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From Satellite to Genes: An Integrative Approach for Timely Monitoring of Harmful Cyanobacteria in Lake Erie Beach Water

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Abstract An integrated approach for quantifying cyanotoxins was investigated using satellite remote sensing with molecular and chemical tools in Lake Erie. Remotely sensed satellite-based water color measurements with Medium Resolution Imaging Spectrometer (MERIS) were compared with *in situ* measurements of cyanobacteria pigments, *M. aeruginosa* populations (total and microcystin-producing subpopulation), and microcystin (MC) concentrations. Water samples were collected from a popular Headlands Beach in Lake Erie during the summer of 2010. The quantitative anomaly of cyanobacterial blooms between the two phycocyanin (PC) measurements demonstrated a good correlation (MERIS vs. *in situ*, r=0.84). PC was a better harmful cyanobacteria indicator than chlorophyll-*a* and correlated significantly with *M. aeruginosa* population ($P<0.05$). MC was detected in 33.8% of the samples and temporal pattern demonstrated that spikes of *mcyA* and PC occurred prior to MC peaks. Successful analysis within the 1 km nearshore region was another remarkable finding, which may be applicable for smaller water bodies.

Keywords: *microcystin, phycocyanin, satellite remote sensing, Microcystis aeruginosa, Lake Erie*

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1. Introduction

Lake Erie, the most southern and biologically productive Great Lake, provides 11 million people with water for drinking, recreation and transportation [1]. As has been the case throughout the world [2,3,4,5], increases in the frequency of cyanobacterial harmful algal blooms (CHABs) have generated health, drinking and recreational water concerns among water quality managers and public health officials in the Great Lakes region. Globally, toxic cyanobacteria can be found in inland as well as coastal water environments. Cyanobacterial blooms were reported to be widely spread and there is a significant association between cyanobacterial blooms and non-alcoholic liver disease in the United States [6]. Although only certain species of cyanobacteria possess toxin-producing capabilities, recent studies suggest that changing aquatic environments, including rising water temperatures and climate-induced eutrophication as part of a global warming scenario [7], will be more conducive for bloom-forming cyanobacteria than current conditions. This is particularly true for the harmful strains of *Microcystis* spp. [8].

Microcystins (MCs) are the most commonly reported cyanotoxins in North America, while others such as anatoxins, saxitoxins and cylindrospermopsin are less frequently reported [9,10]. Because Lake Erie is the shallowest and most nutrient-rich Great Lake, it is the most vulnerable to persistent problems associated with CHABs in an increasingly warming climate [11,12]. A large-scale cyanotoxin monitoring study from 2000 to 2004 reported that Lake Erie MC levels exceeded 1.0 µg/L in 4% of the samples, were 0.1 to 1.0 µg/L in 29% of the samples [13]. Around the turn of century, blooms of *Microcystis aeruginosa* were more commonly reported in Lake Erie's western basin and, in August 2003, one bloom of *M. aeruginosa* persisted for nearly one month [14]. More recently, the record-setting algal bloom was observed

in Lake Erie in 2011 [15] and MC was detected in drinking water in the City of Toledo in Ohio in August 2014.

Current approaches for quantifying HAB-associated toxins in water include the use of expensive antibody-based methods (enzyme-linked immunosorbent assays [ELISAs]), protein phosphatase type 2 inhibition assays, high-performance liquid chromatography coupled with ultra-violet light detection or mass spectrometry [59], and liquid chromatography-tandem mass spectrometry [16]. These approaches often require the shipment or delivery of samples to distant laboratories, increasing sampling costs and delaying the time needed for making management decisions. These two significant problems (high cost, latency) hinder the timely monitoring and communication of CHAB risk for beach users and water plant managers. To address these significant problems, alternative methods, such as using fluorometry for measuring cyanobacteria-specific photosynthetic pigments have gained favor [17,18,19] and have been coupled with remote sensing activities [20,21].

Because of the unique spectral characteristics of photosynthetic pigments, spaceborne remote sensing has been introduced to monitor HABs [22]. Many operational programs now include satellite remote sensing in their detection method portfolios due to its large spatial coverage. However, because of the lack of HAB-specific remote sensing algorithms and the well-established relationship between satellite observation and *in situ* data, remote sensing is mostly used as a screening and warning tool. For example, remote sensing studies have demonstrated significant associations with reflectance [23] and photosynthetic pigments, such as chlorophyll-*a* and phycocyanin PC [24,25,26,27], or the ratio of the two pigments [21]. Agha et al [28] showed the importance of using multiple approaches, including remote sensing, for monitoring of cyanobacteria to reduce uncertainty. To date, hardly any studies have evaluated the linkage between measured toxin concentrations and/or toxin-producing cyanobacteria with remotely sensed satellite data at nearshore beach waters where direct human exposure happens.

Given this significant gap in the literature, the objective of our study was conducting an integrated approach for predicting and quantifying cyanotoxins using satellite water color measurements and molecular and chemical tools so that the findings serve to pave the way to use the link between remote sensing and molecular ecology of CHABs to a greater extent in the future. As in Sridhar and Vincent [23], the focus on *Microcystis* spp. is primarily due to its frequency as the dominant cyanobacterium globally in many lakes and Lake Erie [21,32]. For remote sensing, multi-frequency Medium Resolution Imaging Spectrometer (MERIS) water color observations onboard the European Space Agency's (ESA's) Environmental Satellite (Envisat) were used. For molecular detection, quantitative polymerase chain reaction (qPCR) was used targeting microcystin synthetase gene (*mcyA*), which is one of the major target genes for determining the presence of the microcystin synthetase gene cluster and has been used for the detection of potential microcystin-producing *M. aeruginosa* [29,30]. It also has been used to analyze the diversity of microcystin-producing populations and predict microcystin concentrations [31] and high correlation was observed between toxic *Microcystis*-specific *mcyA* gene number and microcystin concentrations [29,32].

2. Materials and Methods

2.1. *In Situ* Sampling and Measurements

The field and laboratory methods were performed using water samples collected from the popular Headlands Beach located in the central basin of Lake Erie near Cleveland and Painesville in Ohio, USA (Figure 1). For ten weeks in 2010 (July 13 to September 16, 2010), beach water samples were collected 35 times at two locations (East: 41° 45' 33''N, 81° 17' 21'' W; West: 41° 45' 21'' N; 81° 17' 37'' W) following the Ohio Department of Health beach water sampling guidelines (Ohio Department of Health, 2005). Water quality parameters (water temperature, turbidity, pH, dissolved oxygen [DO], conductivity) were measured *in situ* using a YSI water quality data sonde (Yellow Springs Instruments, Yellow Springs, OH, USA) and Hach Turbidimeter (Hach Company, Loveland, CO, USA). Chlorophyll *a* and PC were quantified *in vivo* using intact cells without filtration or extraction as described in our previous study [18] using a two-channel handheld Aquaflour™ flourometer (Turner Designs®, Sunnyvale, CA). Average values were recorded after reading two times. Chlorophyll *a* (excitation at 460 ± 20 nm, emission > 665 nm) was standardized (R^2 = 99.9%) with liquid primary chlorophyll *a* standards (catalog number 10-850, Turner Designs®). PC (excitation at 595 nm, emission at 670 nm) was standardized (R^2 = 99.9%) using a lyophilized powder containing approximately 30% c-PC from *Spirulina* sp. as determined by Sigma-Aldrich® (catalog number P6161, Sigma-Aldrich®, St. Louis, MO). The starting mass of the PC powder used in the stepwise dilution and subsequent standard curve development was multiplied by 0.3 to reflect the true PC mass contained in the powder. To remove debris and zooplankton, water (200 mL) samples were pre-filtered through 20 µm pore size nylon filter membranes (Osmonics, Minnetonka, MN) and then filtered through a sterile 0.45 µm pore size mixed cellulose ester filter membrane (Pall Corporation, Ann Arbor, MI). Membrane filters were transferred to individual 50 mL sterile tubes. Water samples and membrane filters were sent to The Ohio State University main campus laboratory (Columbus, OH) on ice for analysis via (qPCR) and ELISA.

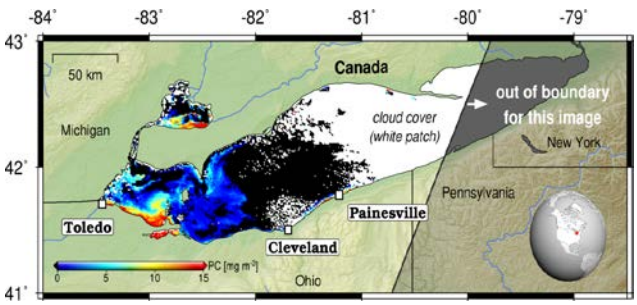

Figure 1. Geographical map of the study sites at Painesville in central Lake Erie, Ohio. The lake surface is color-coded by estimated PC centration on Sep 9, 2010, computed from MERIS L1 FRS data and a nest semi-empirical model [40]

From the samples, 100, 50, and 20 mL of water were filtered through a mixed cellulose ester filter (0.45 µm pore size, 47 mm diameter; Millipore, Bedford, MA) to enumerate fecal indicator bacteria (*E. coli*). *E. coli* was cultured on modified m-TEC agar (Aquacheck Laboratory,

Weathersfield, VT). The plates were pre-incubated at 35°C for 2 h and then at 44.5°C for 18-20 h. Red/magenta-colored colonies were counted as *E. coli* (U.S. Environmental Protection Agency, [USEPA]) [56]. Colorimetric methods were used for the determination of nitrate by the dimethylphenol method (Method 10206, Hach Company, Loveland, CO) and total phosphorus with the USEPA-approved acid persulfate digestion method (Method 8190, PhosVer® 3, Hach Company, Loveland, CO). The information on daily water level was obtained from the National Oceanic and Atmospheric Administration (NOAA) National Data Buoy Center (NDBC) at Station 9063053 located at Fairport Harbor, Ohio.

2.2. Toxin Measurements

MC concentrations were quantified using the US EPA-validated Microcystins/Nodularins (ADDA) ES, ELISA kit in a 96-well format (catalog number PN520011ES, Abraxis®, Warminster, PA) as described previously [18,32]. All samples (including positive and negative controls) were evaluated in duplicate. There was good agreement between the two measured optical densities for each sample (Pearson correlation = 0.881, $p < 0.001$). Samples between 0.10 and 0.15 µg/L were qualified as "detected but not quantified" (DNQ) and samples below 0.10 µg/L were considered "non-detects" according to manufacturer's instruction. For statistical analyses, the 'non-detects' were assigned a value of 0.05 µg/L (half of the detection limit) and the DNQs were assigned a value of 0.125 µg/L, assuming that the microcystin levels in these samples were in the published range of 0.1 µg/L and 0.15 µg/L based on the information provided by ELISA manufacturer (http://www.abraxiskits.com/uploads/products/docfiles/278_Microcystin%20PL%20ADDA%20users%20R120214.pdf, Abraxis, Warminster, PA).

2.3. qPCR Measurements of mcyA and PC-IGS

As a positive control, *Microcystis aeruginosa* NIES-843 was cultured (kindly provided by Dr. Wilhelm, University of Tennessee) on MA medium [33]. DNA from *M. aeruginosa* NIES-843 culture and the membrane filters (filtered water samples) was extracted using the xanthogenate-sodium dodecyl sulfate (XS) DNA extraction protocol [34] with minor modification. Briefly, 100 µL of *M. aeruginosa* NIES-843 culture were centrifuged at 12,000 rpm for 1 min. The pellet was resuspended in 1 mL of XS buffer. Each membrane was transferred to a 2-mL microcentrifuge tube that contained 1 mL of XS buffer and incubated at 70°C for 2 h to release DNA. After incubation, each sample was vortexed for 10 s, put on ice for 30 min, and centrifuged at 13,200 rpm for 15 min at 4°C. The supernatant was transferred to a new tube and mixed with an equal volume of 100% isopropanol. The sample was transferred to a column in the DNeasy® Blood and Tissue Kit (Qiagen, Valencia, CA) and centrifuged for 1 min at 10,000 rpm. The column was washed with 500 µL of buffer AW1 in DNeasy® Blood and Tissue Kit, and centrifuged for 1 min at 10,000 rpm. The column was then washed with 500 µL of buffer AW2 in the DNeasy® Blood and Tissue Kit, and centrifuged for 1 min at 12,000 rpm. One hundred microliters of AE buffer was added to the column and incubated for 1 min. To elute DNA, the column was centrifuged for 1 min at 12,000 rpm.

To quantify the densities of the total *M. aeruginosa* population and potential microcystin-producing *M. aeruginosa* subpopulation, the phycocyanin intergenic spacer (PC-IGS) and *mcyA* gene for *M. aeruginosa*-specific microcystin synthetase were targeted [30,32]. Real-time qPCR was carried out in duplicate with a StepOne™ Real-Time System (Applied Biosystems, Foster City, CA) in a 48-well format with a total reaction mixture volume of 20 µL containing 2 µL of DNA, forward and reverse primers for PC-IGS (Forward: 5'-GCTACTTCGACCGCGCC-3'; Reverse:5'-TCCTACGG TTTAATTGAGACTAGCC-3'; 500 nM each) and for *mcyA* (Forward: 5'-AGCGGTAGTCATTGCATCGG-3'; Reverse:5'-GCCCTTTTTCTGAAGTCGCC-3'; 200 nM each) [30] and 10 µL of SYBR® universal PCR master mix (Applied Biosystems). Thermal cycling consisted of an initial cycle of 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 30 s, and annealing and extension at 62°C for 3 min. A melting curve analysis was performed after amplification to distinguish the targeted PCR product from the false-positive PCR product. The melting curve analysis was performed by heating samples to 95°C for 30 s, cooling to 62°C for 3 min, and then heating the samples at 0.3°C/s to 95°C. Cycle threshold (Ct) values of a real-time PCR result were determined from a fixed threshold (0.2). DNA from *M. aeruginosa* NIES-843 culture and distilled water were used as positive and negative controls, respectively. Samples showing the Ct values in both replicates were regarded as positive detects. The concentrations of PC-IGS and *mcyA* in the positive samples were determined using standard curves as described previously [18,32].

2.4. Satellite Remote Sensing: Water Color Measurements

In order to estimate water quality, early studies used satellite remote sensing to observe watercolor changes [35]. The accessory pigment stored in the cyanobacteria allows us to detect the presence of cyanobacteria apart from other phytoplankton containing mostly chlorophylls [26]. PC, other than chlorophyll *a* and carotenoid, is the most measureable pigment-protein complex in *Microcystis* spp. absorbing visible light near the wavelength at 620 nm [36], although chlorophylls also have considerable absorption features in the similar reflectance spectra [58]. Hence, the spatial quantification of cyanobacteria density in water is thus feasible by estimating pigment occurrence and levels from the optical information obtained by orbital sensors and with an appropriate multispectral algorithm.

Based on optical properties [36], there are currently at least three major algorithms available for the quantification of PC: 1) a semi-empirical model [37]; 2) a single reflectance ratio algorithm [57]; and 3) a nested semi-empirical band ratio model [40]. In this study, the nested semi-empirical model developed by Simis et al. [40] was adopted since it statistically outperforms other algorithms [36]. Among the vast array of spectroradiometers working in space, MERIS onboard Envisat at present provides the best band configuration for the study of PC [35]. MERIS has an approximate spatial resolution of 300 m and operates in the solar reflective spectral range with up to 15 programmable

spectral bands (390 nm to 1040 nm) selected by ground command. Since March 2002, Envisat operated in a near-polar sun-synchronous orbit at a mean orbital altitude of 815 km. However, due to a sudden failure in communication, it ceased its operation on 9 May 2012.

The nested semi-empirical band ratio model used in this study primarily used MERIS bands 6, 7, 9, and 12. According to Simis et al. [40], band 6 centered at 620 nm exactly matches the maximum absorbance of PC, while band 7 is centered at 665 nm and supports the estimation of chlorophyll *a* at an independent spectrum. Because of an overlap of absorbance between PC and chlorophyll *a* at 620 nm, it required additional information in other bands to proportionally subtract the chlorophyll *a* contribution in the total biomass concentration at 620 nm. The absorption by PC, $a_{pc}(620)$, is described as below [40,41]:

$$a_{pc}(620) = \left\{ \begin{bmatrix} \left(\frac{B(705)}{B(620)} \right) \times \left(a_w(709) + b_b \right) \\ -b_b - a_w(620) \end{bmatrix} \right\} \times \delta^{-1} - \left(\varepsilon \times a_{chl}(665) \right) \quad (1)$$

where

$B(705)$ = Band 9 water-leaving reflectance centered at 705 nm for measuring a trough in PC absorbance spectra [unit: dimensionless]

$B(620)$ = Band 6 water-leaving reflectance centered at 620 nm for measuring a peak in PC absorbance spectra [unit: dimensionless]

$a_w(709)$ = pure water absorption at 709 nm [unit: m^{-1}]

b_b = backscattering coefficient estimated by a single band (Band 12 of MERIS centered at 775 nm in this study)

$a_w(620)$ = pure water absorption at 620 nm [unit: m^{-1}]

δ = 0.82, correction factor

ε = 0.24, proportion of chlorophyll *a* absorbance at 620 nm of which measured around 665 nm.

The spectrally neutral backscattering coefficient (b_b) retrieved by MERIS Band 12 around 775 nm, B(775), is formulated as [40]:

$$b_b(775) = a_w(778) \times \frac{B(775)}{0.082 - 0.6 \times B(775)} \quad (2)$$

where $a_w(778)$ = pure water absorption at 778 nm [unit: m^{-1}]

The estimate of chlorophyll *a* via wavelength near 665 nm is described as (40):

$$a_{chl}(665) = \left\{ \begin{bmatrix} \left(\frac{B(705)}{B(665)} \right) \times \left(a_w(709) + b_b \right) \\ -b_b - a_w(665) \end{bmatrix} \right\} \times \gamma^{-1} \quad (3)$$

where

$B(665)$ = Band 7 water-leaving reflectance centered at 665 nm for measuring a peak shoulder in chlorophyll *a* absorbance spectra

$a_w(665)$ = pure water absorption at 665 nm

γ = 0.68, estimated chlorophyll *a* absorption

The pure water absorption coefficients (a_w) at different wavelengths in this study used the values by Pope and Fry [42] and Simis et al. [40,43] and the values are 0.281 (at 620nm), 0.401 (665 nm), 0.727 (709 nm), and 2.71 m^{-1} (778 nm).

Finally, the assessment of PC concentration in [(mg pigment) m^{-3}] is then modeled as:

$$PC = a_{pc}(620) / \hat{a}_{pc}(620) \quad (4)$$

where $\hat{a}_{pc}(620)$ is the absorption coefficient of PC at 620 nm, which converts the absorption characteristic in [m^{-1}] to the actual concentration in [mg/m^3]. In this study, an empirical value of $\hat{a}_{pc}(620)$ equal 0.007 [m^2/mg pigment] was adopted.

2.5. Statistical Analysis

Spearman's rank correlation was used to explore the relationship between bloom-related measurements and environmental parameters. Logistic regression analysis was performed to quantify the relationship between pigment levels and qPCR detections. Further, multivariable linear regression was used to identify the relationships between PC and environmental variables, where PC level was log-transformed due to its skewed distribution. A backward elimination approach was adopted for selecting predictors in multivariable linear regression. Variables not significant at the 0.05 level were eliminated sequentially from the model and then quadratic terms of the selected variables were tested to allow for potential non-linear relationships. All statistical analyses were performed with SPSS® (Release ver. 19.0.0; SPSS Inc.).

3. Results and Discussion

3.1. Calibration of Satellite Observations with *In Situ* Measurements

There are 12 snapshots of MERIS L1B data available with visible water surface information at the location of the 35 daily *in situ* samples since the MERIS optical measurements were affected by cloud cover at the moment of scanning. The MERIS L1B 300m Full Resolution full Swath (FRS) data [38] were preprocessed by NASA's Goddard Space Flight Center (GSFC) Ocean Color Science Team are available from http://oceancolor.gsfc.nasa.gov/. After downloading the data, the Basic ERS and Envisat (A) ATSR and MERIS (BEAM) VISAT toolbox provided by ESA and Brockmann Consult and its supplementary Regional Case-2 Water Processor [44] were used to further refine the coastal regions data. In particular, the Case-2 Regional Processor (C2R) v1.5.2 was applied to convert the top of atmosphere (TOA) radiance archived in the original L1B data to water leaving radiance (R_L_W) above the surface. C2R is a two-fold plug-in processor dedicated to atmospheric correction and water constituents' retrieval [45]. However, unlike the workflow suggested in Chawira [44], the correction subroutine called 'Improve Contrast over Ocean and Land (ICOL) v2.7.4' [46], which is a processor embedded in BEAM VISAT, was not applied. Since the water pixel from the coastal region usually contains an overestimate of

atmospheric radiance and the neighboring land areas backscatter more photons to the sensor, ICOL nominally reduces the adjacency effects [47]; thus, it enhances the water-leaving radiance near the shorelines. However, the test with the 12 concurrent satellite and laboratory measurements showed that the correlation and root-mean-square (RMS) between the two PC quantities are slightly degraded when ICOL correction was applied in this study. Binding et al. [48] also mentioned that the usefulness of ICOL is still uncertain regarding its insignificant improvement in measuring algal pigments. Therefore, the optional ICOL correction step was not applied in this study.

Next, multispectral nested models presented in Eq. 1 to Eq. 4 were used to compute PC at the study area. To prevent potential land contamination and regional irregularity in the reflectance data, all pixels were spatially smoothed with PC>0 in a spatial box bounding 81.32°W–81.26°W in longitude and 41.74°N–41.77°N in geodetic latitude. This box covers about 1 km offshore surrounding Headlands beach (81.29°W, 41.76°N). The comparison of the PC concentration estimated with MERIS and the *in situ* measurements is summarized in Figure 2. The temporal variation corresponded well between the two independent data sets with a bias estimated as 0.15 mg/m^3. Two possible reasons may explain the bias between these two observations. First, the relatively coarse resolution of MERIS in 300 m spatially averages the drifting surface scums along the shore yielding an inaccurate estimate since the actual cyanobacteria only occupy a smaller area. Another potential reason is that other materials, such as colored dissolved organic matter (CDOM), chlorophyll *b*/chlorophyll *c*, detritus, tripton, and water itself may contribute undistinguished absorbance near the same spectral range [37]. However, these potential factors that may contribute to uncertainties were not considered because of the simplification manner of this nested semi-empirical model [43].

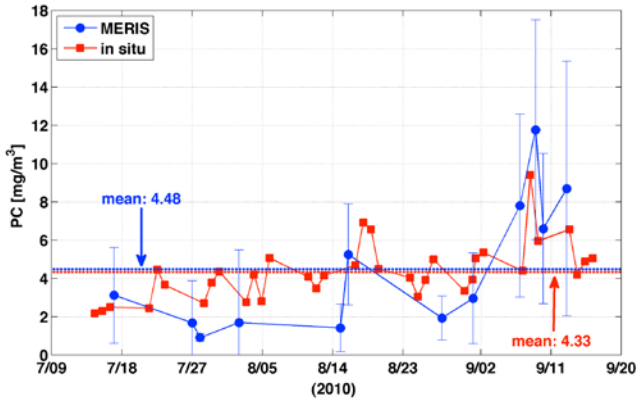

Figure 2. Temporal variation of PC estimated by MERIS and measured with *in situ* samples. *In situ* data collection and laboratory analysis were performed from 7/13/2010 through 9/16/2010 (35 samples). MERIS data were selected during the fieldwork campaign when cloud-free conditions prevailed

By syncing both data sets by a way of linear interpolation of *in situ* quantities at the timing of the MERIS snapshots, a good correlation and excellent agreement was observed between the two PC measurements. As shown in Figure 3, the two PC time series (after removing the relative bias) agree well both in correlation (r = 0.84) and in trend during the *in situ* campaign. The RMS of the difference between the two time series is low at 2.41 mg/m^3, indicating excellent agreement. This result implies that although the spaceborne sensors are incapable of quantitatively capturing the exact PC concentration because they are restricted by their spatial resolution and limited spectral bandwidths, they can efficiently identify the pigment 'anomaly' in the cyanobacterial biomass. As long as there is a pre-calibration between MERIS and *in situ* data to estimate a typical average bias at a certain locale, spaceborne spectral data from MERIS (Envisat) and its enhanced successor (the upcoming OLCI onboard Sentinel-3 satellites) can potentially be used as a monitoring system. This system will provide a timely assessment of the cyanobacteria density, including blooms, over a study region and other larger and smaller water bodies, as well as nearshore environments.

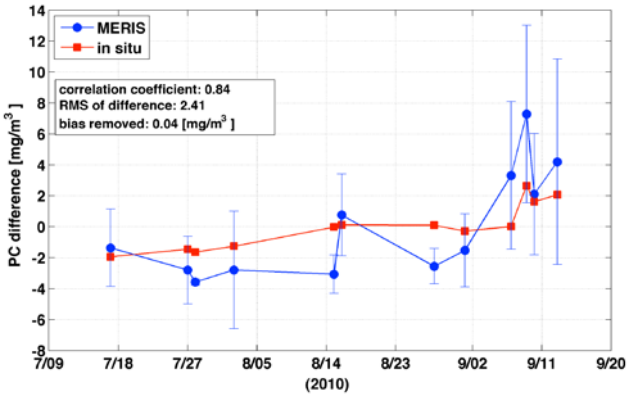

Figure 3. Comparison of the PC anomaly between the *in situ* PC measurements (red) and the MERIS estimates (blue). A relative bias estimated at 0.04 mg/m^3 between the two time series has been removed. The correlation coefficient and RMS are 0.84 and 2.41 mg/m^3, respectively. The error bar for the MERIS estimate is computed based on the standard deviation of all PC>0 pixels in the spatial averaging box

3.2. Accuracy of Satellite Observations

In this study, the MERIS Level 1 data were used to enable the performance of a nested semi-empirical approach similar to Simis et al. [40], whereby excellent agreement was obtained in the comparison or calibration with the *in situ* data. It was concluded that the PC bias between MERIS and *in situ* measurements collected from Headlands Beach in central Lake Erie is estimated to be approximately 0.15–0.04 mg/m^3 (before and after syncing with *in situ* data). This value is much smaller than a bias of ~4 mg/m^3 reported by Ruiz-Verdú et al. [36], the estimated bias >29 mg/m^3 under some extreme conditions [40], and the bias ranging between 8.8–34.8 mg/m^3 reported by Randolph et al. [41] when a fixed $\hat{a}_{pc}(620)$ value was applied. However, it is noted that the RMS difference obtained in this study (~56% to the average of *in situ* PC concentration) is higher than 19.7% reported by Simis et al. [40], presumably due to an overall low level of PC (<50 mg/m^3) in the current study region.

3.3. Water Quality of the Beaches

The average total phosphorus concentration was 0.012 mg/L while the average nitrate concentration was 0.065 mg/L. This suggests that the study sites were in the oligo-mesotrophic condition [49]. The average dissolved oxygen was 10.81 mg/L and the average pH was 8.51. During the

study, HAB-associated variables (e.g. chlorophyll *a*, PC, microcystin, PC-IGS, *mcyA*) and water quality factors varied substantially, as did some of the environmental variables, including water temperature and water level (Table 1). Microcystin was detected in 33.8% of the samples with concentrations ranging from undetectable to 0.62 µg/L. The maximum microcystin level did not exceed the value of 1 µg/L recommended by the WHO [50] as the drinking water standard.

Table 1. Summary of *in situ* measurements including HAB-associated and environmental variables, and other water quality factors from Headlands beach from July to September in 2010*

Variables	Mean ± SE	Min	Max
microcystin (µg/L)	0.10 ± 0.10	ND**	0.62
PC-IGS (gene copies/L)	$2.13 \times 10^4 \pm 7.89 \times 10^3$	ND	4.00×10^5
mcyA (gene copies/L)	$9.59 \times 10^3 \pm 4.10 \times 10^3$	ND	2.40×10^5
phycocyanin (mg/m^3)	4.35 ± 0.19	2.01	9.57
chlorophyll-*a* (mg/m^3)	6.30 ± 0.39	1.52	18.37
water temperature (^{o}C)	24.27 ± 0.30	19.04	27.99
pH	8.51 ± 0.02	8.02	8.84
specific conductivity (µS)	312 ± 3	227	416
dissolved oxygen (mg/L)	10.66 ± 0.47	5.63	16.79
turbidity (NTU)	13.3 ± 2.00	1.2	79.4
E. coli (colony forming units/100 mL)	$6.21 \times 10^1 \pm 8.79 \times 10^0$	3.00	2.94×10^2
total phosphorus (mg/L)	0.012 ± 0.001	0	0.043
nitrate-N (mg/L)	0.065 ± 0.006	0.011	0.241
water level (m above sea level)	174.22 ± 0.01	174.04	174.34
mcyA/microcystin	14942.0 ± 7674.3	0	480138.6

*The values are summarized from all the measurements from East and West Headlands beach. ** non-detect.

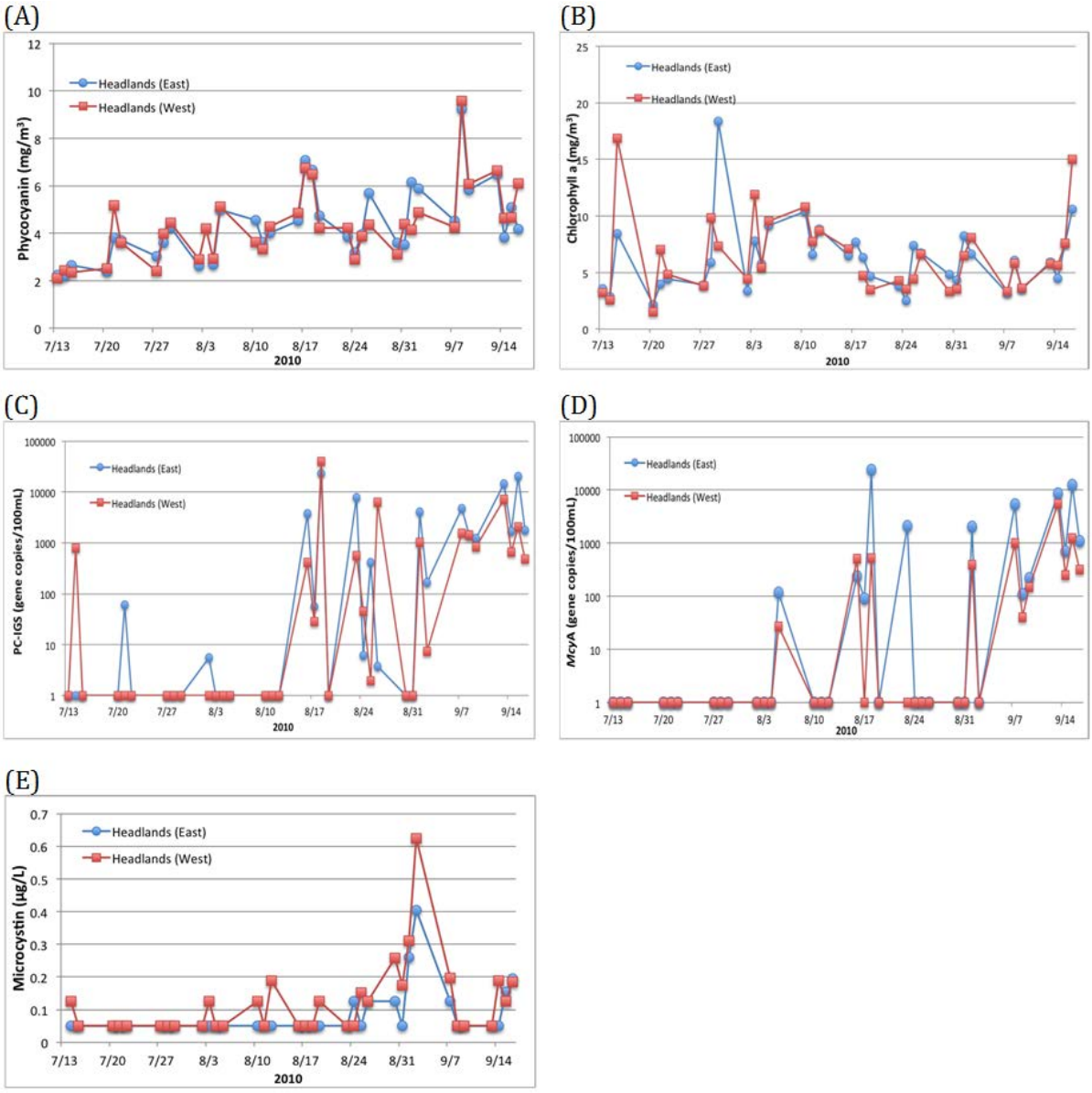

Figure 4. Temporal trends of HAB-related *in situ* measurements from the water samples collected at Headlands Beach (East and West) from July to September in 2010: (A) phycocyanin; (B) chlorophyll-*a*; (C) *M. aeruginosa* phycocyanin intergenic species gene (PC-IGS) measured with qPCR; (D) toxin producing gene (*mcy*A) measured with qPCR; and (E) microcystin

3.4. Temporal Variations of Pigments, Microcystin, and Microcystis aeruginosa

Chlorophyll *a* levels ranged from 1.52 to 18.37 mg/m^3 (mean 6.30 ± 3.25 mg/m^3) whereas PC levels ranged from 2.01 to 9.57 mg/m^3 (mean 4.35 ± 1.54 mg/m^3); the temporal variations are shown in Figure 4 (A) and 4 (B). Significant weekly variations in chlorophyll *a* levels were observed, particularly during the first three weeks, but the overall trend did not change significantly. PC levels increased throughout the study period, with some weekly variation, at both East and West sites.

The temporal variations of PC-IGS and *mcyA* are shown in Figure 4 (C) and 4 (D). Of the total 70 water samples, 34 (48.6%) samples were positive for PC-IGS (Figure 4 (C)) and the levels varied from 0 (non-detects) to 4.0 × 10^5 gene copies/L during the study period with a mean of 2.13 × 10^3 gene copies/L. Between July and early August, only 3 of 32 samples (9.4%) were positive (concentration range: 2.36 × 10^0 - 8.80 × 10^3 gene copies/L). PC-IGS levels began to increase in mid-August, indicating the beginning of *Microcystis* bloom during that time (Figure 4C). *mcyA* was detected in 24 (34.3%) samples with a range from 0 (non-detects) to 2.40 × 10^5 gene copies/L (Figure 4D).

The trend of *mcyA* abundance was similar to that of PC-IGS, indicating that toxin-producing *M. aeruginosa* increased with the total *M. aeruginosa* population. MC was detected in 33.8% of the samples (23 of 68 samples) with concentrations ranging from below detection to 0.62 µg/L. The temporal variation of the MC level is shown in Figure 4 (E). MC was not detectable in July and early August, but began to increase in late August. The mean MC level during the study was 0.10 µg/L with the highest MC concentration detected on September 2, 2010 from Headlands beach (West). The general trend is that the peaks of *mcyA* and PC-IGS occurred earlier and are followed by the MC peaks.

3.5. Relationships between CHAB-associated Measurements and Environmental Variables

The PC level showed a significant correlation with chlorophyll *a*, PC-IGS, *mcyA* and several environmental parameters such as water temperature and turbidity (Table 2). However, the chlorophyll *a* level did not show significant correlation with any CHAB-associated measurements or environmental parameters, except pH. The PC-IGS level was significantly correlated with water temperature, pH, turbidity, water level, and total phosphorus. The *mcyA* level showed a high correlation with PC-IGS (Spearman's ρ=0.7902, P<0.05), which supported the observation that toxic *M. aeruginosa* grew alongside non-toxic *M. aeruginosa*. The MC level was significantly correlated with water temperature and level, *E. coli* density (fecal contamination indicator), and total phosphorus, but surprisingly, a significant relationship with PC was not observed. This lack of significant relationship between these two parameters may be explained by several reasons. First, the relative abundance of toxic cyanobacteria might not have been constant during the study; especially since there was no toxic *Microcystis* as indicated by the level of *mcy*A in July and early August. Second, the amount of PC produced by cyanobacteria varies depending on environmental factors and the stage of their growth [51]. It was reported that the MC production ability by *Microcystis* could also vary by a factor of 2-3 under different environmental conditions and their growth rate [52,53]. The variability discussed above may explain why we did not observe a significant relationship between the PC and MC parameters.

According to Spearman correlation analysis, our data show that the relative abundance of *mcyA* was negatively correlated with water temperature (Table 2). It has been reported that the effects of temperature on the relative abundance of toxic *M. aeruginosa* populations can vary depending upon the geographical locations of the study sites. For example, in an experimental setting, elevated temperatures yielded significantly higher growth rates of toxic *Microcystis* in northeastern US lakes *[8]* and potentially toxic *Microcystis* genotypes were positively associated with the water temperature of a Korean Reservoir [54]. In contrast, other groups reported that there was no significant relationship between the relative abundance of toxic *M. aeruginosa* and water temperature in a Japanese Lake [30], a Chinese Lake [27], and in western Lake Erie [55]. These latter studies found that abundance of toxic *M. aeruginosa* was more closely related with nutrients such as nitrate [30], total phosphorus, and/or *ortho*-phosphate concentrations [27,55] rather than water temperature. Therefore, more research is warranted to understand the effect of water temperature coupled with the nutrients on the relative abundance of toxic *M. aeruginosa*.

Table 2. Spearman correlations between bloom measurements and environmental parameters (* indicates significance at α=0.05).

	phycocyanin	Chlorophyll-*a*	PC-IGS	*mcyA*	microcystin
phycocyanin	1				
chlorophyll *a*	0.398*	1			
PC-IGS	0.558*	-0.060	1		
mcyA	0.670*	0.095	0.790*	1	
microcystin	0.136	0.073	0.225	0.137	1
water temperature	-0.346*	0.284	-0.742*	-0.562*	-0.347*
pH	-0.080	0.302*	-0.450*	-0.221	0.001
dissolved oxygen	0.362*	0.027	0.193	-0.039	-0.253*
turbidity	0.640*	0.016	0.593*	0.599*	-0.122
E. coli	0.058	0.226	-0.164	0.002	-0.341*
total phosphorus	0.017	-0.211	0.292*	0.182	0.288*
nitrate-N	0.011	-0.039	0.193	0.139	0.066
water level	-0.578*	-0.015	-0.666*	-0.617*	-0.492*

3.6. Relationship between Pigment Levels and qPCR Measurements

Because it failed to show a significant correlation with qPCR data (Table 2), chlorophyll *a* concentration, as a general indicator of algal biomass, did not provide enough information on harmful cyanobacteria densities. PC seemed to be a better proxy for determining harmful cyanobacteria densities and predicting CHABs since it was significantly correlated with both *mcyA* and PC-IGS (Table 2). To better understand the relationship between PC concentrations and qPCR results, logistic regression was used and qPCR data were transformed to binary data (0 indicated not-detected and 1 indicated detected). Logistic regression estimated that for every observed 1 mg/m^3 increase in PC there was a 3-fold increased odds of detecting PC-IGS (Odds Ratio = 2.97; 95% CI 1.67, 5.31). Additionally, PC and *mcyA* detection were also positively associated with 4-fold increased odds of detecting *mcyA* for each 1 mg/m^3 increase in PC (Odds Ratio = 3.96; 95% CI 2.00, 7.85). The relationship between PC and *M. aeruginosa*, especially a toxic *M. aerugninosa* subpopulation, supported the conclusion that PC is a good surrogate for CHABs related to *M. aeruginosa*. The significant positive correlations between PC with cyanobacteria, *Microcystis*, toxic *Microcystis*, and MC level were also observed in Lake Erie [55].

4. Conclusions

- Spaceborne spectral images enabled the timely estimation of PC levels at central Lake Erie beaches and these estimated levels were in excellent agreement with *in situ* measured PC levels from beach water samples.
- Satellite and *in situ* observations of factors associated with cyanobacterial blooms were successfully integrated and the results suggest an early warning system is achievable in this nearshore beach environment.
- Concentrations of chlorophyll *a* and PC were positively correlated. However, PC is likely a superior proxy for monitoring cyanobacterial blooms as PC concentrations, unlike chlorophyll *a*, were significantly correlated with PC-IGS and *mcyA* gene levels.
- Phycocyanin (PC) concentrations were significantly correlated with the relative abundances of PC-IGS and the *mcyA* gene from Lake Erie beach water samples.
- Time-series analysis demonstrates an upward trend in measured levels of *mcyA*, PC-IGS and PC throughout the duration of the study with all three having concentrations that peaked in early September at the approximate time that concentrations of microcystin also peaked.
- Since the study sites were relatively clean beaches, future studies evaluating this approach are warranted in environments that have greater cyanobacterial blooms and present higher human health concerns.
- Persistent cloud cover significantly reduced the number of spaceborne spectral images that could be obtained from MERIS for inclusion in this study. This represents a potential drawback of using remotely sensed data as a timely early warning tool form cyanobacterial bloom.

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