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Article *in* Aquatic Microbial Ecology · August 2014

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doi: 10.3354/ame01708

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online August 15

Connecting the blooms: tracking and establishing the origin of the record-breaking Lake Erie *Microcystis* bloom of 2011 using DGGE

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ABSTRACT: Summer blooms of *Microcystis* now occur every year in Lake Erie with varying concentration, duration, and spatial extent. The recording-breaking bloom of 2011 began in the western corner of the lake during early summer, and reached its peak in late summer covering 2968 km^2. Start and peak blooms were offset by 3 mo and separated by 120 km, raising the question: Is *Microcystis* transported across the lake or do separate blooms arise from separate source populations? This study addressed this question by measuring the genetic diversity of *Microcystis* across the lake and throughout the summer. Seven sites separated by about 100 km were sampled monthly during the summer of 2011 for genetic analysis of the *Microcystis* population. Furthermore, 2 major rivers (Maumee and Sandusky) and lake sediments were sampled and collected prior to bloom formation to investigate source populations. Denaturing gradient gel electrophoresis was used to generate *Microcystis*-specific molecular fingerprints of the 16S-23S rRNA internal transcribed spacer region. Dendrograms and principal component analysis were used to investigate similarity among samples. Fingerprints of lake water samples were more similar to the sediments than tributaries, indicating the sediments were a more likely bloom source. All lakes samples collected were >50% similar with several universal bands, indicating *Microcystis* was transported west to east by water currents and that the beginning and peak blooms were not isolated. This information characterizes the origin and movement of this massive and problematic bloom, and can be used to inform management practices aimed at preventing blooms in Lake Erie.

KEY WORDS: *Cyanobacteria* · DGGE · Eutrophication · *Microcystis*

INTRODUCTION

Cyanobacterial blooms have the potential to produce toxic compounds that can harm humans and wildlife, alter aquatic ecosystems, and negatively impact local economies (Huisman et al. 2005, Dodds et al. 2009). Human activities and global climate change have resulted in conditions that favor cyanobacterial blooms that occur worldwide (Paerl & Scott 2010). For example, excessive nutrients originating from agricultural runoff and poor sewage treatment can increase nutrient concentrations in aquatic systems, resulting in conditions that favor the rapid growth of *Cyanobacteria* (Downing et al. 2001). While nutrient abatement programs have been proposed to limit and prevent cyanobacterial blooms (Paterson et al. 2011, Scott & McCarthy 2011), such programs might benefit substantially from understanding the geographic origin of the inoculum of *Cyanobacteria* and how blooms are transported within a lake, especially large lakes.

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Lake Erie is the 11th largest lake on Earth (surface area) (Herdendorf 1982), and the toxic cyanobacterium *Microcystis* spp. is widespread in the lake (Ouellette et al. 2006). *Microcystis* blooms (>10^5 cells ml^{-1} at the surface) occur in the western basin primarily during summer months, when high nutrient concentrations and favorable light conditions create an ideal environment for bloom development (Wilhelm et al. 2003, Conroy et al. 2005, Moorhead et al. 2008, Chaffin et al. 2011, 2012). Since the early 2000s, *Microcystis* blooms have occurred annually in Lake Erie, with large variations in bloom density and spatial distribution (Stumpf et al. 2012, Bridgeman et al. 2013). For example, in years featuring relatively high nutrient loading from the Maumee River (Joosse & Baker 2011), *Microcystis* blooms can extend eastward into the center of the lake (Stumpf et al. 2012) covering nearly 3000 km^2.

All phytoplankton require an inoculum to begin each new growing season, which is most often maintained either by the survival of a low number of propagules in the water column throughout the year, or by the production of resting spores that over-winter on the lake sediments (Reynolds 2006). It follows that *Microcystis* from previous blooms over-winters on the surface of the lake sediments, and once appropriate bloom conditions arise, is available to inoculate the water column, facilitating the next bloom (Preston et al. 1980). Tributaries can also contribute *Microcystis* to lakes. The Maumee River is a likely source of *Cyanobacteria* to Lake Erie, as high *Cyanobacteria* densities are frequently observed throughout the river, especially during the summer months (Conroy et al. 2014). Despite eutrophic conditions, the river waters are frequently turbid, limiting sunlight penetration, and subsequently, the likelihood that blooms will occur in the river. These factors provide support to the 'algal loading hypothesis', which states that tributary phytoplankton are nutrient-replete but light-limited, and once the phytoplankton are loaded into the clear waters of the lake, they take advantage of the more-favorable light climate (Conroy et al. 2008).

Wind and water currents can impact phytoplankton distribution in lakes. Water currents in Lake Erie are driven by tributaries that enter the lake in the western basin, and exit the lake through the easternmost distributaries (Beletsky et al. 2013). Currents and winds impacting large lakes are known to transport *Microcystis* blooms away from their original locations (Hutchinson & Webster 1994, Ishikawa et al. 2002). Furthermore, modeling efforts have shown that the prevailing currents in Lake Erie move from west to east (Beletsky et al. 2013) and would support the movement of source materials and subsequent blooms from the western basin toward the central and eastern basins.

In Spring 2011, high rainfall and phosphorus loading (Stumpf et al. 2012) followed by calm waters during early summer (Michalak et al. 2013) facilitated a cyanobacterial bloom in Lake Erie of unprecedented density and spatial coverage, which was confirmed to be *Microcystis*. Satellite imagery suggested that 2 separate blooms occurred (Michalak et al. 2013), the first occurring during July and August in the western basin, and the second in October in the central basin. Yet to be determined, however, is whether the July and August bloom spread throughout the lake and provided a source of *Microcystis* for the October bloom. Lake Erie water masses generally move from west to east (Swayne et al. 2005); therefore, it is possible that *Microcystis* transported by currents could have served as a source of inoculum for the October bloom. Alternatively, the October bloom could have developed independently of the July and August bloom, fed by a unique source of inoculum. This is a logical assertion, as differing environmental conditions define the western and central basins, and can select for distinct *Microcystis* species. For example, dissolved nutrient concentrations in the central basin are generally lower than those in the western basin (Conroy et al. 2007, Chaffin et al. 2013), and different strains of *Microcystis* could be adapted to the different nutrient concentrations of each basin (Davis et al. 2009).

Determining the origin of inoculum of the annual algal blooms can facilitate the development of management practices that limit bloom frequency and intensity. Denaturing gradient gel electrophoresis (DGGE) analysis is a genetic fingerprinting tool used to describe the structure of microbial communities (Muyzer 1999). Because DGGE analysis features greater resolution than culture-based methods of community analysis (Temmerman et al. 2003), it can be used to provide a descriptive fingerprint of a mixed microbial assemblage, including assemblages of *Microcystis*. If the genetic signatures of *Microcystis* occurring in different parts of the lake are distinct, then it can be assumed that the blooms originated locally, especially if the *Microcystis* was genetically similar to the local source population in sediments or nearby tributaries. Conversely, if blooms featured similar signatures, and if the signatures were different from the potential local sources, then the possibility of locally distinct sources could be ruled out. Because understanding the dynamics of cyanobacterial blooms

can allow for management efforts focused on the source(s) of the bloom, the goals of this study were to use genetic fingerprinting (1) to test the hypothesis that the 2011 *Microcystis* bloom was a single bloom that spread throughout the lake or, alternatively, multiple blooms, and (2) to determine the geographic origin(s) of the *Microcystis*.

MATERIALS AND METHODS

Sample collection

Water and sediment samples were collected from 7 lake locations (Fig. 1). One location in each of the Maumee and Sandusky rivers was also sampled. Sediments were collected on 8 June 2011 from 5 of the sites (MBay, WB_W, WB_E, SBay, and SSB_W, Fig. 1) prior to bloom formation to determine whether the lake bottom was a source of cyanobacteria for the blooms. Surface sediments (top 2 cm) were collected in triplicate (to ensure sufficient sediment was collected) using a ponar dredge and pooled into sterile, 50 ml tubes and then placed on ice. The dredge was disinfected before each new location was sampled by first scrubbing with phosphate-free detergent, and then by submersion in 0.33% bleach solution for at least 20 min, then sodium thiosulfate for 5 min, and finally, deionized water. Upon arrival at the laboratory, sediment samples were stored at −80°C until further analysis.

The 7 bay and lake sites were sampled monthly in July, August, and September. Because of the distance between sites, all sites could not be sampled on the same day, but were always sampled within 2 d of each other. In general, the easterly sites were sampled earlier in each monthly sampling campaign, while the westerly sites were sampled later. Sites SSB_E and CB were sampled on 10 October in response to a cyanobacterial bloom detected in the central basin by satellite. Water samples were collected from the surface to near sediment (Table 1) using an integrated tube sampler, stored in 1 l acid-washed polyethylene bottles and kept on ice prior to analysis, which occurred within 4 h of sampling. An additional water sample was collected and preserved in Lugol's solution for *Microcystis* enumeration (see next section).

To determine whether tributaries were a source of *Microcystis* for the blooms, the Maumee and Sandusky

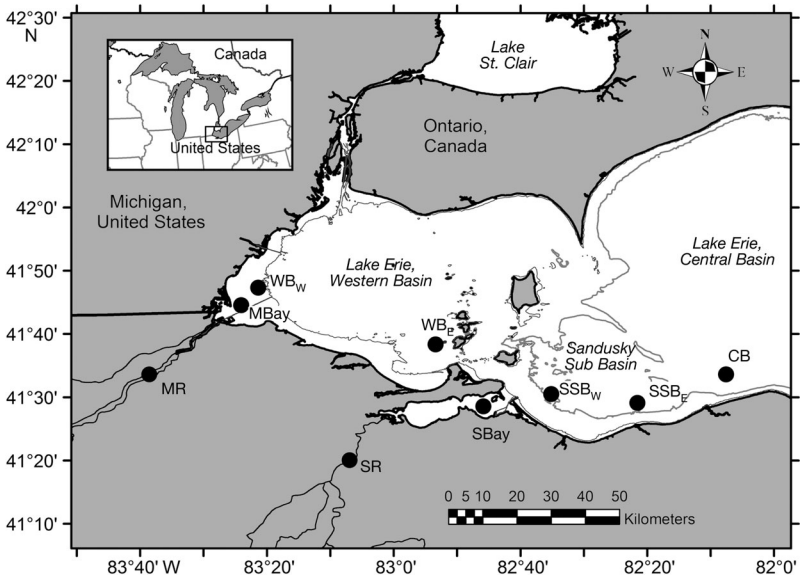

Fig. 1. Sample locations in Lake Erie and 2 major tributaries. See Table 1 for site abbreviations

Table 1. Sample locations with site depth and sample depth of water collected. Notation for site abbreviations is as follows: M = Maumee, WB = western basin, S = Sandusky, SSB = Sandusky sub-basin, R = River, CB = central basin. Subscripts: W = west, E = east. CB was only sampled once on 10 October. Only sediment samples were collected on 8 June

Site	Latitude (° N)	Longitude (° W)	Depth (m)	Sample depth (m)	Dates sampled
MR	41.56020	83.64260	1.3	0–0.25	23 Jun, 11 Jul, 4 Aug, 6 Sep
MBay	41.74250	83.40090	2.3	0–2	8 Jun, 22 Jul, 12 Aug, 14 Sep
WB_W	41.78890	83.35630	5.8	0–5	8 Jun, 22 Jul, 12 Aug, 14 Sep
WB_E	41.63918	82.88988	8.5	0–7	8 Jun, 20 Jul, 11 Aug, 12 Sep
SR	41.33440	83.11570	1.3	0–0.25	23 Jun, 11 Jul, 4 Aug, 6 Sep
SBay	41.47590	82.76404	2.7	0–2	8 Jun, 20 Jul, 11 Aug, 12 Sep
SSB_W	41.50828	82.58571	13.1	0–8	8 Jun, 20 Jul, 11 Aug, 12 Sep
SSB_E	41.48500	82.35920	12.7	0–8	20 Jul, 11 Aug, 12 Sep, 10 Oct
CB	41.56037	82.12592	13.9	0–8	10 Oct

rivers (sites MR and SR, respectively) were sampled approximately 1 wk prior to the lake sampling during each month. Water samples collected from the tributaries were processed identically to those collected from the lake.

Upon arrival at the laboratory, between 250 and 750 ml of sample water was filtered through 0.45 µm pore diameter polyvinylidene fluoride (PVDF) membranes for DGGE analysis. The volume filtered was dependent on the amount of *Cyanobacteria* and/or suspended sediments in the sample, and samples with higher amounts of *Cyanobacteria* required less volume. Filters were stored at −80°C in sterile tubes until analysis.

Microcystis enumeration

The plankton biomass in the fixed water samples (300 ml) was concentrated to between 7 and 30 ml by allowing phytoplankton to settle to the bottom in a 500 ml graduated cylinder for at least 48 h. Each concentrated sample was transferred to a 30 ml amber glass vial. A 1 ml subsample of the concentrated sample was pipetted onto a gridded, Sedgewick-Rafter counting chamber and viewed at 200× magnification. Cells of *Microcystis* in colonies were enumerated using the relationship between colony diameter and numbers of cells (Watzin et al. 2006) and Spot Advanced software was used for the measurements, as in Davis et al. (2012). At least 50 *Microcystis* colonies were measured until a constant average was obtained, and all colonies in the 1 ml subsample were counted.

Molecular methods

DNA was extracted from the PVDF filters and sediments (10 g) using the MoBio Power Water kit (#14900, MoBio Laboratories) and MoBio PowerMax Soil DNA kit (#12988), respectively, and stored at −20°C. PCR amplification of the 16S-23S rRNA internal transcribed spacer (ITS) region was performed using *Cyanobacteria*-specific primers, including 16S rRNA-CSI-F (5′-G(T/C)C ACG CCC GAA GTC (G/A)TT AC-3′) and 23S rRNA-UL-R (5′-CCT CTG TGT GCC TAG GTA TC-3′) (Janse et al. 2003). PCR was performed in a 50 µl reaction volume containing 1 µl of DNA, 0.02 U μl^{-1} *Taq* DNA polymerase, 0.2 µM of each primer, 200 µM of each dNTP, 0.5 mg ml^{-1} of bovine serum albumin, and 2.5 mM $MgCl_2$. PCR products were separated on a 2% agarose gel containing 0.5 µg ml^{-1} ethidium bromide and were visualized, photographed with a Kodak Gel Logic 200 imaging system, and analyzed with Kodak one-dimensional image analysis software. The band containing *Microcystis* PCR products (550 bp; Janse et al. 2003) was excised and purified using the Promega PCR Clean-Up System. The purified DNA was reamplified, as described above, and the diagnostic *Microcystis* DNA band was excised, purified, and reamplifed to verify that the PCR product was the 550 bp *Microcystis* band. For the final amplification, a 40 bp GC clamp was added to the 3′ end of the forward primer (Muyzer et al. 1993) to facilitate separation of the *Microcystis*-specific PCR products during DGGE analysis.

DGGE analysis was performed according to the method of Janse et al. (2003) using an 8% polyacrylamide gel (1.0 mm thick) containing a denaturing gradient of 30 to 40% (7 M urea and 40% formamide represents 100% denaturant concentration). PCR products generated from the sample collected from SSB_W in August were used as a DGGE marker because following DGGE, the fingerprint contained 10 bands that spanned the lane, allowing for lane-to-lane comparisons of the fingerprints. The marker was loaded such that no more than 4 fingerprint lanes separated each marker lane. Electrophoresis was performed at 60 volts for 16 h at 60°C in a DCODE Universal Mutation Detection system (Bio-Rad Laboratories) containing 1× Tris-acetate-EDTA (TAE) buffer. Following electrophoresis, gels were stained for 15 min with 50 ml of a GelStar nucleic acid stain (diluted 1:10 000 in 1× TAE) and visualized as described above. To confirm that bands detected using DGGE originated from *Microcystis* DNA, cores from 11 bands were excised from the gel, and DNA was eluted from each following incubation overnight in 1 ml of 1.0 M Tris-HCl at 4°C. Fifty microliters of the eluted DNA was PCR amplified and visualized, as described above. All excised bands were 550 bp.

Image analysis

All DGGE fingerprint images were analyzed using GelCompar II software (version 4.5, Applied Maths) for pairwise comparisons of fingerprint similarity. Normalization was performed using the DGGE marker as an external reference. Similar bands that occurred in more than half of the samples were used as internal references. Bands with a minimum profiling of 5% were considered. Each band was considered to be an operational taxonomic unit (OTU). The Dice index

(Dice 1945) was used to calculate similarity matrices. Cluster analysis was performed on the resulting similarity matrix using the unweighted pair group method with arithmetic means algorithm, resulting in dendrograms that graphically displayed relationships between *Microcystis* community fingerprints (McLellan et al. 2003). The integrity of the dendrogram organization (branching and structure of the resulting clusters) was validated by the calculation of cophenetic correlation coefficients with GelComparII software, which were always >0.85 (maximum is 1.0). Separate dendrograms were generated to determine the similarity of region-specific *Microcystis* assemblages (Maumee and western basin, and Sandusky and sub-basin) to investigate source–sink relationships among river, lake bottom sediments, and the lake. In general, it is risky to compare different DGGE gels according this method, as it is prone to induce errors due to difficulties of aligning all the DGGE bands across gels. Therefore, from each gel we selected the DGGE bands for which we were fully confident of their *Microcystis* identity and knew their alignment behavior across gels, and created from this selection a database (absence, presence, and intensity of bands) that was subjected to a principal component analysis (PCA). In this way, we were able to determine relationships among *Microcystis* OTU composition across all samples. PCA was conducted using the factor dimension reduction tool in SPSS version 21 (version 21.0, IBM).

RESULTS

Microcystis aeruginosa was the dominant cyanobacterium in all lake sites except for site Sandusky Bay (SBay), which was dominated by the filamentous cyanobacterium *Planktothrix agardhii* (Fig. 2). Other species of *Microcystis* were observed, but their densities were <5% that of *M. aeruginosa*. The species were not differentiated by the molecular methods and are collectively referred to hereafter as *Microcystis*. The July *Microcystis* bloom began in Maumee Bay (MBay) and exhibited 23 000 cells ml^{-1} (integrated throughout the water column), and peaked in the western basin (WB) during August with cell densities exceeding 60 000 ml^{-1} (Fig. 2). Surface scum (top 10 cm) cell densities exceeded 10^6 cells ml^{-1} during peak bloom. Higher *Microcystis* densities were present in the Sandusky sub-basin (SSB) sites during September than in August, and the greatest cell densities for site SBB_E was recorded during October. The central basin (CB) site was sampled only during October and exhibited half the *Microcystis* cell density of SSB_E. *Microcystis* was not present in tributary samples.

DGGE analysis showed that most water samples produced fingerprints containing between 5 and 8 bands (Figs. 3 & 4). Three *Microcystis* phylotypes (bands #2, #4, and #5) were present in all samples that exhibited the high *Microcystis* cell densities (Fig. 2), suggesting that although the blooms were separated in space and time, they were linked by universal *Microcystis* phylotypes. Additionally, cluster analysis of the similarity matrix showed that the *Microcystis* assemblages from both western and eastern sites exhibited increased similarity during common blooms. For example, while the overall *Microcystis* assemblage similarity at all sites shared 43% similarity, assemblages sampled during blooms in July, August, and September were 62, 68 and 72% similar, respectively.

Cluster analysis of *Microcystis* in western Lake Erie (Fig. 4A) showed no clear relationship between assemblages in the Maumee River and those in Maumee Bay and the western basin. For example, *Microcystis* collected from the Maumee River in July and September was 36% similar to the *Microcystis* in all other samples. However, temporal relationships between the assemblages in Maumee Bay (MBay) and the western basin (WB_E) suggested that blooms in those locations were related, as bay and basin *Microcystis* assemblages were at least 70% similar during July, August, and September.

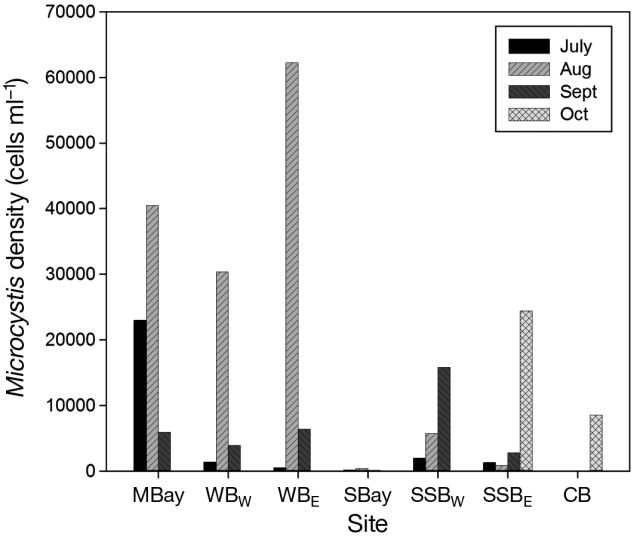

Fig. 2. *Microcystis* cell density in samples collected during summer 2011 in Lake Erie. Only sites SBB_E and CB were sampled in October. *Microcystis* cell density quantification was based on water sampled throughout the water column, whereas surface-only samples would have given cell densities 10 to 100 times higher. See Table 1 for site abbreviations

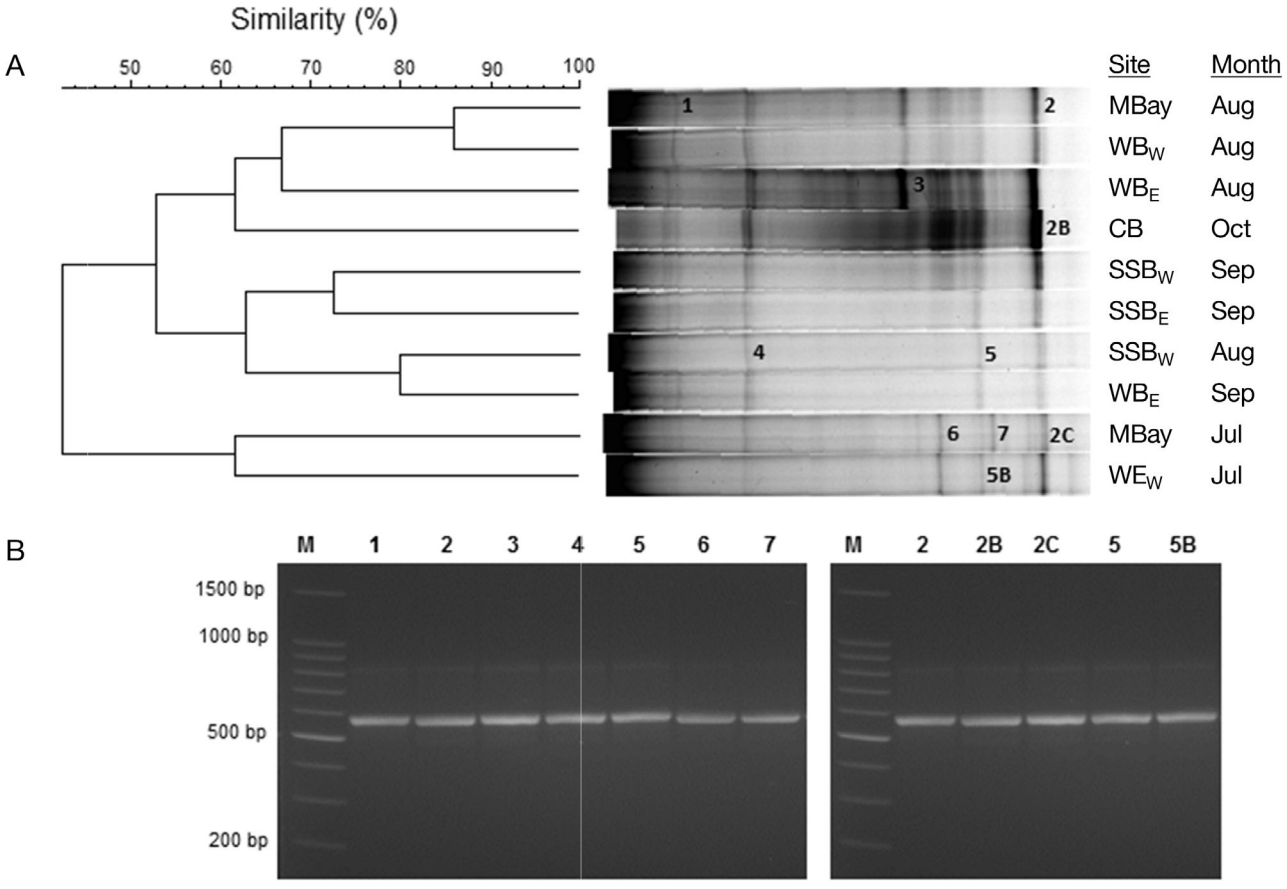

Fig. 3. (A) Dendrogram of peak *Microcystis* cell density samples shows high similarity throughout summer 2011. Dice index (Dice 1945) was used to calculate similarity. Numbers on the DGGE gel correspond to excised bands. (B) Agarose gel (2%) of PCR products from the excised bands verified that excised bands were 550 bp, which corresponds to *Microcystis* (Janse et al. 2004). PCR amplification was conducted using the 40 bp GC clamp, so the products appear to be 590 bp. See Table 1 for site abbreviations

Microcystis assemblages in the Sandusky sites exhibited a source–sink relationship, as the Sandusky River assemblages served as a source for the Sandusky Bay (SBay) sites. Specifically, cluster analysis showed that *Microcystis* assemblages in the Sandusky River were ~70% similar to those in Sandusky Bay and one of the westernmost Sandusky sub-basin sites. A second cluster of Sandusky *Microcystis* assemblage fingerprints included the sediment samples, 2 Sandusky sub-basin sites (August and September), the westernmost western basin (August), and the Sandusky Bay (September) sites, but illustrated no clear relationship between the sites (30% overall similarity).

The PCA of *Microcystis* OTUs analyzed together supported the cluster analysis (Fig. 5). Samples containing high *Microcystis* cell densities exhibited genetically similar *Microcystis* assemblages (such as, July, August, and September MBay, WB_W, and WB_E, and August, September, and October SBB_W, SBB_E, and CB), whereas samples containing low *Microcystis* cell densities (such as all MR, SR, and SBay, and July SSB_E) did not group with other samples. Furthermore, *Microcystis* assemblages in sediments collected before bloom formation from sites MB, WB_W, and WB_E grouped with the in-lake samples that had high *Microcystis* cell densities. The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.625 and the 2 components shown in Fig. 5 explained 39% of the total variation.

DISCUSSION

Our *Microcystis* cell density data concur with the 2011 time series satellite images shown by Michalak et al. (2013), indicating the occurrence of a bloom in the western basin in July and August, and then in the central basin during October. The highest *Microcystis* cell density measurements in the western basin

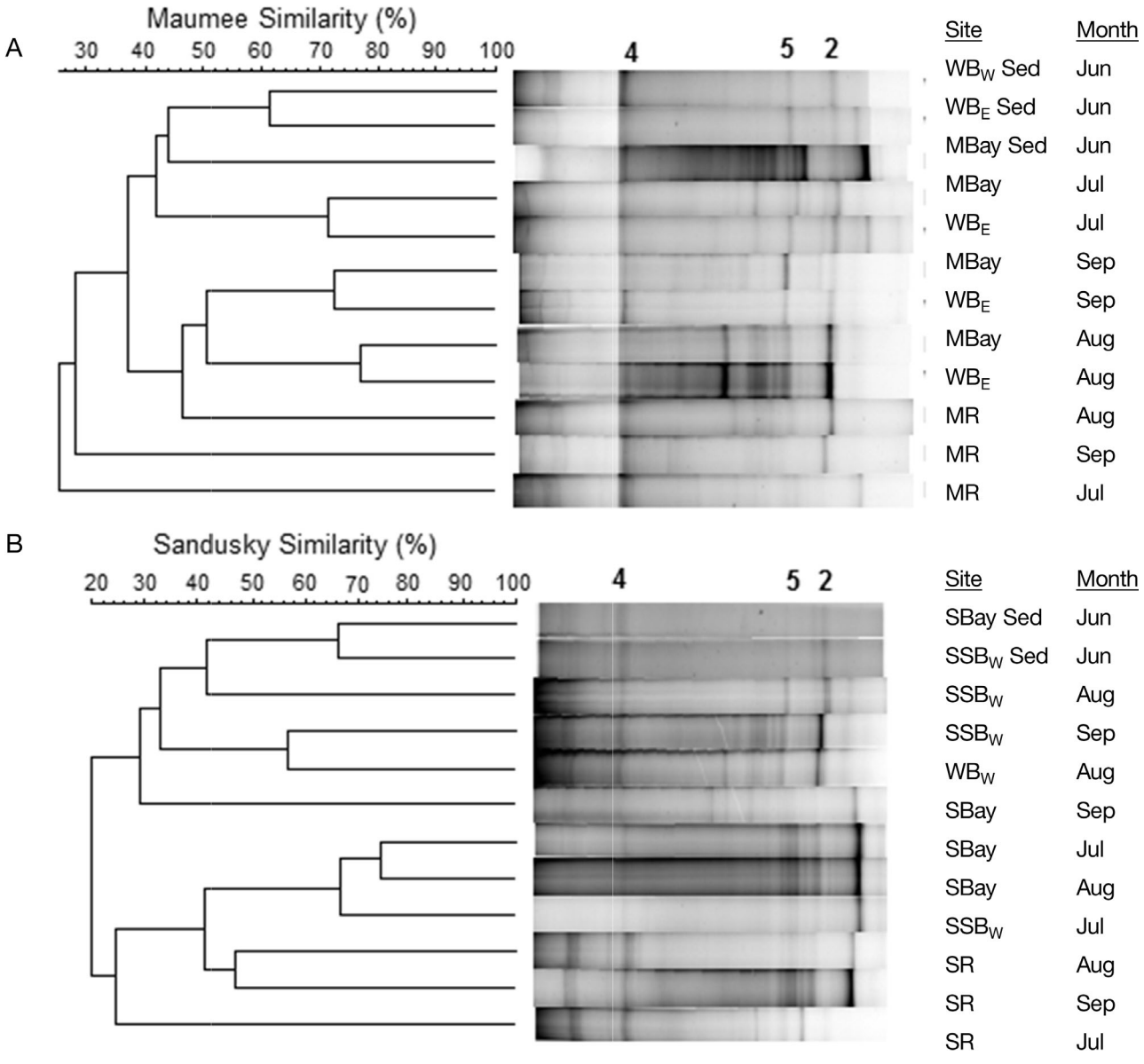

Fig. 4. (A) Dendrogram of the Maumee River–western basin system shows greater similarity between sediment and bloom samples than river and bloom samples. (B) Dendrogram of the Sandusky River–sub-basin system shows 2 main clusters of sub-basin and western basin samples and the river and bay samples. Numbers indicate position of bands shared by most samples from Fig. 3. Note the difference in similarity scale. See Table 1 for site abbreviations

sites occurred in August, whereas the highest cell densities in the Sandusky sub-basin occurred in September and in the central basin in October. Collection of the microbial biomass that was present during the 2 blooms followed by genetic fingerprinting of the *Microcystis* assemblages allowed us to test the hypothesis that a source–sink relationship existed between the 2 tributaries (the Maumee and Sandusky rivers; sources) and the sinks (the western and central basins). PCR-DGGE analysis of the 16S-23S rRNA ITS region of most axenic *Microcystis* cultures will yield 1 band in the fingerprint (Janse et al. 2004). However, our analyses resulted in fingerprints for each site that exhibited complex patterns of multiple bands, suggesting that *Microcystis* blooms in Lake Erie comprise a mixture of *Microcystis* strains. Samples collected during peak *Microcystis* cell densities revealed collective similarity among assemblages. The combination of cell density data and DGGE analyses of samples collected during peak bloom periods supports the hypothesis that the bloom comprised multiple, genetically similar strains of *Microcystis*, and was not the result of discrete assemblages. The bloom that occurred in the central basin

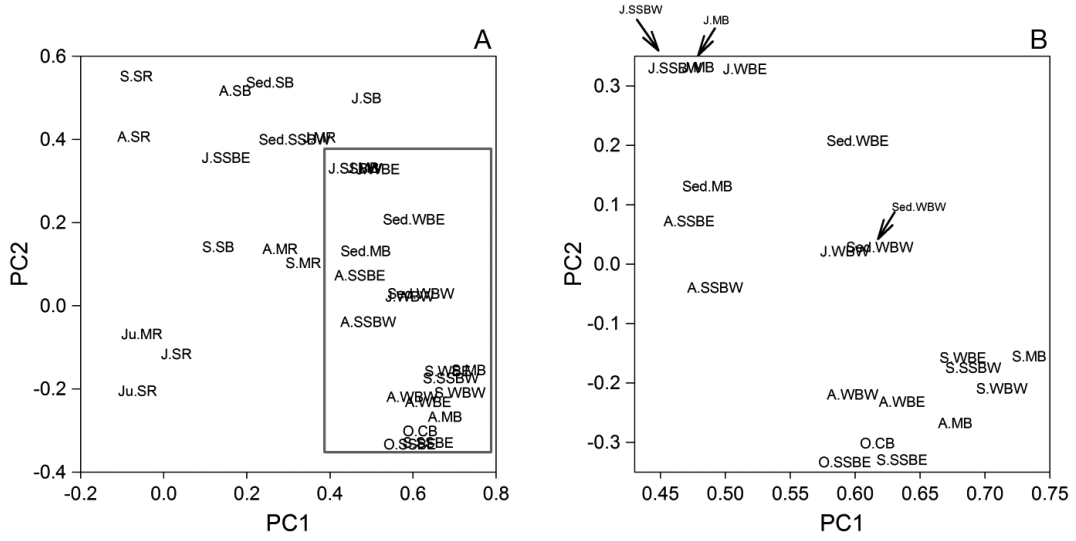

Fig. 5. Principal component analysis of the selected *Microcystis* DGGE bands observed in different DGGE gels (see 'Materials and methods'). This analysis showed greater similarity of sediment and in-lake samples than river and in-lake samples, and that peak *Microcystis* bloom samples were very similar to each other. The 2 components explained 39% of the total variation. The symbols on the plot indicate the month and sample location; for example, 'J.SB' is the July sample collected from Sandusky Bay. (A) All of the samples collected and (B) the area outlined in the gray box in panel A rescaled to allow identification of closely related samples. See Table 1 for site abbreviations

(October) was tracked to the Sandusky sub-basin in September and to the western basin (including Maumee Bay) in August.

Wind and water currents play an important role in determining phytoplankton distribution in lakes. For example, lake sites that would normally not support bloom development can often be impacted by blooms because currents can move the bloom throughout the lake (Ishikawa et al. 2002). Winds can also transport *Microcystis* blooms, which results in the accumulation of biomass at downwind lake sites (Hutchinson & Webster 1994). In Lake Erie, the general water currents are west to east and driven by river inflow in the western basin and outflow in the eastern basin (Beletsky et al. 2013). Therefore, water current models of Lake Erie (Beletsky et al. 2013) and our data together support the hypothesis that the *Microcystis* bloom of 2011 that developed in the western basin spread eastward throughout summer and ultimately impacted the central basin. Follow-up studies that include more frequent molecular analysis combined with time-series satellite imagery (as Wynne et al. 2010 did for the 2008 bloom) would provide deeper insights into how a *Microcystis* bloom is transported across Lake Erie.

Lakebed sediments in Maumee Bay are known to contain historically high densities of *Microcystis*, and have been implicated as a source of *Microcystis* for prior blooms (Gruden 2010). Further evidence supporting the role of sediments as a source of inoculum for *Microcystis* blooms in Lake Erie is provided by Rinta-Kanto et al. (2009) who found that *Microcystis* could be cultured from lakebed sediments sampled 1 mo prior to a bloom event, while sequences of *mcyA* (necessary for microcystin production) sampled from the same lake sediments were similar to those sampled from the water column during the bloom. In the current study, the *Microcystis* assemblages sampled from lakebed sediments (June) in the western basin were most similar to the assemblages sampled during the first (July) bloom event, suggesting that the sediments provided source *Microcystis* for the bloom. Conversely, the Maumee River was not a major contributor of *Microcystis* to the bloom events, as *Microcystis* assemblages sampled from the river in July and September exhibited low similarity (38%) to any other assemblages. The absent or minor role of the Maumee River as a *Microcystis* source has been observed previously, as Kutovaya et al. (2012) showed that *mcyA* gene sequences collected from the Maumee River were associated not with *Microcystis*, but rather with *Planktothrix*. It should be noted that the *Microcystis* assemblage in the river in August exhibited increased similarity (50%) to assemblages from Maumee Bay and the western basin, suggesting a role as a *Microcystis* source. However, because the bloom was already widespread in the lake at the time of the August river sampling, the similarity between the lake and river samples is a likely indicator of reverse flow of the lake into the river. Backflow

events can occur when prolonged northeasterly winds create temporary reverse-flow conditions in the lower Maumee River (Herdendorf 1990).

Microcystis assemblages in the Sandusky River exhibited limited genetic similarity to those in outlying lake sites (Sandusky sub-basin and western basin). Conversely, *Microcystis* assemblages from the river exhibited similarity to samples from Sandusky Bay. Taken together, the results indicate that the Sandusky River is a potential source of *Microcystis* to Sandusky Bay, but not likely to the greater lake. Although our results indicate similarity between the *Microcystis* assemblages in the Sandusky River and Sandusky Bay, the relevance of this relationship with regard to providing source *Microcystis* for a bloom event must be interpreted with caution, as *Microcystis* represents a minor proportion of the total phytoplankton population in Sandusky Bay (Conroy et al. 2007). Lugol's samples indicated high amounts of the filamentous cyanobacterium *Planktothrix agardhii*, which dominates the Sandusky Bay due to the shallow, turbid, and well-mixed conditions that favor filamentous *Cyanobacteria* over colonial *Cyanobacteria* (like *Microcystis*) and eukaryotic algae (Scheffer 1998).

The Maumee and Sandusky rivers are 2 major tributaries for Lake Erie, but did not contribute detectable *Microcystis* to the bloom. Samples collected during previous years to investigate the relationship between the rivers and bloom events also indicated that the Maumee River did not play a role in contributing *Microcystis* to blooms (Kutovaya et al. 2012). However, while the role of the rivers to the bloom formation was minimal with regard to *Microcystis* contribution, the rivers have been shown to impact cyanobacterial bloom ecology through other mechanisms. For example, the Maumee and Sandusky rivers drain watersheds that feature high-intensity agriculture (Han et al. 2011) and contribute high concentrations of phosphorus to the lake that support cyanobacterial growth (Baker & Richards 2002). Local field soils exhibit high clay content, which typically increases the availability of preferential flow pathways (cracks and fissures) during the dry conditions typical during summer. These features aid in the rapid transport of materials through the soil profile during the initial stages of soil rewetting (Jamieson et al. 2002), increasing the likelihood that nutrients will leach through the profile. Conversely, during wet conditions, the clay will swell, constricting the flow pathways, resulting in decreased water (and nutrient) percolation and increased overland runoff, creating chemical conditions in the nearshore zones that favor *Microcystis* growth (Chaffin et al. 2011).

Genetic fingerprinting methods, such as DGGE analysis, can overcome the limitations of enumeration analyses by effectively characterizing the structure of microbial communities (Muyzer 1999, Temmerman et al. 2003), including cyanobacterial assemblages (Sigler et al. 2003). While *Microcystis*-specific primers do not exist for the 16S-23S rRNA ITS region, we are confident that the PCR products that we subjected to DGGE analysis represented *Microcystis* DNA. The PCR products that we used in our analyses were all 550 bp in size, which is a unique, diagnostic size for the *Microcystis* ITS region (vs. other *Cyanobacteria*) (Janse et al. 2003). Additionally, we excised the PCR product from the agarose screening gel, purified, and re-amplified it twice (confirming the size) before performing DGGE analysis. Finally, following DGGE analysis, several bands were excised, purified, and amplified to confirm the correct fragment size (Fig. 3). Hence, we are confident that our analysis was specific to *Microcystis*. Despite evidence that single DGGE bands can represent >1 phylotype (Felske et al. 1999), we maintain that the majority of DGGE bands in our analyses represent a unique *Microcystis* strain; 76% of *Microcystis* colonies isolated from European lakes resulted in a uniquely positioned, single band following DGGE analysis (Janse et al. 2004).

In conclusion, the 2011 *Microcystis* bloom in Lake Erie originated in Maumee Bay and the western basin in July and August, spread eastward to the Sandusky sub-basin in September, and reached the central basin in October. DGGE analysis indicated that the *Microcystis* assemblages were contiguous, as demonstrated by several persistent and universal *Microcystis* phylotypes. Although the composition of the bloom was highly dynamic with phylotypes appearing and disappearing throughout the lake and over time, high genetic similarity among the *Microcystis* populations was maintained in the lake, likely a result of source *Microcystis* originating in lake sediments, and not major tributaries. Therefore, it follows that those management actions that limit *Microcystis* proliferation in the western basin of Lake Erie might limit those that occur in the Sandusky sub-basin and central basin.

Acknowledgements. We are grateful for field and lab assistance during this project from Sarah Panek, Peter Bichier, Courtney Mobilan, Tyler Lawson, Matt Thomas, and Hilary Nightingale. Samples collected on 21 July and 10 October were made possible by Bob Hanko and Mike Chaffin from Cranberry Creek Marina (Huron, Ohio). Samples collected on 11 August and 12 September were aboard Ohio State University Stone Laboratory R/V 'Erie Monitor' by the Ohio

Sea Grant College Program, Project R/ER-072, under award NA06OAR4170020 from the National Sea Grant College Program, National Oceanic and Atmospheric Administration. Molecular work in this project was funded in part through the Lake Erie Protection Fund small grant program award #SG 406-11. Additional support was provided by the National Science Foundation under Grant No. 1313897 and 1039043. Additional support for J.D.C. was provided by a teaching fellowship from National Science Foundation Grant #NSF DGE-0742395 to the University of Toledo's Lake Erie Center. We thank 2 anonymous reviewers who helped improve this manuscript.

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Editorial responsibility: Rutger de Wit, Montpellier, France

Submitted: December 9, 2013; Accepted: June 10, 2014
Proofs received from author(s): August 7, 2014