Standard Operating Procedure for Chlorophyll *a* Sampling Method Field Procedure

LG404

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1.0 SCOPE AND APPLICATION

1.1 This method is used to sample chlorophyll-containing algae from the Great Lakes and Tributary streams.

2.0 SUMMARY OF METHOD

2.1 A representative lake water sample is collected from Niskin bottles from various depths and filtered by vacuum filtration onto a Whatman GF/F filter in dim light. The filter is then placed in a prelabeled 16 mm screw cap glass culture tube which is stored in the dark in a freezer. Extraction and analysis may take place aboard ship during a survey, or after shipment to a land-based laboratory.

3.0 APPARATUS

6 300-mL plastic filter funnels with magnetic base, Gelman Aluminum manifold with space for 3-6 filter funnels

Vacuum system (3-4 psi) ship system or portable vacuum pump Tubing and glass carboy in milkcrate for storing filtrate

GF/F filters, Whatman (47-mm diameter, 0.7 um pore-size) 16 x 100 mm disposable glass screw-cap culture tubes Pasteur short disposable pipettes

Rubber bulb

Plastic wash bottle, 500-mL

Plastic wash bottle with eye dropper, 500-mL, for MgCO3

Filter forceps

Opaque sample bottles, 1000-mL (Nalgene or equivalent)

4.0 REAGENTS

4.1 **Saturated Magnesium Carbonate Solution:** A pre-weighed sample of ten grams of magnesium carbonate are added to 1000 mL of reagent water in a 1 L clear plastic container. The solution is settled for a minimum of 48 hours, after which the clear solution is decanted into a new container for subsequent use. **Only the clear "powder free" solution is used during subsequent steps.**

5.0 SAMPLE HANDLING AND PRESERVATION

5.1 The entire procedure is carried out as much as is possible in subdued light (green) to prevent photo-decomposition. The frozen samples are protected from light during storage for the same reason. During the filtration process, the samples are treated with a MgCO3 solution (section 4.1) to eliminate acid induced transformation of chlorophyll to its degradation product, pheophytin. Samples are grouped by station and completely wrapped in aluminum foil and stored in individual Ziploc bags during storage until the samples are extracted and analyzed aboard ship or transported to a land-based laboratory. Samples to be extracted and analyzed at a land-based laboratory are transported to the laboratory in a

cooler containing dry ice. Analysis is performed as soon as possible following sampling and always within three and a half weeks.

6.0 FIELD PROCEDURE

6.1 A list of all chlorophyll samples along with the corresponding preprinted labels, is prepared ***before the start of each cruise*** by GLNPO staff. It is possible that some listed specific depths are not sampled due to redundancy or the absence of a feature (e.g. DCL). GLNPO staff and marine technicians lead such sample collection decisions through review of the water column profile prior to bottle closure. They also make the decision of what discrete depth samples to combine for the surface integrated samples (INT). All other depth codes are collected at discrete depths within single Niskin bottles. Spring and Summer depth codes (including master's stations) are listed in GLNPO's Water Quality Survey QAPP.

6.2 Opaque Nalgene bottles, permanently labeled with depth codes, are used to carry the water samples from the Rosette Niskin sampling operation to the ship's biology lab. Prior to each station, the appropriate bottles for that station are delivered to the sampling deck in a milkcrate and the appropriate labeled culture tubes for that station are on the benchtop. The labels on the tubes are covered with clear plastic tape to protect against wetness particularly during the sonication process.

6.3 After filling, the opaque bottles are held in the biology lab refrigerator for up to an hour before filtering. Samples should be filtered within 30 minutes, but may sit up to 2 hours in refrigeration, only under unusual circumstances and must be noted on data sheets.

6.4 Filter forceps are used to place 47-mm diameter Whatman GF/F filters, textured side up, on Gelman magnetic filter funnels in a filtration manifold.

6.5 Sample Volume:

Due to differing trophic levels among the Great Lakes, the volume of water filtered varies. For Lake Erie, 150 mL of sample is filtered. For Lakes Ontario, Huron, Michigan, and Superior, a 250-mL sample is used. As each sample is filtered, the volume filtered is entered on the Chlorophyll a Preparation Form with date/time and analyst initials.

6.6 A vacuum pressure of no more than 5 psi (10 in Hg, or 2.5 cm Hg) is used. A valve for the shipboard vacuum system is accessible at the biolab lab bench and portable vacuum pumps are also available.

6.7 The sample bottle is inverted several times to create a uniform mixture, and the 250-mL graduated cylinder is rinsed 3 times with sample water prior to measuring the sample volume. The sample volume is measured from the bottom of the meniscus.

6.8 The sample volume is added to the filter funnel, the valve of the filtration unit is turned on and the graduated cylinder is rinsed with 10 to 20 mL of reagent water which is added to the filter funnel. Valves can be turned on after setting up all six funnels. Keep track of the order of the bottle depth codes.

6.9 When 10-50 mL of sample remains on the filter, 10 drops of the $MgCO_3$ solution are added, using a disposable pipette. The sides of the filter funnel are rinsed with reagent water. The valve on the filtration unit is switched off as soon as the liquid disappears, to prevent the breakage of cells.

6.10 With two filter forceps, the filter is carefully removed from the funnel, folded in half, rolled, and placed into the bottom of the pre-labeled 16 mm culture tube which is then tightly closed. Double check to ensure the depth code on the label is the correct one for that specific sample.

6.11 When all samples for a station have been filtered, the tubes are wrapped as a group in aluminum foil. Using masking tape, the aluminum foil package is labeled with the lake, station and date, and the package is immediately placed in the freezer. All of the above procedures are completed in subdued (green) light. Be sure to empty the glass carboy for filtrate after every series of six samples.

7.0 QUALITY ASSURANCE

7.1 Each of the following audits is collected once per lake basis (approximately 20 samples). The QA samples are listed on the preprinted field sheets.

7.2 Field duplicates are taken from a second Niskin bottle closed at about the same time and location as the regular field sample. It is transported from the Niskin bottle to the onboard biology laboratory in an opaque bottle marked as a duplicate (FDn) sample.

7.3 Laboratory duplicates (LDn) are filtered from the same opaque sample bottle as their corresponding regular field samples.

7.4 Field blanks (Field Blk or SYN for synthetic), consisting of reagent water are carried by an opaque sample bottle from the onboard Barnstead reagent water system to the filtration apparatus. The bottle is used only for blanks and is permanently marked (SYN) as such.

8.0 SAFETY AND WASTE HANDLING

8.1 Refer to GLNPO Safety, Health & Environmental Compliance Manual (Version 9.3) and individual instrument procedural operations manuals for specific details on applicable 1) personal health and safety issues; 2) instrumental, chemical, and waste handling procedures; and 3) accident prevention. This applies to all EPA personnel, EPA contractors or federal, state, or local government agencies, and persons who operate or are passengers onboard US EPA GLNPO vessels during all activities and surveys.

8.2 All applicable safety and waste handling rules are to be followed. These include proper labeling and disposal of chemical wastes. Over-board discharges of chemical wastes are forbidden.

8.3 During sampling, caution, common sense, and good judgement should dictate appropriate safety gear to be worn in any given situation on deck.

8.4 Collecting samples in cold weather, especially around cold water bodies, carries the risk of hypothermia and frostbite. Sampling team members should wear adequate clothing for protection in cold weather, particularly for the spring survey. For specific information regarding sampling during cold conditions, please refer to the Standard Operating Procedures for Winter Operations (available from GLNPO as Appendix N in the *QAPP US EPA Great Lakes National Program Office Open Lake Water Quality Sampling Surveys* (May 2017, revised March, 2019).

8.5 Collecting samples in extremely hot and humid weather carries the risk of dehydration and heat stroke. Sampling team members should carry an adequate supply of water or other liquids for protection against dehydration in hot weather.

8.6 Work vests, hard hats, and steel toed boots must be worn while working on the fantail and Rosette deck.

9.0 SHIPPING

9.1 When samples are not extracted and analyzed aboard ship during a survey, shipment to a land-based lab is necessary. At the conclusion of sampling in a lake, the available samples in individual station bags are wrapped into one complete batch and clearly labeled with survey, lake, and date. These samples are stored in the ship's walk-in freezer until they can be transported to the land-based laboratory. For transport, the batches are packed in a cooler with adequate dry ice to last for the duration of the trip. Upon arrival at the land-based laboratory, the samples are placed in the freezer (-20oC) until they can be analyzed.

10.0 CHLOROPHYLL A PREPARATION FORM

10.1 The field technician should use the hard copy Chlorophyll a Preparation Form to enter the relevant sampling data. A copy of this form can be found in Appendix E of the WQS QAPP and is displayed in Attachment 1 of this SOP. The following table provides guidance on entering data in each field in the form. See LG101 Electronic Field Information Recording for an explanation of Survey ID, Visit ID, Station ID and Sample ID.

Chlorophyll a Preparation Form	
Field Name	**Data Entry Instructions**
Survey ID	[preprinted; example "MI1921"]
Visit ID	[preprinted; example "M017G19"]
Station ID	[preprinted; example "MI 17"]
Preparation Batch ID	Enter the preparation Batch ID
Sample ID	[preprinted; example "19GM40S22"]
Depth Code	[preprinted; examples "SRF", "MEP", "B10"]
QC ID Code	[preprinted; "RFS", "LDn"]
Check Mark	Insert a check mark in this column upon completion of the sample preparation
Depth	Enter the depth at which the sample was taken (in meters)
Temperature	Enter the water temperature associated with the sample (in °C)
Remarks	Record any unusual circumstances associated with the sample or sample preparation
Sample volume	Enter the volume of the filtered sample (in mL)
Preparation Date	Enter the date on which the sample was prepared in "mm/dd/yyyy" format
Preparation Finish Time	Enter the time at which the Chlorophyll a preparation was completed for all samples associated with the station in "UTC, military" format
Personnel (initials)	Enter the initials of the personnel entering the data

Attachment 1:
Chlorophyll a Preparation Form

ER2011 | Chlorophyll a Preparation | Spring 2020 GLNPO's WQS

Survey ID	Visit ID	Station ID	Preparation Batch ID
[preprinted]	[preprinted]	[preprinted]	FC

Sample ID	Depth Code	QC ID	Check Mark	Depth, m	Temperature, °C	Remarks
[preprinted]	[preprinted]	[preprinted]				

Sample volume; mL	Preparation Date (mm/dd/yyyy)	Preparation Finish Time (UTC, military)	Personnel (initials)XXX

Survey ID	Visit ID	Station ID	Preparation Batch ID
[preprinted]	[preprinted]	[preprinted]	FC

Sample ID	Depth Code	QC ID	Check Mark	Depth, m	Temperature, °C	Remarks
[preprinted]	[preprinted]	[preprinted]				

Sample volume; mL	Preparation Date (mm/dd/yyyy)	Preparation Finish Time (UTC, military)	Personnel (initials)XXX

Method: LG 404

Entered into electronic file _____ (Initials)