

For: International Field Year for Lake Erie – 2005, Rapid-Response Funding Opportunity

Lakewide Primary Productivity Survey of Lake Erie Using Advanced Technology

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Executive summary: Recent technological advances in fluorimetry enable aquatic scientists to establish qualitative and quantitative assessments of the phytoplankton community. We propose to use FRRF (Fast Repetition Rate Fluorimetry; Chelsea Instruments) and FluoroProbe (bbe Moldeanke, GmbH) sensors to map the floristic composition and health of the phytoplankton community. Traditional methods to establish the health of a phytoplankton community require intensive water sampling efforts and labor-intensive sample analysis (phytoplankton identification, pigment analysis) and experimentation, e.g. use of light:dark dissolved oxygen method, [^{14}C]- NaHCO_3 method, [^{18}O]- H_2O to measure gross photosynthesis (Ostrom et al. 2005), and time and space consuming experiments to establish photosynthetic efficiency on discrete water samples.

The research groups of Twiss (Clarkson) and Smith, Guildford and Hecky (Waterloo) each possess a FRRF (Mk I) and FluoroProbe (Series 7, and Series 5). We propose to use these instruments in both horizontal and vertical profiling modes. Twiss will immerse the instruments in a ferrybox (40 L) that will be continuously fed with lakewater from a depth of 5 m. Ancillary instruments will measure temperature, conductivity, CDOM, geographical position, and time. Data on floristic composition (Chlorophyceae, Cyanobacteria, Heterokontophyta & Dinophyceae, and Cryptophyceae) as well as photosynthetic efficiency (a measure of phytoplankton health) will be measured at 8 second intervals in the ferrybox. Discrete samples will be taken from the ferrybox every 30 minutes while underway to measure TP, DIN, size fractionated chl-*a*, and material for related projects (e.g. cyanobacterial toxins, bacteria, etc.). At fixed stations, Smith will use a FRRF and FluoroProbe on a hydrowire to characterize the entire water column that will allow real-time profiling to identify regions of interest. Discrete samples and ancillary data will be obtained using the ship's rosette and CTD, respectively.

We propose two cruises (lakewide transects) in late spring and late summer to validate the use of this advanced technology. The R/V *Lake Guardian* is the ideal platform for this project since Twiss has used a pumping system from this vessel while underway, the necessary high speed data links with GPS from the bridge are available, and this ship is in the Clarkson site license for the use of [^{14}C]- NaHCO_3 , which will be used in a limited number of assays to compare photosynthetic efficiency with that obtained by FRRF.

Twiss has five publications (3 in special issue of *J. Great Lakes Res.*, 1 in *Oecologia*, 1 in *Environ. Sci. Technol.*) resulting from the recent Lake Erie Trophic Study.

Scientific Rationale

Project Description:

The need to establish a lake-wide database of phytoplankton and their productivity for specific Lake Erie management purposes was clearly recognized over 25 years ago:

“Phytoplankton species and densities, together with their associated productivities at all depth in the lake, should be determined.”

(Burns et al. 1976).

Burns et al. (1976) acknowledged that data on phytoplankton and primary production would constitute only one facet of a complex predictive lake model. At that time, lake-wide measurements of phytoplankton (Munawar and Munawar 1976) and primary production (Munawar and Burns 1976) were attempted and maps established. Markarewicz (1993) described changes in phytoplankton biomass over the 17 year period from 1970-1987 in each basin of Lake Erie. However, both major earlier floristic studies (Munawar and Munawar 1976, Markarewicz 1993), omitted the picophytoplankton (0.2-2 μm), a plankton size fraction that comprise a significant fraction of the pelagic epilimnetic phytoplankton in Lake Erie (ca. 30%, Twiss and Campbell 1998; DeBryn et al. 2004).

The work of Munawar and co-workers (Munawar and Burns 1976, Munawar and Munawar 1976) provides the most complete dataset on primary productivity and phytoplankton biomass and floristic composition to date. Due to the enormous microscopical and experimental demands required to make the measurements, the maps produced by Munawar and co-workers were based on only 25 stations across Lake Erie that were sampled at 4 week intervals from April to December in 1970. Thus, the resolution of these past efforts does not match our current knowledge of the scale and frequency of physical forcing in this system. In addition, changes to the ecosystem of Lake Erie in the past 35 years resulting from nutrient loading due to phosphorus control measures, exotic species invasion (notably dreissenid mussels), and recognition of the ecological importance of autotrophic picoplankton, force us to re-measure these parameters.

Recently, use of the light:dark dissolved oxygen method, [^{14}C]- NaHCO_3 method, and [^{18}O]- H_2O method for measuring primary production in Lake Erie were compared and shown to be reasonably similar to their estimations (Ostrom et al. 2005). Measurements of photosynthesis and photosynthetic efficiency using these methods require time and space consuming experiments on discrete water samples. In addition, traditional methods to establish the floristic composition of the phytoplankton community require intensive water sampling efforts and labor-intensive sample analysis (phytoplankton identification, pigment analysis).

Recent technological advances in fluorimetry enable aquatic scientists to establish qualitative and quantitative assessments of the phytoplankton community. Submersible *in situ* instruments such as the Fast Repetition Rate Fluorimeter (FRRF; Chelsea Instruments) and the FluoroProbe (bbe Moldeanke, GmbH) are able to measure the floristic composition and health of the phytoplankton community with a temporal resolution of seconds (a more complete description of these instruments is provided below). Therefore, these measurements can be made at degrees of resolution that match hydrodynamic influences and over vast geographical distances.

In summer 2005 we propose to:

1. Obtain the first lake-wide measurements of phytoplankton quantity (plankton size class, taxonomic grouping) and health (photosynthetic efficiency) in Lake Erie using advanced instrumentation;
2. Correlate phytoplankton size class, major phytoplankton divisions, and phytoplankton health to water quality parameters; and,
3. Analyze the variability in phytoplankton community indices (photosynthetic efficiency, composition) to characterize the dominant frequencies of variation for comparison with the dominant scales of physical forcing energy as revealed by parallel studies of hydrodynamics.

We propose to conduct two lake-wide research cruises in 2005 (late spring, late summer). These cruises will provide the opportunity to demonstrate the power of applying this technology to mapping phytoplankton and primary productivity in Lake Erie using advanced instrumentation over traditional methods. As guiding principles, we will use the instrument array and sampling design to test the following hypotheses:

Hypotheses:

1. Surface waters are dominated by cyanobacteria in the western basin, Cryptophytes and Heterokontophyta in the central and east (i.e. basin-sale gradients in phytoplankton exist).
2. Gradients in phytoplankton community composition will be observed in near-shore/offshore transects and in vertical profiles.
3. Photosynthetic efficiency will vary with season;
 - a. Photosynthetic efficiency in pelagic surface waters will be reduced in late summer due to loss of nutrients from surface waters;
 - b. Photosynthetic efficiency will be high in metalimnetic communities due to entrainment of nutrients from hypolimnetic waters.

We expect to obtain a solid dataset that will augur well for a full use of these advanced instruments for lake-wide investigative surveys in 2006 and 2007. These future intensive surveys will provide useful information for predictive lake model applications.

Methodology:

FRRF and FluoroProbe instruments will be used in both horizontal and vertical profiling modes. Two cruises will be conducted in order to observe expected diverse phytoplankton communities and also to make observations of primary productivity prior to (spring) and during (late summer) central basin hypoxia.

The Clarkson group will maintain the instruments immersed in a “ferrybox” (40 L) that will be continuously fed ($4 \text{ L}\cdot\text{min}^{-1}$) with lakewater from the epilimnion while underway (e.g. a depth of 5 m). At fixed stations the Waterloo group will conduct a vertical profiling by attaching a FRRF and FluoroProbe to a hydrowire. The vertical profiling FRRF will have an attached photosynthetically available radiation (PAR) photosensor.

Sampling underway will require the starboard deployment of a “Fish”. Water will be pumped onboard through a Teflon-lined polyethylene tube using a pneumatically-driven Teflon double diaphragm pump (McMaster Carr, Husky Model 307). Water will enter through a bulkhead port into the Primary Productivity container lab onboard the R/V *Lake Guardian* where it will enter the ferrybox. A bypass will allow for discrete sampling on line under a laminar flow hood. This system has been used to pump water onto the R/V *Lake Guardian* on previous cruises although it was done while at station. The use of the depressor plate will allow sampling underway: Twiss has worked with a similar sampling system onboard the R/V *Blue Heron* (Sept. 2000, May 2004) that sampled while underway at 8-10 knots. A multi-sensor (YSI 600XL) and PAR photosensor (4π) will be attached to the Fish to monitor depth, conductivity and sampling temperature, and ambient PAR photon flux density: data will be monitored from the Primary Productivity lab and logged.

The hydraulic residence time of the ferrybox is 10 minutes. Thus, the water in the ferrybox will continually integrate water over approximately 3.1 km (at 10 knots). Water in the ferrybox will be assessed using an array of fluorimetric sensors at 8 second intervals. Water will be pumped into the box and flow out by gravity out of the box after mixing. Mixing will be aided by pumping water through a field fluorimeter (AU-10CE) to measure CDOM and back into the ferrybox. Discrete water samples can be sampled from the ferrybox through a valve. The box will be drained and cleaned at every fixed station. All sensors will be inspected and cleaned during a daily 2 hour scheduled maintenance period.

The instruments collecting data in the ferrybox are listed in the table below. Data will be collected on a fully ruggedized laptop field computer. All data are time stamped to allow for synchronizing and geo-referencing of the data sets.

Instrument	Make, model	Parameter(s) measured
Fast Repetition Rate Fluorimeter (FRRF)	Chelsea Instruments, Mk I	Photosynthetic efficiency, primary productivity
FluoroProbe	Bbe Moldaenke GmbH, Series 7	Total Chl-a and Chl-a attributed to: Cyanobacteria, Chlorophyta, Heterokontophyta & Dinophyta, Cryptophyta; Temperature; CDOM ¹ ; Transmittance (710 nm) ¹
Field fluorometer	Turner Designs, AU-10CE	Colored Dissolved Organic Matter (CDOM)
Multi-sensor (attached to Fish)	YSI-600XL sensor, YSI-610DM interface	Conductivity, Temperature, Depth, Dissolved oxygen, pH
Integrating quantum radiometer (attached to Fish)	Li-Cor LI-188B, with 4π submersible sensor	Photosynthetically Available Radiation (PAR)
Computer (1.3GHz)	Panasonic, CF-29	Geographic position, data basing

¹Use by instrument for self-calibration.

Description of data produced by sensors:

FRRF: The FRRF measures bulk photosynthetic parameters of the entire phytoplankton community (see above) that is exposed to the fluorimeter excitation light and from which the emission light is detected. The detector is open to the water flowing past the head of the FRRF (Fig. 3A). Analysis is conducted on 8 second intervals but each analysis requires only milliseconds to take place, thus there is essentially no risk of mixing in the ferrybox compromising the analysis. F_v/F_m is the measurement of photochemical quantum efficiency of photosystem II: $F_v/F_m = (F_m - F_0)/F_m$, where F_0 and F_m = initial and maximal fluorescence rates. Photosynthetic efficiency, α , and light-saturated photosynthetic rate (primary productivity) P^m_b can also be obtained from the FRRF measurements.

FluoroProbe: Like the FRRF, the FluoroProbe is also open to the water in the ferrybox. Water flowing past the sensor windows (Fig. 3B) is analyzed in sequence by a series of six excitation wavelengths with a fixed emission wavelength (680 nm). The FluoroProbe also measures CDOM, %T, and temperature (internal instrument and water temperature), in order to make corrections to its algorithms that detect the various phytoplankton divisions. Since CDOM is an important factor in operation of the FluoroProbe, discrete water samples will be collected (and filtered <0.45 μm) and stored at 4°C for verification of CDOM background. If any correction is necessary then the CDOM correction parameters can be used to correct (post-collection) the raw data collected by the FluoroProbe. Hence, if CDOM quality changes markedly during transects then the data collected by the FluoroProbe are still valid.

Field fluorimeter: The field fluorimeter will be used to monitor CDOM in the ferrybox. This fluorimeter uses a 30 mm flow-through cell and is calibrated to express CDOM as Suwannee River Fulvic Acid (SRFA) equivalents (standard reference SRFA is available from the International Humic Substances Society, Golden, CO).

Multi-sensor: The YSI-600XL submersible multi-parameter water quality sensor will be used to monitor sampling depth of the Fish while underway to ensure proper sampling depth. In addition, conductivity, water temperature, dissolved oxygen and pH will also be collected.

Integrating quantum radiometer: Will provide a time integrated value for PAR photon flux density at the depth from where the water sample is collected. The time integration will be 10 minutes, the same as the residence time of the ferrybox. Thus, a calculation of photosynthesis measured by FRRF (that uses PAR measured at depth) will be collected into 10 minute bins. Note that the PAR data can only be of use during daylight hours and when the hull of the ship is not shading the PAR sensor on the towed Fish.

Field computer: This computer is designed for field use. The computer has an internal GPS as a redundant back up of the data link to the GPS on the bridge. In the event of computer failure all the instruments above are capable of collecting data and logging data for >22 hours at a sampling frequency of once per 8 seconds. Copies of all software for sensor communications use will be brought onboard with an additional laptop computer in the event of field computer failure.

Instrument redundancy: Note that these expeditions will bring onboard two (2) each of FRRF and FluoroProbe. In the event failure of one of these instruments then the option of using a backup is available. Moreover, instrument cross-calibration will be possible. An additional fluorimeter (Turner Designs, TD-700) will be brought onboard for measurement of chlorophyll-*a*. The TD-700 is also capable of measuring discrete samples of CDOM; accordingly, the AU-10CE can be fitted to measure chl-*a*.

Description of discrete water sampling from the ferrybox:

At 30 minute intervals during transects, 2 L of water will be drawn from the ferrybox for measuring size fractionated chlorophyll-*a* (0.2-2 μm , 2-20 μm , >20 μm), Phosphorus (particulate, dissolved), POC and PON (by CHN analysis), and microscopical examination of selected samples. At selected stations, primary productivity will be assessed using [^{14}C]- NaHCO_3 . In addition, samples will be taken for total bacteria, cyanobacterial pigment and toxins, and analysis by flow cytometry. These samples can be made available to other collaborators for analysis.

Size fractionated chlorophyll-*a*: Parallel filtrations (in duplicate) will be made using polycarbonate membrane filters at low filtration vacuums. Chl-*a* will be extracted in 5 mL of 90% acetone at 4° C in the dark and measured fluorimetrically (Welschemeyer 1994) onboard the ship.

P speciation: Three hundred mL of whole water will be filtered (onto combusted GF/F filters) for measuring particulate P and total dissolved phosphorus. Samples will be conducted in duplicate. Upon return to the lab, phosphorus will be measured colorimetrically following a persulfate digestion (antimony/molybdate/tartrate method [Wetzel & Likens 2000] and a 10 cm pathlength cuvette.

POC and PON: Particulate C and N will be determined using an Exeter Analytical CE-440 Elemental Analyzer, with 1000 ml samples filtered on pre-combusted glass fiber filters (GF/F).

Primary productivity: At 2-3 times per day, photosynthetic efficiency of phytoplankton will be assessed by the [^{14}C]- NaHCO_3 method (Wetzel and Likens 2000) using a photosynthetron (Hiriart et al. 2002). The photosynthetron method uses 18 different irradiance levels to determine total organic fixation rates as a function of irradiance, at near *in situ* temperatures. Alkalinity will be measured by Gran titration onboard the ship (Wetzel & Likens 2000).

Ancillary data: Water samples can be collected from the ferrybox or through the online water sampler in the laminar flow hood at any frequency required by other researchers. For example, samples can be collected and fixed for bacteria and cyanobacterial toxin analysis (S.W. Wilhelm, G.L. Boyer; Rinto-Kanto et al. in press), fixed for analysis by flow cytometry and microscopy (H.J. Carrick), and frozen for pigment analysis by HPLC. In addition, comparison of gross primary productivity determined by use of light:dark dissolved oxygen method, [^{14}C]- NaHCO_3 method, and [^{18}O]- H_2O methods can build on a published data set for Lake Erie (Ostrom et al. 2005) and strengthen our confidence in the various proxies currently used to assess primary production.

Data analysis:

Data will be tabulated for use in correlative analyses. The horizontal transect data set will contain the following data in two Excel spreadsheets per transect (A = sensor data, B = discrete data):

A (collected every 8 seconds): time, depth, latitude, longitude, water temperature at depth, water temperature in ferrybox, conductivity at depth, dissolved oxygen at depth, pH at depth, total chl-*a*, Chlorophyta (mg/m³ chl-*a* equivalent), Cyanobacteria (mg/m³ chl-*a* equivalent), Heterokontopyta & Dinophyta (mg/m³ chl-*a* equivalent), Cryptophyta (mg/m³ chl-*a* equivalent), photosynthetic efficiency (F_v/F_m), and primary production (mmol O/m³/h; note: primary production will be integrated over 10 minutes – see description in text above).

B (collected every 30 minutes): time, depth, latitude, longitude, total chl-*a*, chl-*a* (0.2-2 µm), chl-*a* (2-20 µm), chl-*a* (>20 µm), PP, DP, POC, PON.

The data here will be in a format that is amenable to automated collection and data basing (FieldWorker Enterprise 6.0; FieldWorker Products Ltd., Toronto, Ontario), mapping (ArcGIS 8.2; Environmental Systems Research Institute, Redlands CA), and correlation analysis by standard statistical software. Frequency distribution analysis can be used to test for factors affecting phytoplankton community composition and health.

Project Relevance:

None of the current expertise in primary productivity measurements (e.g. G.L. Fahnenstiel) at NOAA-GLERL is currently tasked with measuring primary production throughout Lake Erie. Thus, the work described here should satisfy a knowledge gap and provide the necessary personnel and instrumentation to allow data needed to address, in part, the open questions posed by NOAA-GLERL research scientists:

- *How does hypoxia influence species composition, abundance, diversity, and production of microbes, phytoplankton, ...?*
- *Are there particular biological attributes (e.g., phytoplankton production...) that influence the timing, magnitude, and duration of hypoxia?*

Collaboration/Other Project Linkages:

Twiss and Smith are members of the Erie at the Millenium research group, and have contributed to the USEPA-GLNPO funded Lake Erie Trophic Study. The PIs have *ad hoc* collaborations with the following research groups: MERHAB, MELEE, and the University of Waterloo Lake Erie Project. The research proposed here will complement the NOAA-GLERL research activity of Fahnenstiel (HABs) and Ruberg (real-time measurement by remote sensors). The instrumentation described here is applicable for use in survey or moored use in an Integrated Coastal Observing System for the Great Lakes.

Governmental & Societal Relevance:

The instrumentation described herein, and its proposed application, has the potential to rapidly assess primary productivity on a lake and basin-wide scale in Lake Erie. *The same data set collected by more standard experimental techniques* (e.g. C-14 methodology, dissolved oxygen budgets, O-18 incubations; Ostrom et al. 2005) *would simply not be feasible on this vast*

scale. The data set that will be collected in these cruises will be invaluable to the design of future research cruises in 2006 and 2007. Other techniques for measuring primary productivity are required to investigate certain assumptions of the FRRF technique and thus, our proposal will augment measurements of primary productivity by other university and government scientists.

Given the movement underway for remote sensing applications in the Great Lakes, field testing of FRRF and FluoroProbe techniques will be invaluable to the acceptance of these methods.

From a societal perspective, the application of these advanced technologies has the potential to partially satisfy social demands on environmental scientists to understand how the lake functions so that it can be understood and managed properly.

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3. Project FLIPPER 2005* Timeline

Date	Activity
Late March, early April 2005	First planning meeting of PIs.
Late June 2005	Research cruise No.1 of FLIPPER project.
July-August 2005	Analysis of data and samples from FLIPPER I. Modify data collection streams, if necessary.
July 11-15, 2005	Participate in cruise onboard CCGS <i>Limnos</i> to refine methods prior to September FLIPPER cruise.
Early September 2005	Research cruise No.2 of FLIPPER project.
September - October 2005	Analysis of data and samples from FLIPPER II; compilation of data sets from FLIPPER I & II.
November 2005	PI meeting to report on research findings.
February 2006	Present results at ASLO-2006 winter meeting.
March 2006	Submit proposals (NYSG, NSF, USEPA-GLNPO) to continue investigations on interesting aspects of primary productivity identified by the FLIPPER surveys.

* **FLUORIMETRIC INVESTIGATION OF PRIMARY PRODUCTIVITY – ERIE 2005**

