Study Plan for the U.S. Coast Guard Survey of Great Lakes in Winter 2012-13

1. Project Objective

The biogeochemical processes of the Laurentian Great Lakes during the winter are relatively unknown and represent an important uncertainty in our understanding of this important system. U.S. 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 USCG 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 USGC *MACKINAW*.

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.

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).

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 Commanding Officer. 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. Designated crew members of *MACKINAW* trained in sampling and supervised by LT Stephen Elliott, Operations Officer, will deploy the stainless steel sampling bottle to a depth of 1 m and collect the water grab sample. On occasion where BGSU personnel have joined the vessel, they will assist with this operation.

c. Sub-samples for particulate chlorophyll *a* and dissolved nutrient concentrations will be processed by LT Elliott or 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 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) or as detailed in Twiss et al. (2012; size-fractionated PCTE membranes). For the latter, size-fractionated Chl *a* biomass is measured in triplicate for total (>0.2 μm)- and microphytoplankton (>20 μm) size classes (parallel filtrations), using the corresponding nominal pore-size, 25-mm diameter polycarbonate filters.

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) 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 an accompanying file.

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 each water grab sample.

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

Twiss, M.R., R.M.L. McKay, R.A. Bourbonniere, G.S. Bullerjahn, H.J. Carrick, R.E.H. Smith, J.G. Winter, N.A. D'Souza, P.C. Furey, A.R. Lashaway, M.A. Saxton, and S.W. Wilhelm. 2012. Diatoms abound in ice-covered Lake Erie: Investigation of offshore winter limnology in Lake Erie over the period 2007 to 2010. *J Gt Lakes Res,* **38:** 18-30.

Welschmeyer, N.A., 1994. Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. *Limnol Oceanogr* **39:** 1985–1992

Sampling Checklist (Instructions to Coast Guard personnel)

1. 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 "Nuts".
    b. In the "TP" bottle, add 50 mL of whole water from the sample.
    c. Syringe-filter 50 mL of sample into the "Nuts" 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 (9) foil squares for storing filters.
    c. Filter triplicate water samples through GF/F, 0.2 μm, and 20 μm filters.
        1. For GF/F and 0.2 μm filters, sample volume is 50 mL.
        2. For 20 μm filters, sample volume is 100 mL.
    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 $MgCO_3$ 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 $MgCO_3$ (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 $MgCO_3$ 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.