Standard Operating Procedures - Water Sample Collection for Toxin Analysis

SCOPE

This document, prepared by the Surface Water Ambient Monitoring Program (SWAMP), is a standard operating procedure (SOP) for collecting cyanobacteria and water for cyanotoxin measurements conducted in the laboratory. Monitoring and measuring the concentration of cyanotoxins is critical to assessing human health risks and making decisions about posting advisories.

CAUTIONS AND INTERFERENCES

- Samples should be stored in controlled environments during field visits and when being shipped to the laboratory. Many **cyanotoxins are sensitive to high temperatures and bright light**. Store samples under cool temperatures 2-6° C (up to 8° C) in dark or amber-colored containers.
- **Do not add sodium thiosulfate** to water samples, it will degrade some classes of cyanotoxins.
- The choice of sample bottle material should be considered for toxins analysis. Cyanotoxins, particularly microcystins, adsorbs to most plastic materials and lowers measured toxin concentration. SWAMP **recommends use of amber glass or polyethylene terephthalate glycol (PETG, recycle symbol #1) bottles.** To streamline supplies among all procedures in this guide, these two bottle materials are consistent in all supply lists.
- To further prevent loss of cyanotoxins, consider the material of devices used to collect water samples. It is recommended that devices (e.g. churns) should be made of fluorocarbon polymers, such as Teflon®; metals, such as stainless steel; or glass (Graham et al. 2008).
- If lab analyses for anatoxin and saxitoxin will be conducted, the addition of a preservative solution is **recommended**; this optional step is described under Procedures, section 4 (pg. 8).

PERSONNEL QUALIFICATIONS/RESPONSIBILITIES

All personnel conducting this procedure must be familiar with the health and safety protocols within the Health and Safety Guide before visiting a site, and should also possess a demonstrated proficiency in the use of each instrument. Personnel are responsible for the implementation of the procedures outlined in this SOP and to ensure that the data generated meets the standards and objectives of the monitoring project. The project or field crew leader should conduct periodic review of field personnel to ensure that personnel are following procedures in accordance with this SOP.

EQUIPMENT AND SUPPLIES

When selecting equipment, consider project needs and study objectives and select your equipment accordingly. Be sure that any materials that are used are in accordance with a lab's analytical method (contact the testing lab prior to sampling to meet their requirements). The following is a general list of equipment for the field:

___ Copy of this SOP
___ Site Dossier (See Reconnaissance Guide)
___ SWAMP Cyanobacteria Field Data Sheet
___ Chain of Custody Form
___ Sample label
___ Clear tape
___ Deionized water
___ Resealable zipper plastic bags
___ Gloves of appropriate length
___ Global Positioning System (GPS) receiver or smart phone capable of displaying coordinates
___ Pen/Pencil and clipboard
___ Sampling device (see Sample Types on pg.2)
___ Cooler and gel ice packs
___ Sample bottles: PETG (preferred) or amber glass
___ Composite sample container of adequate volume (see procedures for "algal mat" and "depth integrated" samples to determine needed volume)
___ Algal mat collection tools (see pg. 6)
___ Liquinox detergent (or other phosphate free detergent
___ Wash basin
___ Soft brush or sponge

PROCEDURE

Depending on a study's objectives, the characteristics of the waterbody, and the observed distribution of the bloom, a variety of sample types can be collected to characterize a potential harmful algal bloom (HAB). **For the purpose of this SOP, the following sample collection methods are intended for human and animal health risk studies in recreational areas (as described by Graham et al., 2008). The sample types in this SOP are defined as "surface water grab," "surface scum," "algal mats" and "depth integrated;" consider the sample type prior to selecting equipment (Pgs. 2-3).** Monitoring results are used to make decisions about posting and de-posting health advisories.

At each monitoring site, collection of a "surface water grab" should be prioritized and an additional sample type should be collected to characterize the greatest risk of exposure when the following is observed (a) visible scum then collect surface scum sample; (b) visible detached or attached algal mats then collect algal mat sample; (c) monitoring of an open water area then collect depth integrated sample.

Recommended monitoring sites for planktonic and benthic bloom sampling include: shorelines or beaches; open water areas used for full-body contact recreation; bays, coves, lagoons, or backwater areas; and all other publicly accessible waterbodies. Due to the spatial and temporal variability of blooms, sampling should be routinely conducted during periods of recreational use of the waterbody, at a frequency of daily to weekly. The cost of monitoring can limit the frequency and number of samples collected so sampling sites should be in areas where the maximum density of cyanobacteria is present (i.e. target "hot spots"). Visual indicators may include: discoloration, opaque or soupy appearance, greater turbidity, surface scum, and algal mats (attached/detached)**.** If these visual indicators are absent, other evidence such as chemical factors, satellite imagery, and historic evidence is appropriate to target sample sites. **Cyanotoxins can be present in water during low density blooms that do not produce scum or after bloom material has decayed.**

Distribution of planktonic cyanobacteria (visually on surface or subsurface) is affected by hydrology (depth, flow direction, mixing, inlets/outlets), local conditions (wind, temperature, stratification), light intensity (time of day). In addition, planktonic cyanobacteria cells are buoyant and can self-regulate their depth in the water column throughout the day. Knowledge of these conditions improves selection of sites for monitoring (detailed guidance for lakes/reservoirs found in Graham et al., 2008).

Sample Types

Surface Water Grab (pg. 4) samples characterize the potential exposure from wading, swimming, and other water contact. The intent of this procedure **is to obtain a whole water sample from a single point** using an open mouth bottle held by a gloved hand or suspended on a pole. The sample will capture material accumulated both on the water's surface and in the water column and may include visual bacterial colonies, algal material, and some scum. The intent is **not** to collect a surface scum sample or average toxin concentration by compositing samples.

Surface water grab samples are the most common sample type used to measure cyanotoxin concentration. A water grab sample should be collected at each sampling site on all waterbody types (stream, lake, reservoir, wetland, etc.), but exceptions occur for benthic blooms and non-surface blooms. To characterize risk of exposure by water contact, **samples are collected from sites where the most visible indicators of a bloom are present to determine the maximum concentration of cyanobacteria and potential toxins (i.e. target "hot spots")** (Graham et al., 2008).

Surface Scum (pgs. 5) sample collection is **highly recommended** when scum is observed near recreational areas to characterize the worst case cyanotoxin concentration. The information provided by scum samples further supplements surface water grab results. **Scum samples should not be labeled surface water grab samples** - these samples should be collected separately. Many genera of cyanobacteria can accumulate on the surface of water, and this dense accumulation of cells forms scum. This accumulation can contain the greatest concentration of toxins in a bloom, and

pose higher risks to humans and animals that come into contact with the material. Scum can be found floating at the surface of water and frequently swept back into the water column by the current; scum can also be swept on to the shoreline by current or wind. **Note - scum is not the only indicator that a bloom is present.**

Algal Mat (pg. 6) sample collection is **highly recommended** when benthic bloom is observed near recreational areas (including upstream/downstream) to characterize the risk of exposure; surface water grab sample may not adequately characterize risk of exposure in these instances. Benthic blooms may form dense material that appear as film (biofilm), clumps of colonies, and mats which may include non-toxic filamentous algae. Collectively, these materials are referred to as "algal mats" in this SOP (refer to the Visual Guide for more information). Both attached and detached algal mats pose significant risk of exposure. Algal mats that are comprised of cyanobacteria can concentrate toxins within the material and release toxins continuously, and when they decay. Children and animals that can access these algal mats are at the greatest risk; dogs in particular are documented to increase their exposure by preferentially consuming these algal mats, resulting in illness and death.

The procedure for this sample type recommends collection of a composite sample. Researchers have documented high variability of cyanobacteria species within a single algal mat, as well as variable concentration of cyanotoxins within small portions of the algal mat (Wood et al, 2010; Wood et al., 2012). Due to the variable toxin concentration within a single mat, collection and analysis of a small portion of a single mat may not fully characterize the risk to public health. For these reasons, the procedure describes collection of composite sample to reduce the chance of missing toxic algal mats. A composite sample, consisting of multiple portions of different algal mats, will provide an average toxicity of algal mats within a survey area. Note - composite samples may dilute a toxin 'hotspot'. Additionally, toxin analysis of separate algal mats is cost prohibitive; by collecting a composite sample the total number of samples per site visit will be reduced. It is recommended to direct cost savings to fund more frequent site visits.

Depth Integrated (pgs. 7-8) samples should be collected to characterize the cyanobacteria community present in the photic zone and **highly recommended** when subsurface bloom is present. It is important to note that cyanobacteria communities vary in site and distribution, so collecting composite or depth integrated samples can dilute concentrations of toxins and fail to characterize the greatest exposure risk. Sample site should target open-water areas where contact recreation, such as jet skiing, paddle boarding, inner tubing, and swimming may occur. Most cyanobacteria maintain a position in the photic zone, regardless of mixed depth; therefore, if cyanobacterial distribution in the water column cannot be determined, an integrated photic zone sample is generally representative of the cyanobacteria community (Graham et al., 2008). The photic depth can be estimated as approximately 2.5 times the Secchi depth; without a Secchi disk the default depth is 3 meters (Cyanoscope, 2017; Graham et al., 2008).

Common sampling devices to obtain a depth-integrated sample include: Thief Samplers (Kemmerer bottle or VanDorn bottle), Bailers (Teflon stop-cock bailer), or Pumps (diaphragm or peristaltic). The feasibility of collecting depth-integrated samples depends on the depth of the waterbody and access to watercraft. This sample type is not applicable to shorelines and wadeable areas due to depth limitations. Shallow, well-mixed water should be sampled using a surface water grab sample.

(The following methods were developed with input from Wilde (2008), Bowling (2012), Gregor (2005), Graham et al. (2008), Graham and Van Dyke (2017), and can be performed in a variety of habitat types (e.g. streams, estuaries, lakes).

Sample Collection

1. Prepare for sample collection

1.1. Contact the laboratory prior to shipping samples and coordinate shipping timeframe, shipping conditions, and accompanying paperwork (i.e. Chain of Custody).

1.2. Setup sampling equipment and supplies in staging area near sample site. The staging area can be located along the shoreline, in a boat, or other applicable area.
1.3. Record applicable information on the Field Data Sheet and Site Reconnaissance Form.

2. Prepare label for containers

2.1. If using a waterproof pen, pre-label container at each site.
2.2. If a waterproof pen is not supplied, complete labels at each site and apply to container after sampling. Cover label with clear tape to prevent loss of text from moisture during storage.
2.3. When using glass containers, cover the label with clear tape to prevent peeling during storage.
2.4. Complete labels with the following information:
- Site Code -**or-** Site Name
- Date, Time
- Sample Type
- Preservative

2.5. Complete labels at each site and apply to container immediately after sampling. Cover label with clear tape to prevent loss of text from moisture during storage.
2.6. Special care should be taken to ensure the tape covers the labels when using glass sampling containers so that moisture does not remove the information during cold storage and transportation.
2.7. Begin to fill out the Chain of Custody Form and ensure all information on the labels, is correct.

3. Collect sample

3.1 Refer to the Health and Safety Guide for appropriate protection. When sampling blooms, assume that the water contains toxin-producing cyanobacteria and avoid direct contact with skin.
3.2 Record each sampling site's coordinates using a GPS receiver or smart phone. Refer to Reconnaissance Guide for instruction on capturing coordinates.

3A. Collection Method: Surface water grab sample

- To obtain a surface water grab sample from the **shoreline or wadeable area - use step 3A.1 -or- 3A.2**
- To obtain a surface water grab sample from a **dock, boat, or other non-wadeable area - use step 3A.2**

3A.1 For a surface water grab sample using a hand-held bottle

1. Put on new elbow-length (recommended) gloves and obtain a clean bottle.
2. New sampling bottles should not be rinsed with surface water prior to sample collection
3. If sampling in a stream or river, ensure the sample is collected facing upstream. If wading in, disturbed sediment may resuspend loosely attached benthic cyanobacteria into the water column.
4. Remove cap from bottle and hold in opposite hand from bottle
5. Grasp bottle from the bottom and submerge bottle (mouth first) into surface of water, sink bottle downwards 2-4 inches below the surface in a U-shaped motion, then pull the bottle out of water with the mouth facing up. Do not submerge sample bottle so low that water goes into glove
6. Try to avoid overfilling the bottle; pouring out the sample is discouraged because it is not homogenous. If the sample container is overfilled, shake gently at the elbow 5 times, and then pour out a small volume of water (for streams, pour downstream of the sampling site).
7. Immediately cap the bottle.
8. Wipe off exterior of sample bottle and attach label.
9. Place bottle into a cool ice chest. The sample containers should remain in the dark and be cooled to 4-6° C (do not freeze) during the remainder of the field sampling day. To maintain cool ice chests, store in the shade.

3A.2 For surface water grab sample using suspended bottle mounted on pole sampling device

1. Obtain a pole sampler device and ensure that the sample bottle can be securely mounted to the pole.
2. Put on clean gloves and obtain a clean bottle.
3. Mount bottle onto the pole sampler device.

4. Remove cap from bottle and place cap in a clean area. Use both hands to hold pole sampler device.
5. Submerge bottle (mouth first) into surface of water using swinging motion, sink bottle downwards 2-6 inches below the surface in a U-shaped motion, then lift bottle with mouth facing up.
6. Immediately cap bottle.
7. Wipe off exterior of sample bottle and attach label.
8. Place bottle into a cool ice chest. The sample containers should remain in the dark and be cooled to 4-6° C (do not freeze) during the remainder of the field sampling day. To maintain cool ice chests, store in the shade.
9. (*This section adapted from Graham and Van Dyke, 2017)*

3B. Collection Method: Surface Scum Sample

- To obtain a scum sample from the shoreline or wadeable area, use step 3B.1 -**or-** 3B.2
- To obtain a scum sample from a dock, boat, or other non-wadeable area, use steps 3B.2

3B.1 For scum sample using hand held bottle:

The top layer of scum is exposed directly to UV that produces a layer of desiccated or lysed cells that release internalized pigments and toxins. These molecules are sensitive to UV and degrade over time, therefore, the sample should be collected at the scum-water interface while avoiding the very top of scum layer. When scum is dense, mixing of the scum is needed to capture a representative scum sample.

1. Put on new elbow-length gloves and obtain a clean bottle.
2. Remove cap from bottle and hold in the opposite hand from the bottle.
3. If scum is *not* too thick, mixing of the scum layer is not needed prior to sampling. Hold the bottle nearly parallel with the surface and scoop up the surface scum **and** top 1-2 inches of water (scum-water interface) -**OR-**
4. If the scum layer is thick, mix the scum layer by rinsing the bottle 3 times with surface water (this mixes dense scum). Hold the bottle near the bottom and submerge into surface of water to scoop up the surface scum and water, then pour the bottle out in the same area previously disturbed. Repeat bottle rinsing 2 more times. After rinsing the bottle 3 times, collect the sample. Hold the bottle nearly parallel with the surface and scoop up the surface scum at sufficient depth to collect scum along with top 1-2 inches of water (scum-water interface).
5. Do not submerge sample bottle so low that water enters glove. Try to avoid overfilling the container; pouring out a sample is discouraged because it is not homogenous. If the sample container is overfilled, shake gently at the elbow 5 times, then pour out a small volume of water (for streams, pour downstream of the sampling site).
6. Immediately cap the bottle.
7. Wipe off exterior of sample bottle and attach label.
8. Place bottle into a cool ice chest. The sample containers should remain in the dark and be cooled to 4-6° C (do not freeze) during the remainder of the field sampling day. To maintain cool ice chests, store in the shade.

3B.2 For scum sampling using suspended bottle mounted on pole sampling device:

1. Obtain pole sampler device and ensure that the sample bottle can be securely mounted to pole.
2. Put on clean gloves and obtain a clean bottle.
3. Mount bottle onto pole sampler device.
4. Remove cap from bottle and place cap in clean area. Use both hands to hold pole sampler device.
5. Follow steps 3-5 of section 3B.1.
6. Carefully reach for the bottle and immediately cap it.
7. Wipe off exterior of sample bottle and attach label.
8. Place bottle into a cool ice chest. The sample containers should remain in the dark and be cooled to 4-6° C (do not freeze) during the remainder of the field sampling day. To maintain cool ice chests, store in the shade. (This section adapted from Kudela and Van Dyke, 2017)

3C. Collection Method: Algal Mat Sample

Benthic blooms may form dense material that appear as film (biofilm), clumps of colonies, or mats which may include non-toxic filamentous algae. Benthic blooms may also form a thin, diffuse layer of material on the bottom substrate (benthos) of the waterbody, particularly on fine gravel or sand. Collectively, **these materials are referred to as "algal mats" in this SOP**. These algal mats can consist of a single specie or diverse community of cyanobacteria and filamentous algae. Researchers have documented a high variability of cyanobacteria species within a single algal mat, as well as variable concentrations of cyanotoxins (Wood et al, 2010; Wood et al., 2012). The following procedure was designed to capture these concentrations through the collection of composite samples. A composite sample, consisting of portions from various algal mats, will provide an average toxicity of algal mats within the survey area.

3C.1 For a composite algal mat sample

1. Survey visible algal mats (biofilm, colonies, mats) within the sample reach, avoiding disturbed areas within recreation areas. For channels, extend survey upstream of recreational area to a maximum of 200 linear feet. For lakes or ponds, extend survey beyond target recreational area in both directions to a maximum of 200 linear feet. For larger areas of interest (greater than 200 linear feet), repeat this procedure to collect an additional sample.
2. Record GPS coordinates of area of interest to be sampled.
3. Before collecting bloom material, fill the sample container (250 mL-500 mL)with ambient water to 1/4 the volume to maintain moisture during collection, storage, and shipping.
4. **Collect a composite sample:** collect a sample of approximately 2 cm^2 from each algal mat and place into a single collection container. Repeat bloom material collection until entire area of interest is sampled. The proportion of material collected should represent the percentage of cyanobacteria community present; for example, if approximately 70% of bloom material consists of reddish-brown rough mats *(e.g. Phormidium sp.)* and approximately 30% of bloom material consists of dark green smooth mats (e.g. *Anaebena sp.*), then the final container should contain approximately 70% of the first type and approximately 30% of the second type.
   Note - *For cyanobacteria blooms that appear to be be visually similar (color, texture), a single dominant genus of cyanobacteria may be responsible for the bloom.* ***It is not*** *recommended to collect a single portion of algal mat assuming it to be representative of the entire bloom (i.e. the toxin concentration is not homogenous). Rather, collect small portions of multiple algal mats.*
   Note - *For cyanobacteria blooms that appear to consist of visually distinct algal mats, a diverse community of cyanobacteria and filamentous algae may be responsible for the bloom.* ***It is*** *recommended to collect small portions of each type of algal mat to obtain a composite sample that represents the entire bloom.*
5. When collecting **attached** algal mats, remove a portion of the mat using tools (e.g. gloved hand, forceps, turkey baster, trowel) that are appropriate for the bottom substrate.
6. When collecting a **detached** algal mat**,** use forceps, turkey baster, or a gloved hand.
7. Do not collect desiccated material (cells are likely to have lysed, releasing toxins).
8. Close container and ensure all material is submerged in ambient water. If it is not covered, pour ambient water from a clean separate container to avoid losing material.
9. Wipe off exterior of sample bottle and attach label. Label sample container as "composite algal mat."
10. Place container into a cool ice chest. The sample containers should remain in the dark and be cooled to 4-6**°** C (do not freeze) during the remainder of the field sampling day.
11. Repeat this procedure to collect a 2nd algal mat sample if the area of interest is greater than 200 linear feet. *(This section adapted from Fadness and Van Dyke, 2017)*

3D. Collection Method: Depth Integrated Sample

The following is general guidance. Refer to the manufacturer's instructions for information specific to the device used. When choosing sampling equipment for depth integrated sampling, ensure that the equipment will not rupture or deform cyanobacteria cells. This damage interferes with pigment analysis, taxonomy, and toxin analysis.

3D.1 For depth integrated sampling using Thief Samplers (e.g. Kemmerer sampler, Van Dorn sampler)

1. Triple rinse sampler with ambient water prior to use, rinse away from sampling location
2. Set the sampler in the vertical position, open the sampler, and suspend on the line.
3. Lower the sampler vertically through the water column to the desired depth within the photic zone. Care should be taken to prevent turbidity in the water while lowering the device; do so by slowly and gently lowering it.
4. Allow enough time to fill sampler.
5. Trigger mechanism to close sampler.
6. Retrieve sampler.
7. Transfer the sample slowly from the sampler drain valve into a sample bottle.
8. Repeat the above steps until the volume of water required for analyses has been collected.
9. Wipe off exterior of sample bottle and attach label.
10. Place bottle into a cool ice chest. The sample containers should remain in the dark and be cooled to 4-6° C (do not freeze) during the remainder of the field sampling day. To maintain cool ice chests, store in the shade.

3D.2 For depth integrated sample using a Tube Sampler (e.g. Cyanoscope kit tube sampler, PVC tube)

1. Hold tube sampler head in one hand and retrieval rope in the other.
2. Slowly lower the sampler head and tubing into the water column to the 3 meter depth mark.
3. Slowly retrieve the sampling tube by pulling retrieval rope towards chest.
4. Repeat the process 3 times allowing sample-tubing volumes to rinse the sample tubing with ambient water before collecting the sample.
5. After rinsing, slowly lower the sampler head and tubing directly down into the water column until the 3 meter depth indicator line on the tubing is at the surface. Care should be taken to ensure the tubing extends downward in the water and does not curve off to the side.
6. Kink the end of the sampling tube in order to create a vacuum in the tube.
7. Retrieve the sampler by pulling the retrieval rope towards your chest rapidly until the bottom opening is at the same height as the top of the tube.
8. Place the sampler head over the collection bottle and drain the sample directly into the collection bottle.
9. Repeat the above steps until the volume of water required for analyses has been collected.
10. Wipe off exterior of sample bottle and attach label.
11. Place bottle into a cool ice chest. The sample containers should remain in the dark and be cooled to 4-6° C (do not freeze) during the remainder of the field sampling day. To maintain cool ice chests, store in the shade.
12. A video demonstrating the use of the Cyanoscope kit tube sampler can be found here.

3D.3 For depth integrated sample using a Bailer (e.g. Teflon stop-cock bailer)

1. Triple rinse sampler with ambient water prior to use, rinse away from sampling location
2. Select a Teflon bailer that is long enough to incorporate the entire area of the water column that is of interest. For example, if the photic zone extends to a depth of 3 meters and an integrated photic-zone sample is being collected, then the bailer needs to be at least 3 meters in length.
3. Lower the bailer slowly, until the base (the check-valve intake) is at the bottom of the desired depth interval.
4. Bring the bailer to the surface.
5. Draw off the water into a Teflon churn by means of a bottom-emptying device that releases the check valve.
6. Repeat the above steps until the volume of water required for analyses has been collected.

7. Wipe off exterior of sample bottle and attach label.
8. Place bottle into a cool ice chest. The sample containers should remain in the dark and be cooled to 4-6° C (do not freeze) during the remainder of the field sampling day. To maintain cool ice chests, store in the shade.

3D.4 For depth integrated sample using a Pump (diaphram, not peristaltic)

1. Slowly lower the pump intake to the desired depth.
2. Rinse the sample tubing three times with ambient water before collecting the sample.
3. Pump the sample directly into the laboratory supplied sample bottle or compositing vessel.
4. Repeat the above steps until the volume of water required for analyses has been collected.
5. Wipe off exterior of sample bottle and attach label.
6. Place bottle into a cool ice chest. The sample containers should remain in the dark and be cooled to 4-6° C (do not freeze) during the remainder of the field sampling day. To maintain cool ice chests, store in the shade.

4. Post Sample Collection

4.1. Bring sample container back to staging area and add preservative solution (recommended, not required). SWAMP currently recommends addition of the Abraxis® ana-sax preservative liquid to prevent degredation during storage.

4.2. The final concentration of the preservative liquid should be 10%. For a final sample volume of 250 mL, add 25 ml of preservative to 225 mL of surface water. For a final sample volume of 500 mL, add 50 mL of preservative to 450 mL of surface water.

4.3. Use pipette dropper to transfer preservative from working bottle to sample bottle -**OR-** Use pre-dosed vial of preservative and add to sample bottle.

4.4. Mix sample container by slowly inverting sample bottle repeatedly (bend at elbow a minimum of 4 times) to mix sample. Return bottle to cool ice chest.

4.5. Finish recording information on field data sheet including sample types (e.g. 3A.1, 3C.1), sampling depths, site, time, others.

4.6. Clean equipment (refer to the “Equipment Cleaning” section below).

4.7. For the final steps after field sampling, refer to the “Sample Handling and Shipping” section below.

SAMPLE HANDLING AND SHIPPING

Samples should be cooled to 2-6°C as soon as possible after sampling. On hot days, replenish gel packs in the ice chest prior to shipping to ensure that samples remain cool. Note that wet ice, loose or bagged, should not be used for shipping due to possibility of contamination and leaks during shipment. **Contact testing lab to confirm that sample processing (handling and preservation temperatures) meets the needs of the lab’s analytical method.** Ship samples to the testing lab by the end of sampling day with adequate gel packs to maintain cool temperatures (<6°C up to 8°C). If shipping is delayed up to 5 days post collection, store samples in in the dark and freeze. Freezing samples for short term storage, until analyses can be conducted, will ensure sample integrity but lyses the cells. **Glass bottles should not be frozen unless ½-¾ of their volume is empty in order to allow for expansion during freezing.**

Glass sampling bottles should be packed with extra cushioning to prevent breakage (e.g. bubble wrap, packing peanuts). Ship samples overnight with a courier to the testing lab to maintain temperature. Place chain of custody form within plastic bag (to prevent damage) and include within shipping box. Make a copy of the chain of custody form for your own records (a photo from smartphone is appropriate). If the shipping container must be returned, include pre-paid shipping label or arrange for the laboratory to return the container for an additional fee.

LABORATORY COMMUNICATION AND ANALYSIS

For analysis of all samples: Request the laboratory to **measure the total fraction of toxins** when conducting water monitoring for studying health risk in recreational water. The total fraction includes free toxins dissolved in water and

any toxins contained within cyanobacteria cells or particulates. All water samples should be processed using appropriate cell lysis procedure (e.g. sequential 3x freeze/thaw cycles) to ensure cells are lysed prior to measuring toxins.

For algal mat sample:When interpreting results from lab analysis of algal mat sample consider sampling design of compositing the sample. The resulting toxin concentration from the composite represents an average of the area sampled and does not represent toxic hot spots that may have been present in an individual algal mat.

EQUIPMENT CLEANING

If the sampler will not be reused during a field trip, rinse its components thoroughly with deionized water before they dry and place the sampler in a plastic bag for transport to the office/laboratory for cleaning. If the sampler will be reused during the field trip, rinse its components with deionized water before they dry. Place all components of sampling equipment into a plastic bag for transport to the next sampling site. Field-clean all of the sampling equipment at the next sampling site using 0.2 % Liquinox™ detergent (or other phosphate free detergent); soak and scrub parts in wash basin; rinse with deionized water; then rinse with native water before use (EPA, 2015; Graham, 2008). For detailed instructions on field equipment decontamination, refer to Appendix A. For any watercraft or wading equipment, general decontamination procedures should be followed.

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Appendix A - Equipment Cleaning Procedure

INTRODUCTION

All sampling equipment used for sample collection, processing, and handling must be cleaned before use. Plan to clean equipment before the first use each day and, if multiple sites are to be sampled in a day, re-clean equipment before use at any subsequent sampling sites. Cleaning will prevent contamination of samples.

CAUTION

Avoid use of sampling devices with plastic components since plastic adsorbs cyanotoxins and may contaminate samples. In addition, if equipment will be used for inorganic sampling (i.e. trace metals, mercury), avoid wrapping equipment in aluminum foil.

EQUIPMENT AND SUPPLIES

When selecting equipment, consider project needs and study objectives and select your equipment accordingly. The following is a general list of equipment for the field:

___ Deionized water
___ Resealable zipper plastic bags
___ Gloves, appropriate length
___ Sampling device(s)
___ Composite sample container (if used)
___ Liquinox detergent (or other phosphate free detergent)
___ Wash basins for each step
___ Nonmetallic, non-colored brush
___ Sponge
___ Wading equipment or any other equipment in contact with waterbody at previous sampling sites (if used)

Procedure

1. If the sampling equipment **will not** be reused during the same sampling trip, triple-rinse the sampler components thoroughly with clean water (tap or deionized water) before they dry, and place the sampler in a plastic bag for transport to the laboratory for cleaning.
2. If the sampling equipment **will** be reused during the sampling trip, triple-rinse the sampler components with deionized water before they dry. Field-clean the sampler at the next sampling site before use.
3. In addition to sampling equipment, efforts should be made to apply these cleaning procedures to any watercraft to be used in the collection of samples. For further information regarding decontamination, please see the SWAMP site here. Non-motorized watercraft should follow the procedures below to the extent possible.
4. Be sure to clean equipment away from the sampling site. Be mindful of where the waste from cleaning ends up.

The following instructions should be used to clean equipment for harmful algal bloom sampling. The sequence of steps is important. *(Instructions adapted from US EPA , 2015 & Graham et al. 2008.)*

1. Use new gloves for each step.
2. Scrub the equipment/tubing by using deionized or tap water with a nonmetallic, non-colored brush to remove visible debris. It is advisable to store water for cleaning in a squeezable water bottle.
3. Soak the equipment and tubing for 30 minutes in 0.2% Liquinox™ solution or other phosphate-free detergent. Use portable wash basins when cleaning in the field.
4. Thoroughly rinse the equipment and tubing with tap water.
5. Then rinse the equipment 3 times with deionized water. Allow everything to air dry completely.
6. Reassemble equipment and wrap in plastic bags for storage and transport.