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Late-summer phytoplankton in western Lake Erie (Laurentian Great Lakes): bloom distributions, toxicity, and environmental influences

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Abstract Phytoplankton abundance and composition and the cyanotoxin, microcystin, were examined relative to environmental parameters in western Lake Erie during late-summer (2003–2005). Spatially explicit distributions of phytoplankton occurred on an annual basis, with the greatest chlorophyll (Chl) *a* concentrations occurring in waters impacted by Maumee River inflows and in Sandusky Bay. Chlorophytes, bacillariophytes, and cyanobacteria contributed the majority of phylogenetic-group Chl *a* basin-wide in 2003, 2004, and 2005, respectively. Water clarity, pH, and specific conductance delineated patterns of group Chl *a*, signifying that water mass movements and mixing were primary determinants of phytoplankton accumulations and distributions. Water temperature, irradiance, and phosphorus availability delineated patterns of cyanobacterial biovolumes, suggesting that biotic processes (most likely, resource-based competition) controlled cyanobacterial abundance and composition. Intracellular microcystin concentrations corresponded to *Microcystis* abundance and environmental parameters indicative of conditions coincident with biomass accumulations. It appears that environmental parameters regulate microcystin indirectly, via control of cyanobacterial abundance and distribution.

Keywords Algae · Chlorophytes · Cyanobacteria · Microcystin · *Microcystis* · *Pandorina* · *Planktothrix*

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Introduction

The Laurentian Great Lakes (USA and Canada) constitute the largest system of fresh surface water on earth and are an invaluable natural resource. Lake Erie, the shallowest and smallest of the Lakes by volume, is divided into eastern, central, and western basins, each quite different in physical, chemical, and biological aspects. Lake Erie has a long history of anthropogenic perturbation (Burns 1985; Munawar et al. 2002). Excessive nutrient loading during the mid-twentieth century [e.g., 1.61×10^5 and 2.73×10^4 metric tons of total nitrogen (N) and phosphorus (P), respectively, from 1966 to 1967 alone; Sly 1976] resulted in eutrophic waters in the western and central basins. Phytoplankton biomass increased with hypoxia/anoxia a common occurrence during the summer (Davis 1964; Rosa and Burns 1987).

The establishment of P-reduction directives and the invasion of non-indigenous dreissenid mussels (in the early 1970s and late 1980s, respectively) led to declines in primary production and algal biomass, ultimately providing for enhanced water clarity and bottom-water oxygen (O_2) throughout western Lake Erie (Nicholls and Hopkins 1993; Madenjian 1995; Fahnenstiel et al. 1998). Nuisance blooms were absent throughout the western basin during the early 1990s, but phytoplankton abundance increased during the mid-1990s, with summer blooms dominated by the cyanobacterium, *Microcystis aeruginosa* Kützing (Vanderploeg et al. 2001). The blooms have been recurrent with toxic populations a common occurrence (Budd et al. 2002; Ouellette et al. 2006; Dyble et al. 2008). Concentrations of the cyanotoxin, microcystin, intermittently have exceeded recommendations for drinking water and low-risk recreational exposure (1 and 2–4 $\mu g\ l^{-1}$, respectively, World Health Organization 2003, 2004).

The instantaneous rates of phytoplankton growth and potential for biomass accumulation throughout western Lake Erie are controlled by nutrient availability, light, wind events, and grazing pressure, acting singularly or in combination (Nicholls and Hopkins 1993; Fahnenstiel et al. 1998; Wilhelm et al. 2003; Porta et al. 2005; Conroy et al. 2005). However, the mechanisms underlying toxicity events are unknown. Cyanotoxins are assumed to be secondary metabolites—compounds derived from primary metabolism, but not necessary for cell development and thought to influence fitness and/or defense against herbivores (Babica et al. 2006; Leflaive and Ten-Hage 2007). Given that secondary metabolites are produced by diverse cyanobacteria encompassing varied ecological niches, it is plausible that microcystin synthesis is associated with and/or regulated by environmental factors affecting cell metabolism, growth, and ultimately, abundance (Paerl and Millie 1996). Reports that state microcystin production and/or cell quota are influenced by select abiotic parameters, including temperature, irradiance, macro/micronutrients, and pH (van der Westhuizen and Eloff 1985; Lukac and Aegerter 1993; Song et al. 1998; Downing et al. 2005), support this premise.

Five million people depend upon the waters of western Lake Erie for consumption, transportation, power, recreation, and a host of other uses (Fuller et al. 1995). The occurrence of cyanobacterial blooms and their deleterious consequences upon water quality, utilization of affected waters, and human health (Paerl 1988; Pilotto et al. 1997; Hitzfield et al. 2000) have raised concerns to the sustainability of western Lake Erie as a natural resource. Our understanding of the factors influencing bloom proliferation requires knowledge of the synergistic interactions and/or feedbacks between community composition and water-column properties, as well as a comprehension of the bloom-forming taxa within the context of the overall community (Millie et al. 2008). Here, we characterize phytoplankton composition/abundance and the environmental conditions influencing communities during late-summer, a period when bloom proliferation typically is greatest throughout the basin. We hypothesized that (1) environmental conditions vary, both intra-annually and across western Lake Erie, and (2) these variances provide for spatially and temporally explicit patterns of phytoplankton composition/abundance and toxicity that can be explained by proximate causes (e.g., physical, biological forcing).

Materials and methods

Study site and sample acquisition

Lake Erie's western basin (Fig. 1) comprises ca.13% of the Lake's total surface area (ca. 3,300 of

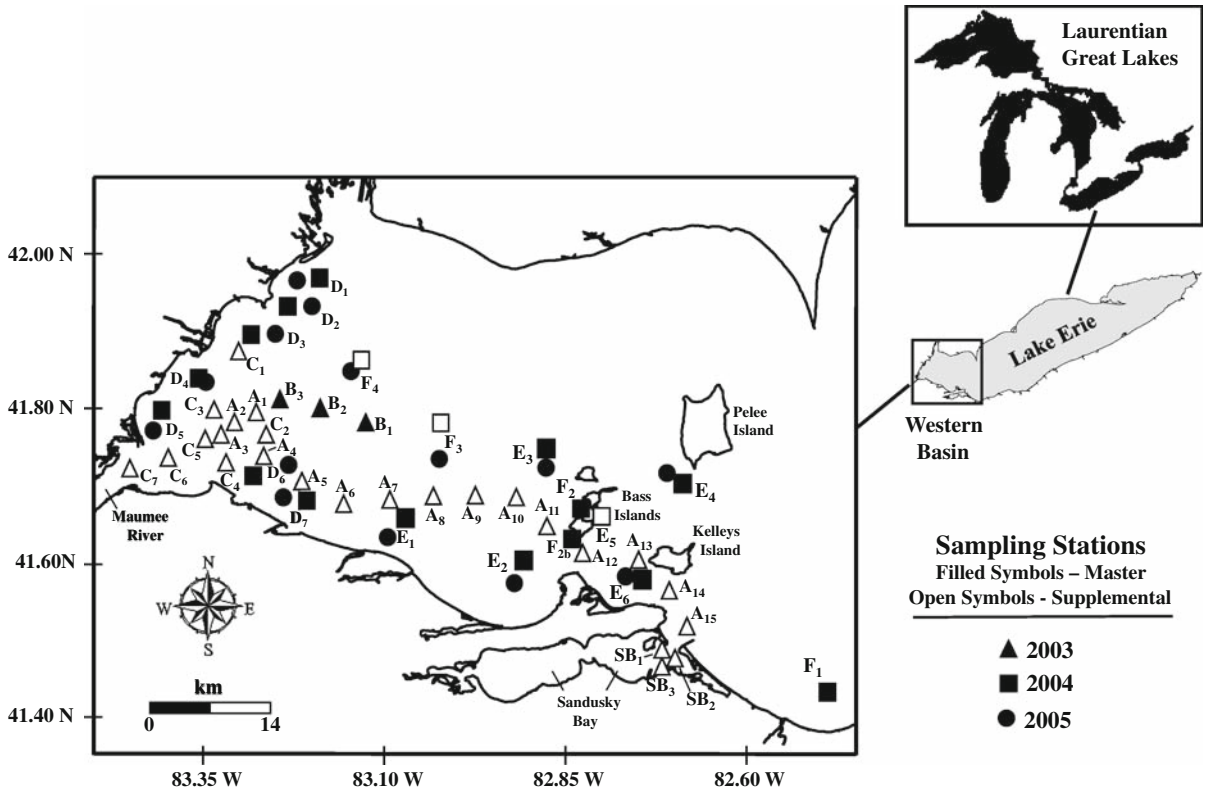

Fig. 1 Sampling stations throughout Lake Erie's western basin. *Inset* Study area relative to the Laurentian Great Lakes and Lake Erie. *Symbols* for stations are labeled as sampling transect (*A*–*F*) and station number (*subscript*), whereas supplemental stations are delineated only as *symbols*

25,690 km^2, Hartman 1973). Western Lake Erie has a water retention/replacement time of 51 days (considerably less than the 2.6 years for the entire lake) and resembles a sub-littoral zone with respect to limnological conditions. The extreme shallowness of the basin (mean depth of 7.4 m, maximum depth of 19 m) allows waters to warm rapidly upon the onset of high temperatures and water-column stratification only occurs during wind-free periods (Chandler 1940; Bolsenga and Herdendorf 1993). Water from the Detroit River (draining the oligotrophic 'upper' Great Lakes) flows into northwestern Lake Erie and constitutes 80% of the basin's total inflows. Water from the Maumee River flows into the southwestern portion of the basin and contributes ca. 10% of the basin's total inflows. The Maumee River drains a large watershed (ca. 17,100 km^2) dominated by agricultural activities and is the tributary contributing the largest storm runoff (ca. 25% of the total) within the Lake Erie basin (Bolsenga and Herdendorf 1993).

Synoptic survey cruises throughout the western and southern regions of Lake Erie's western basin were conducted from 9 to 11 August 2003, 28 to 30 August 2004, and 21 to 23 August 2005. Master or supplemental sampling stations were established along distinct transects (A, B, C in 2003 and D, E, F in 2004 and 2005; Fig. 1). At master stations, Secchi depth was measured and the water column characterized using a specific conductance (SpCond) –temperature (Temp)–water column depth (Z_{WC}) profiler (Sea-Bird Electronics, Inc.; Bellevue, WA, USA), additionally equipped with sensors for downwelling photosynthetic active radiation (PAR), O_2, hydrogen ion (pH), fluorometry (Fluor), and transmissometry (Trans). A LI-190SA cosine sensor interfaced with a LI-1000 data logger (Li-Cor Biosciences, Inc.; Lincoln, NE, USA) was mounted on the ship's infrastructure for continuous acquisition of surface PAR. Modified Niskin bottles (all rubber parts were replaced with Teflon-coated or silicone;

Fahnenstiel et al. 2002) were cast to collect sub-surface water (1 m depth—midpoint of bottle) for abiotic and biotic characterization. Sub-surface sampling was based upon chlorophyll (Chl) fluorescence profiles (obtained via CTD casts) indicating that phytoplankton abundance throughout the upper water column (1–2 m depth) was uniform. At supplemental stations (in 2003), water for selected biotic analyses only was collected.

Physical and chemical parameters

Light parameters were determined using equations from Wetzel (2001) and Fahnenstiel et al. (2000). Diffuse attenuation coefficients (K_D) were calculated from PAR profiles. The euphotic depth (Z_E) was assumed to equal the depth of 1% of surface irradiance (I_0) and considered equivalent to Z_{WC} for instances where values of Z_E exceeded Z_{WC}. Depth-specific irradiance (I_Z) was calculated as:

$$I_Z = I_0 e^{-K_D Z_{WC}} \quad (1)$$

and water column irradiance (I_{ZWC}) was calculated by integrating Eq. 1 over the mixed layer, or:

$$I_{ZWC} = (I_0/K_D Z_M)\left(1 - e^{-K_D Z_M}\right) \quad (2)$$

where Z_M is the depth of the mixed layer. Because of the shallow depths at all stations (see "Results") and the usual isothermal nature of the water column, Z_M was considered equivalent to Z_{WC}. A 3-day mean ($\bar{I}_{ZWC}$) was calculated (from values of I_{ZWC} for the day of sampling and the preceding 2 days) to 'average out' single-day variation and to be more consistent with respect to phytoplankton generation times (Fahnenstiel et al. 2000).

Dissolved nutrient concentrations in filtrates were determined after drawing aliquots of water under low vacuum through pre-rinsed, tared 0.7-µm glass fiber filters or 0.4-µm polycarbonate filters. Total-, particulate-, and soluble-phosphorus (TP, PP, and SP) were measured using standard colorimetric procedures on an Auto Analyzer II (Davis and Simmons 1979). Aliquots for TP initially were stored in acid-cleaned Pyrex test tubes at 5°C, later digested in an autoclave (after addition of potassium persulfate), and measured as SP (Menzel and Corwin 1965). Particulate organic carbon (POC) and particulate organic nitrogen (PON) concentrations of whole water samples were determined using a Model 1110 carbon (C)–hydrogen (H)–N elemental analyzer (CE Elantech, Inc., Lakewood, NJ, USA). Total carbon dioxide concentration was derived from alkalinity and pH measurements (Vollenweider 1974). Particulate (molar) N:P, C:P, and C:N ratios were calculated from PON, PP, and POC concentrations, respectively.

Biological parameters

Total phytoplankton within whole-water aliquots were both preserved (using Lugol's iodine solution) in amber bottles and drawn under low vacuum (no greater than five in. Hg) onto glass fiber filters. Cyanobacterial colonies and trichomes within whole-water aliquots were isolated onto 53-µm nitex mesh and 'washed' with distilled water onto glass fiber filters under low vacuum (Vanderploeg et al. 2001; Dyble et al. 2008; Fahnenstiel et al. 2008). Dissolved microcystin within aliquots of 0.7-µm filtered water was concentrated onto Waters Oasis HLB C_{18} solid-phase extraction (SPE) cartridges (in 2004 only). Filters and SPE cartridges were immediately frozen and stored at −80°C for later analyses.

Phytoplankton abundance and composition were assessed using photopigment characterization and microscopic enumeration. For photopigment analyses, frozen filters were placed in 100% acetone, sonicated, and extracted in darkness at −20°C for 12–16 h. Filtered extracts were injected directly into a Model 600E HPLC (Waters Corp., Milford, MA, USA) equipped with a single monomeric (Microsorb-MV; 100 × 4.6 mm, 3 µm; Varian, Inc., Palo Alto, CA, USA) and a single polymeric (Vydac 201TP, 250 × 4.6, 5 µm; The Separations Group, Inc., Hesperia, CA, USA) reverse-phase C_{18} columns, in series. Pigments were quantified by integrating chromatographic peaks generated by an in-line, Waters 2996 photodiode array detector and calibrated from authentic pigment standards. Mobile phases, solvent flow rates, and column temperature followed that are described in Pinckney et al. (1996).

Qualitative microscopic examination of plankton at time of sampling revealed that basin-wide phytoplankton communities were comprised in all study years of bacillariophytes, chrysophytes, cryptophytes, pyrrophytes, chlorophytes, and cyanobacteria. Photopigment suites useful as chemotaxonomic biomarkers for these phylogenetic groups included: fucoxanthin,

diadinoxanthin, Chl $c_{1/2}$—bacillariophytes and chrysophytes, alloxanthin, β, ε-carotene, Chl $c_{1/2}$—cryptophytes, peridinin—pyrrophytes, neoxanthin, violaxanthin, lutein, Chl *b*—chlorophytes and euglenophytes, and zeaxanthin—cyanobacteria (Millie et al. 1993).

Absolute and relative phylogenetic-group Chl *a* concentrations were derived from the pigment suites using chemical taxonomy (CHEMTAX) matrix factorization (Mackey et al. 1996, 1998). CHEMTAX optimizes group-specific carotenoid:Chl *a* ratios via iteration and a steepest descent algorithm to identify the minimum residual unexplained by the solution (as root mean square error) and from which, group abundances are calculated (Mackey et al. 1996, 1998). Because carotenoid:Chl *a* ratios within phytoplankton communities might be expected to vary depending upon species composition, cell physiological state, irradiance, etc., the data set was divided into annual and transect subsets, prior to independent CHEMTAX calculations (Mackey et al. 1998; Schlüter et al. 2000). CHEMTAX calculations are sensitive to the initial carotenoid:Chl *a* ratios identified for each group; as such, initial ratios were chosen from those previously derived for and/or applicable to Great Lakes phytoplankton (Millie et al. 2002; Schlüter et al. 2006). To further minimize error arising from ratio variation, data subsets were assessed independently using the initial ratio matrix and an additional 59 matrices generated randomly as the product of the initial values and a randomly determined factor (*F*):

$$F = 1 + S \times (R - 0.5) \quad (3)$$

where *S* is a scaling factor (=0.7) and *R* is a randomly generated number between 0 and 1. The best 10% of results (i.e., exhibiting the least root mean square error) then were used to estimate mean abundances (Millie et al. 2008).

Cyanobacteria were enumerated microscopically via Utermöhl's (1958) sedimentation technique, with cell densities converted to biovolumes by means of geometric figures best approximating the shape of individual taxa. The type species of *M. aeruginosa* accounted for 94.04 + 4.61(standard error) % of the total *Microcystis* abundance; accordingly, the type species was combined with its morphospecies (i.e., *Microcystis novacekii* (Komárek) Compère, *Microcystis wesenbergii* (Komárek) Komárek, and *Microcystis botrys* Teiling) to produce a unified species complex (Otsuka et al. 2001; Fahnenstiel et al. 2008). *Microcystis natans* Lemmermann ex Skuja, *Microcystis firma* (Kützing) Schmidle, and *Microcystis smithii* Komárek et Anagnostidis were retained as distinct taxa.

Carbon-specific growth of bloom-forming cyanobacteria in 2004 were estimated by Chl *a*-^{14}carbon labeling, following the technique detailed by Redalje (1993) and modified by Fahnenstiel et al. (2000) for Great Lakes phytoplankton. Briefly, whole-water aliquots in polycarbonate bottles were inoculated with $NaH^{14}CO_3$ and the bottles incubated for 24 h (dawn to dawn) in a simulated in situ incubator. Cyanobacterial colonies and trichomes were isolated and collected, after which photopigment analyses of these samples were conducted. Following fraction collection of the ^{14}C-labeled Chl *a* eluting from the HPLC column and the counting of the eluant's activity with a liquid scintillation counter, growth rates were derived from the rate of Chl *a* synthesis, using equation six from Goericke and Welschmeyer (1993).

Intra- and extra-cellular microcystins were quantified by commercially available Abraxis ELISA kits (in 2003 and 2004) and HPLC/tandem mass spectrometry (MS-MS, in 2005). 'Clean' analytical technique and supplies (Hyenstrand et al. 2001) were utilized. For intracellular microcystins, filters with cyanobacterial colonies/trichomes were placed in 70:30 methanol:water, sonicated, and toxins extracted for 12 h at −20°C. After removing the solvent extract, extraction was repeated twice again (each for 1-h). In order to optimize purification of microcystins and to increase analytical sensitivity for microcystins, the toxin extracts were combined and passed through SPE cartridges. Microcystins retained on the SPE cartridges were eluted with 90:10 methanol:water buffered 0.1% trifluoroacetic acid (Lawton et al. 1994). Extracellular microcystins concentrated onto SPE cartridges at the time of sampling were similarly processed.

For HPLC/MS-MS, extracts were injected directly into a Model 200 HPLC (PerkinElmer Life and Analytical Sciences, Inc., Wellesley, MA, USA) equipped with a reverse-phase C_{18} (Gemini, 150 × 3 mm, 3 μm; Phenomenex, Inc., Torrance, CA, USA) column. Microcystin congeners were separated via a binary solvent gradient, following Lawton et al. (1994): 1 to 5 min—10:90 [acetonitrile-0.02% TFA (solvent A)]:[H_2O-0.02% TFA (solvent B)] to 95:5 [A]:[B]; 5–12 min—95:5 [A]:[B] at a flow rate of 0.40 ml min^{-1} and 40°C. Congeners were quantified via electrospray ionization (Navigator MS; Finnigan

Mat Corp., San Jose, CA, USA), operated in single ion monitoring mode and using nodularin as an internal standard. ELISA and HPLC/MS-MS analyses were calibrated with authentic toxin standards.

Statistical analyses

Data were transformed prior to statistical analysis (where appropriate) to stabilize variance and to increase homogeneity of normalcy. An ANOVA or *T*-test assessed annual differences for physical/chemical parameters, total Chl *a* concentrations, and intracellular microcystin concentrations. For data not drawn from a normally distributed population and/or exhibiting dissimilar variance between subjective groups, a Kruskal–Wallis ANOVA on Ranks was used. A Holms–Sidak or a Mann–Whitney Rank Sum Test assessed differences between mean pairs. The correspondences among microcystin cell quotas/concentrations, cyanobacterial cell densities/growth rates, and abiotic parameters were determined using a Pearson Product Moment Correlation Analysis.

Multivariate gradient analyses represented abiotic (physical/chemical) and community (phylogenetic group Chl *a*/cyanobacterial biovolume) relationships. Such ordination approaches allow the simultaneous visualization of data encompassing multiple dimensions in a low-dimensional 'space' and affords comparisons of environmental–biota relationships and/or gradients in their entirety, while simultaneously diminishing data 'noise' (Gauch 1982; McCune and Grace 2002). A Principal Component Analysis (PCA), utilizing euclidean distances, ordered sampling sites with respect to abiotic variables. Prior to PCA, pairwise scatter plots were constructed to illustrate symmetric distribution of/or linear relationships among variables (Clarke and Gorley 2006).

The between-sample similarity (*S*) for phylogenetic-group Chl *a* concentrations and cyanobacterial biovolumes was assessed using the Bray–Curtis coefficient:

$$S_{ih} = 100\left\{1 - \frac{\sum_{j=1}^{p}\left|Y_{i,j} - Y_{h,j}\right|}{\sum_{j=1}^{p}\left(Y_{i,j} + Y_{h,j}\right)}\right\} \quad (4)$$

for matrices (as $Y_{i,j}$), comprised of phylogenetic groups or cyanobacterial taxa ($j = 1, \ldots, p$) across multiple samples ($i = 1, \ldots, h$). The between-sample dissimilarity was the complement of similarity (i.e., $100 - S_{ih}$; Clarke and Warwick 2001; McCune and Grace 2002). For each of the community matrices, between-sample coefficients were assigned a rank order and ordination in two-dimensional space was constructed iteratively, via non-metric multi-dimensional scaling (MDS). Non-metric MDS is the 'method of choice' for ordination of community data in its entirety—the use of ranked distances tends to linearize the relationship between distances of species and environmental space, thereby reducing problems associated with single species–environmental relationships (Clarke 1993; McCune and Grace 2002). The resulting graphical presentation depicted among-sample similarities/dissimilarities in the same rank order as the relative coefficient dissimilarities (i.e., sampling stations plotted close together represented comparable compositional structure, whereas those positioned far apart represented different compositions). The departure from monotonicity between the similarity/dissimilarity distances in the rank order matrix and those in the ordination (i.e., 'stress'; McCune and Grace 2002) was calculated according to Clarke and Warwick (2001). For each of the community matrices, MDS was repeated (250×) to ensure that the lowest 'stress' solutions generated by the ordination algorithm were the best solutions. A 'stress' value of <0.20 represented a useful two-dimensional representation of the data (Clarke and Gorley 2006).

A one-way Analysis of Similarity assessed community differences among annual sample groups and a Similarity Percentage Analysis determined contributions for each phylogenetic group/taxon to the mean group similarity/dissimilarity (Clarke and Gorley 2006). A forward/backward stepwise search analysis identified the subset of taxa that best approximated the ordination using all taxa (as determined by a Spearman rank correlation coefficient, $\rho > 0.95$; Clarke and Warwick 2001). The association between abiotic and biotic rank matrices was assessed by calculating ρ between elements of the matrices and then comparing the value to a frequency histogram created by randomly permuting samples and recalculating ρ after each permutation. Abiotic variables were matched, singly and in combination, to ordination plots in order to select subsets of variables (in increasing complexity) that maximized ρ between biotic/abiotic rank matrices (see Clarke and Gorley 2006).

Results

Water-column depths at (master) sampling stations throughout the western basin ranged from 4.5 to 10.6 m. Based on sub-surface physical/chemical variables, annual groupings (2003/2004 and 2005) of master stations were apparent (Fig. 2) and suggested temporal variability of meteorological and/or hydrological conditions. The initial two components of the PCA included hydrological descriptors that together explained the majority of total variability among master stations; values of water-column clarity (K_D, Trans, Z_E) and SpCond and values of Temp, $\bar{I}_{ZWC}$, and P availability (N:P and C:P ratios) explained 35 and 21% of the total variability within the first and second component, respectively.

Concentrations of SP and PP comprised ca. 21 and 72%, respectively, of basin-wide mean TP concentrations (mean ± standard error; 43.14 ± 15.72 µg TP l^{-1}). Concentrations of TP and PP were greatest during 2003, likely attributable to the occurrence of a large phytoplankton bloom. Mean annual particulate values of (molar) N:P and C:P ratios ranged from 11.55:1 to 28.91:1 and 80.04:1 to 207.85:1, respectively, with ratios greater in 2003 and 2004 than in 2005 ($P \leq 0.001$; Table 1). Mean annual particulate (molar) C:N ratios ranged from 7.31:1 to 7.77:1 and were similar among years. Concentrations of POC corresponded to Chl *a* concentrations ($r = 0.98$, $P \leq 0.0001$). Mean annual ratios of particulate C:Chl *a* ranged from 63.77:1 to 179.08:1 µg C:µg Chl, with mean ratios less in 2003 than during 2004 and 2005 ($P \leq 0.05$; Table 1). Ratios corresponded positively ($r = 0.36$–0.43, all $P \leq 0.05$) with $\bar{I}_{ZWC}$, Trans, and Z_E, whereas ratios corresponded negatively ($r = -0.42$ to -0.72, all $P \leq 0.05$) with K_D and variables indicative of phytoplankton biomass and production (O_2, pH, PP, PON, Fluor). Values for Temp and SpCond were greatest during 2003, whereas mean K_D, Secchi, Z_E, and Fluor values and mean particulate C:N ratios were similar among years.

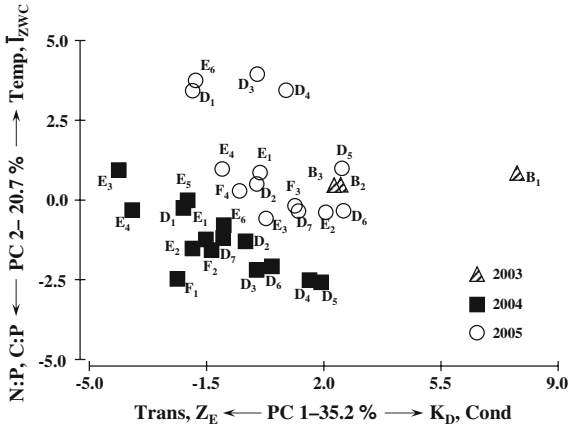

Fig. 2 Two-dimensional principal components (*PCs*) ordination of master stations based on water-column physical/chemical variables. Stations denoted as a function of sampling year. Refer to Fig. 1 for station locations and label identifications. Percent of total variability explained by physical/chemical variables is indicated for each corresponding principal component (see text for variable abbreviations)

No evidence of an algal surface 'scum' was observed at any station. Concentrations of Chl *a* throughout the basin ranged from 16.42 to 832.31, 2.50–28.72, and 2.95–16.52 µg l^{-1} in 2003, 2004, and 2005, respectively (Fig. 3), with the greatest basin-wide, mean concentration occurring in 2003 (Fig. 3, inset). Concentrations were greatest offshore the mouth of the Maumee River and decreased with increasing distance (eastward) from the River. Throughout the southwestern portion of the basin, concentrations in near-shore waters (at stations within C and D transects) generally were ca. 1.5 to 3-times greater than concentrations in offshore waters (stations F_3 and F_4, Figs. 1, 3). Concentrations of Chl *a* within lower Sandusky Bay (sampled only during 2003) ranged from 7.24 to 141.63, with concentrations greater than 100 µg l^{-1} occurring at the innermost stations (SB_2 and SB_3).

Absolute and relative group Chl *a* concentrations were dissimilar among years ($P \leq 0.001$, Figs. 4a, 5). Chlorophytes, cyanobacteria, and bacillariophytes contributed the greatest mean Chl *a* concentrations in 2003, 2004, and 2005, respectively (Fig. 4b). The greatest bloom event occurred in 2003 and resulted from an accumulation of chlorophytes (ca. 727 µg chlorophyte Chl *a* l^{-1}) offshore the mouth of the Maumee River (Figs. 1, 3, 4b). Microscopic examination at the time of sampling revealed *Pandorina morum* Bory to almost solely comprise the chlorophyte component. Significant biomass of cyanobacteria (up to 100 µg cyanobacterial Chl *a* l^{-1}) also occurred within this bloom, with lesser accumulations occurring throughout the basin in 2004 and 2005 (Fig. 4b). Concentrations of cyanobacterial Chl *a* at the innermost stations within Sandusky Bay during 2003 were ca. 100 µg l^{-1}.

Table 1 Physical/chemical parameters at master stations (see Fig. 1) throughout western Lake Erie. Data are means ± standard error (2003, $n = 3$; 2004, $n = 15$; 2005, $n = 14$). See text for variable abbreviations

Variable (units)	2003	2004	2005
Temp (°C)***	25.53 ± 0.22[a]	22.78 ± 0.08[b]	25.14 ± 0.15[a]
Secchi (m)	1.01 ± 0.26	1.39 ± 0.12	1.36 ± 0.13
K_D (m^{-1})	1.28 ± 0.24	0.88 ± 0.06	0.89 ± 0.08
Z_E (m)	3.81 ± 0.61	5.52 ± 0.35	5.26 ± 0.36
$\bar{I}_{ZWC}$ (mol quanta m^{-2} d^{-1})	7.20 ± 1.41	7.18 ± 0.55	8.28 ± 0.86
TP (μg l^{-1})***	191.00 ± 167.00[a,b]	17.49 ± 1.40[b]	38.95 ± 3.67[a]
PP (μg l^{-1})***	184.67 ± 162.67[a]	10.61 ± 0.97[b]	30.94 ± 3.24[a]
SP (μg l^{-1})*	6.33 ± 4.49[a]	4.83 ± 0.64[b]	7.60 ± 1.70[a,b]
PON (mg l^{-1})*	0.84 ± 0.60[a]	0.14 ± 0.02[b]	0.16 ± 0.02[a,b]
POC (mg l^{-1})*	6.93 ± 5.70[a]	0.85 ± 0.09[b]	0.95 ± 0.13[a,b]
Particulate N:P (molar)***	19.23 ± 5.52[a,b]	28.91 ± 2.09[a]	11.55 ± 0.99[b]
Particlate C:P (molar)***	128.49 ± 21.72[a,b]	207.85 ± 12.83[a]	80.04 ± 5.88[b]
Particlate C:N (molar)	7.48 ± 1.49	7.31 ± 0.20	7.77 ± 0.22
C:Chl *a* (μg:μg)*	63.77 ± 1.57[a]	179.08 ± 13.67[b]	174.60 ± 14.04[b]
pH ([H^{-1}])***	9.22 ± 0.12[a]	8.26 ± 0.04[b]	8.36 ± 0.04[b]
O_2 (mg l^{-1})***	10.57 ± 0.61[a]	6.25 ± 0.23[b]	6.98 ± 0.20[c]
SpCond (μs cm^{-1})**	308.43 ± 17.46[a]	255.83 ± 4.98[b]	269.68 ± 6.61[b]
Fluor (Sea Tech units)	0.26 ± 0.06	0.25 ± 0.03	0.28 ± 0.03
Trans (%)***	14.13 ± 2.46[a]	42.59 ± 2.69[b]	31.11 ± 3.79[c]

*, **, *** Annual means are different at the 0.05, 0.01, and 0.001 probability levels, respectively

[a, b, c] Variable means with distinct letters are different at the 0.05 probability level

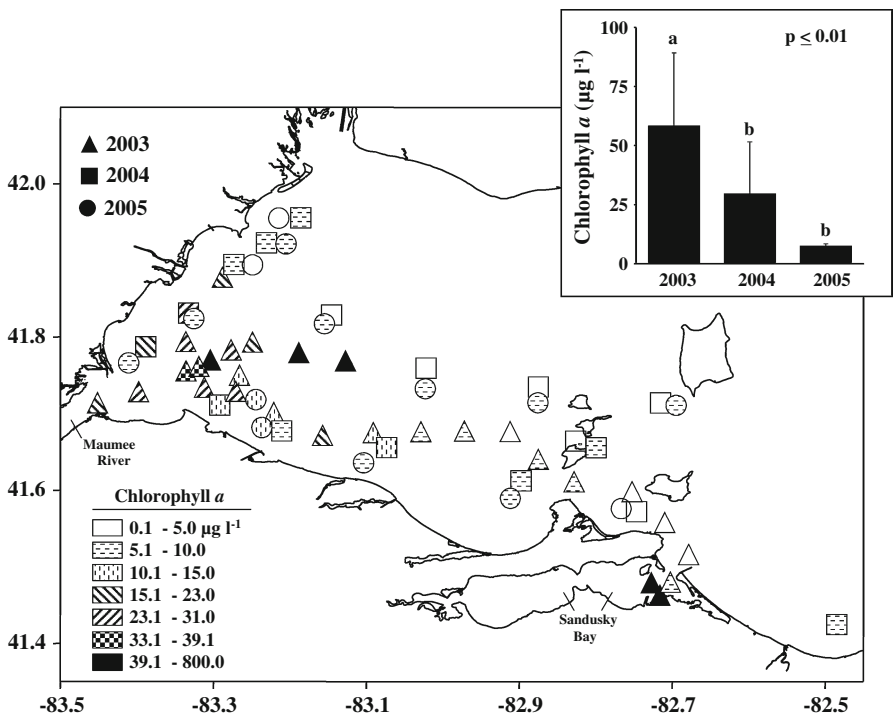

Fig. 3 Total Chl *a* concentrations at master/supplemental stations. Refer to Fig. 1 for station identification. *Inset* Basin-wide concentrations of the lake proper (excluding Sandusky Bay) as a function of sampling year. Data are means ± standard error (2003, $n = 25$; 2004, $n = 18$; 2005, $n = 14$). Means with distinct letters are different at the 0.05 probability level

Chlorophytes, bacillariophytes, and cyanobacteria contributed the greatest relative concentrations basin-wide in 2003, 2004 and 2005, respectively. These phylogenetic groups also comprised the maximal relative abundances observed (greater than 90%, as group Chl *a*: total Chl *a*) at any one station. Relative

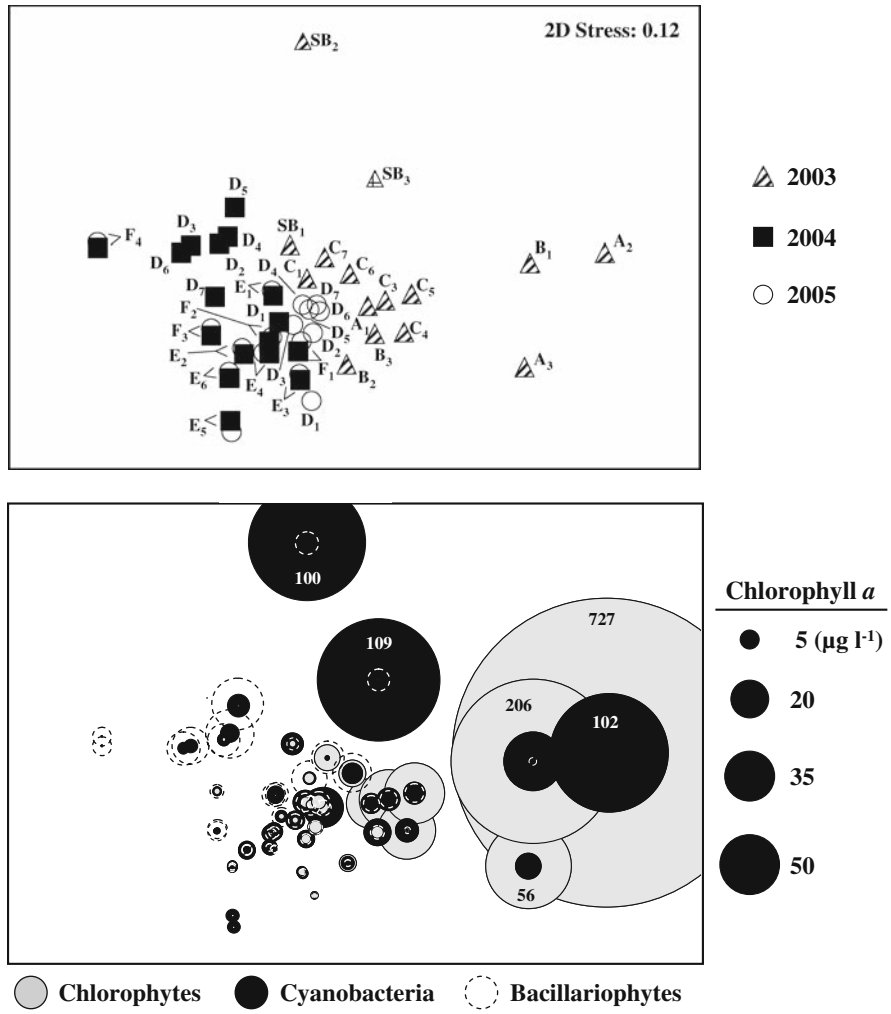

Fig. 4 MDS ordinations of sampling stations based on Bray–Curtis similarities of phylogenetic-group Chl *a* concentrations. **a** Stations denoted as a function of sampling year. *Symbols* for master stations are labeled as sampling transect and station number. **b** Phylogenetic-group Chl *a* concentrations for chlorophytes (*shaded*), cyanobacteria (*filled*), and bacillariophytes (*dashed-clear*) *superimposed*, as *circles* of differing sizes, on stations. Concentrations exceeding scale values are labeled

cryptophyte and chrysophyte Chl *a* were similar among years and typically less than those of chlorophytes, bacillariophytes, and cyanobacteria. Intermittent accumulations of cryptophytes and chrysophytes provided for relative abundances up to 75 and 47%, 69 and 34%, and 57 and 56% in 2003, 2004, and 2005, respectively, and afforded episodic importance to the composition of the late-summer community. Although pyrrophytes displayed a relative abundance of ca. 10% at an isolated station during 2003 (data not shown), the overall contribution of this group to late-summer communities was negligible. In 2003, cyanobacteria dominated the community in Sandusky Bay (Fig. 5, inset). Water-column optical properties (i.e., K_D, Secchi), SpCond, pH, and combinations thereof, maximized ρ (Table 2) and were deemed parameters that 'best' delineated community patterns in a manner consistent with that of all physical/chemical parameters throughout western Lake Erie.

With respect to the biovolumes, cyanobacterial communities were dissimilar ($P \leq 0.001$; Fig. 6a) among years. To examine apparent annual differences in cyanobacterial composition, stations were classified as 2003/2004 and 2005 sample groups (see Fig. 6b). *M. aeruginosa* was the dominant and most-widespread cyanobacterium within the 2003/2004 sample group (Table 3; Fig. 6b), contributing ca. 80% of the similarity among stations and greater than 80% of the relative biomass throughout the Lake proper (Fig. 6c). In 2005, *Planktothrix agardhii* (Gomont) Anagnostidis and Komárek was dominant

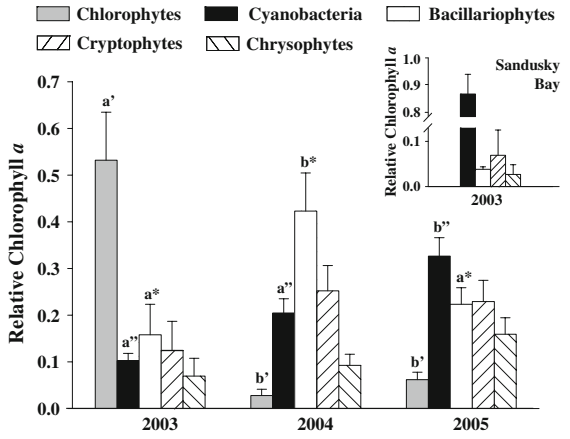

Fig. 5 Relative, phylogenetic-group Chl *a* concentrations throughout the western basin as a function of sampling year. Data are means ± standard error (2003, $n = 12$; 2004, $n = 16$; 2005, $n = 16$). Group means (designated by $'$, $''$, and *) with distinct *letters* are different at the 0.05 probability level; note: if letters are not provided, variable means were not different among years. *Inset* Relative, phylogenetic group concentrations within Sandusky Bay in 2003

(Table 3). Although its mean relative biomass only was ca. 40% of the cyanobacterial biomass basin-wide, *P. agardhii* accounted for up to 90% of the biomass at select stations (refer to Fig. 6b, c). The stepwise search analysis identified the cyanobacteria, *M. aeruginosa, P. agardhii, Aphanizomenon flos-aquae* (L.) Ralfs ex Bornet and Flahault, *Chroococcus limneticus* Lemmermann, and *Merismopedia tenuissima* Lemmermann, to comprise the subset that 'best' approximated the ordination patterns derived using all cyanobacteria ($\rho = 0.96$, $P \leq 0.001$), thereby validating the results of the SIMPER analysis (Table 3). In 2003, qualitative microscopic examination (at the time of sampling) revealed *P. agardhii* to be the dominant taxon within the Sandusky Bay community, with little *M. aeruginosa* present. Water Temp, N:P stoichiometry, SP concentration, and $\bar{I}_{ZWC}$ (Table 2) were deemed parameters to 'best' delineate patterns of cyanobacterial biovolumes in a manner consistent with that of abiotic parameters.

Intracellular and extracellular microcystin were determined by ELISA (in 2003/2004) and intracellular microcystin by LC-MS/MS (in 2005). As such, reported concentrations represent the sum of all microcystin congeners (inclusive of microcystin-LR, -LA, -RR, and -YR, see Dyble et al. 2008). In 2003, the intracellular microcystin concentration associated with the bloom event offshore the Maumee River was 0.13 μg l^{-1}, whereas intracellular concentrations up to 3.2 μg l^{-1} occurred within Sandusky Bay (corresponding with the greatest observed cyanobacterial biomass, see Figs. 3, 4b). Intracellular microcystin concentrations in 2004 ranged from 0.04 to 1.64 μg l^{-1} (Fig. 7a) and were considerably greater than extracellular microcystin concentrations ($P < 0.01$; Fig. 7a). However, extracellular concentrations approximated or exceeded intracellular concentrations at select offshore stations (stations

Table 2 Environmental variables, taken *k* at a time, and producing the greatest correspondence between biotic (phylogenetic-group Chl *a* concentrations and cyanobacterial biovolumes) and abiotic (PCA) rank matrices for each k, as measured by a weighted Spearman Rank Correlation Coefficient, ρ

Biotic variable	*k*	Variable combination (ρ)
Chl *a*	1	pH (0.32); SpCond (0.22)
	2	**pH and TP (0.33)**; K_D and pH (0.32)
	3	**pH, TP and SpCond (0.33); K_D, pH and SpCond (0.33)**
	4	K_D, pH, TP and SpCond (0.33); pH, TP, SpCond and $\bar{I}_{ZWC}$ (0.33)
	5	Secchi, pH, TP, SpCond, and $\bar{I}_{ZWC}$ (0.32); Secchi, K_D, pH, TP and SpCond (0.32)
Cyanobacterial biovolumes	1	Temp (0.38); $N{\cdot}P^{-1}$ (0.36)
	2	SP and $N{\cdot}P^{-1}$ (0.49); Temp and SP (0.45)
	3	Temp, SP and $N{\cdot}P^{-1}$ (0.50); SP, $N{\cdot}P^{-1}$ and $\bar{I}_{ZWC}$ (0.47)
	4	**Temp, SP, N:P and $\bar{I}_{ZWC}$ (0.51);** Temp, SP, N:P and SpCond (0.47)
	5	Temp, SP, N:P, Z and $\bar{I}_{ZWC}$ (0.49); Temp, SP, N:P, SpCond and $\bar{I}_{ZWC}$ (0.48)

Bold type indicates overall optimum value for each biotic matrix. The two best variables or combinations thereof, for each *k*, are listed

See text for variable abbreviations

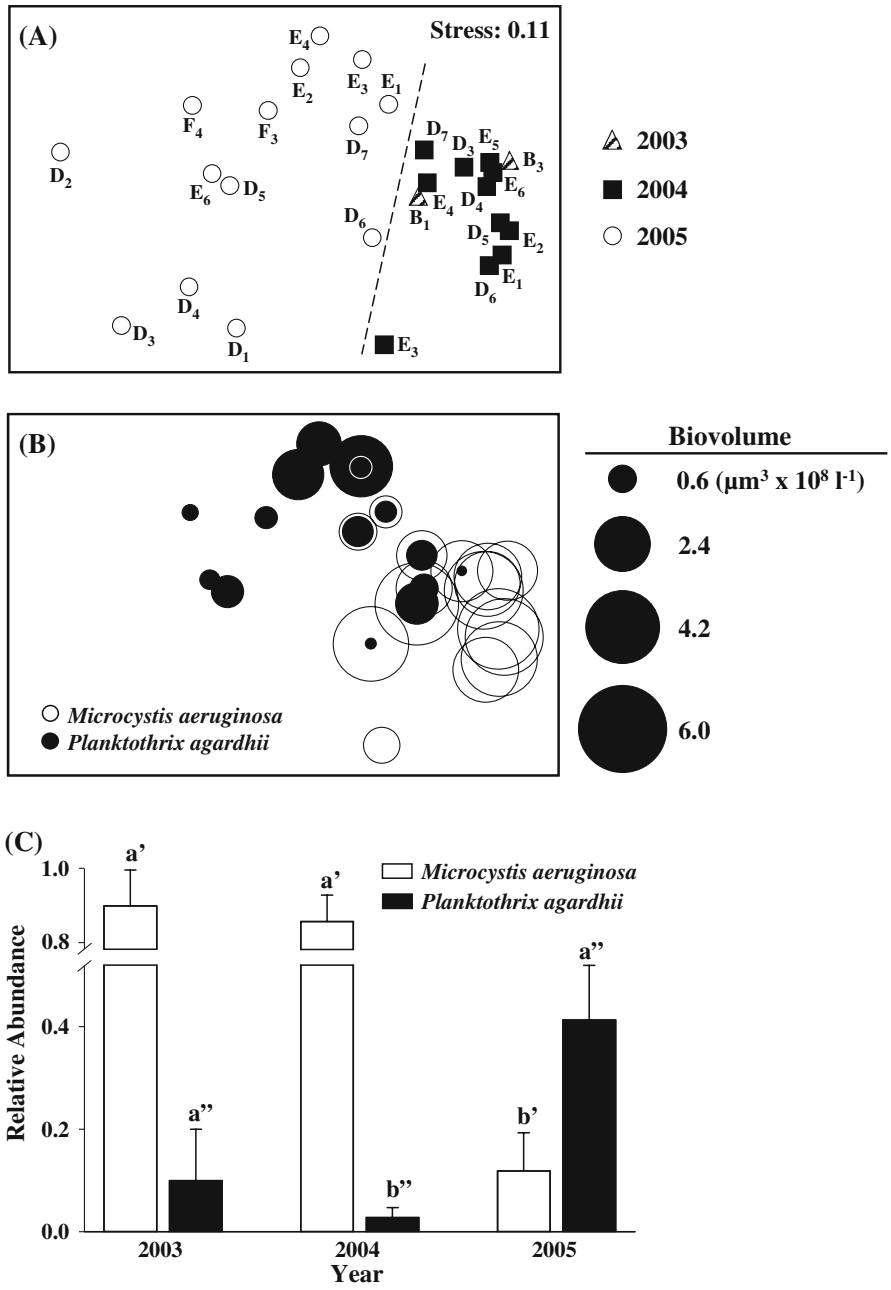

Fig. 6 **a**, **b** MDS ordinations of sampling stations based on Bray–Curtis similarities of cyanobacterial biovolumes. **a** Stations denoted as a function of sampling year. *Dashed line* separates subjective 2003/2004 and 2005 sample groups. Refer to Fig. 1 for station locations and label identifications. **b** Absolute *Microcystis aeruginosa* and *Planktothrix agardhii* biovolumes *superimposed* (as *symbols* of differing sizes) on stations. **c** Relative cyanobacterial biovolumes (for *Microcystis aeruginosa* and *Planktothrix agardhii*) as a function of sampling year. Data are means ± standard error (2003, $n = 2$; 2004, $n = 11$; 2005, $n = 14$). Taxon means (designated by ′and ″) with distinct *letters* are different at the 0.05 probability level

E_3 to E_6, refer to Fig. 1). In 2005, intracellular microcystin concentrations ranged from non-detectable to 0.14 µg l^{-1} (mean + standard error; 0.04 + 0.01 µg l^{-1}).

Annual associations between intracellular microcystin and cyanobacterial Chl *a* concentrations were distinct (Fig. 7b). Intracellular concentrations corresponded to *Microcystis* cell abundance ($r = 0.65$, $n = 11$ and 0.58, $n = 14$ in 2004 and 2005, respectively; both $P \leq 0.05$), but not with *Planktothrix* cell abundance. In 2004, intracellular concentrations corresponded positively with SpCond, pH, K_D, TP, PP, SP, POC, POC, and PON and negatively with Z_E and Secchi ($P \leq 0.05$, $n = 15$).

Because *M. aeruginosa* and *P. agardhii* are known producers of microcystin throughout Lake Erie (Rinta-Kanto and Wilhelm 2006; Dyble et al. 2008), normalizations for microcystin cell quotas used the collective

Table 3 Mean cyanobacterial biovolume ($\mu m^3 \times 10^7\ l^{-1}$) within and taxon contributions to total mean dissimilarity (=83.11) between 2003/2004 and 2005 sample groups (refer to Fig. 5a)

Taxon	2003 and 2004	2005	Contribution (%)	Cumulative %
Microcystis aeruginosa Kützing	35.13	4.78	33.32	33.32
Planktothrix agardhii (Gomont) Anagnostidis	3.05	9.59	12.78	46.09
Aphanizomenon flos-aquae (L.) Ralfs ex Bornet and Flahault	3.28	3.60	8.89	54.98
Microcystis smithii Komárek et Anagnostidis	1.97	0.01	6.71	61.69
Chroococcus limneticus Lemmermann	0.00	1.10	5.17	66.86
Anabaena spp.	0.02	2.50	4.97	71.83

Taxa are ordered in decreasing contributions, as determined by Similarity Percentage Analysis. Taxa contributing to the initial 70% of the total dissimilarity are listed

cell densities for these taxa. In 2004, values of microcystin cell quotas ranged from 5.03×10^{-8} to 8.51×10^{-7} μg cell^{-1} (mean ± standard error; $2.62 \times 10^{-7} \pm 6.65 \times 10^{-8}$ μg cell^{-1}) and cyanobacterial growth rates from 0.03 to 0.33 d^{-1} (mean ± standard error; 0.10 ± 0.02 d^{-1}). Microcystin cell quotas corresponded positively and negatively to pH and $\bar{I}_{ZWC}$, respectively ($P \leq 0.05$, $n = 11$), but did not correspond to growth rates ($P > 0.05$). The minimal cell quotas (mean ± standard error; $7.47 \times 10^{-8} \pm 3.07 \times 10^{-8}$ μg cell^{-1}) during 2005 resulted from the negligible toxin concentrations during this sampling period.

Discussion

Phytoplankton and environmental influences

Basin-wide dominance of late-summer phytoplankton varied on an annual basis; chlorophytes, bacillariophytes, and cyanobacteria contributed the majority of the total Chl *a* in 2003, 2004, and 2005, respectively. Bloom events were spatially episodic, with the greatest accumulations occurring along the basin's southwestern shoreline. The maximal observed biomass arose from an immense bloom of *P. morum* (up to 727 μg chlorophyte Chl *a* l^{-1}), within waters directly impacted by the Maumee River. Throughout this bloom's expanse, *M. aeruginosa* and *P. agardhii* also were present (reaching 99 and 20%, respectively, of up to ca. 100 μg cyanobacterial Chl *a* l^{-1}). Although the magnitude of the *Pandorina* bloom event was surprising, accumulations of chlorophytes and cyanobacteria, or both, throughout the western basin were not unexpected. Both phylogenetic groups thrive in nutrient-enriched waters (Moss 1977; Paerl 1988) and historically have been abundant throughout western Lake Erie (Munawar and Munawar 1996; Nicholls 1997; Makarewicz et al. 1999). Chlorophytes are characterized by high growth/loss rates and a high demand for nutrients, whereas cyanobacteria typically have lower growth/loss rates and a lower nutrient demand (than chlorophytes; Jensen et al. 1994; see Paerl and Millie 1996). As such, intermittent (albeit, short-term) occurrences of localized chlorophyte dominance might be expected for phytoplankton blooms 'fueled' by nutrient-rich Maumee River inflows.

The spatial- and temporal-variability of phytoplankton abundance/composition throughout the western basin appears to be controlled by hydrologic processes (also see Wallen and Botek 1984). Water clarity (K_D, Secchi), pH, and SpCond were environmental parameters that 'best' delineated patterns of phylogenetic-group Chl *a* throughout western Lake Erie. Such parameters are conservative and together act as useful tracers for water mass movements (water transport and mixing, tributary inflows, etc., e.g., Vandelannoote et al. 1999). Hydrological conditions throughout the western basin largely are influenced by inflows of the Detroit River (mean annual discharge of ca. 5100 m^3 s^{-1}) and to a lesser extent, the Maumee River (mean annual discharge of ca. 135 m^3 s^{-1}; Herdendorf 1987). Water clarity and SpCond within inflows of these tributaries are distinct from those of waters within the western basin (see Sonzogni et al. 1978; Herdendorf 1987; Richards 2006). Water masses of the Detroit River reach ca. halfway into the western basin, before typically turning north- and/or east-ward, whereas the Maumee River discharges into the basin's most southwestern portion, with flows

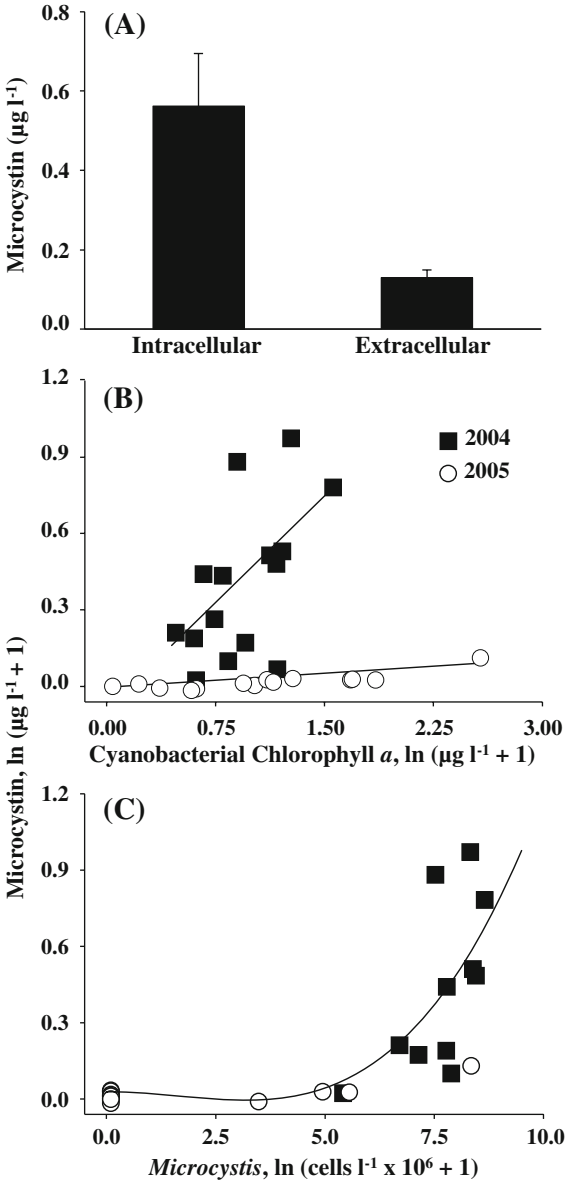

Fig. 7 **a** Intracellular and extracellular microcystin concentrations in 2004. Data are means ± standard error ($n = 15$). Intracellular microcystin concentrations as a function of **b** cyanobacterial Chl *a* concentrations, and **c** *Microcystis* cell densities. *Trend lines* represent best-fit relationships ($P \leq 0.05$), as predicted by linear and polynomial least-squares regression

predominantly directed eastward. During periods of persistent north or northeastern winds, Detroit River flows may reach the western and southern shorelines of the basin and Maumee River flows can 'pile up' offshore the river mouth and/or be directed along the basin's southern shoreline (Kovacik 1972; Herdendorf 1987). Mixing of such high-volume water masses (coincident with the basin's shallow depth) ensures the formation of transitory and spatially explicit environmental gradients, or ecotones, where species-specific growth responses enable opportunistic taxa to accumulate large biomass. Water circulation then can disperse or transport phytoplankton biomass throughout the basin.

The combination of P-availability, $\bar{I}_{ZWC}$, and Temp as parameters delineating cyanobacterial patterns suggested that biotic processes (most likely, resource-based competition) controlled cyanobacterial abundance and compositional dynamics throughout the western basin. Healey and Hendzel (1980) designated stoichiometric 'thresholds' of ≥50.82:1 and >100.43:1 µg C:µg Chl *a* to denote moderate and severe nutrient deficiency, respectively. Given this, mean basin-wide ratios of C:Chl *a* indicated phytoplankton to be moderately nutrient limited in 2003 and severely nutrient limited in 2004 and 2005 (refer to Table 1). Phosphorus is the most important nutrient regulating the phytoplankton growth and biomass increases, including those of cyanobacteria, in western Lake Erie (Hartig and Wallen 1984; Fahnenstiel et al. 1998; Wilhelm et al. 2003; c.f.; Fitzpatrick et al. 2007). During this study, phytoplankton communities likely were controlled by P-availability, although occurrences of both P- and N-deficiency were noted. In 2004, the mean basin-wide ratios of ca. 29:1 N:P and 208:1 C:P indicated that phytoplankton were P-limited (based on the stoichiometric criteria of >22:1 N:P and 129–258:1 C:P by Healey and Hendzel 1980, to denote severe and moderate P-deficiency, respectively). Although the use of mean values to denote basin-wide nutrient status of phytoplankton can be problematic—in that extreme states of nutrient sufficiency and deficiency are over- and under-stated, respectively (after Hecky et al. 1993)—the large standard errors (relative to mean values) associated with N:P and C:P ratios signified a considerable variability in P status (including instances of P deficiency) associated with communities in 2003. Healey and Hendzel (1980) designated particulate ratios of <8.3:1 C:N to denote N-sufficiency. Because mean basin-wide C:N ratios ranged from 7.31:1 to 7.77:1, phytoplankton appeared N-sufficient in all years. However, instances of moderate N-limitation (i.e., C:N ratios of ≥8.3:1 to ≤14.6:1; Healey and Hendzel 1980) were observed at select stations in 2003 and 2004. Such variable

instances of contrasting nutrient-limitation are consistent with the findings of Guildford et al. (2005) that although phytoplankton in western Lake Erie often appear nutrient sufficient (overall), P and N can, at times, be limiting.

During periods of nutrient sufficiency, light and temperature become the primary factors regulating cyanobacterial growth and accumulations. Climatic conditions during late-summer throughout western Lake Erie include intense yet variable irradiance, low wind speeds, and high temperatures. *Microcystis* and *Planktothrix* optimize and exploit light-harvesting (to the exclusion of other taxa) within near-surface, static waters via gas-regulated buoyancy alterations and dynamic photoadaptive capacities (Paerl 1984; Paerl et al. 1985; Millie et al. 1990). Ratios of C:Chl *a* were positively associated with $\bar{I}_{ZWC}$ and Z_E and negatively associated with variables indicative of algal biomass and production, suggesting that alteration(s) of Chl *a*:cell within phytoplankton occurred across distinct light environments. As a phylogenetic group, cyanobacteria prefer warmer waters than diatoms and chlorophytes (Paerl and Huisman 2008). Although lower water temperatures generally favor the growth of *P. agardhii* over *Microcystis* spp. (Roberts and Zohary 1987), basin-wide water temperatures during 2005 (when *Planktothrix* was dominant basin-wide) were similar to those of previous years. However, temperature effects are secondary to the interacting effects of water-column mixing, light availability, and nutrients in determining the 'species succession' of bloom-forming cyanobacteria (Paerl 1996) and the relationship between light and nutrient availability in controlling phytoplankton is not straightforward (Fahnenstiel et al. 2000). Further, it must be remembered that phytoplankton communities were assessed in late-summer, when bloom proliferation throughout the basin typically is greatest. Identified associations of phytoplankton abundance and composition with nutrient limitation and/or abiotic variables only reflect outcomes of (short-term) environmental forcing and could not be used to infer causality for inter- and intra-annual succession and/or the timing of bloom initiation (c.f. Millie et al. 2008). Nevertheless, phytoplankton composition and distribution throughout western Lake Erie appeared to be regulated by physical forcing and nutrient availability, acting synergistically and differentially upon phylogenetic groups (c.f. Lean et al. 1983).

Cyanobacterial toxicity

Microcystin concentrations were within the range of values typically reported for western Lake Erie and other eutrophic waters of the Great Lakes, including Saginaw Bay (Lake Huron) and Lake Ontario (Vanderploeg et al. 2001; Murphy et al. 2003; Rinta-Kanto et al. 2005; Dyble et al. 2008; Fahnenstiel et al. 2008). In 2004, microcystin bound within cells contributed the majority of total microcystin; intracellular concentrations were ca. five-times that of dissolved (extracellular) concentrations. Extracellular concentrations did approximate or exceed (up to four-times that of) intracellular concentrations at select offshore stations, signifying a localized release of cellular toxin within a senescing cyanobacterial community (e.g., Ross et al. 2006). Alternatively, as suggested by Kaebernick et al. (2000), an increase in extracellular microcystin may arise from active toxin release upon cell exposure to an unidentified (high) irradiance threshold. Nevertheless, the episodic occurrence of equivalent or greater extracellular concentrations (than intracellular concentrations) refutes the dogma that most cyanotoxins are maintained within cells (see Rinta-Kanto et al. 2005), and confirms the need to assess both particulate and dissolved microcystins for realistic appraisals of toxicity.

The physiological basis for microcystin synthesis is unknown, and it is difficult to unequivocally identify endogenous and/or environmental variables that inhibit or induce its production and accumulation. If microcystin synthesis is tightly coupled to cell growth, the controls of toxin accumulation might be related to the (immediate) environment within which cyanobacteria exist, as they compete with other taxa for nutrients, energy, and/or habitats essential for optimizing growth (Orr and Jones 1998; White et al. 2003). In 2004, microcystin concentrations and cell quotas corresponded to multiple environmental variables. However, such variables may indicate conditions coincident with blooms caused by nutrient-laden tributary inflows (i.e., light environment, P availability, pH, SpCond). Notably, growth rate and microcystin cell quota did not appear to be associated. From this, it appears that environmental factors regulate microcystin indirectly, via control of cyanobacterial abundance and distribution (see Orr and Jones 1998; Wu et al. 2006; Fahnenstiel et al. 2008).

Any attempt to delineate factors promoting toxic phenomena must consider the phylogenetic- and genomic-constraints of microcystin production. Although greater microcystin concentrations often occur in systems dominated by *P. agardhii* (than in those dominated by other cyanobacterial species; Fastner et al. 1999), intracellular concentrations within western Lake Erie corresponded to *Microcystis* abundance, but not with *P. agardhii* abundance. Both the lower microcystin concentrations and the cell quotas in 2005 than in 2004 might be explained by a 'shift' in cyanobacterial dominance from *M. aeruginosa* to *P. agardhii*. Conversely, the highest observed microcystin concentrations (in Sandusky Bay, 2003) were associated with *Planktothrix* dominance, with little *Microcystis* present. Morphologically identical toxic and non-toxic populations of *M. aeruginosa* and *P. agardhii* simultaneously occur (Kurmayer et al. 2002, 2004; Via-Ordorika et al. 2004) and the co-occurrence of these taxa has been documented for western Lake Erie (Rinta-Kanto and Wilhelm 2006; Dyble et al. 2008). Within potentially toxic populations, considerable variability in the gene cluster associated with microcystin production exists (e.g., Kaebernick et al. 2000). Consequently, microcystin cell quota can fluctuate by several orders of magnitude (Blackburn et al. 1997; Carmichael 1997). Presumably, intra- and inter-annual variation in toxin production and/or accumulation naturally occur throughout the western basin, dependent upon the proportion of toxic genotypes within a mixed population and the 'shifts' in the dominance of genotypic 'strains' and/or species with different toxicities (Rapala and Sivonen 1998; Briand et al. 2002).

Potential ramifications of blooms

Expansive chlorophyte and cyanobacterial blooms throughout the basin may have ecological and/or health ramifications. Due to their toxicity, low nutritional value, and/or a cell size/morphology that is difficult to ingest, *Pandorina, Microcystis*, and *Planktothrix* are not 'preferred' prey items for most grazing predators (Blom et al. 2001; Łotocka 2001; Kampe et al. 2007). Microcystins can accumulate within the food web, with subsequent developmental abnormalities within and/or whole-scale elimination of biota postulated to arise from the toxicity (Ernst et al. 2001; Katagami et al. 2004). Yet, zooplankters and dreissenid mussels successfully prey upon *Pandorina, Microcystis*, and *Planktothrix* (Stutzman 1995; Epp 1996; Dionisio-Pires et al. 2005) and as a result, blooms of these taxa may have a greater role in food web functioning in western Lake Erie than generally believed (after Nicholls 1997). Given the magnitude of the chlorophyte/cyanobacterial bloom (ca. 830 μg Chl *a* l^{-1}) in lake waters directly impacted by nutrient-laden inflows of the Maumee River in 2003, a significant amount of fixed C and evolved O_2 would be added to the system. Experiments revealed that photosynthetic productivity within the bloom to be 1,781 ± 102 μg C l^{-1} h^{-1} (mean + standard error, $n = 4$), as determined from 1 h, ^{14}C incubations (G. Fahnenstiel, unpublished data). Given this rate and assuming a 10 h period of optimal PAR within a representative late-summer day (of 14 h photoperiod; c.f. Hiriart-Baer and Smith 2004), daily production of the surface bloom may reach ca. 16–20 mg C l^{-1} d^{-1}. Such a high productivity rate (and resultant daily production) associated with localized, surface blooms within nutrient-enriched waters is (are) not unique. Robarts (1984) and Roberts and Sephton (1989) observed carbon-fixation rates up to ca. 5,900 and 8,900 μg C l^{-1} h^{-1}, respectively, for phytoplankton communities (predominantly *M. aeruginosa*) in hypertrophic African lakes. Conversely, bloom-forming cyanobacteria typically are poor oxygenators of the water (Paerl and Tucker 1995). Instances of diel and short-term hypoxia/anoxia arising from night-time cell respiration and senescence of the bloom could result and create (localized) hazardous conditions for fauna. Further, the export of epilimnetic algal biomass from the Lake's western to central basin and its subsequent implications upon hypolimnetic oxygen budgets in the central basin may be noteworthy (Edwards et al. 2005).

On an annually intermittent basis and/or at specific locales, cyanobacterial Chl *a* concentrations throughout the western basin exceeded guideline concentrations of 10 and 50 μg total Chl *a* l^{-1} (under conditions of cyanobacterial dominance) to denote potential allergenic and moderate health concerns, respectively, related to exposure in recreational waters (World Health Organization 2003). Although microcystin concentrations typically were less than the guideline concentration for human consumption (of 1 μg l^{-1}, see World Health Organization 2004),

microcystin concentrations for the *Planktothrix* bloom in Sandusky Bay during 2003 and *Microcystis* accumulations along the western shoreline of the basin during 2004 exceeded this 'threshold' concentration. In a concurrent study, Dyble et al. (2008) reported 58 μg microcystin l^{-1} to be associated with a *Microcystis* bloom entrapped within a swimming/boating basin (station F_{2b}, see Fig. 1).

Conclusions

Basin-wide phytoplankton dominance throughout western Lake Erie varied, with chlorophytes, diatoms, and cyanobacteria contributing the majority of the late-summer total Chl *a* in 2003, 2004, and 2005, respectively. Bloom events were annually and spatially episodic, with maximum Chl *a* concentrations occurring within waters impacted by Maumee River inflows and in Sandusky Bay.

The maximal observed biomass resulted from an immense bloom of the chlorophyte, *P. morum* (up to 727 μg chlorophyte Chl *a* l^{-1}), and the cyanobacteria, *M. aeruginosa* and *P. agardhii* (up to 100 μg cyanobacterial Chl *a* l^{-1}), in 2003. *Microcystis* was the most-widespread and dominant cyanobacterium throughout western Lake Erie in 2003 and 2004, whereas *Planktothrix* accounted for up to 90% of the relative cyanobacterial biomass at select sites in 2005.

Phytoplankton abundances were regulated by physical factors and P-availability, acting synergistically and differentially upon phylogenetic groups. Water mass movements and mixing were the primary determinants for (overall) phytoplankton accumulations and distributions. Taxon-specific optimization of nutrients, light availability, and water temperature controlled cyanobacterial composition dynamics and bloom potentials.

Intracellular microcystin concentrations corresponded to *Microcystis* abundance (but not to *Planktothrix* abundance) and to environmental parameters (i.e., light environment, P-availability, pH, SpCond), indicative of conditions coincident with cell accumulations arising from nutrient-laden tributary inflows. Because cyanobacterial growth rates did not correspond to microcystin cell quotas, environmental parameters appeared to regulate microcystin indirectly, via control of cyanobacterial species composition, abundance, and distribution.

Bloom events of significant magnitude could impact primary production basin-wide and potentially alter food web functioning. Cyanobacterial Chl *a* and microcystin concentrations intermittently exceeded the World Health Organization's guideline concentrations denoting human health concerns. The episodic occurrence of sizeable extracellular microcystin concentrations confirmed the need to assess both particulate and dissolved cyanotoxins for realistic appraisals of toxicity.

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