Wyoming Department of Environmental Quality, Water Quality Division
Watershed Protection Program

Harmful Cyanobacterial Blooms: Cyanobacteria Collection (Lentic)

Introduction	Cyanobacterial blooms have the potential to produce toxins or other irritants that pose health risks for people, pets and livestock. The primary goal of collecting cyanobacteria is to identify and enumerate the cyanobacteria present to determine potential health risks within a waterbody. When choosing the appropriate type, location and frequency of samples, the sampler must be knowledgeable of cyanobacteria characteristics as well as local conditions that affect the temporal and spatial variability of blooms.
Quality Control	Samplers follow the SOP. Include field quality control samples during sample collection (see *Field Duplicates* below).
Equipment	Hand-held open mouth sampler (optional) Plastic sample bottle (250 mL Nalgene®) Elbow length or shoulder-length gloves (protection from irritation) Goggles and mask to cover nose and mouth (if spray is unavoidable) Plastic knee boots, hip waders or chest waders (if collection requires wading) Disposable, powderless gloves Glutaraldehyde or Lugol's solution Pipettes and bulb Cooler with wet ice or ice packs (if not preserving sample immediately) Digital camera to record appearance of bloom Pens and permanent markers Data sheet Bags for shipping Packing Tape Mailing Label Laboratory forms (e.g., chain of custody)
Field Duplicates	Include duplicate samples in at least ten percent (10%) of all collected samples. Since cyanobacteria sampling is generally the result of complaints and therefore unpredictable, collect a duplicate sample with the first sample in each series (i.e., a duplicate for the 1st of 10 samples, another duplicate for the 11th of 20 samples). Duplicate sampling consists of one sampler using two bottles simultaneously.
Preservative	Glutaraldehyde stored in an opaque plastic bottle. Under field conditions, glutaraldehyde-based solutions can be stored for 6-12 months; or Lugol's solution stored in an opaque plastic bottle. See the SOP for *Periphyton Sample Preservative – Lugol's Solution* for details.
Holding Time	Add glutaraldehyde or Lugol's solution to samples within 8 hours of collection. If not preserving immediately, store samples on ice and in the dark. Generally, samples for investigating potential health risks of a bloom are transported or shipped immediately to the laboratory for identification and enumeration. If not

investigating an immediate health risk, preserved samples may be stored indefinitely in the dark. Do not freeze the samples as freezing may lyse cells and make identification difficult.

Safety Precaution

Wear shoulder-length gloves when collecting cyanobacteria samples. Wear googles to prevent possible toxin exposure to the eyes during windy conditions or when spray is unavoidable. Avoid inhalation of spray by wearing a mask. Use chest waders and personal floatation devices if wading offshore. Never ingest water or allow skin contact. Always wear disposable, powderless gloves when processing and preserving samples. Do not touch hands to mouth or other exposed areas of the body before washing. Wash hands with soap and water as well as rinse all equipment with water after collections.

Procedure

Analytical laboratories may have specific protocols in place for the processing, preservation, and shipping of samples. Accordingly, the procedures presented here may be modified.

1. Review *Safety Precaution* for information on safety equipment and protocols to prevent exposure to cyanobacteria.

2. To determine potential health risks, collect samples at locations with the greatest potential for human and/or animal exposure to cyanobacteria. Depending on the location of the bloom, sampling locations may include beaches, shoreline access areas, boat ramps, docks, marinas, or open water. Be mindful of wind direction as cyanobacteria may accumulate in downwind areas. Note sample location on data sheets and sample labels.

3. Wear shoulder-length gloves during sample collection. Collections will generally be made by wading from the shoreline or sampling from a dock or boat, depending on the location of the bloom. When cyanobacteria are concentrated in nearshore or littoral zones, wade into the waterbody to reach the densest portion of the bloom that also represents the area of greatest exposure potential. Sample non-wadeable or open water from a boat or other reliable structures (e.g., dock) and target areas of greatest exposure potential.

4. When collecting a sample, use an open-mouth sampler or sample bottle. If a surface scum is present, hold the bottle parallel to the water surface and collect both scum material and surface water immediately below the scum (i.e., top 1-2 inches). For diffuse blooms or those with cyanobacteria distributed throughout the water column, invert and submerge the bottle to elbow depth. Once at elbow depth, revert the bottle and raise to the water surface such that the bottle samples the water column as evenly as possible. If using a sampler, draw off each sample into the sample bottle until desired volume is reached. Ensure there is sufficient room for preservative.

5. Wear disposable, powderless gloves when preserving and processing samples. Preserve samples immediately after collection or keep on ice until preserved. Always keep samples out of light. Glutaraldehyde is the preferred preservative

for identifying and enumerating cyanobacteria cells, however, Lugol's iodine may also be used if glutaraldehyde is not available. The required amount of preservative is as follows:

a. Preserve samples with glutaraldehyde to a 1% final concentration. If standard 25% strength glutaraldehyde is used, 4 mL are added to every 100 mL of sample volume or 10 mL to each 250 mL sample.
b. For Lugol's iodine solution, use a ratio of 1:100 (1 mL per 100 mL of sample or 2.5 mL per 250 mL of sample) or add until the sample turns the color of weak tea. Samples with abundant organic matter may require more preservative (see SOP for *Periphyton Sample Preservative – Lugol's Solution*).

6. Label sample bottles according to laboratory guidelines. The following information is required (see SOP for *Sample Labeling*):

   a. Site name and/or location (e.g., beach, dock, boat ramp)
   b. Sample ID (Initials-YY-Julian Day-Sample No.)
   c. Date (mm-dd-yyyy) and time (24 hr)
   d. Preservative type and volume (mL)
   e. Type of analysis (e.g., identification & enumeration)

7. Prior to shipping, contact analyzing laboratories to confirm shipping protocol and schedule. In most cases, samples are shipped on the same day as collection. Samples are not to be shipped on Fridays, Saturdays, or the day before a holiday as recipient laboratories will likely be closed. Include paperwork required by the recipient laboratory in all shipments. Check all samples for correct labelling. Ensure that samples are kept cool and in the dark during shipping. Typically, samples are packed in double bags, placed in coolers, and shipped overnight to arrive at the analyzing laboratory the next morning.

8. Sample frequencies are determined on a case by case basis and depend on study objectives. If multiple samples are collected to monitor cyanobacteria over time, it is recommended that each sample be collected a minimum of 24 hours apart.

References

Ohio EPA, 2014. Public Water System Harmful Algal Bloom Response Strategy (Draft). Ohio Environmental Protection Agency, Columbus, Ohio.

US Geological Survey, 2008. Cyanobacteria in Lakes and Reservoirs: Toxin and Taste-and-Odor Sampling Guidelines. Cyanobacteria, Version 1.0, Chapter A7, Biological Indicators.

Utah DEQ/DWQ, 2016. Recommended Standard Procedures for Phytoplankton Collection to Determine Harmful Algal Blooms. Utah Department of Environmental Quality, Division of Water Quality, Salt Lake City, Utah.

Wyoming Department of Environmental Quality, Water Quality Division
Watershed Protection Program

Harmful Cyanobacterial Blooms: Cyanotoxin Collection (Lentic)

Introduction	Cyanobacteria can produce cyanotoxins that can affect the skin, liver and central nervous system in people, pets and livestock. Common cyanotoxins include microcystin, cylindrospermopsin, anatoxin and saxitoxin. The type of toxin produced is dependent on the cyanobacteria present. As such, cyanobacteria identification and enumeration is generally recommended prior to selecting a cyanotoxin analysis. The procedure for cyanotoxin sample collection for laboratory quantitative analysis follows the general steps in the SOP for *Harmful Cyanobacterial Blooms: Cyanobacteria Collection (Lentic)*. Specific instructions for cyanotoxin sample preparation, processing, and preservation are included below.
Quality Control	Samplers follow the SOP. Include field quality control samples during sample collection (see *Field Duplicates and Blanks* below).
Equipment	Hand-held open mouth sampler (optional) Plastic sample bottle (30-60 mL amber PETG or bottles recommended by lab) Amber glass container/vial for anatoxin analysis Elbow length or shoulder-length gloves (protection from irritation) Goggles and mask to cover nose and mouth (if spray is unavoidable) Plastic knee boots, hip waders or chest waders (if collection requires wading) Disposable, powderless gloves Cooler with dry ice (wet ice or ice packs if samples will be frozen within 24-36 hours) Digital camera to record appearance of bloom Pens and permanent markers Data sheet Packing tape Bags for shipping Mailing label Laboratory forms (e.g., chain of custody)
Containers	Per request of analyzing laboratory. *NOTE*: Cyanotoxins are organic compounds that can adhere to certain sample containers, resulting in absorptive loss of toxin. Therefore, sample containers must be made of fluorocarbon polymers such as PETG, metals such as stainless steel, or glass.
Field Duplicates And Blanks	Include duplicates and field blanks in at least ten percent (10%) of all collected samples. Since cyanotoxin sampling is generally a result of complaints and therefore unpredictable, collect a duplicate and field blank with the first sample in each series (i.e., a duplicate and field blank for the 1st of 10 samples, another duplicate and field blank for the 11th of 20 samples). Duplicate sampling consists of one sampler using two bottles simultaneously. See SOP for *Blanks* for field blank information.

Preservative

Preservatives are only required for ambient anatoxin and saxitoxin samples as well as treated drinking water samples; ambient microcystin and cylindrospermopsin samples do not need to be preserved. Ambient anatoxin and saxitoxin samples should be preserved with 10X Concentrated Sample Diluent (i.e., buffer solution) immediately following collection to prevent absorptive loss of toxin. Preserve samples to a 1:10 ratio (e.g., 3 mL of preservative per 30 mL of sample).

Holding Time

Microcystin, cylindrospermopsin and saxitoxin samples can be stored on ice or refrigerated up to five (5) days while anatoxin-a samples can be stored on ice or refrigerated up to 28 days. Cyanotoxin samples must not exceed 10^{o}C while being stored or shipped. If microcystin, cylindrospermopsin and saxitoxin samples will not be analyzed within five (5) days, or anatoxin-a samples within 28 days, samples should be frozen. Frozen cyanotoxin samples must be analyzed within 180 days.

NOTE: Freezing will cause cyanobacteria cells to lyse, hence frozen samples are only appropriate for total-concentration analysis (i.e., sum of intracellular and extracellular toxins). If freezing, ensure complete mixing of samples and sufficient room for thermal expansion within the sample container. To determine total cyanotoxin concentrations, laboratories generally submit samples to three (3) freeze-thaw cycles to lyse cells. Accordingly, it saves laboratory processing time to freeze samples immediately after collection by storing and shipping on dry ice. Check with analyzing laboratory to determine if samples should be immediately stored and shipped on dry ice.

Safety Precaution

Wear shoulder-length gloves when collecting cyanotoxin samples. Wear googles to prevent possible toxin exposure to the eyes, especially under windy conditions or when spray is unavoidable. Avoid inhalation of spray by wearing a mask. Use chest waders and personal floatation devices if wading offshore. Never ingest water or allow skin contact. Always wear disposable, powderless gloves when processing and preserving samples. Do not touch hands to mouth or other exposed areas of the body before washing. Wash hands with soap and water as well as rinse all equipment with water after collections.

Procedure

The procedure for collecting cyanotoxin samples for total concentration analysis is presented below. Outsourced laboratories may have specific protocols in place for sample processing, preservation, and shipping. Accordingly, the procedures presented here may be modified.

1. Review *Safety Precaution* for information on safety equipment and protocols to prevent exposure to cyanotoxins.

2. To determine potential health risks, collect samples at locations with the greatest potential for human and/or animal exposure to cyanotoxins. Depending on the location of the bloom, sampling locations may include beaches, shoreline access areas, boat ramps, docks, marinas, or open water. Be mindful of wind direction as cyanobacteria and therefore cyanotoxins may accumulate in downwind areas. Note sample location on data sheets and sample labels.

3. Wear shoulder-length gloves during sample collection. Collections will generally be made by wading from the shoreline or sampling from a dock or boat, depending on the location of the bloom. When cyanobacteria are concentrated in nearshore or littoral zones, wade into the waterbody to reach the densest portion of the bloom that also represents the area of greatest exposure potential. Sample non-wadeable or open water from a boat or other reliable structures (e.g., dock) and target areas of greatest exposure potential.

4. When collecting a sample, use an open-mouth sampler or sample bottle. If a surface scum is present, hold the bottle parallel to the water surface and collect both scum material and surface water immediately below the scum (i.e., top 1-2 inches). For diffuse blooms or those with cyanobacteria distributed throughout the water column, invert and submerge the bottle to elbow depth. Once at elbow depth, revert the bottle and raise to the water surface such that the bottle samples the water column as evenly as possible. If using a sampler, draw off each sample into the sample bottle until desired volume is reached.

5. Wear disposable, powderless gloves when preserving and processing samples. Preserve anatoxin and saxitoxin samples with 10X Concentrated Sample Diluent immediately after collection. The required amount of preservative for anatoxin and saxitoxin samples is as follows:

    a. A ratio of 1:10 (3 mL of 10X Concentrated Sample Diluent per 30 mL of sample).

6. Store all samples out of light and on ice. Freeze microcystin, cylindrospermopsin and saxitoxin samples if they will not be shipped and analyzed within five (5) days and if anatoxin-a samples will not be shipped and analyzed within 28 days. Frozen cyanotoxin samples must be analyzed within 180 days. If freezing, ensure sufficient room for thermal expansion in the sample bottle. To determine total cyanotoxin concentrations, laboratories generally submit samples to three (3) freeze-thaw cycles to lyse cells. Accordingly, it saves laboratory processing time to freeze samples immediately after collection by storing and shipping on dry ice. Check with analyzing laboratory to determine if samples should be immediately stored and shipped on dry ice.

7. Label samples bottles according to laboratory guidelines. The following information is required (see SOP for *Sample Labeling*):

    a. Site name and/or location (e.g., beach, dock, boat ramp)
    b. Sample ID (Initials-YY-Julian Day-Sample No.)
    c. Date (mm-dd-yyyy) and time (24 hr)
    d. Preservative type and volume (mL) if applicable
    e. Number of freeze-thaw cycles (if frozen prior to sending to laboratory)
    f. Type of analysis (e.g., microcystin, saxitoxin, etc.)

8. Prior to shipping, contact analyzing laboratories to confirm shipping protocol and schedule. In most cases, samples are shipped on the same day as collection. Samples are not to be shipped on Fridays, Saturdays, or the day before a holiday as recipient laboratories will likely be closed. Include paperwork required by the recipient laboratory in all shipments. Check all samples for correct labelling. Ensure that samples are on ice and in the dark during shipping. Typically, samples are packed in double bags, placed in coolers on wet or dry ice, and shipped overnight to arrive at the analyzing laboratory the next morning.

9. Sample frequencies are determined on a case by case basis and depend on the overall objective of the study. If multiple samples are collected to monitor cyanotoxins over time, it is recommended that each sample be collected a minimum of 24 hours apart.

References

Abraxis Algal Toxins, Fresh Water (Cyanotoxins), Fresh Water Kits User Guides, accessible at https://www.abraxiskits.com/products/algal-toxins/

Ohio EPA, 2014. Public Water System Harmful Algal Bloom Response Strategy (Draft). Ohio Environmental Protection Agency, Columbus, Ohio.

US EPA, 2016. Method 546: Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay. US Environmental Protection Agency, Office of Water, Washington, DC.

US EPA, 2017. Detection of Algal Toxins in Surface Water Samples Using Abraxis Cyanotoxin Automated Assay System. Standard Operating Procedure BIOLM-008. US Environmental Protection Agency, Region 8 Laboratory, Golden, Colorado.

US Geological Survey, 2008. Cyanobacteria in Lakes and Reservoirs: Toxin and Taste-and-Odor Sampling Guidelines. Cyanobacteria, Version 1.0, Chapter A7, Biological Indicators.