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Seasonal Hypoxia and the Genetic Diversity of Prokaryote Populations in the Central Basin Hypolimnion of Lake Erie: Evidence for Abundant Cyanobacteria and Photosynthesis

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ABSTRACT. *The reoccurring region of seasonal hypoxia in the central basin of Lake Erie* ("the dead zone") *has been of significant interest to researchers over the past several years. Surprisingly however, no efforts to characterize the endemic microbial community, responsible for the consumption of oxygen in this system, have been published. To understand how the microbial community may be interacting with this event, we have begun to characterize microbial members by using molecular tools. Phycoerythrin-rich cyanobacteria appear abundant and active in a narrow region (~ 1.5 m) below the thermocline during hypoxic conditions, reaching abundances of greater than 10^5 mL^{-1} and being the primary agent releasing 1.5 mg O_2 L^{-1} above the daytime demands in this region. Sequencing of 16S rDNA amplicons, generated with universal eubacterial primer sets, from the Lake Erie's hypolimnion during seasonal oxygen depletion demonstrated that cyanobacteria, most closely related to phycoerythrin-rich* Synechococcus *spp., dominate during rapid drawdown of oxygen (0.083 mg L^{-1} d^{-1} in 2004) in this region. Analyses of another conserved marker of phylogeny (RuBisCO) has been used to confirm the presence of these cell types. Numerous distinct taxa of heterotrophic bacteria are also represented in the 16S library. The results of this study suggest that novel groups of cyanobacteria may persist within the Lake Erie* dead zone *during hypoxic conditions and, along with the heterotrophic community, strongly influence system geochemistry.*

INDEX WORDS: Synechococcus*, dead zone, hypoxia, molecular microbiology, bacteria.*

INTRODUCTION

The Laurentian Great Lakes are no doubt an important resource. With more than 30 million people in their watershed, they are estimated to contain ~18% of the world's potable water supply (Fuller *et al.* 2002). Lake Erie, the smallest of these lakes, has seen significant effects from the ~ 13 million residents that use it as a potable water supply as well as a recreational and economic resource. During the past several decades, alterations due to both anthropogenic and natural factors have led to a renewed interest in the function of this system. One specific area of focus has been the seasonal hypoxia which occurs in the central basin of this lake. The formation of this hypoxic region has been recognized for over 30 years (Charlton and Milne 2004); recent work however has suggested that changes within

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the system (*e.g.,* nutrient loading, lake level, climatology) may be leading to an expansion of this so-called *dead zone*.

Lake Erie is functionally divided into three separate basins (denoted the western, central, and eastern basin). Hypoxia in the hypolimnetic region of the central basin is primarily a function of lake morphology: primary production in the central basin fuels biological carbon export to a physically constrained (1–4 m thick) hypolimnion, which leads to the rapid drawdown of dissolved oxygen when gas exchange is constrained by a stable thermocline. Indeed, much of the formation of this hypoxic region can be explained by limnetic physics. However, given that oxygen concentration in the hypolimnion is a function of net oxygen consumption (respiration > photosynthesis) it becomes necessary to better understand the metabolic potential of the microbial community. To address this, we first must understand the composition and dynamics of both the phototrophic and heterotrophic members of this consortium.

Insight into the microbial ecology of the Lake Erie ecosystem seriously lags behind studies of both coastal and offshore marine systems (*e.g.,* DeLong 1997) as well as many other lakes (*e.g.,* Giri *et al.* 2004). While historical information on the diversity and activity of nano- and microplankton based on classic morphological studies are rife in the literature, characterization of prokaryotic picoplankton (*e.g.,* Xu and Tabita 1996, Ouellette *et al.* 2006) and heterotrophic prokaryotes—including Archaea (Keough *et al.* 2003) are at best limited, and generally constrained to estimates of abundance (*e.g.,* DeBruyn *et al.* 2004) and activity (Hwang and Heath 1997, Wilhelm and Smith 2000). Indeed, among these studies we are unaware of any information on the genetic diversity of hypolimnetic prokaryotic communities, which is potentially quite different from surface populations. To this end we felt that it was important to gain some insight into the prokaryotic community structure of the central basin hypolimnion during the transition to hypoxia. As such phylogenetic analysis, based on the metabolic capabilities of known microorganisms, is a useful tool that can provide a first glimpse into the biogeochemical potential of the community.

In this study we provide information on water samples collected in 2002 which were used to generate a large 16S rDNA library. Results from this work as well as from samples collected during research cruises in 2003, 2004, and 2005 were analyzed and compared to physicochemical and biological parameters collected in the central basin. Conclusions drawn from these data provide a first step toward understanding the critical linkage between oxygen producers and consumers in the central basin hypolimnion.

METHODS AND MATERIALS

Samples were collected during research cruises on the *CCGS Limnos* in July 2002, 2003, 2004, and 2005 (Fig. 1—Map) using 10-L Niskin bottles mounted on a rosette system. Details of water column structure and physiochemistry (temperature, dissolved oxygen, transmissometry) were collected with the ship's CTD equipment at each location. Integrated contour plots of temperature and oxygen were generated from samples collected on 13 different sampling dates (between 15 April and 13 September 2004) using SigmaPlot 9.0 (Systat Software Inc). Shifts in daily water column oxygen concentrations were determined from samples taken ~ hourly in August 2005 at station 84. Additional casts were made with a SeaBird SBE-9 equipped with a SeaTech fluorometer to characterize the vertical distribution of chlorophyll *a* fluorescence.

Nutrient Measurements

Nutrient concentrations (NO_3^- + NO_2^-, NO_2^-, total dissolved [< 0.45-µm] nitrogen, total dissolved [< 0.45-µm] phosphorus) were measured in whole water samples. Measurements of nutrient concentration were made at the National Laboratory for Environmental Testing (Environment Canada) using standardized techniques (NLET 1994). Sample pre-processing (filtration) was completed on the ship and samples were stored at –20°C prior to analysis.

Epifluorescent Enumeration of PE-rich *Synechococcus*

Total PE-rich *Synechococcus* were determined in samples collected in July and August 2005 by direct enumeration of autofluorescent cells as previously described (Caron *et al.* 1985). In brief, cells from 10 mL of glutaraldehyde fixed samples (2% v/v, final) were collected onto 25 mm-diameter, 0.20-µm nominal pore-size black polycarbonate filters and these filters mounted on glass slides. Cell densities were determined using a Leica DMXRA epifluorescence microscope: in each case at least 20 fields of view or 200 cells were enumerated for duplicate preparations of individual samples.

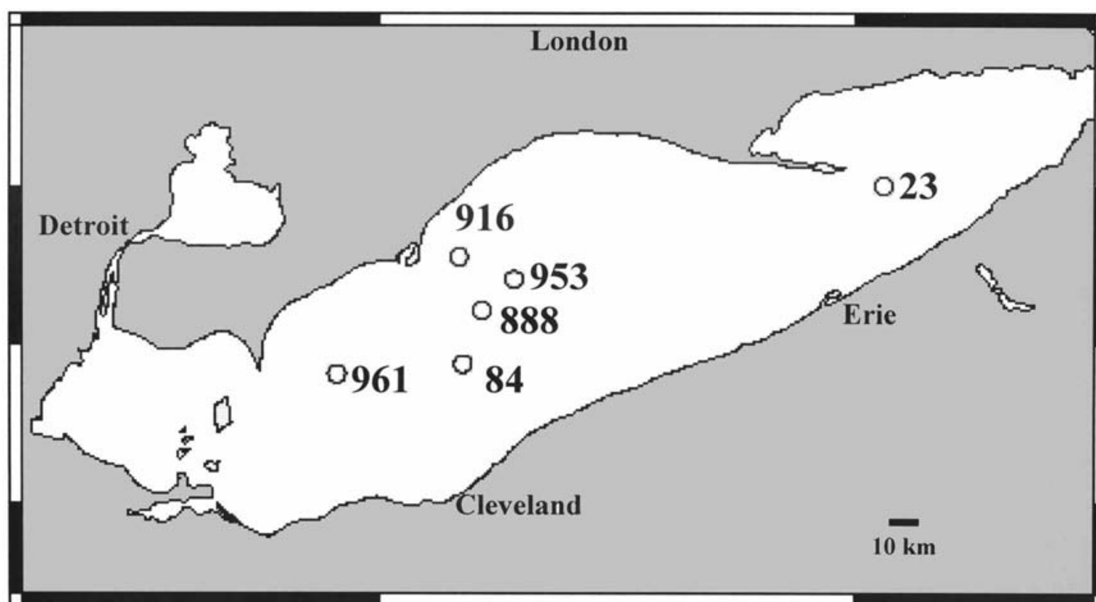


FIG. 1. Location of stations within Lake Erie that were occupied for this study.

Microbial Community Structure

Samples (100 mL) for the analysis of microbial community structure were collected onto 0.2-μm polycarbonate filters by filtration and samples stored at –20°C until analysis. High molecular weight DNA was quantitatively extracted from filters as previously described (Rinta-Kanto *et al.* 2005). Sequence analysis for community diversity was performed on a 2002 sample using universal 16S rDNA primer sets. PCR was performed using universal eubacterial primers 46F (GCYTAACACATGCAAGTCGA) (Kaplan *et al.* 2001) and 519R (TTATTACCGCGGCKGCTG) (Lane 1991; W. Jeffrey, *pers. comm.*), which amplify a fragment of the 16S rDNA gene (using the *Escherichia coli* numbering system) approximately 473 bases long. The primer pair 46F and 536R (nearly identical to 519R of Lane 1991) listed in Kaplan *et al.* (2001) has been demonstrated to hybridize with 90% of 1,500 bacterial sequences tested (90% for 46F and 99% for 519R).

For the analysis of specific subgroups, PCR primers specific to the Ribulose-1, 5-bisphophate carboxylase-oxygenase (RuBisCO) large subunit gene (*rbcL*) sequences (Bachoon *et al.* 2001) were employed. For the quantification of cyanobacteria 16S rDNA genes in samples, the primers and TaqMan probe set described by Rinta-Kanto *et al.* (2005) was employed. Samples were processed using a MJ DNA Engine Opticon System (BioRad) and standardized to the single copy plasmid standard previously described (Rinta-Kanto *et al.* 2005).

Sequences for the 16S rDNA library were determined at the Clemson University Genomics Institute while sequences from *rbcL* were obtained from the Molecular Biology Resource Facility at the University of Tennessee. Sequences have been deposited in GenBank under accession numbers DQ167589 to DQ167722.

After the removal of primers, sequences were aligned with Clustal W (Thompson *et al.* 1994), manually checked and edited in BioEdit (Hall 1999). BLAST analysis was conducted through the NCBI website (Altschul *et al.* 1990) to compare individual sequences to known sequences. Neighbor-joining analysis was conducted using Kimura 2-parameter distance and complete deletion, using the Mega 3.1 software package (Kumar *et al.* 1994). Only positions that were unambiguous and present for all sequences were utilized. Bootstrapping employed 5,000 replications. Phylogenetic reconstructions was also completed and bootstrapped (100 iterations) using the Maximum Parsimony analysis within the Mega 3.1 software package.

RESULTS

Water Column Characteristics

Profiles of water column structure at master station 84 in the central basin of Lake Erie, collected

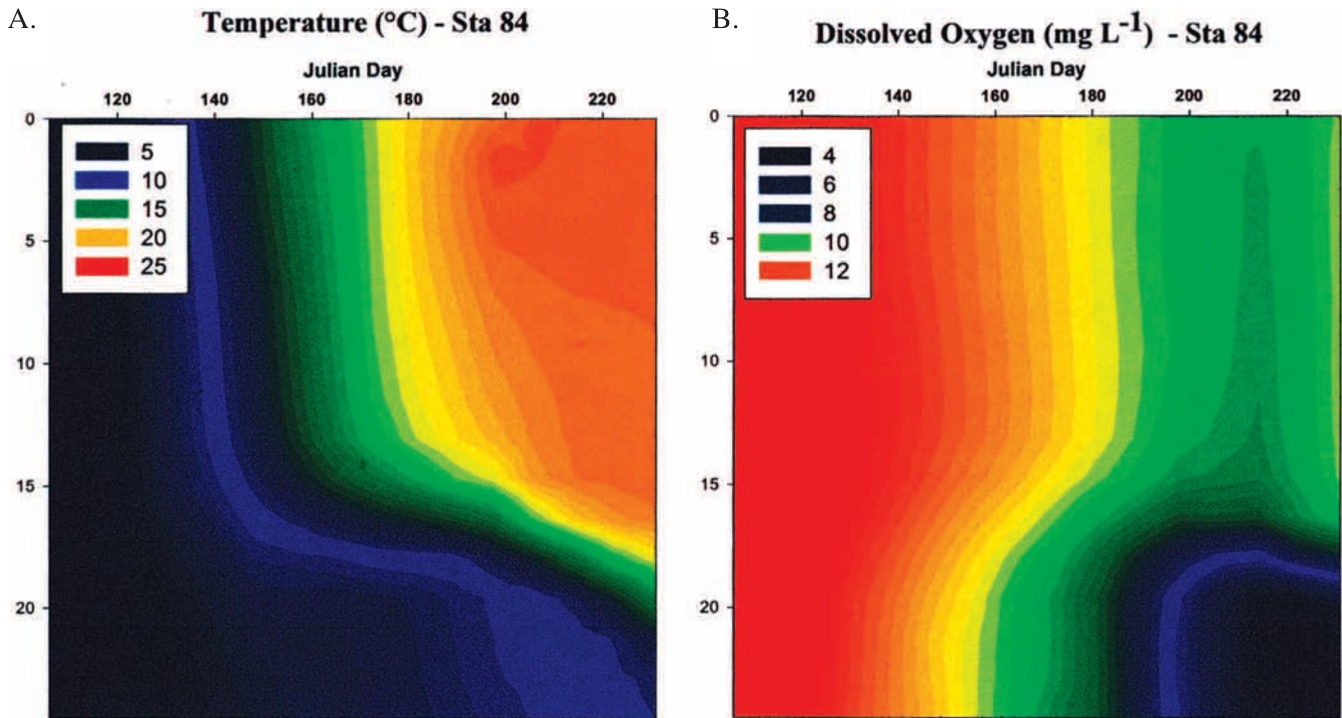


FIG. 2. Seasonal water column oxygen depletion in the central basin of Lake Erie during April–September 2004. Water column oxygen profiles were used to generate integrated contour plots from data collected on 13 separate dates for temperature (A.) and dissolved oxygen (B.) during the 2004 field season.

between 15 April and 26 August 2004, have allowed for a reconstruction of the seasonal onset of both stratification and hypoxia in the system (Fig. 2). As typical of our observations since 1998, thermal stratification of the water column became apparent by mid-June at both the master station (Sta. 84) and other stations in the central basin (data not shown). Coupled with the onset of stratification, water column oxygen levels decreased to hypoxic (< 2 mg L^{-1}) levels. Water column oxygen levels returned to oxic by our 13 September 2004 sample (data not shown). Water column thermal structure had dissipated by this point and profiles of both temperature and oxygen suggested a homogenously mixed water column.

To characterize net oxygen consumption rates during this period, mean hypolimnetic oxygen concentrations for the bottom 4 meters of water (up to 0.5 m from the sediments) were determined for each cast (Fig. 3) and converted to percent saturation based on direct estimates of temperature. Analysis of the rate of decrease (*vis a vis* drawdown) of oxygen concentrations suggested a net oxygen consumption rate of 83 µg O_2 L^{-1} d^{-1} (r^2 = 0.977) during the 15 April to 26 August period.

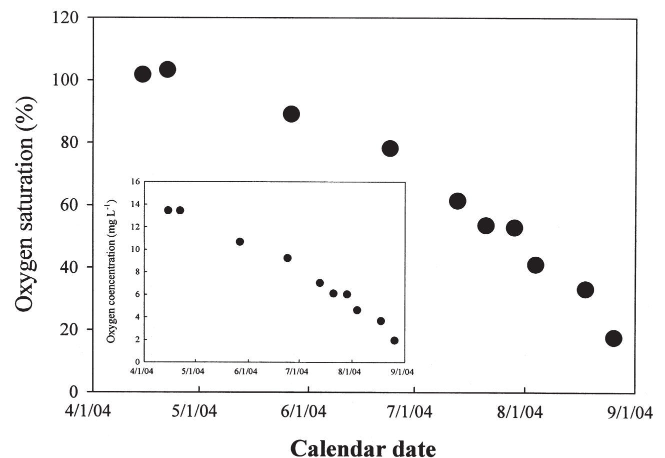


FIG. 3. Hypolimnetic oxygen (% saturation) determined from a regression analysis of the mean dissolved oxygen concentration in the water column for the bottom 4 m (not including the last 0.5 m due to the potential for bioturbation). Inset provides the same information with oxygen given in concentration. These data were used to determine the rate of net oxygen depletion, which was 0.083 mg L^{-1} d^{-1} (r^2 = 0.977).

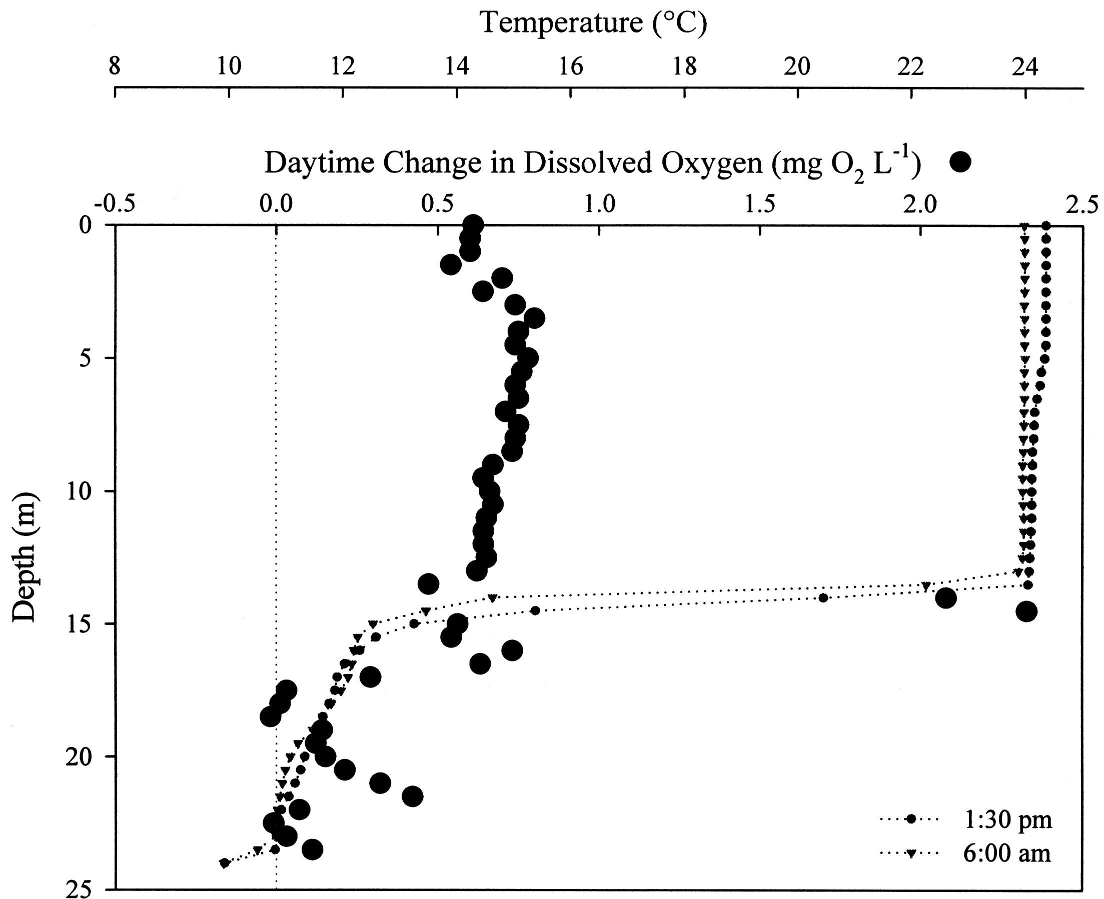


***FIG. 4.** Differential oxygen concentrations between near solar noon (13:30) and early morning (06:00). Significant increases in water column oxygen during daylight hours are noted at* **ca** *15 m. Water column thermal structure for both times (small inset symbols) is also given to demonstrate water column stability during observations.*

As well as considering the seasonal process of net oxygen consumption in the hypolimnion, we were also interested in diel variations that may be occurring due to biological processes. Figure 4 illustrates the analysis of one set of samples collected during August 2005. The difference in dissolved oxygen in water column profiles collected at ~ solar noon (1:30 pm local time) and just at sunrise (6:00 am) was calculated to determine if oxygen production in the water column was significant. As expected, water column oxygen production in the epilimnion was significant, with the concentration during the daytime being 0.5 mg L^{-1} higher than the concentration at sunrise. Perhaps more interesting, the oxygen concentration directly below the thermocline was 2–2.3 mg L^{-1} higher during the daytime, indicative of a rapidly photosynthesizing population. Examinations of the rate of probe response to changes in the water column oxygen suggested that the probe could respond to net changes of 4 mg L^{-1} within 0.5 m depth, (not shown) implying that these differences were not a function of instrument limitation.

Cyanobacterial Abundance in the Water Column

Direct counts of *Synechococcus* from samples collected in July and August 2005 demonstrate their abundance in the water column (Table 1). *Synechococcus* were consistently detected, with higher abundances near the thermocline and in the hypolimnion relative to the epilimnion noted for samples collected during July. This distribution is illustrated in Figure 5, where the peak in *Synechococcus* direct counts corresponds with both the fluorescence maximum in the hypolimnion as well as the peak in oxygen production associated with the base of the thermocline.

TABLE 1. Densities of* Synechococcus *from direct counts of samples collected at stations in Lake Erie during July and August of 2005. Estimates are mean of duplicate samples (± range). BQL – cell densities are below quantifiable levels* (ca *240 cells mL*$^{-1}$*).

	JULY 2005 SAMPLES			AUGUST 2005 Samples	
station	Z (m)	***Synechococcus* (mL^{-1})**		Z (m)	***Synechococcus* (mL^{-1})**
84 (Jul 13)	5	9.95 (± 3.67) × 10^3	84 (24 Aug)	1	2.83 (± 0.09) × 10^5
	13.5	5.14 (± 2.71) × 10^4		5	1.20 (± 0.08) × 10^5
	15	4.73 (± 3.52) × 10^5		15	1.02 (± 0.10) × 10^5
	16.5	2.46 (± 0.41) × 10^5		16.3	2.70 (± 0.05) × 10^4
	20	3.87 (±0.03) × 10^4		22	1.23 (± 0.16) × 10^4
888 (Jul 14)	5	6.49 (± 9.18) × 10^2	888 (25 Aug)	1	2.15 (± 0.30) × 10^5
	17	5.13 (± 0.89) × 10^4		16	1.64 (± 0.09) × 10^5
				20	2.74 (± 0.11) × 10^4
916 (Jul 14)	5	BQL	953 (24 Aug)	1	1.81 (± 0.03) × 10^5
	11	5.63 (± 0.61) × 10^3		10	1.60 (± 0.05) × 10^5
	19.5	3.35 (± 0.21) × 10^4		17	7.27 (± 0.10) × 10^4
				20	1.47(± 0.10) × 10^4
23 (Jul 11)	5	BQL	961 (25 Aug)	1	3.04 (± 0.18) × 10^5
	12	BQL		9.5	2.50 (± 0.40) × 10^5
	17	BQL		11	3.48 (± 0.23) × 10^4
	25	1.75 (± 0.21) × 10^5		15	1.25 (± 0.11) × 10^4
	40	1.11 (± 0.89) × 10^5			

As a separate line of evidence for cyanobacterial abundance in the hypolimnion, we employed a quantitative PCR approach for total cyanobacteria with samples collected in 2004 (Fig. 6). This approach is independent of and not skewed by potential observational biases of a microscopist. As with the direct count data, the cyanobacterial abundance appears higher in the hypolimnion of the central basin in July and August samples. Hypolimnetic peaks were also observed in chlorophyll *a* fluorescence in the water column during these periods, suggesting the presence of a significant picoplankton community at depth (Fig. 6).

Microbial Diversity—16S rDNA Library

As a first attempt to characterise the microbial community from the central basin hypolimnion, we cloned and sequenced multiple 16S rDNA amplicons from DNA collected during O_2 drawdown at station 84 in July 2002. This sample was chosen primarily due to a red discoloration associated with the filter after collection of water samples at 18 m depth. Lake Erie dead zone (LEDZ) sequences most closely associated with heterotrophic and autotrophic prokaryotes were seen in this library. The observation of filter discoloration and microscopic counts (above) suggested that this region was dominated by phycoerythrin-rich picocyanobacteria, which was confirmed using these molecular tools (Fig. 7, Table 2) as 98 of the 134 sequences most closely aligning with *Synechococcus* MH301, a PE-rich cyanobacterium isolated from Lake Mondsee (Crosbie *et al.* 2003). Among these, 33 identical sequences typified by LEDZ11 (accession number DQ167597) as well as 16 identical sequences typified by LEDZ100 (accession number DQ167591) represented > 36% of the total library. Additional cyanobacterial LEDZ sequences fall within the picocyanobacterial clades defined by marine and freshwater *Synechococcus* spp. and *Cyanobium* spp. (Fig. 7). Twelve other sequences clustered tightly together with two other *Synechococcus* isolates (BO9404 and MW10). Outside of the cyanobacteria the amplicon sequences were most similar to a broad range of heterotrophic bacteria having diverse metabolic capabilities (see Table 2). The taxonomic classes represented are common to freshwater aquatic environments and lake sediments, and likely contain facultative anaerobes capable of tolerating seasonal shifts in oxygen levels. Within this group, most sequences had high homologies to known isolates of heterotrophic bacte-

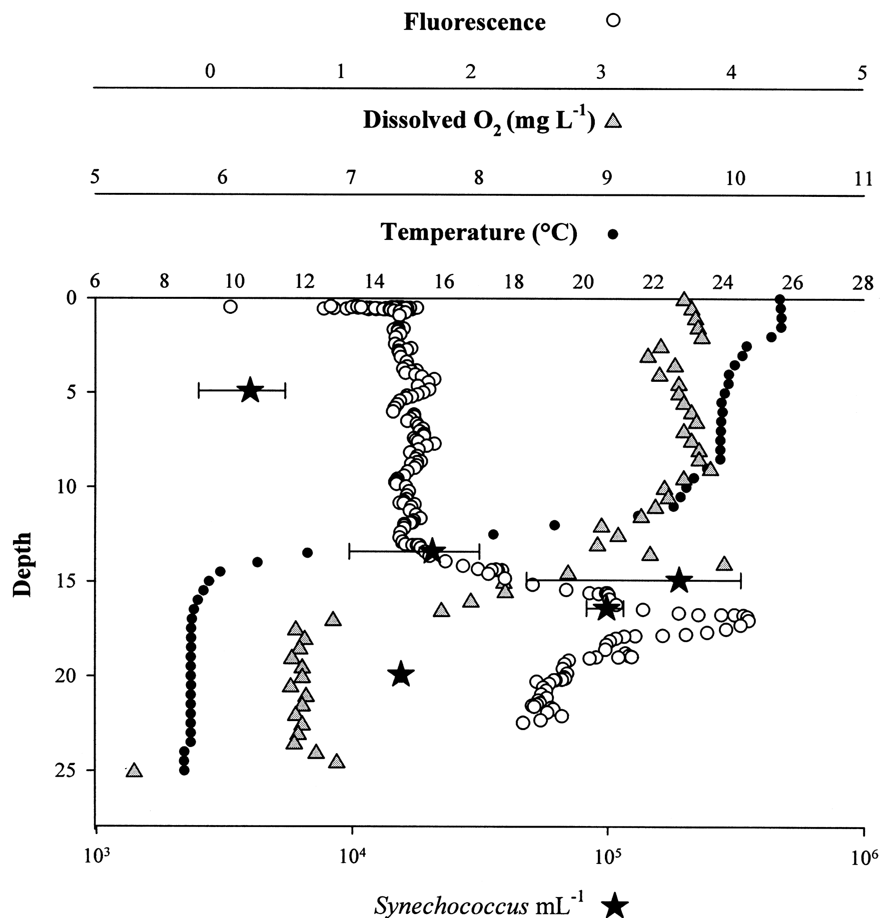


FIG. 5. Profile from July 2005, demonstrating the distribution of* Synechococcus *(determined by direct counts of autofluorescent cells containing phycobilins). Peaks in water column chlorophyll* a *fluorescence and hypolimnetic oxygen (determined on different profiling instruments) coincide with the peak abundance of cells.

ria. However, sequences which were most closely related to sequences common to the environmental genetic databases (uncultured actinobacter and uncultured planctomyces) were also found.

Cyanobacterial Diversity—rbcL

The above data, combining phylogenetics with qPCR and direct counts, indicated that a substantial number of cyanobacteria may be present in the central basin hypolimnion. As such we felt it prudent to confirm their presence using an unrelated genetic marker. Specifically, due to the phylogenetic affinity of some 16S sequences to marine clade cyanobacteria, we tested for the presence of sequences diagnostic for marine-like *Synechococcus* spp. Sequence analysis was undertaken using primers for the (RuBisCO) large subunit gene (*rbcL*). Although only two sequences from the July 2002 sample were analyzed, the results clearly indicate that the amplicons were from the marine form 1A cluster that also includes marine *Synechococcus*, freshwater *Cyanobium,* and *Prochlorococcus* (Fig. 8).

DISCUSSION

At the onset of this study we posed the following question: Do the microbial communities of the Lake Erie "*dead zone*" represent unique populations that are active during periods of favorable dissolved oxygen concentrations, or do they represent a meta-

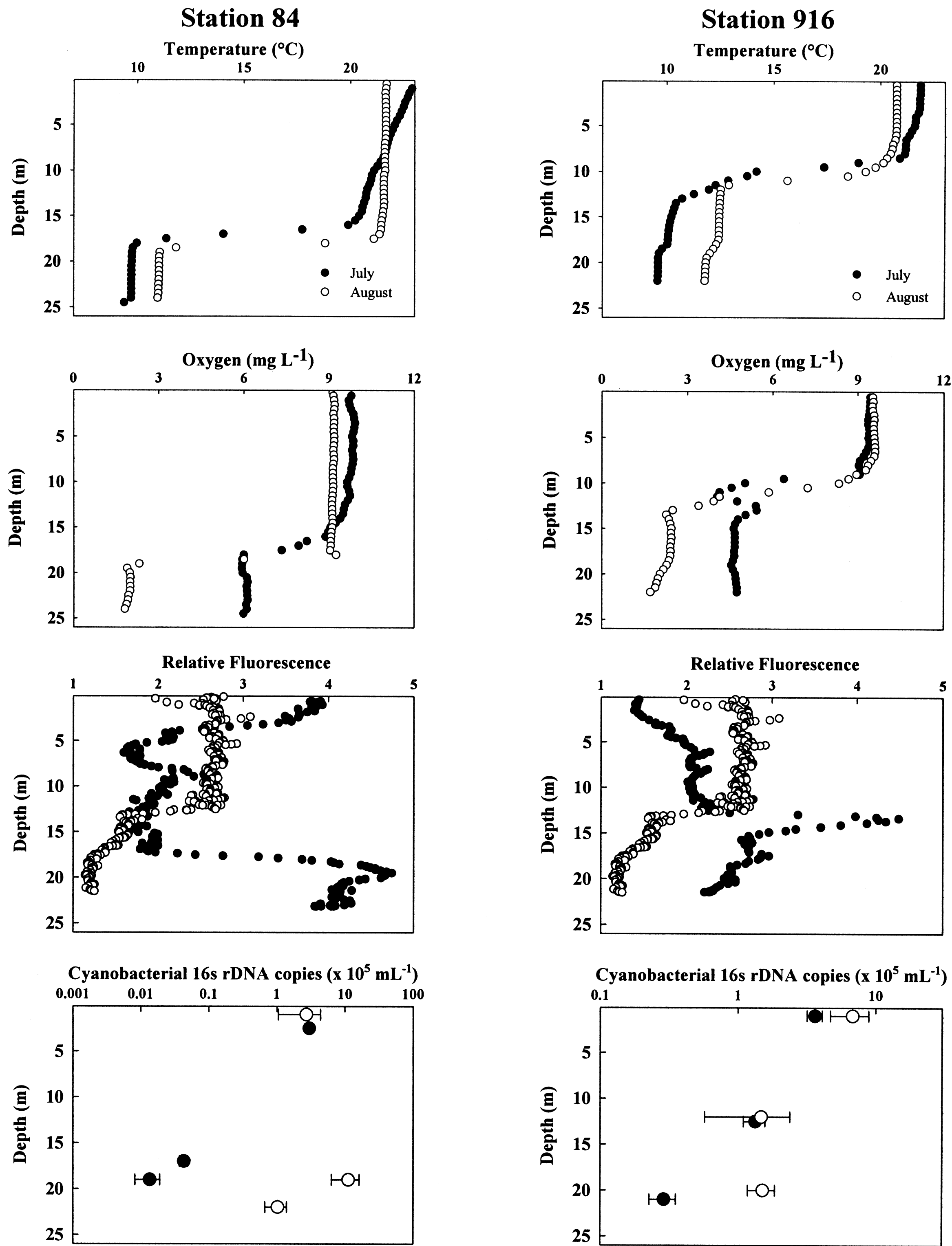
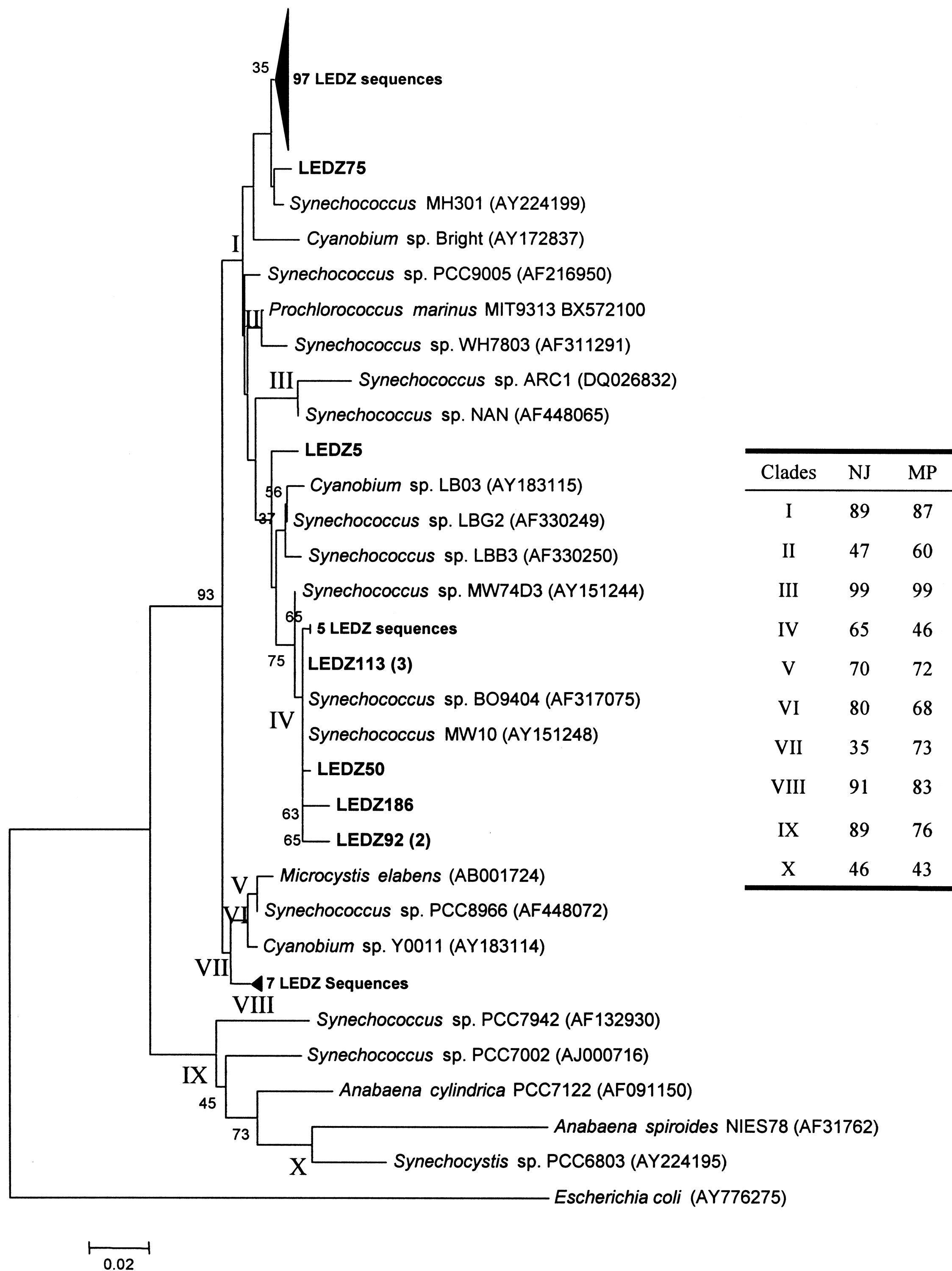


FIG. 6. Water column parameters and quantification of cyanobacteria during 2004 observations of the central basin. Temperature, chlorophyll a, in situ *fluorescence, and cyanobacterial abundance (inferred from 16S rDNA sequence abundance) during sampling in July and August of 2004.*



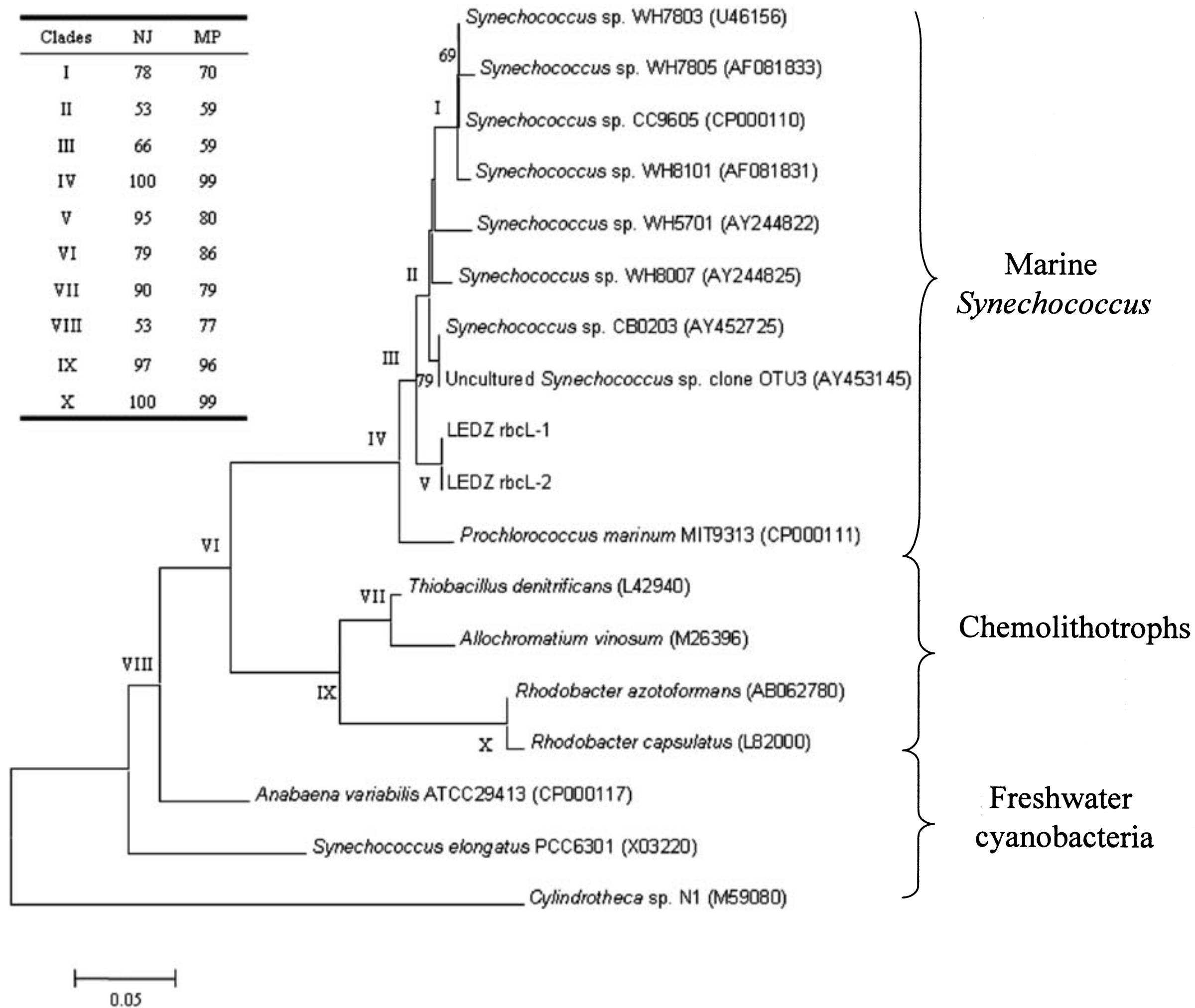
Clades	NJ	MP
I	89	87
II	47	60
III	99	99
IV	65	46
V	70	72
VI	80	68
VII	35	73
VIII	91	83
IX	89	76
X	46	43

FIG. 7. Neighbor-joining analyses of cyanobacterial sequences generated using universal eubacterial primers. PCR was performed on each sample in triplicate, using universal eubacterial primers 46F (Kaplan et al. *2001) and 519R (Lane 1991, W. Jeffrey,* pers. comm.*), which amplify a fragment of the 16S rDNA gene. Results demonstrate a high frequency of a single group of* Synechococcus *in the library. At nodes where dendrogram structure was conserved, bootstrap values are given in the inset table for estimates using neighbor-joining (NJ) and maximum parsimony (MP) approaches. Other values are bootstrap values from NJ method with percentages < 30 removed.*

TABLE 2. Phylogenetic affiliations and metabolism of heterotrophic prokaryotes identified in a universal eubacterial 16S rDNA library.

LEDZ Sequence	Organism	% identity	Taxonomic group	Growth	Comments
LEDZ 14	Uncultured actinobacteria	93%	Firmicutes (high GC, gram positive)	facultative anaerobic*	Produce secondary metabolites (*e.g.,* antibiotics)
LEDZ 18	Uncultured planctomycete clone LiUU-5-103	99%	Planctomycetes (gram negative)	anaerobic	ANAMMOX metabolism—generates N_2 in the presence of NH_4^+ and NO_2^-
LEDZ 19	*Terrabacter* sp.	96%	Firmicutes (high GC, gram positive)	aerobic	Can degrade chlorinated aromatics
LEDZ 27	*Nordella oligomobilis*	95%	α-proteobacteria	aerobic	Rhizobiales; *Nordella* sp. often symbiotic with amoebae
LEDZ 77	*Brevundimonas* spp.	99%	α-proteobacteria	aerobic	Caulobacteriaceae family
LEDZ 95	*Acidobacterium* sp.	97%	Firmicutes (gram positive)	aerobic	Sediment / soil bacteria, prefers low pH
LEDZ 114	*Methylobacter psychrophilus*	99%	γ-proteobacteria	aerobic	Type I methanotroph—consumes methane aerobically
LEDZ 118	*Acinetobacter* spp.	100%	γ-proteobacteria	facultative anaerobic*	Can be opportunistic pathogen
LEDZ 127	*Caulobacter* sp.	97%	α-proteobacteria	aerobic	Growth largely in oligotrophic environments
LEDZ 133	*Chryseobacterium* sp.	97%	flavobacteria	aerobic	Can be opportunistic pathogen
LEDZ 150	*Gledibacter algens*	99%	flavobacteria	aerobic	*G. algens* is psychrophilic (Antarctic lakes), although mesophilic *Gelidibacter* have been described
LEDZ 151	*Cryptosporangium aurantiacum*	94%	Actinomycetales	aerobic	Common in soil and lake muds

*some species exhibit anaerobic growth



Clades	NJ	MP
I	78	70
II	53	59
III	66	59
IV	100	99
V	95	80
VI	79	86
VII	90	79
VIII	53	77
IX	97	96
X	100	99

FIG. 8. Phylogeny from Ribulose-1, 5-bisphophate carboxylase-oxygenase (RuBisCO) large subunit gene (*rbcL*) sequences. Neighbor-joining tree constructed from aligned amino acids (138 residues). Results suggest that hypolimnetic populations of cyanobacteria in Lake Erie have an affinity to the* Synechococcus *Marine Cluster and as such present a second line of independent evidence that these* Synechococcus *are in the monophyletic marine group. At nodes where dendrogram structure was conserved, bootstrap values are given in the inset table for estimates using neighbor-joining (NJ) and maximum parsimony (MP) approaches. Other values are bootstrap values from NJ method with percentages < 50 removed.

bolically plastic group that undergoes biochemical switches to compensate for dissolved oxygen levels? The results of this study provide, for a first time, insight into the phylogenetic profile of the microbial communities that persist during the development of seasonal hypoxia in Lake Erie. Several key observations, including the mixture of abundant phototrophs with heterotrophic aerobes and facultative anaerobes, suggest that biogeochemical processes in this system are tightly coupled—indeed the observations that significant populations of oxygenic cyanobacteria persist in this region and that oxygen is actively produced just below the thermocline during hypoxia suggest that rapid oxygen cycling must occur. This idea is supported by the presence of obligate aerobes in the system in spite of low levels of dissolved oxygen, and the daily shift in oxygen concentration that we have documented during high resolution profiling of the water column. We discuss these preliminary obser-

vations below with respect to the potential role of picocyanobacteria in the central basin hypolimnion.

Hypolimnetic Oxygen Drawdown and Diel Production in Lake Erie's Central Basin

Significant interest in the popular press has focused on the seasonal formation of the "*dead zone*" in Lake Erie. Somewhat a misnomer (but now an accepted name), microbial activity is sufficient to consume most of the oxygen in this constrained volume of water within a few weeks of thermal stratification: indeed the term "*too alive*" may be more appropriate. As such, the central basin hypolimnion establishes the potential for a series of unique metabolic activities to occur: sufficient light for photosynthesis (especially in recent years as the central basin has moved toward oligotrophic status (Charlton and Milne 2004), combined with ample inorganic nutrients and transient oxygen, establishes a series of possibilities for system metabolism.

During our observations seasonal oxygen consumption in the central basin occurred at a rate of 83 µg L^{-1} d^{-1} (0.11 mmol m^{-3} h^{-1}). Given that the hypolimnetic volume (*i.e.*, < 20 m) of the central basin of Lake Erie can be estimated at *ca* 2.03×10^{10} m^3 (W.M. Schertzer, personal communication), this translates into a total consumption 5.35×10^7 mol O_2 d^{-1} in the central basin hypolimnion. One assumption is that the majority of this oxygen demand is driven by the vertical transport of epilimnetic produced carbon into the hypolimnion. Estimates for primary production rates in the epilimnion vary dependent on the method used to make them, but are more than sufficient (*ca* 0.1–90 mM O_2 m^{-3} h^{-1}) across a volume of 2.9×10^{11} m^3 (assuming the volume above 20 m) or 2.4×10^{11} m^3 (assuming the volume above 15 m) to provide this much carbon (Ostrom *et al.* 2004). It should be noted that at least some of this primary production is potentially exported laterally to the eastern basin of the Lake (Boegman *et al.* 2001) or consumed by the active microbial community in the epilimnion while being vertically exported (DeBruyn *et al.* 2004). Regardless, the excess of epilimnetic C production suggests that O_2 produced in the hypolimnion by photosynthetic organisms may never accumulate, as primary production settling into this confined system will fuel bacterial oxygen consumption.

To determine if there was a diel signature in hypolimnetic oxygen production, we carried out a series of hourly profiles of oxygen concentrations in the water column to see if we could detect hypolimnetic oxygen production. In previous years we had noticed a small but consistent increase in oxygen concentration just below the thermocline during the daytime, and this was apparent again during the July cruise at *ca* 15 m (see Fig. 5). To determine the potential effect of this production, we wanted to determine whether significant oxygen could accumulate during the daytime (when photosynthesis would occur). To do this we determined the difference in solar noon water column oxygen concentration relative to early morning (pre-sunrise) oxygen concentrations (bacterial activity through the night should have significantly depleted oxygen concentrations). One concern with this approach is in the calibration of equipment between profiles: indeed it could be argued that the 0.5 mg L^{-1} higher oxygen concentrations in the epilimnion during the daytime sampling relative to the pre-sunrise sampling may represent a calibration error. If we assume this though, there was still an enhancement of oxygen concentrations across 1.5 m of depth (from *ca* 15–16.5 m, Fig. 4) above and beyond the surface difference. Averaged across the cross-sectional area of the lake's central basin for these depths (a volume of 1.25×10^{10} m^3, W.M. Schertzer, personal communication) this would represent a production of 1.875×10^{13} mg (18,750 tonnes) of O_2 above the demand in this region that would be available to the rest of the hypolimnion *via* diffusion, or within this region after sunset. As such, not only does this information represent an important insight for those interested in modeling Lake Erie physiochemistry, it also suggests the presence of an active photosynthetic population.

Cyanobacteria in the Hypoxic Hypolimnion—Three Lines of Evidence

Perhaps the most significant result of this study is the demonstration that a significant population of cyanobacteria persist within a thin layer at the interface of the thermocline and hypolimnion of the central basin. Indeed, the high percentage of cyanobacterial 16S rDNA sequences within the entire library during the onset of hypolimnetic hypoxia in 2002 strongly suggested that hypoxia stimulates the establishment of the picocyanobacterial deep chlorophyll layer. As evidenced by fluorometric profiles and direct counts, it appears that the majority of these cells are constrained to a narrow band that persists just below the thermocline. At

this depth the low level of PAR (*ca* 1% of ambient) combined with ample inorganic nutrients from the mineralization of carbon exported from the surface could drive this community. During the drawdown of oxygen, we have observed historically a decrease in ambient nitrate and an increase in ammonium that could fuel this population of cells. For example, during the 2004 field season dissolved NH_4^+ concentrations in the hypolimnion of sta. 84 (1.41 µM) and sta. 916 (2.17 µM) exceed surface values by *ca* 2.2-fold (0.65 and 1.00 µM, respectively). In contrast the nitrate concentrations were 0.55- to 0.76-fold lower in the hypolimnion than in surface waters. As such, the NH_4^+: NO_3 ratio in the hypolimnion (0.6–0.9) may favor NH_4^+ assimilation while the NH_4^+: NO_3 ratio in the epilimnion of these stations (0.2–0.3) would likely favor NO_3 assimilation. This is in contrast to station 23 in the eastern basin. With a fully oxygenated hypolimnion, NO_3 concentration was higher in the hypolimnion than surface waters (5.37 µM *vs.* 3.68 µM) while the ammonium concentration was inverse (1.59 µM in the hypolimnion *vs.* 2.11 µM in the epilimnion). Given evidence that some cyanobacteria (*Synechococcus* and *Prochlorococcus* spp., Moore *et al.* 2002) cannot use nitrate, changes in water column N speciation might drive community shifts during the onset of seasonal hypoxia.

Numerically, PE-rich *Synechococcus* were abundant in samples analyzed by epifluorescence, ranging from 10^3–10^5 cells per mL in our samples. The overall abundance of cyanobacteria (which would also include phycocyanin rich and filamentous strains not included in the above estimate) was also high, with > 10^5 copies of their 16S rDNA gene sequence detected by quantitative PCR during visits to two sites in 2004. Given that most picoplanktonic genomes completed to date in GenBank contain *ca* 2–3 copies of the 16S rDNA gene, the total genomic equivalents (*i.e.,* cell densities) in our samples is on par with that measured for total cyanobacteria using this approach in other studies (*e.g.,* Becker *et al.* 2002).

Heterotrophic Prokaryotes in the Central Basin Hypolimnion—Preliminary Insight

Given the predominance of cyanobacterial sequences in our data set, we are left with only a minimal number of sequences which represent the heterotrophic prokaryotes in this system. During our 2002 sampling, water column oxygen was at *ca* 70% saturation: as such we expected a mix of aerobic and facultative anaerobic microbes which we anticipated would be driving oxygen consumption. One potential problem with this hypothesis is that it excludes the role of benthic microbes in the consumption of hypolimnetic oxygen—indeed, it has previously been suggested that the activity of microbes in the sediment may be sufficient to account for the consumption of the hypolimnetic oxygen (Leutheuser 1979).

Among the sequences, perhaps the most intriguing is the presence of a sequence indicative of of a *Planctomycetales*-related clone in the sample. Members of this phylogenetic group have been previously demonstrated to be capable of a series of novel biogeochemical reactions, including the ability to oxidize ammonia under anaerobic conditions (Strous *et al.* 1999). This process requires both ammonia and nitrite in an anaerobic environment, resulting in the production of dinitrogen gas (Strous *et al.* 1997). Although the ANAMMOX reaction still remains a novel observation in natural systems, similar studies, citing conditions of low oxygen and abundant ammonia and nitrite, have suggested the presence of these organisms and the potential for this process to occur (Mills *et al.* 2004). Indeed, ammonia oxidation to N_2 may in part explain why Lake Erie has not seen the accumulation of dissolved nitrogen (presumably due to anthropogenic effects) that has been observed in Lake Superior (Ivanikova *et al.* 2005).

Yet another interesting cluster of sequences (LEDZ 19 and LEDZ 174) appears to be closely related to a *Terrabacter* sp. Organisms in this group have been shown to de able to metabolize chlorinated-aromatic compounds (Aislabie *et al.* 1999). These anthropogenic compounds have been historically shown to accumulate in the Laurentian Great Lakes, including Lake Erie (Czuczwa and Hites 1986). Research has demonstrated that two distinct processes (one aerobic and one anaerobic) are involved in the breakdown of polychlorinated biphenyls (PCBs) (Abramowicz 1995). Anaerobic PCB dechlorination, has been shown to convert highly chlorinated PCBs to lightly chlorinated *ortho*-enriched congeners. The products from this anaerobic process are then readily degradable by a wide range of aerobic bacteria. As such, the presence of the *Terrabacter*-like sequences and those of other potential PCB biotransformers (J.L. Bouzat, pers. com.) suggest that the aerobic / anaerobic cycling within the central basin during seasonal hypoxia may indeed play a role in the natural bioremediation of anthropogenic contaminants.

Moreover, the production of trace amounts of oxygen by oxygenic phototrophs (*vis a vis* the abundance cyanobacteria we have identified) suggests that during seasonal hypoxia there may be a sufficient redox gradient within the hypolimnetic water column for these processes to occur simultaneously. Whereas this remains hypothetical at this point, it obviously begs the need for further study of the microbial community both before and during seasonal hypoxia.

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REFERENCES

Abramowicz, D.A. 1995. Aerobic and anaerobic PCB biodegradation in the environment. *Environ. Health Perspectives Suppl.* 103:S5.

Aislabie, J., Davison, A.D., Boul, H.L., Franzmann, P.D., and Jardine, D.R. 1999. Isolation of *Terrabacter* sp. strain DDE-1, which metabolizes 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene. *Appl. Environ. Microbiol.* 65:5607–5611.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. 1990. Basic local alignment tool. *J. Mol. Biol.* 15:403–410.

Bachoon, D., Chen, F., and Hodson, R.E. 2001. RNA recovery and detection of mRNA by RT-PCR from preserved prokaryotic samples. *FEMS Microb. Letters* 201:127–132.

Becker, S., Fahrbach, M., Böger, P., and Ernst, A. 2002. Quantitative tracing, by Taq nuclease assay, of a *Synechococcus* ecotype in a highly diversified natural population. *Appl. Environ. Microbiol.* 68: 4486–4494.

Boegman, L., Loewen, M.R., Hamblin, P.F., and Culver, D.A. 2001. Application of a two-dimensional hydrodynamic reservoir model to Lake Erie. *Can. J. Fish. Aquat. Sci.* 58:858–869.

Caron, D.A., Pick, F.R., and Lean, D.R.S. 1985. Chroococcoid cyanobacteria in Lake Ontario: vertical and seasonal distributions during 1982. *J. Phycol.* 21:171–175.

Charlton, M.N., and Milne, J.E. 2004. *Review of thirty years of changes in Lake Erie water quality.* National Water Research Institute Contributions 04-167.

Crosbie, N.D., Poeckl, M., and Weisse, T. 2003. Rapid establishment of clonal isolates of freshwater autotrophic picoplankton by single-cell and single-colony sorting. *J. Microbiol. Meth.* 55:361–370.

Czuczwa, J.M., and Hites, R.A. 1986. Airborne dioxins and dibenzofurans: sources and fates. *Environ. Sci. Technol.* 20:195–200.

DeBruyn, J.M., Leigh-Bell, J.A., McKay, R.M.L., Bourbonniere, R.A., and Wilhelm, S.W. 2004. Microbial distributions and the impact of phosphorus on bacterial activity in Lake Erie. *J. Great Lakes Res.* 30:166–183.

DeLong, E.F. 1997. Marine microbial diversity: The tip of the iceberg. *Trends in Biotechnology* 15:203–207.

Fuller, K., Shear, H., and Wittig, J. 2002. *The Great Lakes: an environmental atlas and resource book.* Toronto, ON. U.S. EPA and The Government of Canada.

Giri, B.J., Bano, N., and Hollibaugh, J.T. 2004. Distribution of RuBisCO Genotypes along a Redox Mono Lake, California. *Appl. Environ. Microbiol.* 70:3443–3448.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). *Nucl. Acids Symp. Ser.* 41:95–98.

Hwang, S.-J., and Heath, R. 1997. Bacterial productivity and protistan bacterivory in coastal and offshore communities of Lake Erie. *Can. J. Fish. Aquat. Sci.* 54:788–799.

Ivanikova, N.V., McKay, R.M.L., and Bullerjahn, G.S. 2005. Construction and characterization of a cyanobacterial bioreporters capable of assessing nitrate assimilatory capacity in freshwaters. *Limnol. Oceanogr. Methods* 3:86–93.

Kaplan, C.W., Astaire, J.C., Sanders, M.E., Reddy, B.S., and Kitts, C.L. 2001. 16S ribosomal DNA terminal restriction fragment pattern analysis of bacterial communities in feces of rats fed *Lactobacillus acidophilus* NCFM. *Appl. Environ. Microbiol.* 67:2935–2939.

Keough, B.P., Schmidt, T.M., and Hicks, R.E. 2003. Archael nucleic acids in picoplankton from Great Lakes on three continents. *Microb. Ecol.* 46:238–248.

Kumar, S., Tamura, K., and Nei, M. 1994. MEGA: Molecular Evolutionary Genetics Analysis software for microcomputers. http://www.megasoftware.net/. *Comput. Appl. Biosci.* 10:189–191.

Lane, D. 1991. 16S/23S rRNA sequencing. In *Nucleic acid techniques in bacterial systematics, eds.* E. Stackebrandt and M. Goodfellow, pp. 115–175. New York: John Wiley and Sons.

Leutheuser, F.C. 1979. Lake Erie sediment oxygen demand. MS thesis. The University of Michigan, Ann Arbor, Michigan.

Mills, H.J., Martinez, R.J., Story, S., and Sobecky, P.A. 2004. Identification of members of the metabolically active microbial populations associated with *Beggia-*

toa species mat communities from the Gulf of Mexico cold-seep sediments. *Appl. Environ. Microbiol.* 70:5447–5458.

Moore, L.R., Post, A.F., Rocap, G., and Chisholm, S.W. 2002. Utilization of different nitrogen sources by the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. *Limnol. Oceanogr.* 47:989–996.

NLET (The National Laboratory for Environmental Testing). 1994. *Manual of Analytical Methods; Volume 1. Major Ion and Nutrients.* Burlington, Ontario, Environment Canada.

Ostrom, N.E., Carrick, H.J., Twiss, M.R., and Piwinski, L. 2004. Evaluation of primary production in Lake Erie by multiple proxies. *Oecologia* 144:115–124.

Ouellette, A.J.A., Handy, S.M., and Wilhelm, S.W. 2006. Toxic *Microcystis* is widespread in Lake Erie: PCR detection of toxin genes and molecular characterization of associated microbial communities. *Microb. Ecol.* 51:154–165.

Rinta-Kanto, J.M., Ouellette, A.J.A., Twiss, M.R., Boyer, G.L., Bridgeman, T.B., and Wilhelm, S.W. 2005. Quantification of toxic *Microcystis* spp. during the 2003 and 2004 blooms in Western Lake Erie. *Environ. Sci. Technol.* 39:4198–4205.

Strous, M., vanGerven, E., Kuenen, J.G., and Jetten, M. 1997. Effects of aerobic and microaerobic conditions on anaerobic ammonium-oxidizing (Anammox) sludge. *Appl. Environ. Microbiol.* 63:2446–2448.

Thompson, J.D., Higgins, D.G., and Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucl. Acids Res.* 22:4673–4680.

Wilhelm, S.W., and Smith, R.E.H. 2000. Bacterial carbon production in Lake Erie is influenced by viruses and solar radiation. *Can. J. Fish. Aquat. Sci.* 57:317–326.

Xu, H.H., and Tabita, F.R. 1996. Ribulose-1,5-biosphosphate carboxylase/oxygenase gene expression and diversity of Lake Erie planktonic microorganisms. *Appl. Environ. Microbiol.* 62:1913–1921.

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