

Study Plan for the Canadian Coast Guard Survey of Lake Erie in Winter 2016

1. Project Objective

The biogeochemical processes of Lake Erie during the winter are poorly understood and represent an important uncertainty in our understanding of this important system. Coast Guard operations on the Great Lakes during the winter offer a valuable opportunity for data collection to fill this gap in our knowledge. This project uses current Coast Guard operations as a sampling platform to measure the distribution of phytoplankton biomass and dissolved nutrients through the Great Lakes in the winter.

2. Project Design

The project consists of synoptic sampling of the near-surface waters of the Great Lakes during normal operations of the CCGS *GRIFFON*.

3. Project Parameters

a) Sampling location, time, and local conditions

The latitude, longitude (decimal degree format) and time will be recorded from the ship's navigational suite at every sampling location. Local environmental conditions, including air and water temperature, wind direction and strength, cloud cover, ice cover, and ice thickness will also be recorded when possible.

*b) Particulate chlorophyll *a* concentration*

Near-surface water (1 m depth) will be collected using a stainless steel sampling bottle and processed using the chlorophyll *a* standard operating procedure (Appendix A). We have adopted this approach rather than use of Go-Flo bottles to accommodate working in ice. These bottles were custom made (welded stainless steel) by Fletcher Manufacturing, Bowling Green, OH).

Since the sampling bottle is re-used, it is subject to a cleaning regimen including washing with tap water and phosphate-free detergent followed by rinsing with tap water and de-ionized water.

Equipment blanks will be processed at a frequency of 5% (1 of 20 samples). For these blanks, the sampling bottle is rinsed and filled with reagent water and then treated as a normal sample. This is done to verify that cross contamination does not occur between samples. The reagent water used will be de-ionized water.

c) Dissolved and particulate nutrient concentrations

Near-surface water (1 m depth) will be collected using a stainless steel sampling bottle and stored frozen until analysis at Heidelberg University (National Center for Water Quality Research). Heidelberg follows established U.S. Environmental Protect

Agency methods for analysis of nutrients and major ions (Appendix B).

4. Sampling Design

- a. The ship will reduce speed to 1-2 knots as it approaches the sampling station, chosen at regular time intervals or by the discretion of the Captain as described by Beall et al., (2016). Stations will not be biased on the basis of ice conditions or location. The location and time of the sampling station will be recorded along with the environmental conditions.
- b. BGSU science personnel will deploy the stainless steel sampling bottle to a depth of 05-1 m and collect the water grab sample.
- c. Sub-samples for particulate chlorophyll *a* and dissolved nutrient concentrations will be processed by BGSU personnel according to the Standard Operating Procedures and stored under the appropriate conditions until collection by BGSU personnel for shipping and analysis.
 - i. Triplicate replicates of particulate chlorophyll *a* will be prepared from each water grab sample.
 - ii. Duplicate replicates of at least 10% of samples for dissolved nutrients will be prepared from each water grab sample.
- d. Particulate chlorophyll *a* samples will be transported back to BGSU with dry ice and processed according to the EPA standard 445.0 method for chlorophyll *a* analysis (GF/F membranes).

Analysis will be completed prior to the 3.5 week hold time allowed under this procedure.

For analysis by fluorometry, samples are extracted in 90% acetone (24 h at -20° C) with sonication and chlorophyll measured in a TD-700 fluorometer (Turner) using the non-acidified approach (Welschmeyer, 1994)

- e. Dissolved nutrient samples will be transported back to BGSU following which they will be shipped to the National Center for Water Quality Research at Heidelberg University (Tiffin, OH) for analysis. Analytical methods used by Heidelberg are described in Appendix B.

5. Quality Assurance & Quality Control

Sampling, sample preparation and analysis will follow the methods in the Manual of Ohio EPA Surveillance Methods and Quality Assurance Practices (Ohio EPA, 2009) and the Inland Lakes Sampling Procedure Manual (Ohio EPA, 2010).

a. *Particulate chlorophyll a concentration*

- i. Triplicate laboratory replicates will be prepared from each water grab sample.
- ii. Replicate field blanks will be prepared daily from de-ionized water.
- iii. Samples will be handled under low light conditions and frozen immediately. Samples will be kept at -20°C or under dry ice (transport) until analysis at

BGSU.

b. Dissolved nutrients

- i. Duplicate laboratory replicates will be prepared from at least 10% of grab samples.
- ii. QA/QC procedures are conducted by the National Center for Water Quality Research at Heidelberg University (Tiffin, OH), the contract laboratory conducting the analyses for dissolved and particulate nutrients.

6. References

- Beall, B.F.N., M.R. Twiss, D. Smith, B. Oyserman, M.J. Rozmarynowycz, C. Binding, R.A. Bourbonniere, G.S. Bullerjahn, M. Palmer, E.D. Reavie, M.K. Waters, W.C. Woityra, and R.M.L. McKay. 2016. Ice cover extent drives phytoplankton- and bacterial community structure in a large north-temperate lake: Implications for a warming climate. *Environ Microbiol* **18**: 1704–1719.
- Welschmeyer, N.A., 1994. Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. *Limnol Oceanogr* **39**: 1985–1992.
- U.S. Environmental Protection Agency. 1979. Methods for Analysis of Water and Wastes. EPA 600/4-79-020, Cincinnati, OH.

Sampling Checklist and Instructions

Record station and environmental conditions.

- a.* Date, time, latitude, longitude, and bottom depth
 - b.* Ice conditions, estimated ice thickness, weather.
 2. Collect 1 L of water using the stainless steel bucket into labeled 1L opaque plastic bottle.
 3. Nutrient sampling
 - a.* Complete two (2) paper labels for each station and stick onto 60 mL plastic bottles. Under “Analysis” on the labels, one bottle should be labeled “TP” and the other “<0.2”.
 - b.* In the “TP” bottle, add 50 mL of whole water from the sample.
 - c.* Syringe-filter 50 mL of sample into the “<0.2” bottle.
 - d.* Store in freezer.
 4. Chlorophyll *a* sampling
IMPORTANT: Minimize light exposure of chlorophyll samples
 - a.* Prepare triplicate paper labels with pencil for each filter type with sampling information (station, date, depth, filter type) for a total of nine (9) labels.
 - b.* Prepare nine (3) foil squares for storing filters.
 - c.* Filter triplicate water samples (50 mL) through GF/F filters.
 - d.* Use hand-pump to generate vacuum.
 - e.* When ~10 mL of sample is left in the filtration funnel, add 10 drops of MgCO_3 solution, then filter down.
 - f.* Rinse funnel twice with distilled water, and turn off vacuum as soon as the water disappears
 - g.* Fold filter in half, then place in foil square with paper label.
 - h.* Close foil packet and label outside of packet with Sharpie, details station, date, and filter type.
 - i.* Store in freezer.
 5. Rinse equipment with tap water, then distilled water, then store.

Appendix A

Standard Operating Procedure for Chlorophyll-*a* Sampling Method: Field Procedure for Use by U.S. Coast Guard

1.0 Scope and Application

This method is used to filter chlorophyll-*a* samples from the Great Lakes and Tributary streams.

2.0 Summary of Method

A grab lake water sample is collected from a stainless steel sampling bottle at various depths and filtered by vacuum filtration in dim light. The filter is then placed in a screw cap polyethylene culture tube in the dark. The tube is stored in the dark at sub-freezing temperatures and transported to the BGSU laboratory for extraction and analysis. The BGSU laboratory will follow protocol LG405, developed by the EPA's Great Lakes National Program Office (GLNPO) for water quality surveys of the Great Lakes (appended).

3.0 Apparatus

Plastic filter funnel, Pall Filtron (250 mL capacity)
Vacuum manifold system to accommodate 3 filter funnels
Vacuum system (3-4 psi)
GF/F filters, Whatman (25 mm)
Screw cap polyethylene tubes
Graduated polystyrene pipettes (25 mL; disposable)
Pasteur short disposable pipets
Rubber bulb
Plastic wash bottle, 500 mL
Plastic wash bottle, 500 mL, for MgCO₃
Filter forceps
Opaque sample bottles, 1000 mL (Nalgene or equivalent)

4.0 Reagents

Saturated Magnesium Carbonate Solution Add 10 grams magnesium carbonate to 1000 mL of deionized water. The solution is settled for a minimum of 48 hours. Decant the clear solution into a new container for subsequent use. *Only the clear "powder free" solution is used during subsequent steps.*

5.0 Sample Handling and Preservation

Sample collection and preservation will follow the procedures described in the Manual of Ohio EPA Surveillance Methods and Quality Assurance Practices (Ohio EPA, 2009) and the Inland Lakes Sampling Procedure Manual (Ohio EPA, 2010). The entire procedure should be carried out as much as is possible in subdued light to prevent photodecomposition. The frozen samples should also be protected from light during storage for the same reason. During the filtration process, the samples are treated with MgCO₃ solution (section 4) to eliminate acid induced transformation of chlorophyll to its degradation product, pheophytin. Samples are stored by station in aluminum foil and transported to the BGSU laboratory in a cooler with dry ice. Analysis should be performed as soon as possible following sampling.

6.0 Field Procedure

- 6.1 Following sample collection with the stainless steel sampling bottle, samples are transferred to 1000 mL opaque Nalgene bottles, labeled with the station, sample depth, *eg.* Surface, representing a surface sample
- 6.2 Place filters, using forceps, textured side up. Assemble the filtration apparatus just prior to filtration.
- 6.3 Due to differing trophic levels among the Great Lakes, the volume of water filtered varies. For

Lake Erie, 25 mLs of sample are filtered. After inverting the sample bottle several times to create a uniform mixture, carefully draw 25 mL into a pipette and distribute contents into filtration funnel.

6.4 Turn vacuum pressure on, not exceeding 3 psi. Our plans call for use of a hand pump.

Check Frequently During Filtration to Insure Pressure Does Not Go Above 3 PSI!!!

6.5 When approximately 10 mL of sample remains on the filter, add 10 drops of the MgCO₃ (section 4.1) solution using a disposable pipet. Thoroughly rinse the filter apparatus and graduated cylinder, using a squirt bottle, with deionized water. Turn off vacuum pressure as soon as the liquid disappears to prevent the breakage of cells.

6.6 Using the forceps, fold and remove the filter and carefully place it into the bottom portion of the prelabeled culture tube (see section 10) and close tightly. Lay all tubes flat and completely wrap in aluminum foil. Clearly label the Lake, station and date on masking tape and attach to above mentioned aluminum foil package. Immediately freeze. All the above procedures should be completed in subdued light.

7.0 Quality Control

7.1 Each of the following audits is collected once per lake transect.

7.2 Field duplicates are taken from a second stainless steel sampling bottle collected 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 duplicate sample.

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

7.4 Field blanks, consisting of reagent water are carried by an opaque sample bottle from the onboard reagent water supply to the filtration apparatus. The bottle is used only for field blanks and is permanently marked as such.

8.0 Waste Disposal

Follow all laboratory waste disposal guidelines regarding the disposal of MgCO₃ solutions.

9.0 Shipping

Once a transect has been completed or a batch of 35 samples has been completed, wrap all samples into one complete batch and clearly label with date. Pack tightly in a medium sized cooler and fill all spaces with enough dry ice to last 24 hours. Dry ice is considered a hazardous chemical by most shipping companies and has to be accompanied by authorizing paperwork. Once transported to BGSU, the samples should be immediately placed in the freezer.

10.0 Labeling

Sample identification information is provided on printed labels both prior to and during the survey. The labels are affixed to the side of the 16 × 100 mm chlorophyll tube. The sample identification number is covered with clear tape in case the tube becomes wet.

Appendix B

1.c. Analytical Methods

Sample Preparation Overview

Analyses of total phosphorus and Total Kjeldahl nitrogen are done on whole water samples that include both dissolved and particulate materials (i.e. suspended solids). Analyses of the remaining nutrients are done on filtrates that have passed through a 0.45 micron membrane filter.

Pesticide analyses are completed following solid phase extraction of whole water samples. For the immunoassay procedures, whole water samples are used.

For the metals analysis, whole water samples are digested with nitric/hydrochloric acid and decanted prior to analysis.

Analytical Procedures

The specific methods currently used in the tributary loading program are shown in the adjacent table. In the early days of the program, all nutrients were done using Autoanalyzer II systems. Subsequently, we shifted to Technicon TRACCS systems for all but TP and TKN. Later, the anions were switched to Dionex Ion Chromatography.

Analytical Group	Equipment	Method Reference*
Suspended Sediment	Mettler Balance	EPA Method 160.2
Nutrients and major ions Total phosphorus Total Kjeldahl nitrogen Ammonia nitrogen Soluble reactive phosphorus Silica Specific Conductance Nitrate nitrogen Nitrite nitrogen Chloride Fluoride Sulfate	Technicon AAll Technicon AAll Technicon TRAACS Technicon TRAACS Technicon TRAACS Technicon TRAACS Dionex Ion Chromatograph Dionex Ion Chromatograph Dionex Ion Chromatograph Dionex Ion Chromatograph	EPA Method 365.3 EPA Method 351.2 EPA Method 350.1 EPA Method 365.3 EPA Method 370.1 EPA Method 120.1 EPA Method 300.1 EPA Method 300.1 EPA Method 300.1 EPA Method 300.1 EPA Method 300.1
Current generation pesticides EPTC, Butylate Phorate, Simazine Atrazine, Terbufos Fonofos, Metribuzin Alachlor, Linuron Metolachlor, Chlorpyrifos Cyanazine, Pendamethalin Acetochlor	Gas Chromatography/ Mass Spectroscopy (GC/MS) using a Varian Saturn II	EPA Draft Method 507, solid phase extraction
Current generation herbicides Atrazine, Alachlor Metolachlor, Cyanazine	Immunoassay, Ohmicron RPA1 reader and tubes	Ohmicron Methods
Metals (major) Calcium, Magnesium Sodium, Potassium Strontium, Barium Aluminum, Iron Trace metals Copper, Cadmium Lead, Manganese Zinc	Varian Liberty 100 ICP, with ultrasonic nebulizer	Standard Methods for the Examination of Water and Wastewater, 17 th edition, Method 3120

*Methods for Analysis of Water and Wastes, EPA 600/4-79-020, Cincinnati, OH, 1979

Some Views of the WQL's Analytical Equipment and Laboratories

Since the onset of its tributary loading programs, the WQL has used automated analytical systems for analysis of nutrients and subsequently for pesticides and metals. In the early years of the program, bookkeeping was done manually. However, relatively early in its history, the WQL switched to electronic transfer of all analytical data to its computer data base. Several components of our quality control program are also automated, either by the commercial software that accompanies the analytical equipment or by software developed within the WQL.

In December 2004, the WQL moved into laboratories on the third floor of the newly constructed Gillmor Science Hall. Previously, the laboratory had occupied space in the basement of Bareis Hall.



Varian ICP-MS used for trace metals analyses.



Technicon TRAACS Autoanalyzer for analyses of ammonia, soluble reactive phosphorus, silica, and specific conductance.



Dionex Ion Chromatograph for nitrate, nitrite, chloride, fluoride and sulfate.



GC-MS system and autosampler for volatile organic compounds.



A general view of the suspended sediment laboratory, showing filtering racks, drying oven and balances.



Solid phase extraction filters for pesticides.



Technicon Autoanalyzer II systems are used for the analysis of total phosphorus and total Kjeldahl nitrogen (TKN).