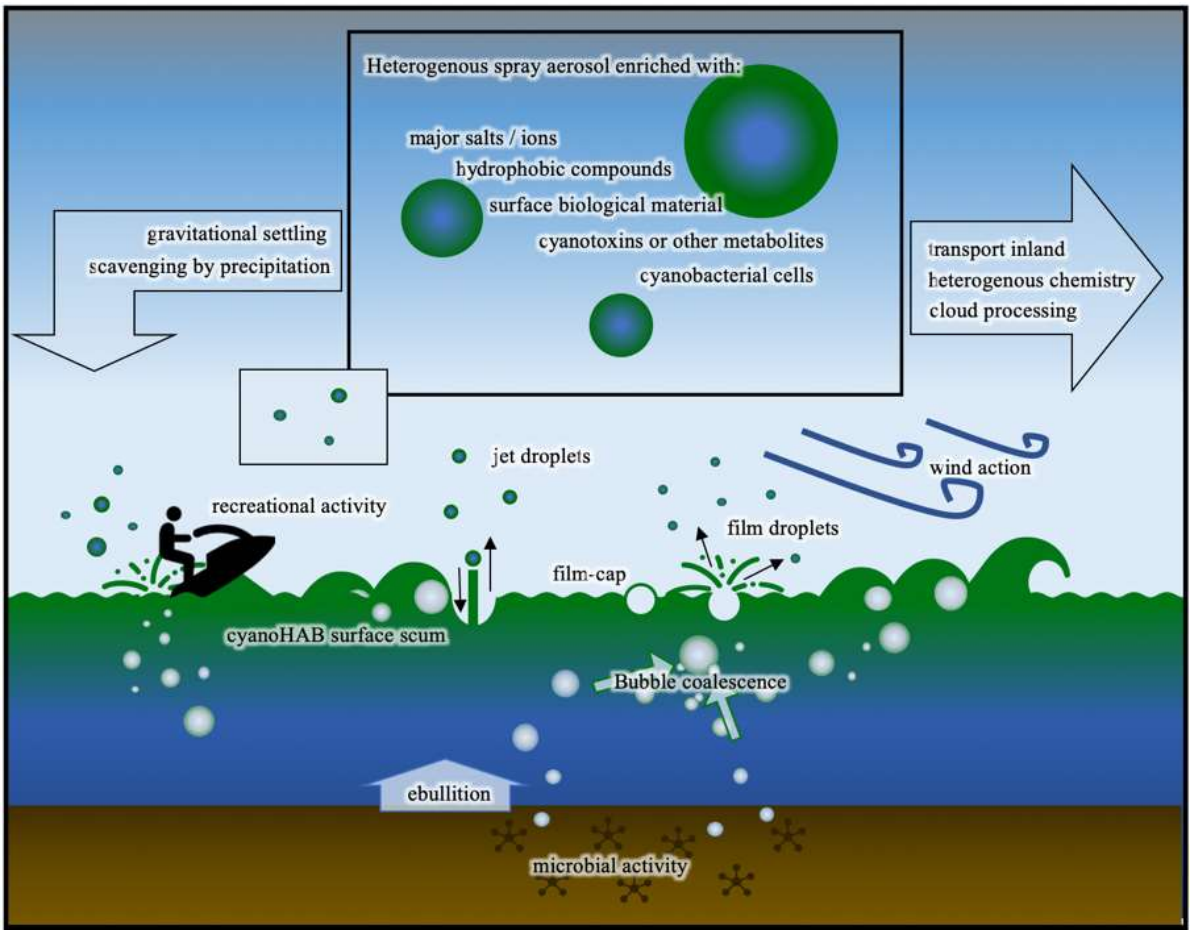


strains of CyanoHABs, but our focus is on *Microcystis,* which has recently been proliferating in the CR-AS. The toxin of concern in the CR-AS is microcystin, a liver toxin frequently

produced by *Microcystis*. Human exposure to microcystin is regulated by the World Health Organization and US Environmental Protection Agency because of its negative impact on human and animal (e.g. pet, livestock, and wildlife) health. The primary route of microcystin exposure is through ingestion, but inhalation is another potential public health concern which is less understood.



What is spray aerosol? Algal cells and their metabolites can be incorporated into aerosol at the water-air interface (May et al., 2018; Murby & Haney, 2016; Yung et al., 2007) . As wind stress or recreational activity generates waves, air bubbles are entrapped beneath the surface. As these bubbles rise, chemical and biological matter in the water column can be adsorbed to the bubbles' surfaces (O'Dowd et al., 2004). When the bubbles reach the surface and pop, a spray is ejected into the air, forming aerosols that may carry cyanobacteria and cyanotoxins. This aerosol can travel for several miles depending on its size, shape, and chemical properties.



The goals of this study are to (1) characterize spray aerosol formed during cyanoHAB conditions to inform potential human exposure to airborne microcystin, and (2) better understand the environmental factors promoting CyanoHAB growth and toxin production. To achieve this, we will collect and quantify (1) microcystin and cyanobacterial DNA in both aerosols and in the water column, and (2) measure environmental parameters in the air and water (e.g. nutrients, chlorophyll a, phytoplankton pigmentation, ambient weather conditions) to understand controls on CyanoHAB growth dynamics, microcystin production, and potential microcystin aerosol formation.

II. WATER AND AIR QUALITY PARAMETERS

Chlorophyll *a* (Chl *a*) is a photosynthetic pigment that is essential for photosynthesis in cyanobacteria (as well as many other algae and plants). It absorbs sunlight and converts it into energy to be used by the plant cell. In water quality monitoring, chlorophyll *a* is easy to measure and is a useful proxy for algal biomass in a water sample. During cyanobacterial bloom conditions, chlorophyll *a* readings, in conjunction with other measurements, can indicate the relative cell concentration of phytoplankton in the sample.

High Performance Liquid Chromatography (HPLC) is a technique with many applications across many fields of research. HPLC separates a mixture of compounds to identify, quantify, and purify the individual components of a mixture, in our case, phytoplankton pigments (Jeffrey et al. 1997). This provides a sense of the types of phytoplankton (algae, bacteria) present in the sample based on the relative abundance of each pigment; for instance, phycocyanin is the pigment responsible for the "blue-green" coloration and nickname of cyanobacteria.

Phytoplankton Morphology is the size, shape, and visible physical characteristics of phytoplankton in a sample. We collect a small sample of water (20 mL), and treat it with Lugol's Iodine solution. This preserves the cells and stains them so that we may look at them under a microscope. The characteristics of the algae we see with microscopy can be paired with our findings from HPLC to reveal more traits about the phytoplankton.

Flow Cytometry (FCM) is a specialized type of cell counting using fluidics without filters or a microscope. It provides a method to both quantify and identify phytoplankton in a heterogenous sample, based upon the specific light scattering and fluorescent characteristics of each cell. It is a useful scientific instrument, as it provides fast, objective and quantitative recording of fluorescent signals from individual cells as well as physical separation of cells of particular interest. Similar to HPLC, it can reveal the relative abundance of phytoplankton pigmentation in a sample but it can do so for individual cells, providing more fine-tuned measurements. It can also count cells, giving us a cell concentration per volume. Sometimes, if a sample is too densely populated, we cannot accurately use FCM, hence the need for multiple methods.

Cyanobacterial DNA will reveal the species of cyanobacteria present in the sample. By extracting DNA from a sample, we can amplify the gene sequences we choose with a PCR (Polymerase Chain Reaction) kit. For this study, we will be amplifying the 16S rRNA sequence from cyanobacteria, which is a segment of the genetic code that is highly conserved across all bacteria. We will also amplify *mcyA*, a gene sequence that is linked to microcystin synthesis when it is actively transcribed. We will be using these samples to look at cyanobacterial species, but also examine the relative abundance of toxin genes in comparison to measured toxin concentrations. This will help us understand which cyanobacterial species in the Chowan are producing toxins.

Nutrients are the major essential inorganic compounds that cyanobacteria and other phytoplankton use for energy alongside sunlight. Nitrogen (N) and phosphorus (P) are the main two elements needed from nutrients to promote plant growth. Different cyanobacteria can uptake and regulate several variable forms of N and P, some can even use forms from atmospheric gases, but others require more accessible forms, like the type we utilize in fertilizers. Other nutrients required for favorable algal growth are iron, sulfur, potassium, magnesium and micronutrients or cofactors (vitamins). We will analyze dissolved nutrients (in the water) for ammonium (NH_4), nitrate (NO_3), phosphate (PO_4), silicate (Si), and total dissolved nitrogen (TDN). We will analyze the particular nutrients (within biological matter or adsorbed to sediments) from the GF/F, establishing the Carbon to Hydrogen to Nitrogen ratio (CHN). The particulate organic matter (POM) you can see on the filter is made up of living (plankton) and detrital material (fragments of waste, dead organisms). Analyzing the POM and dissolved nutrients suspended in water can reveal the trophic state of aquatic ecosystems.

Microcystin is a cyanotoxin produced by several strains of cyanobacteria; it is amongst the most widespread and frequently detected cyanotoxins (Dawson, 1998). One particularly hardy and widespread genus of microcystin producers is *Microcystis,* which has been detected in the CR-AS in recent summers. Both acute and chronic exposure to microcystin are linked to several adverse human health outcomes. As of 2015, the EPA criterion for microcystin in drinking water is < 0.3 µg/L, and only recently (2019) were guidelines implemented for recreational use of water containing microcystin: <8 µg/L for swimming (US EPA). Last summer, microcystin in the Chowan River was measured in one sample at an alarming 650 µg/L in August and several other samples > 300 µg/L. We are interested in measuring the intracellular (toxin within a cyanobacterial cell) and extracellular (toxin that has been released by the cell through stress or cell death), because this could influence its transport in the environment and aerosolization capability. Furthermore, estuarine gradients are one of the few phenomena demonstrated that cause cyanobacteria to prematurely lyse cyanotoxins into the water column, and thus there may be significant concentrations of both extra/intracellular toxin in our samples. We will analyze the toxin with LC-MS/MS (Tandem Liquid Chromatography-Mass Spectrometry) because it is a high resolution technique that allows us to determine the specific microcystin chemical structures.

Spray Aerosol An aerosol, also referred to as particulate matter or particle, is a solid or liquid suspended in a gas. One of the largest sources of algal and bacterial particles in the atmosphere is spray aerosol (SA), formed at the surface of the ocean, lakes, and rivers, etc. (Aller et al., 2005; De Leeuw et al., 2011). We are interested in quantifying the cyanobacterial DNA and toxins in spray aerosol. The aerosol equipment we are using for this project is on loan from the EPA—so only UNC-CH students and faculty will be operating the equipment. These aerosol samplers operate over long periods of time, so we may ask for your help to make sure the samplers are still operating smoothly and are not bothered during the course of the sampling campaign. If the noise becomes an issue, please let us know and we can adjust our sampling plan accordingly.

Environmental Parameters and ambient weather conditions, including air temperature, water temperature, wind speed, wind direction, wave action, relative humidity, light, and salinity, can influence aerosolization and bloom growth dynamics. We will be recording these factors in order to understand how they may influence ultimate human exposure to CyanoHABs. To measure and report these factors at time of sampling, we will be using YSI Sondes, a meteorological station to collect data on water quality and weather conditions.

III. SAFETY & GENERAL INFORMATION

Do not sacrifice your safety during this process. Some chemicals provided are hazardous and should be treated with care. Some objects in the kits may be sharp and could cut the skin if broken or misused. Some water may be sampled around slippery or unstable docks and shorelines. Some water may contain toxigenic algae or other pollutants to be mindful of touching. Some vials contain hazardous chemicals that should not be touched or ingested, and you must make sure they do not spill in the freezer. No data is worth an accident, or in light of the current global COVID-19 pandemic, exposure to illness. Please take your time, wear PPE, practice caution, and use your best judgement (and common sense ☺).

Many CEEG members have been a part of successful research campaigns for several years, and thus, you already know it is very important that you gather your data precisely, neatly, and accurately! If any small errors occur (which they always do at some point or another), please report this in some notes so we can correct for them upon analysis. I want to reiterate how thankful I am that you are taking the time to volunteer with this study and that I trust your judgement fully. The data you collect is indispensable and makes a large difference in expanding our field campaign.

Safety Guidelines:

1. Volunteers should read the entire manual including specific protocols before beginning sampling. Do not feel overwhelmed, this manual is just available for your reference as needed. Contact Haley Plaas at (913)-634-9176 or hplaas@live.unc.edu if any methods are unclear. Text, email, or call are all welcome.

2. Sample with a partner if your COVID-19 permits, but wear a mask to prevent the spread of any illness. Sampling with a buddy is a safety precaution and reduces errors, allowing everyone to double check each other's work.
3. Keep all equipment and reagent chemicals (specifically Lugol's and the FCM vials with pre-added glutaraldehyde fixative) out of the way of small children or pets. These chemicals are poisonous!
4. In the unlikely event of a chemical accident, contact Haley. It is likely not a big deal, but remain calm and call the American Association of Poison Control Centers at: (800) 222-1222 if necessary. In the event of an emergency, call 911.
5. We recommend that you wear gloves when you conduct all tests. This prevents the samples from exposure to you and also prevents your exposure to chemicals and potentially polluted water containing cyanobacterial toxins. At a minimum, always wash skin with full lather when skin contacts polluted water or chemicals.
6. If sampling from a property that is not yours or is public, always obtain written permission from the landowner or municipal contact. Please be courteous and respectful of individual property rights.

General Sampling Guidelines:

1. Protect equipment from prolonged exposure to direct sunlight and extreme temperatures. Store it in the blue boxes provided between sampling times.
2. When collecting a sample, please take your time and follow the steps of each respective protocol. Get familiar with the instructions and procedures before going out in the field. Reference this manual when necessary.
3. Always, ALWAYS homogenize your water sample before pouring. When transferring from the carboy to a graduated cylinder, ensure to mix the carboy thoroughly by gently inverting so that the water is uniform before measuring out the necessary volume for a sample. This is especially important during bloom conditions, when some algae may float or sink in the carboy.
4. Ensure to leave a visible sign/note on the aerosol equipment indicating what the equipment is there for, your contact information, Haley's contact information, and when you will be returning. We do not want to alarm any passersby or attract unwanted attention in case of vandalism.
5. Be careful not to contaminate any samples, especially those for nutrients and DNA processing. Use clean equipment, gloves, and be mindful of other potential contaminants.
6. Carefully seal samples in their respective storage apparatuses, and secure them in Ziploc bags prior to freezing them. Make sure key information like the site, sample number, date, and time collected are recorded on the bags.

7. You may be exposed to cyanobacterial toxins during sampling, and by volunteering, you are agreeing to take full responsibility for your potential exposure to any pollutants that may exist in the sampling region. However, no data or guidelines currently exist to suggest that the air you are breathing will be immediately harmful during bloom events (despite our research goals ☺).

IV. INSTRUCTIONS FOR MONITORING

To reduce any miscommunication on my part, I provided fine-tuned details as if you have never collected a water quality sample before. I apologize in advance for the oversimplification and repetition. The methods provided are what I would follow, but you may find another rhythm that works better for you. All filtration protocols are optimally performed under subdued lighting in an indoor setting to avoid changes in the sample from exposure to sunlight or additional nutrients, but they can also be performed in the field on site if necessary.

Sampling Sites

A. Chowan Beach

- 36°13'33.3"N 76°42'22.7"W
- Contact:

B. Pembroke Creek

- 36°03'31.1"N 76°37'32.4"W

Sample Labeling and Storage Information

Materials:

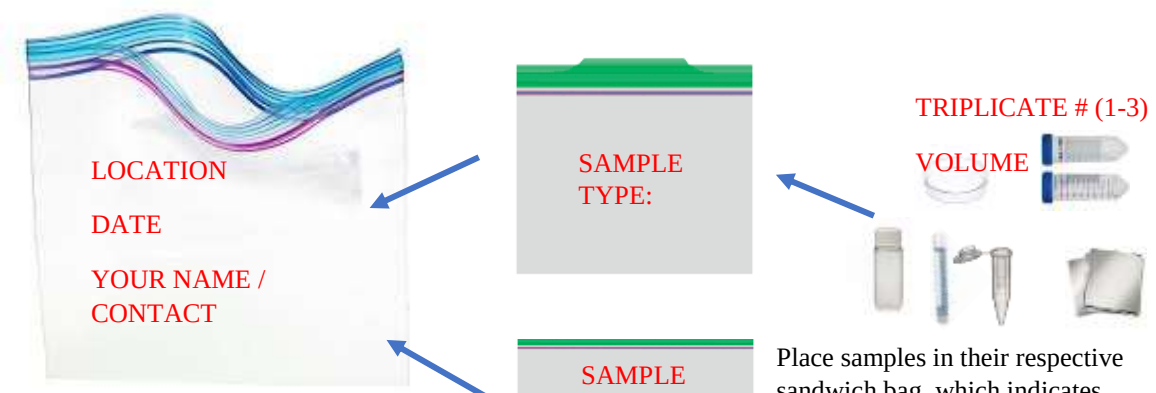
- Waterproof Sharpie
- Ziploc Bags
- Aluminum Foil Squares
- Microcystin and Nutrient Tubes
- Nutrient (CHN) petri dishes
- DNA centrifuge tubes
- Phytoplankton vials
- FCM vials

Sample Labeling Scheme: Familiarize yourself with the labeling scheme samples *ahead of time*. This is so that you can be organized during filtration and are familiar with the samples to be collected beforehand. If any information changes at the time of filtration it is OK to alter the label or add additional information. I will ask you to fix any dates that I have incorrectly pre-labeled. The more information, the better, so that upon lab analysis, we get the best sense of when, where, and how the sample was collected. Always use a wide-tipped waterproof sharpie on the foils and if labeling after the fact, be careful not to press greatly on the filter with the sharpie. This could lead to rips in the foil or filter or push the biomass off the filter.

Specific Labeling Information: Note for every type of sample, each apparatus should be labeled with the volume filtered and **a running number scheme to indicate triplicates (i.e. 1-3, 4-6).** Time ON samples vs. time OFF samples can be stored together for the same sampling date, but make sure the time ON samples are the first 3 numbers in the scheme.

To clarify, the first sampling date in June will be the first six samples (1-3 will be the TIME ON water sample, and 4-6 will be the TIME OFF water sample). The next sampling date, two weeks later, will be the next six samples (7-9 for TIME ON, 10-12 for TIME OFF). Make sure the date and time on and time off recorded are reported somewhere on the Ziploc bags.

Try to place the samples back in the bags they came in to keep them organized so I do not confuse samples stored in aluminum foils or falcon tubes since there are multiple types that are stored in those apparatuses. **Volumes are subject to change** based on the amount of particulate matter and biomass in the water sample. If there is too much clogging up the filter so that water is not easily passing, start over, and use your best judgement to reduce the volume filtered. Just make sure you record the final volume on the storage material. When folding the aluminum squares, ensure not to touch the inside of the packet with bare hands. **THE VOLUME, LOCATION, TIME COLLECTED, AND DATE ARE THE MOST CRUCIAL ASPECTS TO RECORD AND MUST BE INHERENT FROM THE LABELS.**



Place small baggies in gallon bag indicating the sampling site and date. This will go in the freezer.

Place samples in their respective sandwich bag, which indicates sample type (HPLC, FCM, etc.) This is especially important for the foils and nutrient tubes.

Reference the "Cheat Sheet" Chart for this information during filtration:

1. **Chlorophyll *a:*** (abbreviate CHLA) Stored in an aluminum foil square. Filtered on 25 mm glass fiber filters (GF/F) on filtration tower. Volume = 50 mL.
2. **HPLC:** (abbreviate HPLC) Stored in an aluminum foil square. Filtered on 25 mm glass fiber filters (GF/F) on filtration tower. Volume = 100 mL x2.
3. **Phytoplankton Morphology:** (no abbreviation necessary – vial is indicative of sample type). Stored in 25 mL vials with 4 mL Lugol's solution to fix phytoplankton. Volume is flexible. This is the only sample that will NOT be stored in the freezer, and should be stored in a dark box at room temperature.
4. **FCM:** (no abbreviation necessary – vial is indicative of sample type). Stored in 5 mL vials with 50 uL fixate, EM grade glutaraldehyde (pre-added).
5. **DNA:** (no abbreviation necessary – tube is indicative of sample type). Filtered on SUPOR filters. Volume = 50 mL.
6. **Nutrients:** (abbreviate NUTS) Filter is stored in in a petri dish, filtrate is stored in a Falcon tube. Filtered on glass fiber filters (GF/F) on the Erlenmeyer flask so that filtrate may be saved. Volume = 50 mL.
7. **Microcystin (MC):** (abbreviate MC) Filter is stored in an aluminum foil, filtrate is stored in two 50 mL Falcon tubes. Filtered on glass fiber filters (GF/F) on the Erlenmeyer flask so that filtrate may be saved. Volume = 100 mL.

Collecting Water Samples

Materials:

- Diaphragm Pump
- Funnel
- Carboy

Collection Protocol:

1. At the water, open the carboy and place funnel at the mouth.
2. Plug the pump in and switch it on. Place the pump in the water (~2 feet beneath the surface) and place the tubing into the carboy.
3. Rinse the carboy with the sample three times. (Place the lid on, swish it around, pour it out, do it again)
4. Fill the carboy with the water sample.
5. Turn off the pump. Secure the lid.

Chlorophyll *a* and HPLC Filtration

Materials:

- Vacuum filtration manifold with filtration tower(s) and 25mm bases.
- Vacuum pump (electric)
- Carboy for waste
- Graduated cylinder (50 or 100 mL)
- Forceps
- Whatman GF/F, 25mm
- Aluminum foil packets (see sample labeling information)

Filtration Protocol:

1. Set up your filter apparatus (vacuum pump, towers, waste apparatus, etc). ** see diagram in appendix for correct assembly
2. Wearing gloves, use the forceps to place a GF/F onto the tower base. Secure the tower in place. Ensure that the filter is centered and intact (no visible rips). If you are having issues, turning on the pump helps suction the filter to the base, and you can double check with this to make sure they are secure.

3. Invert the sample bottle gently several times. This mixes the sample so it is uniform.
4. Then pour and measure 50 mL (Chl *a*) or 100 mL (HPLC) in graduated cylinder (with bottom of meniscus touching 50 mL line).

5. Before pouring sample into the tower, be sure the tower and filter are secured, otherwise the sample water will leak. If it does leak, no worries, but you will have start over and get a new filter.

6. Pour the water into the tower. Measure 50 more mL and repeat for the triplicates. You can fill every funnel before turning on the pump.
7. Turn on vacuum source and open valves on filter tower manifold. Vacuum pressure should be around approximately 5 psi. Try not to exceed 8 psi since high pressure may break the pigments.
8. You may have to adjust sample volumes based on conditions or losses of sample (spills / accidents). If you spill water out of the base of the funnel, start over with the same volume. If the water will not go down after ~30 seconds, make sure the waste lid is tight and that water is actually filling. If it is not going through the funnel based on the amount of algae clogging up the filter, reduce volumes and try again. Try cutting the volume in half at first but then by 10 (50→25→15mL) Just make sure to record this on the foil!
9. Close valves as the water finishes filtering and turn off the pump as soon as samples have filtered.

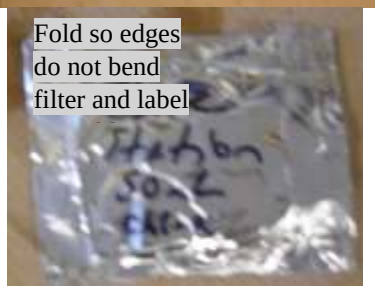
10. Using the forceps, grab a filter and place it on a stack of paper towels (face up, with particulate matter NOT touching the paper towel).

11. Using the forceps, carefully fold filters in half on the paper towel. Try not to touch the sample with the forceps, use the white, untouched edges to do so. The particulate matter should be folded *inside* the filter (like a taco or empanada!) and make the best half circle possible when you fold over. An uneven fold will allow samples to leach out. The folded

edges should be even to prevent filter matter from rubbing off onto the towel or foil. **note: You will get the hang of using the wetness from the filter with the paper towel to fold. I usually drag the filter along the paper towel with my forceps so that it sticks and then I can easily fold the top over to make my filter "taco". You can also use two pairs of forceps if this works best for you.

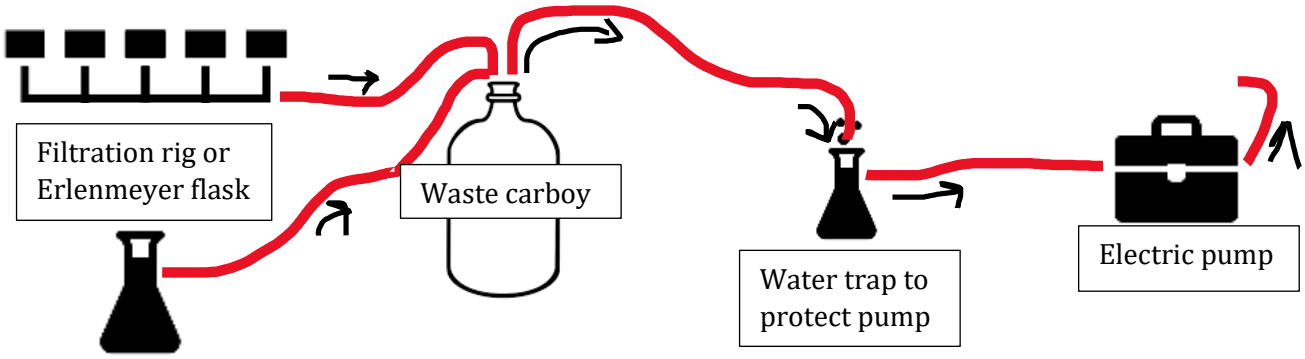
12. Place folded filter between paper towels and gently blot dry by gently pressing it with a PVC roller, like rolling out dough gently. This squeezes the excess water onto the paper towel.

13. Place the folded filter in its designated labeled foil. You can place both HPLC filters in the same packet. Fold each open edge of the foil to make a closed packet but try not to fold the filter itself. Again, if sample volume was different than intended (e.g. due to heavy biomass/ loss of sample) report this on the packet.



14. Place the foil in its respective Ziploc bag. Once the bag is filled with the triplicate samples, place the sealed bag in a freezer ASAP or on ice.

**Filtration Diagram



Phytoplankton Morphology Sample Preparation

Materials:

- Three 25 mL glass vials
- Lugol's Solution
- Pipet / Eye dropper

Phytoplankton Sample Fixing Protocol:

1. Invert sample bottle gently several times. This mixes the sample so it is uniform.
2. Carefully pour the sample into the vial. Leave a bit of headspace.
3. Using the eyedropper and wearing gloves, put 4 mL of Lugol's solution into the vial.
4. Seal the vial.
5. Store sample in designated Tupperware at room temperature.

FCM Sample Preparation

Materials:

- Three 5 mL FCM vials + glutaraldehyde fixative (pre-added)
- Tupperware storage

FCM Sample Fixing Protocol:

1. Add 5mL of sample to vial with eye-dropper. Invert tube several times.
2. Adjust the label your tube if the date / volume / anything significantly deviates from the original label.
3. Leave vials at room temp, in the dark for at least 10 min.
4. Put vials in the freezer within their Tupperware.

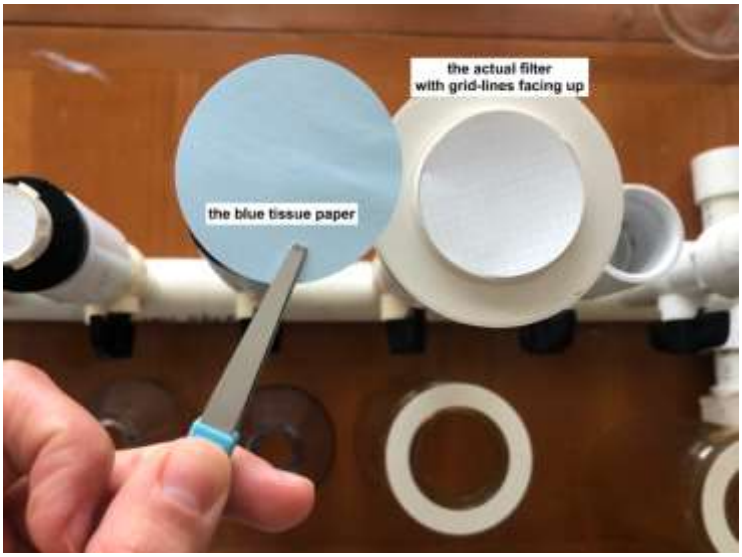
Cyanobacterial & Microbial DNA Filtration

This procedure mirrors much of the chl a / HPLC filtration procedure but **crucially requires the use of different filters, 47 mm PALL SUPOR filters with 47 mm filter towers.*

Filtration Protocol:

1. Set up your filter apparatus (vacuum pump, towers, waste apparatus, etc).

2. Use forceps to place a SUPOR filter onto the tower base. The blue tissue paper is NOT a part of the filter. Remove this from either side of the white filter. Some SUPOR filters have a grid on them. If this is the case, place the grid facing up.



3. Secure the tower in place. Ensure that the filter is centered and intact (no visible rips). If you are having issues, turning on the pump helps suction the filter to the base, and you can double check with this to make sure they are secure.
4. Invert the sample bottle gently several times. This mixes the sample so it is uniform.
5. Then pour and measure 25 mL in graduated cylinder (with bottom of meniscus touching 25 mL line).

6. Before pouring sample into the tower, be sure the tower and filter are secured, otherwise the sample water will leak. If it does leak, no worries, but you will have start over and get a new filter.
7. Pour the water into the tower. Measure 25 more mL and repeat for the triplicates. You can fill every funnel before turning on the pump.
8. Turn on vacuum source and open valves on filter tower manifold. Vacuum pressure should be around approximately 5 psi. Try not to exceed 8 psi since high pressure may break the pigments.
9. You may have to adjust sample volumes based on conditions or losses of sample (spills / accidents). If you spill water out of the base of the funnel, start over with the same volume. If the water will not go down after ~30 seconds, make sure the waste lid is tight

and that water is actually filling. If it is not going through the funnel based on the amount of algae clogging up the filter, reduce volumes and try again. Try cutting the volume by 10 (25→15mL) Just make sure to record this on the foil!

10. Close valves as the water finishes filtering and turn off the pump as soon as samples have filtered.
11. Before pouring sample into the tower, be sure the tower and filter are secured, otherwise the sample water will leak. If it does leak, no worries, secure the tower then restart with another measured volume.
12. Turn on vacuum source and open valves on filter tower manifold. Vacuum pressure should be around approximately 5 psi. Try not to exceed 8 psi since high pressure may break cells.
13. Once all the water has gone through the funnel, switch off the vacuum, and close the tower valves. Remove the funnels.
14. Using two pairs of clean forceps, carefully fold the filters in half on the filter base. Try not to touch the central material with forceps; instead use the white, untouched edges to fold them. The particulate matter should be folded *inside* the filter (like a taco or empanada!) and make the best half circle possible when you fold over. **Use two pairs of forceps to achieve multiple folds so that the filter will fit in the DNA tube.**





15. Securely place folded filter (probably 2-4 folds) into a prelabeled screw cap or snap cap plastic tube. Make sure the cap is fitted properly.
16. Place tubes into the freezer as soon as possible, in their designated baggie.

Nutrients and Microcystin Filtration

This procedure mirrors much of the chl a / HPLC filtration procedure but **crucially requires the use of the Erlenmeyer flask instead of filtration rig so that you can save the filtrate.*

Materials:

- Single 250 mL Erlenmeyer flask with filtration tower(s) and base(s) (small-25 mm)
- Vacuum pump (handheld or electric)
- Graduated cylinder (50 or 100 mL)
- Forceps

- Whatman GF/F glass fiber filters, 25mm
- Nutrient tubes and petri dishes (see sample labeling information)

Filtration Protocol:

1. Set up your filter apparatus (vacuum pump, tower, flask, waste apparatus, etc.)**. Make sure the Erlenmeyer flask is clean. You will be collecting the filtrate that ends up in the flask.

2. Wearing gloves, use the forceps to place a GF/F filter onto the tower base. Secure the tower in place. Ensure that the filter is centered and intact (no visible rips). If you are having issues, turning on the pump helps suction the filter to the base, and you can double check with this to make sure they are secure.

3. Invert the sample carboy gently several times. This mixes the sample so it is uniform.

4. Then pour and measure 50 mL in the graduated cylinder (for nutrients, but 100 mL for microcystin) (with bottom of meniscus touching the 50 or 100 mL line). You may have to adjust sample volumes based on conditions or losses of sample (spills / accidents). Reduce volumes if filters are bright green and you are unable to filter 50 mL. Try cutting the volume in half at first but then by 10 (50→25→15mL) Just make sure to record the final volume!

5. Before pouring sample into the tower, be sure the tower and filter are secured, otherwise the sample water will leak. If it does leak, no worries, but you will have start over and get a new filter.

6. Turn on vacuum source. Vacuum pressure should be around approximately 5 psi. Try not to exceed 8 psi since high pressure may break the pigments.

7. Turn off the vacuum as soon as the sample has filtered. Unscrew the funnel.

8. Using the forceps, grab a filter and place it directly onto the bottom side of the petri dish for nutrients, the aluminum foils for microcystin (face up, with particulate matter NOT touching either apparatus).

9. After collecting the triplicate filters for nutrients and microcystin, take off the filter tower, and carefully pour the filtrate into the designated Falcon tubes. Leave a bit of headspace for expansion during freezing: each tube should hold ~40-45 mL of filtrate. It does not have to be exact.

10. Seal the Falcon tubes, petri dishes, and foil samples in their respective bags.

11. Once the bag is filled with the triplicate samples, place the sealed bag in a freezer ASAP or on ice.

Cleaning and storing your equipment

Between TIME ON and TIME OFF carboys, you must DI rinse and sample rinse the equipment. This is not a full cleaning, rather, rinse the towers, graduated cylinders, forceps, and Erlenmeyer flask with DI water 3 times, then 3 times with the next water sample itself. This will ensure no cross contamination between TIME ON and TIME OFF samples.

At the very end of your filtration:

Materials:

- Bottle with acid solution
- Bottle with DI water
- Spray bottle for acid solution
- Spray bottle for DI water
- Paper towels

Cleaning Protocol:

1. Set up a make-shift paper towel drying rack next to a sink.
2. Fill the spray bottles with their respective solutions.
3. Rinse the inside of the equipment in question (i.e. a flask or graduated cylinder) with the acid. Pour down the sink with the water running to dilute. Do this 3 times.
4. Rinse the inside of the equipment with the DI water 3 times in the same fashion.
5. Set on the drying rack to dry.
6. Rinse the filtration rig with hot water (can be from the sink)
7. Dispose of all liquid in the waste carboy.
8. Disassemble the equipment necessary, and once completely dry, store in a safe, room temperature, dry area away from direct sun.

V. SUMMARY

Final Remarks: Thank you for your invaluable volunteer time to make this research a success. Please reach out to Haley Plaas at any time over the course of this study with questions, concerns, or recommendations. Some details that were not mentioned previously:

1. You can be reimbursed for gas money for any travel associated with your water sampling trips at your request.

2. Your contribution to this work will be noted in the acknowledgements section of all resultant publications and professional presentations.

3. In addition to the research this summer, an undergraduate science communication intern will be working closely with me to shoot a short documentary about the impact of CyanoHABs on the environment and livelihood in eastern North Carolina. All outreach products (videos, etc.) will be available for the CEEG to utilize through media platforms.

4. I am happy to attend future CEEG meetings to discuss our ultimate findings, the implications of this research, and further collaboration!

Thanks again, and I look forward to working with you and learning much about the Chowan from you this summer!

VI. FILTERING CHEAT SHEET

SAMPLE	FILTER TYPE	FILTER APPARATUS	VOLUME	BLOT / FOLD ON PAPER TOWEL?	STORAGE
Chlorophyll a	25 mm GF/F	Filter rig	50 mL	YES	Aluminum foil packet -> freezer
HPLC	25 mm GF/F	Filter rig	100 mL x 2	YES	1 Aluminum foil packet -> freezer
Particulate (intracellular) MC	25 mm GF/F	Erlenmeyer flask	100 mL	YES	Aluminum foil packet -> freezer
CHN	25 mm GF/F	Erlenmeyer flask	50 mL	NO	Petri dish -> freezer
NUTS	Filtrate	Erlenmeyer flask	~ 45 mL*	n/a	50 mL Falcon tube -> freezer
Dissolved (extracellular) MC	Filtrate	Erlenmeyer flask	~ 90 mL* (45 mL *in two tubes)	n/a	50 mL Falcon tubes -> freezer
DNA	47 mm SUPOR (grid up, no blue)	Filter rig	25 mL	NO	DNA tube -> freezer
Phytoplankton	n/a	n/a	~25 mL* + 4mL Lugol's ~~Lugol's~~	n/a	Vial -> tupperware -> room temperature
FCM	n/a	n/a	5 mL, add water with eye droppers	n/a	5mL vials -> ~~tupperware~~ -> freezer

These can be done in a row. Pour filtrate at end.

* volume does not need to be exact. Otherwise assume volume must be accurate and recorded.

VII. REFERENCES

Aller, J. Y., Kuznetsova, M. R., Jahns, C. J., & Kemp, P. F. (2005). The sea surface microlayer as a source of viral and bacterial enrichment in marine aerosols. *Journal of Aerosol Science*, *36*(5–6), 801–812. https://doi.org/10.1016/j.jaerosci.2004.10.012

Bullerjahn, G. S., McKay, R. M., Davis, T. W., Baker, D. B., Boyer, G. L., D'Anglada, L. V., Doucette, G. J., Ho, J. C., Irwin, E. G., Kling, C. L., Kudela, R. M., Kurmayer, R., Michalak, A. M., Ortiz, J. D., Otten, T. G., Paerl, H. W., Qin, B., Sohngen, B. L., Stumpf, R. P., … Wilhelm, S. W. (2016). Global solutions to regional problems: Collecting global expertise to address the problem of harmful cyanobacterial blooms. A Lake Erie case study. In *Harmful Algae* (Vol. 54, pp. 223–238). Elsevier B.V. https://doi.org/10.1016/j.hal.2016.01.003

Dawson, R. M. (1998). The toxicology of microcystins. In *Toxicon* (Vol. 36, Issue 7, pp. 953–962). https://doi.org/10.1016/S0041-0101(97)00102-5

De Leeuw, G., Andreas, E. L., Anguelova, M. D., Fairall, C. W., Lewis, E. R., O'Dowd, C., Schulz, M., & Schwartz, S. E. (2011). Production flux of sea spray aerosol. *Reviews of Geophysics*, *49*(2). https://doi.org/10.1029/2010RG000349

Galbreath, G.D., Wright-Creech, S., Moore, B. L., & Clark, A. H. (1993, updated in 2003). *ALBEMARLE-PAMLICO CITIZENS WATER QUALITY MONITORING MANUAL.*

Jayarathne, T., Sultana, C. M., Lee, C., Malfatti, F., Cox, J. L., Pendergraft, M. A., Moore, K. A., Azam, F., Tivanski, A. V., Cappa, C. D., Bertram, T. H., Grassian, V. H., Prather, K. A., & Stone, E. A. (2016). Enrichment of Saccharides and Divalent Cations in Sea Spray Aerosol during Two Phytoplankton Blooms. *Environmental Science and Technology*, *50*(21), 11511–11520. https://doi.org/10.1021/acs.est.6b02988

May, N. W., Olson, N. E., Panas, M., Axson, J. L., Tirella, P. S., Kirpes, R. M., Craig, R. L., Gunsch, M. J., China, S., Laskin, A., Ault, A. P., & Pratt, K. A. (2018). Aerosol Emissions from Great Lakes Harmful Algal Blooms. *Environmental Science and Technology*, *52*(2), 397–405. https://doi.org/10.1021/acs.est.7b03609

Murby, A. L., & Haney, J. F. (2016). Field and laboratory methods to monitor lake aerosols for cyanobacteria and microcystins. *Aerobiologia*, *32*(3), 395–403. https://doi.org/10.1007/s10453-015-9409-z

O'Dowd, C. D., Facchini, M. C., Cavalli, F., Ceburnis, D., Mircea, M., Decesari, S., Fuzzi, S., Young, J. Y., & Putaud, J. P. (2004). Biogenically driven organic contribution to marine aerosol. *Nature*, *431*(7009), 676–680. https://doi.org/10.1038/nature02959

Olson, N. E., Cooke, M. E., Shi, J., Birbeck, J. A., Westrick, J. A., & Ault, A. P. (2020). Harmful Algal Bloom Toxins in Aerosol Generated from Inland Lake Water. *Environmental Science & Technology*, *0*. https://doi.org/10.1021/acs.est.9b07727

Paerl, H. W., Hall, N. S., & Calandrino, E. S. (2011). Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. *The Science of the Total Environment*, *409*(10), 1739–1745.

https://doi.org/10.1016/j.scitotenv.2011.02.001

Paerl, H. W., & Otten, T. G. (2013a). Blooms bite the hand that feeds them. In *Science* (Vol. 342, Issue 6157, pp. 433–434). American Association for the Advancement of Science. https://doi.org/10.1126/science.1245276

Paerl, H. W., & Otten, T. G. (2013b). Harmful Cyanobacterial Blooms: Causes, Consequences, and Controls. *Microbial Ecology*, *65*(4), 995–1010. https://doi.org/10.1007/s00248-012-0159-y

US EPA. (2016a). *Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin* (Issue December). https://www.federalregister.gov/documents/2019/06/06/2019-11814/recommended-human-health-recreational-ambient-water-quality-criteria-or-swimming-advisories-for

US EPA. (2016b). *Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin* (Issue December). https://www.epa.gov/sites/production/files/2016-12/documents/draft-hh-rec-ambient-water-swimming-document.pdf

Yung, S. C., Zhou, Y., Irvin, C. M., Kirkpatrick, B., & Backer, L. C. (2007). Characterization of aerosols containing microcystin. *Marine Drugs*, *5*(4), 136–150. https://doi.org/10.3390/md20070010