

**IFYLE05: MICROZOOPLANKTON AND PHYTOPLANKTON DISTRIBUTION  
AND DYNAMICS IN RELATION TO SEASONAL HYPOXIA IN LAKE ERIE**

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**Executive Summary:**

Lake Erie is experiencing extensive hypoxic zones in its central basin despite the reductions in external P loading. The exact mechanisms of hypoxia development and its effects on biological communities including phytoplankton remain unclear. In marine and freshwater environments, the microbial food web (including microzooplankton) plays a key role in hypoxic environments. Microzooplankton have been indicated as an important component of Lake Erie ecosystem, but no data on their response to the seasonal hypoxia and trophic interactions with phytoplankton is available. This project aims to examine the composition, abundance, and growth and grazing-mortality rates of microzooplankton and phytoplankton in conjunction with the planned/ ongoing NOAA research on zooplankton and fish distribution in the three basins of Lake Erie. We will combine real-time flow-cytometric examination of plankton along four nearshore-offshore transects and diel vertical sampling with ship-board experiments at selected contrasting sites to determine plankton response to hypoxia and other environmental factors. Microscopic and molecular tools will be used to verify taxonomic determinations. The project will provide critical information for increasing the knowledge Lake Erie biological processes, improving the existing and future models, and enhancing the government's ability to manage Lake Erie resources.

## SCIENTIFIC RATIONALE:

**Project Description:** Hypoxia caused by terrestrial nutrient loading is an emerging national and global problem (Anderson and Taylor 2001; Rabalais et al. 2002). Lake Erie, the warmest and most productive of the Laurentian Great Lakes, had experienced extensive anoxic zones and fish kills due to excessive P loading from municipal sewage and agricultural runoff in the 1960s (Bolsenga and Herdendorf 1993). Subsequently, P loading has been reduced, but the area and extent of late-summer hypoxia in the central basin have not diminished since the late 1980s (U.S. EPA GLNPO long-term monitoring program). The central basin hypolimnion of Lake Erie generally exhibits hypoxia, and occasionally anoxia, at the end of the summer. The volume of hypolimnion, the duration and stability of stratification, and water temperature correlate with the extent of hypoxia in Lake Erie (Murray Charlton, CCIW, pers.comm.). However, our ability to forecast, model, and manage this phenomena is limited by the incomplete understanding of the exact mechanisms of its development and the effects on biological processes.

In marine and freshwater environments, hypoxic/anoxic boundaries are associated non-random aggregation of planktonic protists at and below the oxicleine in various aquatic systems (Finlay et al. 1991; Zubkov et al. 1992; Detmer et al. 1993; Fenchel et al. 1995; Park and Cho 2002). In addition, benthic microaerophilic protists often migrate into the water column where they reach high numbers following the onset of thermal stratification and O<sub>2</sub> depletion in sediments (Bark and Goodfellow 1985; Lavrentyev and Maslevtsov 1988; Beaver and Crisman 1989). These data indicate that the microbial food web plays a key role in hypoxic environments and should be included in the hypoxia monitoring programs and models.

The pelagic microbial food web is now viewed as a continuum of herbivorous, bacterivorous, and omnivorous trophic pathways rather than the incomplete but traditionally considered chain-loop dichotomy (Legendre and Rassoulzadegan 1995). The microbial food web creates and processes the bulk of primary production in the ocean (e.g. Sherr and Sherr 2000 and references therein). In the Great Lakes, abundant assemblages of microbial grazers, characterized by short turnover times (Carrick et al. 1992; Lavrentyev et al. 1995, 2004) and biomass comparable to planktonic crustaceans, inhabit the pelagic zone (Fahnenstiel et al. 1998; Munawar and Lynn 2002; Gardner et al. 2004). They exert considerable grazing pressure on picoplankton (Fahnenstiel et al. 1991) and bacteria (Hwang and Heath 1997; Lavrnetyev et al. 1997; 2004). *Importantly, microbial grazers, particularly ciliates, form direct trophic links to crustacean mesozooplankton* (Carrick et al. 1991; LeBlanc et al. 1997; Bundy et al. 2005).

In Lake Erie, microbial plankton have the largest biomass among the five Great Lakes (Fahnenstiel et al. 1998). Small phytoplankton biomass increases 20-fold in summer, and the proportion of microbial grazers in total plankton biomass also increases during stratification. Despite more than a century of microbiological studies in this region (Landacre 1908 and references therein), the lake remains understudied with respect to microbial plankton dynamics. Major eukaryotic microbial plankton components (other than phytoplankton) have been characterized only partially and the relationships between structure and function of the MFW remains to be examined. To date, detailed taxonomic information on heterotrophic microplankton in Lake Erie is limited to shallow nearshore waters (Lavrentyev et al. 2004).

Within the MFW concept, microzooplankton (i.e. grazers between 20 and 200 µm) herbivory is well established as an important mechanism controlling phytoplankton primary production in pelagic (Levinsen and Nielsen 2002) and estuarine environments (Murrel and Hollibaugh 1998). With the exception of one study on a *Synechococcus*-based food web in Lake Michigan (Fahnenstiel et al. 1991), no data on microzooplankton herbivory in the Great Lakes is

*available in literature.* In a set of preliminary dilution experiments conducted in western and central Lake Erie in the summer of 2004, we measured microzooplankton herbivory rates matching epilimnetic phytoplankton growth rates (up to 0.95 d<sup>-1</sup> and 1.1 d<sup>-1</sup>, grazing and growth, respectively; Moats et al. ALSO Meeting, Feb. 20-26, 2005, Salt Lake City, UT). These experiments have also revealed differential responses to dilution in diverse algal assemblages and the effects of microzooplankton dynamics and composition on the outcome of experiments. Therefore, herbivory studies in productive coastal waters should include more detailed phytoplankton and microzooplankton analyses than the routinely used chlorophyll *a* that can mask trophic interactions between specific algal and ciliate populations.

Phytoplankton (i.e. autotrophic and mixotrophic prokaryotes and protists) form the base of the microbial food web and directly and indirectly fuel the higher trophic levels in pelagic ecosystems. Phytoplankton show a fast response to the changing levels of nutrients, grazing, light-, temperature- and turbulence conditions and pollutants. Therefore, phytoplankton analyses are relevant in bio-indicator assessments, bio-effect monitoring, surface water quality monitoring. In addition, harmful algal blooms (HAB's) can cause serious impacts upon humans, coastal ecosystems and economy. Lake Erie phytoplankton have been the subject of a number of recent studies which examined phytoplankton composition, distribution, responses to nutrient additions and the zebra mussel invasion (e.g. Makarewicz et al. 1999; Frost and Culver 2001; Barbiero and Tuchman 2004; DeBruyn et al. 2004). *However, the relationships between Lake Erie phytoplankton and seasonal hypoxia remain unclear.* For example, the rate of hypolimnetic oxygen depletion in the eastern basin has not changed despite the occurrence of subsurface and benthic algal assemblages (Carrick 2004).

Phytoplankton remain one of the most difficult environmental parameters to determine. General practice is taking discrete water samples followed by preservation, concentration, transport and storage until microscopic determination of species and counting of individuals can be done. Microscopic analysis is a time consuming and expensive procedure. Consequently, this puts a restriction on the number of samples in a survey and thus limits its temporal and spatial resolution. Automation of sampling, analysis and classification via *flow-cytometry offers a practical way to intensify phytoplankton monitoring.* The high speed of analysis (a few minutes per sample) and the real time availability of raw data allow measurements with high frequency and/or spatial detail and improve the reliability and reproducibility of time series and transects. This approach may yield information on phenomena that would have been missed by a fixed sampling regime and enhance the functionality of monitoring and field experiments.

The available information on microbial plankton dynamics and distribution in Lake Erie and other aquatic systems leads to the following testable hypotheses:

(A) *Microzooplankton relative contributions to zooplankton biomass and pelagic secondary production increase from spring to summer and reach its maximum in the hypoxic zone;*

(B) *Phytoplankton composition shifts from the predominance of large diatoms in the spring to nano-picoplankton in the summer. As a result microzooplankton herbivory controls phytoplankton (i.e. is equal to at least 50% of their daily production) in offshore water during the summer stratification, but not in the spring, when phytoplankton sedimentation losses exceed their grazing mortality.*

Testing the above hypotheses across the spatial and temporal gradients in the lake will allow us to answer the following key questions:

(A) *What structural characteristics of microzooplankton communities are associated with structural/numerical shifts in the higher trophic levels (zooplankton/fish) and seasonal stratification and hypoxia?*

(B) *How do these characteristics affect microzooplankton-phytoplankton trophodynamics (production, herbivory)?*

**Project objectives:** Examine the composition, abundance, and trophodynamics (growth and grazing-mortality rates) of microzooplankton and phytoplankton in conjunction with the planned/ongoing NOAA research on HAB's, hypoxia, and zooplankton and fish distribution in the three basins of Lake Erie.

**Project Approach/Methods:** To address these questions and objective, three research cruises in Lake Erie are proposed in May-September 2005. The exact timing and duration of these cruises will be coordinated with the NOAA GLERL plankton/fish group (Vanderploeg and Ludsin). During each cruise, phytoplankton abundance and composition will be examined in an *underway survey*. In both the west and east basin, we will sample a single nearshore-to-offshore transect (Figure 1). In the central basin, we will sample two nearshore-to-offshore transects, one in the west-central portion, which typically becomes hypoxic (< 2 mg/l), and one in the east-central portion which typically remains oxic (> 4 mg/l) in the hypolimnion year-round. All transects will be sampled both during the daytime and nighttime to explore diel distribution patterns related to the changing habitat conditions (e.g., dissolved oxygen, temperature, etc.). Additional diel sampling will be conducted at the offshore endpoint of both central basin transects (Figure 2) during June (pre-hypoxia), August (during low-oxygen event) to examine how oxygen availability influences vertical distribution and composition of phytoplankton and microzooplankton. During each of these two periods, we will sample both central basin locations (~4 km transects) over a 24-h period (collections made every 4 hours). Diel-sampling locations will be located nearby moorings in the central basin such that we have a continuous, real-time record of physical (e.g., temperature, dissolved oxygen, turbidity, currents) and biological (e.g., chlorophyll, sediment traps) data at our sampling stations.

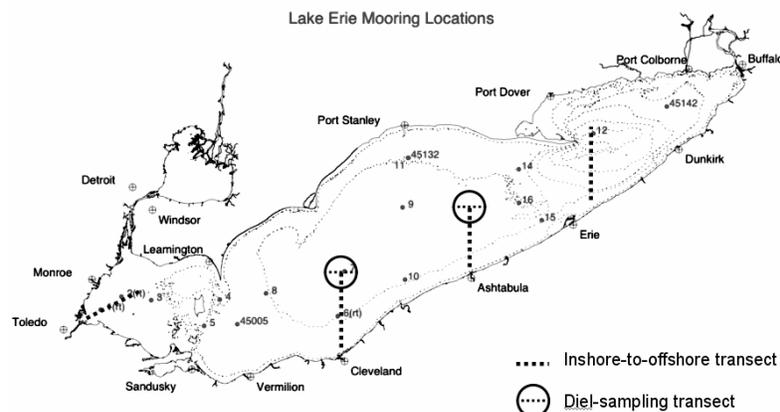


Figure 1. Sampling locations in Lake Erie

We will measure the following parameters: phytoplankton abundance, including cyanobacteria and eukaryotes, and microzooplankton (ciliates and rotifers) abundance and composition. Plankton will be enumerated using an imaging-in-flow system (FlowCAM; Sieracki et al. 1998). This system represents a combination of a flow cytometer and a progressive scan digital camera. Every cell passing through the flow chamber is measured by the FlowCAM and has data

describing ten different characteristics, including time of passage through the laser beam, cell dimensions, equivalent spherical diameter, light-scattering patterns, and chlorophyll and phycoerythrin content. The FlowCAM image processor is aided by a computer program that displays cell data in real time and connects the images to data spreadsheets (Fig.2). All count parameters and images will be stored in software libraries, . We will use a custom redesigned FlowCAM IV equipped with an argon ion blue laser and 0.85 numerical aperture apochromatic optics. This combination provides improved fluorescence detection and resolution. In our test runs, we were able to detect most algal cells  $>1 \mu\text{m}$  (Lavrentyev et al. 2004). The FlowCAM will be used shipboard to provide continuous measurements and quick feedback from surveys and experiments. *At selected sites, the flow-cytometry data will be verified using microscopy and “conventional” high-resolution flow-cytometry (see below).*

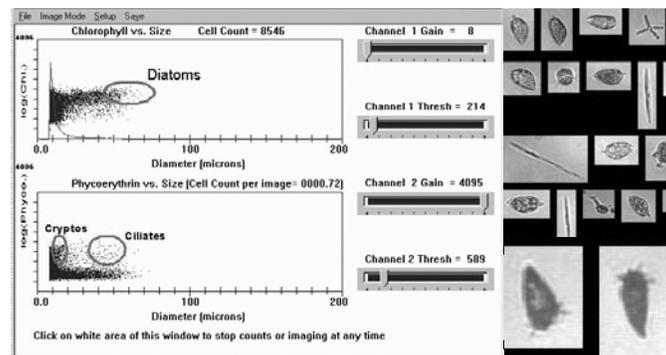


Fig. 1. The FlowCAM scattergram and image library.

*Trophodynamics:* Growth (production)/grazing experiments will be conducted at selected sites/depths representing contrasting conditions with respect to dissolved  $\text{O}_2$ , stratification, and trophic status. We envision at least one detailed experiment per cruise. In each experiment, we will measure the rates of growth and grazing mortality of different groups of phytoplankton and the entire microbial heterotrophic community including microzooplankton, heterotrophic nanoflagellates (HNF), and bacteria. We will combine serial dilution (Landry and Hassett 1982) and conventional size-fractionation (chlorophyll a) with flow-cytometric, microscopic, and fluorometric measurements of plankton. This combination will facilitate data cross-validation and interpretation and allow examination of the responses of natural phytoplankton to grazing gradient. The experimental protocol has been tested and optimized at several nearshore and offshore sites in Lake Erie and the Gulf of Mexico (Jochem et al. 2004; Lavrentyev et al. 2004). Preparation of serial dilution experiments from hypoxic/anoxic waters can alter  $\text{O}_2$  concentrations/re-oxygenate samples. Although aeration of hypoxic samples to  $\text{O}_2$  saturation did not change grazing rates of hypoxic protists (Park and Cho 2002), the manipulations may affect other microbial processes. Therefore, the feasibility of hypoxic serial dilution experiments will be tested using a protocol employed in anoxic Baltic Sea deep-water (Detmer et al. 1993).

*Sample analysis:* HNF's and pigmented nanoflagellates will be preserved with 1.0% (final conc.) formalin, concentrated onto black  $0.8 \mu\text{m}$  pore-size polycarbonate membrane filters, and counted by epifluorescence microscopy following staining with DAPI. A double-band filter set will visualize their DNA and photopigments simultaneously. Microplankton (algae, microzooplankton) will be collected from discrete sites/depth, preserved with 2% Lugol's iodine (final conc.), and counted on an inverted differential interference contrast (DIC) microscope. In addition, colonial protists and micrometazoa (rotifers), not present in sufficient numbers to be

counted in whole water, will be counted from larger samples pre-concentrated by gentle gravity reverse filtration using a 20 µm net. Taxonomic composition of protists will be determined by silver staining (Skibbe 1994). Plankton will be documented using a digital camera.

Heterotrophic bacteria and phototrophic picoplankton from selected water-column samples and serial dilution grazing experiments will be quantified by a FACSort flow cytometer with FACS Loader autosampler from 1% formalin-fixed samples stored in liquid N<sub>2</sub>. Heterotrophic bacteria will be stained with Sybr Green after RNase treatment (Jochem et al. 2004). Cytometric cell counts will be combined with image analysis biovolumes for calculation of bacteria biomass. Preserved bacteria will be stained with Sybr Green, filtered onto 0.2 µm black polycarbonate filters, and cells measured with an image analysis system consisting of a SPOT-2 cooled digital camera (Grade 2 chip) mounted to an epifluorescence microscope and Image Pro software. Phototrophic picoplankton (e.g. *Prochlorococcus* spp., *Synechococcus* spp, small eukaryotic phytoplankton) will be counted unstained on the FACSort and differentiated by their pigment autofluorescence and light scatter signals (Veldhuis and Kraay 2000).

*Data Analysis:* Multivariate statistics (PCA, cluster analyses) will be used to analyze plankton parameters with respect to temporal and spatial variation of major abiotic factors, including seasonal hypoxia. Initial and final concentrations of plankton in experiments will be compared using Student t-test for dependent samples. The growth rates of microbes in control and experimental treatments will be compared using t-test for independent samples. Relationships between pairs of variables (e.g. growth vs. dilution) will be analyzed using linear regression analysis. Bivariate scatterplots will be generated for each regression to check the linearity. If necessary, we will apply non-linear statistics to test the results of dilution experiments. All statistical analyses will be performed using Systat 8.0 (SPSS, Inc).

### **Project Relevance:**

This study will generate a unique data set on the continuous temporal and vertical distribution and dynamics of microplankton in all three basins of Lake Erie and will address the following IYFL goal: “*Determine how hypoxia, exotic species, and HAB’s influence the distribution and productivity of native species, fishery, and biodiversity*”. Microzooplankton have been identified as one of the key research priorities by Lake Erie Research Planning Workshop (March 4-5, 2004, GLERL/NOAA Ann Arbor, MI).

### **Collaboration/Other Project Linkages:**

This study will be conducted in collaboration with GLERL’s scientists, Drs. Henry Vanderploeg and Stuart Ludsin. This collaboration will allow us to compare our underway and vertical plankton measurements with the data obtained using the undulating Plankton Survey System (PSS: optical plankton counter, CTD, fluorometer, and PAR sensor) and BioSonics DT-X acoustics system. In this way, we will generate a continuous record of physical (temperature, oxygen, light levels) and biological (phytoplankton, microzooplankton, zooplankton, forage fishes) variables that could be used in a model. As a part of the ongoing NSF-sponsored microbial observatory project (Lavrentyev and Dr. Joel Duff, University of Akron), we will examine plankton genetic diversity at selected sites. We will also measure growth and grazing mortality rates of picophytoplankton and high- and low-DNA bacteria in collaboration with Dr. Frank Jochem, Florida International University. Additionally, we are planning a set of experiments to examine the hypoxia-nitrogen cycling link in collaboration with the University of Texas scientists, Dr. Wayne Gardner and Mark McCarthy.

**Government/Societal Relevance:**

This project will provide information that is necessary to increase the knowledge Lake Erie key biological processes, improve the existing and future models, and enhance the government's ability to manage Lake Erie resources. Given the size and hydrology of Lake Erie, the results of this study may improve our ability to predict complex and dynamic processes associated with hypoxia in other Great Lakes and the coastal ocean. The scientific community and general public will gain access to the obtained information via electronic media (WWW), seminars, and publications. The proposed research will have an impact beyond the value of the data collected. Students at UA involved in the project will experience a wide range of professional activities and will be involved in all aspects of the research enterprise. At least three relevant courses at UA would draw information from the project (Limnology, Aquatic Ecology, and Environmental Microbiology). The UA is an urban minority-serving institution and has a strong history of recruiting students from under-represented groups in science. The PI is a senior investigator on an NSF-sponsored K-12 education/biodiversity program that focuses on promoting research collaboration between graduate students and high school teachers, which will be able to take part in the laboratory portion of this research.

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### Project timeline

Activity	2005		2006		2007						
	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct-Dec	Jan-Apr	May	Feb
Research/sampling	X	X									
Planning											
Field sampling and experiments			X	X	X	X	X				
Initial sample processing			X	X	X	X	X				
Initial data report									X		
Process samples							X	X	X	X	
Analyze the results							X	X	X	X	
Present the results at IAGLR										X	
Publish the results											X