Study Plan for the U.S. Coast Guard Survey of Lake Erie in Winter 2016

1. **Project Objective**

The biogeochemical processes of Lake Erie during the winter are relatively unknown and represent an important uncertainty in our understanding of this Great Lake. U.S. Coast Guard operations on the Lake 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 Lake Erie in the winter.

2. **Project Design**

The project consists of synoptic sampling of the near-surface waters of Lake Erie during normal operations of the USGC *NEAH BAY*. The cutter *NEAH BAY* operates throughout Lake Erie during the winter season and offers an unparalleled platform for sampling. With the support and leadership of LTJG Daniel Jones, the Executive Officer of *NEAH BAY*, water samples will be collected when mission permits to provide a spatial and temporal survey of the Lake for the concentrations of particulate chlorophyll *a* and dissolved nutrients.

The varied and unpredictable nature of USCG operations on Lake Erie in the winter season necessitates a flexible sampling strategy to maximize the spatial and temporal coverage of the survey. A pilot study conducted in the winter of the 2009-2010 showed sampling at regular time intervals, hourly in the case of ship transits between duty stations, provided ample spatial resolution. This project will use a similar strategy of hourly samples, or regularly spaced stations on the discretion of the command of *NEAH BAY*, to provide adequate spatial resolution for the survey. The project will continue throughout the winter operations season of *NEAH BAY* and will thus provide temporal resolution for the survey.

3. **Project Parameters**

   a) **Sampling location, time, and local conditions**

   The latitude, longitude (decimal degree format with 4 decimal minimum) 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). Heidelberg follows established U.S. Environmental Protection Agency methods for analysis of nutrients and major ions (Appendix B).

4. **Sampling Design**

a. The ship will come to a stop at the sampling station, chosen at regular time intervals or by the discretion of the ship’s command. 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 *NEAH BAY*, trained in sampling and supervised by LTJG Jones, will deploy the stainless steel sampling bottle to a depth of 1 m and collect the water grab sample.

c. Water samples are transferred to 1 L opaque acid-cleaned polyethylene storage bottles and stored at 4 °C until picked up by personnel by BGSU (same day). Samples are transported to BGSU in cooler containing ice packs.

d. 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 shipping and/or 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.

e. Particulate chlorophyll *a* samples will be processed according to the EPA standard 445.0 method for chlorophyll *a* analysis. 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)

f. Dissolved nutrient samples 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**

   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 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. **Credible Data Collection**
   The PI (Dr. McKay) submitted credentials for consideration of renewal of Level 3 Qualified Data Collector status and was approved by Ohio EPA in December 2015. A training session led by Dr. McKay on USCGC NEAH BAY was held on 17 December, 2015 for LTJG Jones to establish sample collection methods and quality criteria.

   USCG servicemen, under the direct supervision of LTJG Jones are responsible for sample collection. Upon arrival at port, BGSU personnel will meet the cutter to pick up samples for same day transport to BGSU.

7. **Qualified Personnel**
   Project Manager
   Level 3 Certification: Chemical Water Quality Assessment
   Effective: 12/28/2015
   QDC # 00519

   Dr. Robert Michael L. McKay
   Department of Biological Sciences
   Bowling Green State University
   Bowling Green, OH, 43403
   Phone: (419) 372-6873
   Email: rmmckay@bgsu.edu
8. References


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 prior to 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 MgCO\(_3\)
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, *e.g.* 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.

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

*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.

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

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

Varian ICP-MS used for trace metals analyses.
A general view of the suspended sediment laboratory, showing filtering racks, drying oven and balances.

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

GC-MS system and autosampler for volatile organic compounds.

Solid phase extraction filters for pesticides.