EFFECTS OF LIGHT ON MICROCYSTIN SYNTHETASE GENE EXPRESSION IN THE TOXIC CYANOBACTERIUM *MICROCYSTIS AERUGINOSA* IN A CONTROLLED FIELD STUDY

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

The toxin-producing cyanobacteria *Microcystis aeruginosa* are ubiquitous residents of lakes, rivers and reservoirs. Some strains of this genus are known to produce the potent hepatotoxin microcystin. The ubiquity of these organisms, and threat of harm to humans and wildlife, has motivated efforts to understand what triggers toxin production. This study examined the cells of *M. aeruginosa* for the synthesis of enzymes responsible for microcystin production, specifically looking at the effect of light intensity on the upregulation of genes for the McyB synthetase protein.

In situ microcosm experiments were performed at two sites along the Hudson River estuary to test the light-induced genetic regulation of cyanobacterial toxin (microcystin) synthesis in *M. aeruginosa*. Specifically, transcript levels of the microcystin synthetase gene, *mcy*B, were quantified using Real Time Reverse Transcriptase Polymerase Chain Reaction amplification in a Lightcycler®. Toxic cultures were suspended in sealed diffusion chambers at three depths: the Secchi depth, just below the surface, and midway between these depths. Samples were also collected at parallel depths in Van Dorn samplers to test for *mcy*B mRNA in ambient waters. mRNA transcripts of the *mcy*B gene as well as microcystin concentrations were analyzed.

In *M. aeruginosa* strain PCC 7806, transcript levels were increased in cells suspended near the water's surface while no definite differences in toxin concentration were observed among cells exposed to different light conditions. These findings demonstrate that upregulation of the microcystin synthetase enzyme operon *mcy*B is stimulated by higher light intensities found near the surface of the water. Further studies testing the prevalence and consistency of this response among various *M. aeruginosa* strains may prove useful in designing bloom mitigation strategies.

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INTRODUCTION

Toxic Cyanobacteria

Cyanobacterial blooms are not only unsightly and/or foul-smelling, but can also be the source of metabolites that may be toxic to higher organisms. These toxins range from skin irritants or substances that induce gastrointestinal inflammation to compounds that cause death via hepatic hemorrhage or neural dysfunction (Kuiper-Goodman et al. 1999, Sivonan and Jones 1999, Kaebernick and Neilan 2001). One toxin that has received considerable attention since the 1980's is the hepatotoxin, microcystin. Microcystin is primarily synthesized by strains of the genera *Microcystis*, *Oscillatoria* (*Planktothrix*), and *Anabaena* (Paerl and Millie 1996, Sivonan and Jones 1999, Kaebernick and Neilan 2001). The compound, a seven-residue highly modified peptide, is referred to as a secondary metabolite as it is not essential to life and provides no hitherto discovered advantage over non-toxic strains in the environment. Nonetheless, microcystin has been implicated in numerous poisonings and deaths of fish, livestock, domestic animals, and humans in Australia, China, the United States, and Brazil, among others (Kuiper-Goodman et al. 1999, Fischer and Dietrich 2000). Incidents such as the deaths of 76 hemodialysis patients in Caruaru, Brazil in 1996, have underscored the need to investigate toxin production and toxic bloom formation in order to protect public health (Carmichael et al. 2001). In addition, further exploration of internal injury to fish and wildlife due to toxin exposure is necessary to appreciate the environmental impact of cyanobacterial toxins.

Cyanobacteria and the Hudson River Estuary

The Hudson River estuary is currently characterized as a cyanophyte-deficient water body owing to the invasion of the exotic benthic bivalve, the zebra mussel (*Dreissena polymorpha*). Thorough surveys carried out by researchers at the Institute for Ecosystem Studies (Millbrook, NY), revealed a consistent and dramatic diminution in the phytoplankton biomass in the Hudson from 1992-1997 (Caraco et al. 1997, Smith et al. 1998). Most notable was the shift in the dominant phytoplankter from cyanobacteria to diatoms (Smith et al. 1998, Bastviken et al. 1998, Strayer et al. 1999). However, the appearance of cyanobacterial blooms at several sites within the tributaries and main channel of the river in 2001 and 2002, after a nearly ten year absence, highlight the capriciousness of estuarine ecology (Caraco, pers. comm.). Irregular climactic, hydrological, and biotic conditions make it difficult to predict when and where cyanobacterial dominance will take hold.

Nevertheless, as recently as 2003, using the highly sensitive and specific polymerase chain reaction (PCR) amplification to detect one member of the Hudson River phytoplankton community, *Microcystis* sp., I was able to confirm not only the presence of these cyanobacteria but also their toxin-producing capacity (Langer Atkinson, unpublished data). Therefore, with the appropriate alliance of physical, chemical, and biological conditions, the estuary remains a site with toxic bloom potential. Also, chronic low level exposure to microcystin has been linked to liver and colon cancer in humans and hepatocyte damage in fish (Fischer and Dietrich 2000, Lun et al. 2002, Zagura et al. 2003). Clearly, there are health risks for humans and potentially for wildlife, although the populations of these microorganisms might not reach bloom proportions in

the Hudson River estuary. Thus, especially in ecosystems such as the Hudson River estuary, the use of molecular detection methods such as PCR may be favored over traditional methods to identify cyanobacteria because of exceptionally low detection limits, and the potential ability to forecast bloom events.

Microcystin Toxin Production

This research focused on the toxin-producing capacity of *M. aeruginosa* by examining the regulation of enzymes involved in toxin synthesis. Analysis of the regulatory mechanisms underlying toxin production is one way to further our understanding of the complex processes governing toxic bloom development. The majority of research to date has focused on isolating which environmental factors, such as nutrient ratios, light exposure and micronutrients, contribute to increased toxin production in laboratory-based studies (Vezie et al. 2002, Wicks and Thiel 1990, Utkilen and Gjolme 1992, Oh et al. 2000, Long et al. 2001). Most studies indicate that optimum conditions for growth result in the highest cellular toxin concentrations. Only with the recent identification of genes involved in toxin production have researchers been able to give attention to the precise regulatory mechanisms for toxin production and examine features of toxin producers *in situ*. In laboratory cultures, elevated light intensity has been found to increase the expression of genes encoding the microcystin synthetase enzyme complex (Kaebernick et al. 2000). Importantly, this response was independent of changes in cellular growth rate and, therefore, quantification would be disconnected from changes in cell numbers.

Study Approach

Our objective was to assess the light-dependent expression of microcystin synthetase genes, *in situ,* using reverse transcriptase real-time polymerase chain reaction amplification (RT/RT-PCR). We aimed to unite quantification of gene expression with the effect of a physical parameter, light, in a natural setting. This approach sought to provide data on *in situ* gene expression by a significant estuarine phytoplankter. The hypothesis put forth was that relative light intensity will be reflected in the *in situ* level of transcription of these light-regulated microcystin synthetase genes.

METHODS

***Microcystis aeruginosa* cultures**

Axenic *M. aeruginosa* cultures obtained from the Pasteur Culture Collection (PCC 7813, 7806, 7005) were used in method optimization and field trials. Cultures were grown at room temperature in BG-11 medium (Rippka 1988) while shaking at 150rpm and on a light dark cycle (12:12) under a light intensity of 5μmol m^{-2} s^{-1}. Toxic cultures PCC 7806 and PCC 7813 and the non-toxic culture PCC 7005 were acclimated to Hudson River nutrient conditions prior to sampling. These cultures were grown in filter-sterilized and autoclaved Hudson water supplemented with BG-11 medium to a final concentration of 0.1X. Cultures were grown to late-log phase, (A_{600}~ 0.800), and transferred at least twice prior to use. All cultures for *in situ* incubations were used at an optical density (A_{600}) of ~0.4 to 0.6 *M. aeruginosa* PCC 7813 and PCC 7806 were used in Esopus Creek and Stockport Flats diffusion chambers, respectively. *M. aeruginosa* PCC 7005 was used as a negative control at both sites.

Sample collection

Two sites, Esopus Creek in Saugerties, NY, and Stockport Flats, NY, were chosen based on experiments using PCR-based detection of toxic *Microcystis* sp. (Langer Atkinson, unpublished data). These locations had the appropriate physical and hydrological conditions to support toxic strains in studies conducted during the 2003 season.

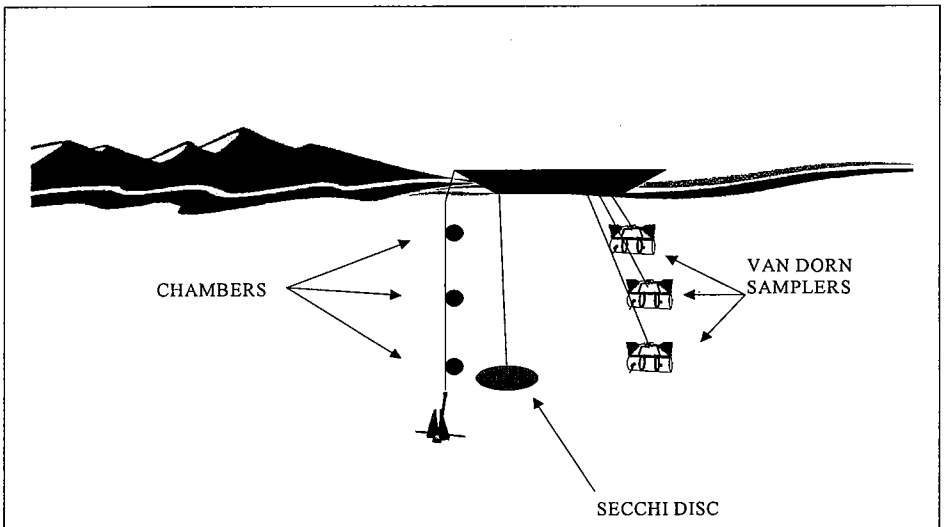

Figure 1. Field sampling set-up, samples drawn from low, medium and high light intensities from cultures in chambers and water collected at the same three depths.

Every effort was made to sample during clear or consistently overcast skies at least three days after the most recent precipitation event. Data were collected on four

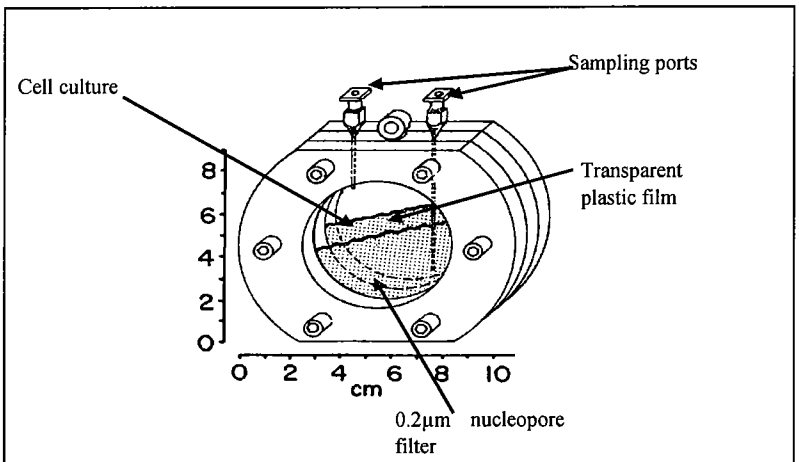

Figure 2. Environmental chamber for in situ culture incubation

dates: July 1, 2004, July 3, 2004, August 4, 2004, and August 6, 2004. All sampling was performed during ebb tide, from a canoe, anchored aft and astern.

General water chemistry (pH, temperature, specific conductance, dissolved oxygen and salinity) was measured using an Hydrolab Surveyor 3 (Hydrolab Corp., Austin, TX). Diffusion chambers were suspended on the sunny, downstream side of the vessel at three depths: the Secchi depth, just below the surface and midway between these depths (Figure 1).

Both diffusion chambers (containing laboratory-grown cultures) and horizontal (Van Dorn) samplers (containing ambient water) were used in this study. Diffusion chambers were assembled according to Figure 2, and suspended with the 0.22 μm polycarbonate filters (Osmonics Inc., Minnetonka, MN) oriented toward the river basin and a transparent plastic film facing upward (McFeters and Stuart 1972). Each chamber was inoculated with ~30 ml of nutrient-acclimated culture. Both the chambers and Van Dorn samplers were pre-incubated at the deepest (darkest) depth for one hour and then moved to their final position. Natural populations collected in the sealed Van Dorn samplers, as well as from diffusion chambers, were held at each depth for a minimum of one hour to allow time for transcription initiation.

The entire contents of the chambers were evacuated immediately following withdrawal from the water. Cells were placed on ice in the dark and duplicate 5 ml aliquots were filtered, in subdued light, onto 25 mm, 3.0 μm mixed cellulose ester filters (Millipore Corp, Billerica, MA). Cells captured on the filters were then sealed in cryogenic tubes and flash frozen in a dry ice/100% ethanol bath for RNA preservation. For toxin analysis, duplicate 5 ml aliquots were collected from each chamber and

maintained on ice, in the dark, in polystyrene Corning tubes until further processing at the laboratory.

Van Dorn samplers were treated similarly. One liter of the sampler contents was collected in a plastic graduated cylinder. Of this, 500 ml were filtered in darkness onto a 47 mm, 3.0 μm mixed cellulose ester filter (Millipore Corp, Billerica, MA) in a sterile, stainless steel chimney filtration apparatus using a hand-operated vacuum pump (Nalgene, Rochester, NY). Filters were flash-frozen for RNA preservation as described. The remaining 500 ml of ambient water sample were placed on ice in the dark for transport to the laboratory for microcystin analysis using ELISA (Enzyme-linked Immunosorbant Assay).

Samples for RNA analysis were transferred to a -80 °C freezer immediately upon arrival at the laboratory. For microcystin detection, cells from the diffusion chambers were pelleted by centrifugation at 5,000 rpm, 4 °C, for 10 min and stored frozen at -20 °C. Horizontal sampler aliquots were filtered onto 3.0 μm polycarbonate filters (Osmonics, Minnetonka, MN). Filters were ultrasonicated for 5 min to release captured cells. The suspension was then pelleted by centrifugation at 5,000 rpm, 4 °C, for 10 min and stored frozen at -20 °C.

ELISA toxin measurements

Microcystin extractions followed the method of Haney and Ikawa (2000). Briefly, cell pellets were freeze/thawed three times before extraction overnight in 250 μl 80% methanol at room temperature. After extraction in methanol, 750 μl of phosphate buffer saline (PBS, pH= 7.4) were added, and 300 μl of sample were filtered through a 0.45 μm

filter syringe (Corning Inc., Corning, NY). An additional 450 µl PBS were then added to the filtrate, for a final methanol concentration of 8%. Chamber sample extracts were diluted 1:1000 in PBS (pH= 7.4) to obtain readings within the range of the standards provided with the kit (see below). Therefore, the final sample concentrations of microcystin measured by the ELISA (Enzyme-Linked Immunosorbant Assay), which is provided in ppb, were adjusted accordingly.

Microcystin toxin analysis was performed using Microcystin (ELISA) Plate Kits available from Envirologix Inc. (Portland, ME). The standard protocol accompanying the kits was followed. All samples were analyzed in duplicate. Reactions were analysed at 450 nm with a 630 nm reference wavelength on an EL 808 Ultra Microplate Reader (Biotek Instruments) using KC4 software in the Biochemistry Core of the Wadsworth Center. Final concentrations were determined by accounting for the 8.3-fold dilution of all extracts and the 5-fold or 250-fold concentration of chamber samples and Van Dorn samples, respectively.

RNA extraction

Total RNA was extracted using a High Pure RNA Isolation Kit (Roche Diagnostics Corp., Indianapolis, IN). The protocol for isolation of total RNA from Gram-positive and Gram-negative bacteria was used. Briefly, cell pellets were resuspended in 200 µl Tris buffer (pH= 8.0) and 4 µl lysozyme (50 mg/ml), and incubated for 10 minutes at 37 °C. Four-hundred microliters of Lysis/Binding buffer were then added, prior to pipetting the entire sample into the glass fiber-based filter and centrifuge tubes provided with the kit. The rest of the extraction followed the standard protocol, with the exception

of an hour-long room temperature incubation with DNase I. Sixty to 75 µl of elution buffer were used to remove the RNA from the filter tubes. Extracted RNA was then stored at -80 °C until further analysis. Total RNA concentrations were determined spectrophometrically from the ratio of 260/280 nm UV absorbance or using the Agilent RNA 6000 Nano assay for the Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA).

Real Time Reverse Transcriptase Polymerase Chain Reaction Amplification

Quantitative RT-PCR was performed in a Light Cycler® (Roche, Indianapolis, IN) using the Qiagen One-Step RT-PCR Kit (Qiagen, Valencia, CA). Primers were chosen from the *M. aeruginosa* PCC 7813 microcystin synthetase (*mcy*B) gene (accession no. AY034601) using the Primer 3 program (Rozen and Skaletsky 2000), available at http://www-genome.wi.mit.edu/genome_software /other/ primer3.html). The forward primer used for detection of *mcy*B was 5'- AGG CAA GCA GAA ATT CAG GA- 3' and the reverse primer was 5'- ATA GCA ACC ACC GTC AAA GG -3'. The resulting product size was 212 base pairs.

Initial trials with forward primer mcyB30F 5'- CCT ACC GAG CGC TTG GG -3' and reverse primer mcyB108R 5' - GAA AAT CCC CTA AAG ATT CCT GAG T -3', using both SYBR® Green I (Molecular Probes, Eugene, OR) and a dual-labeled probe [5' 6-carboxyfluorescein (FAM) / 3' Tetramethyl Rhodamine (TAMRA); Integrated DNA Technologies, Coralville, IA], failed to generate an adequate standard curve (Kurmayer and Kutzenberger 2000). Difficulty amplifying dilute concentrations of RNA in the standard curve using this primer set was attributed to low transcript copy numbers of

*mcy*B mRNA, low initial total RNA concentrations, and the small (80 base pair) size of the amplified product.

All Qiagen One-Step kit reagents were used at the recommended concentrations. Each primer was added to the reaction mix at a 0.5 μM concentration. Between 5-6 ng/μl of total RNA were added to each capillary tube. The Lightcycler® program included a reverse transcription step at 50 °C for 30 minutes and denaturation, RT deactivation and *Taq* polymerase activation at 95 °C for 15 minutes. Amplification was for 45 cycles with denaturation at 95 °C for 15 seconds, annealing at 60 °C for 15 seconds and extension at 72 °C for 30 seconds. For product detection and quantification, the DNA binding dye SYBR® Green I (Molecular Probes, Eugene, OR) was added to the 15 μl reaction volume at a 1/5000 dilution. Melting curve analysis was performed between 56 °C and 95 °C. Samples were run in duplicate at least two times. Also, all amplicons were examined using 2.5% ethidium bromide stained agarose gels to confirm product size. Specificity of the primers was confirmed by RT-PCR amplification of RNA from the non-toxic strain PCC 7005. Also, to verify the absence of contaminating DNA, reactions were performed without the reverse transcription step.

The standard curve for *mcy*B mRNA was generated using dilutions of mRNA extracted from log phase laboratory cultures of PCC 7806 and PCC 7813 grown under standard light conditions using software provided with the Lightcycler®. Dilutions were added to the standard Qiagen master mix and amplified in the Lightcycler® as described above. Calculations of the relative levels of experimental *mcy*B mRNA were performed using Lightcycler® software.

About 65 ml of axenic culture PCC 7806, A_{600}= 0.684, were divided into two equal portions. Both were incubated in darkness for one hour. Subsequent to this incubation, one half was incubated in a flask under the mixing and light regime described above, while the other was aliquoted into 5 ml portions and placed in foil-wrapped corning tubes. At 10, 60, and 120 min time intervals, 5 ml samples of each were withdrawn, filtered onto 3.0 μm polycarbonate filters andd frozen immediately at -20 °C. RNA extractions and Real Time RT-PCR were performed as previously described.

RESULTS

Time course

To validate the use of a 60 min incubation period *in situ*, a time course examining relative transcript abundance over a 120 minute period was performed in the

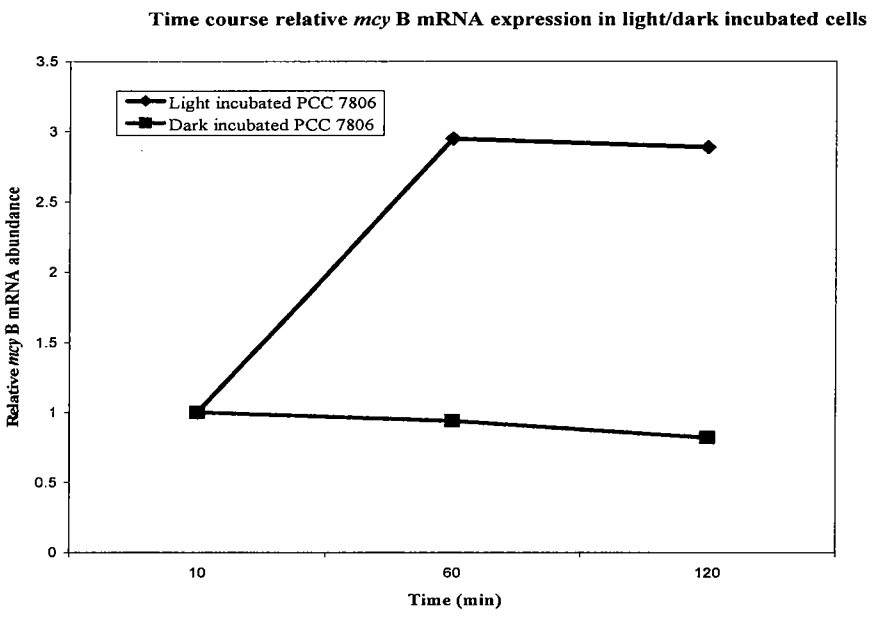

Figure 3. Relative transcript levels of *mcy*B from light and dark exposed cells over a time course measured using Real Time RT-PCR in a Lightcycler®.

laboratory. The time course, which was carried out using the PCC 7806 toxic strain of *M. aeruginosa,* showed no change in transcript abundance for the dark incubated cells (Figure 3). The light incubated cells showed an increase in relative transcript quantityafter 60 minutes incubation and then, the increase appeared to plateau (Figure 3).

Weather and water conditions

Sampling on both dates in Esopus Creek was carried out under partly cloudy skies. Secchi depth in the tributary was 1.2 m on both dates. Skies were clear for the July sampling at Stockport Flats, while in August the sky was overcast. At Stockport Flats, Secchi depths were 0.9 m and 0.76 m for July and August, respectively. Water chemistry values measured were unremarkable for the two sites and sampling dates (Table 1).

	Stockport Flats July 3, 2004	Stockport Flats August 6, 2004	Esopus Creek July1, 2004	Esopus Creek August 4, 2004
DO (mg/L)	9.85	12.03	ND	9.36
% Sat (O_2)	112.4	115	ND	115
Specific Conductance (µS/cm)	0.284	0.251	0.239	0.289
Temperature (°C)	22.9	24	23.63	26.34
Salinity (pss)	0.14	0.13	0.12	0.14
pH	8.17	7.96	8.0	8.28

Table 1. Water chemistries measured using an Hydrolab Surveyor 3. (ND = not determined)

Microcystin concentrations

In agreement with a previously published laboratory-based study of light regulated *mcy*B expression, (Kaebernick et al. 2000), no consistent differences in toxin concentration were observed between cultures exposed to different light levels. This was

true regardless of date or site sampled. Toxin concentrations measured in the chambers on all dates ranged from 7.5-19.1 ppm (Table 2).

Chamber	JULY Final Mcyst concentration (ppm)	AUGUST Final Mcyst concentration (ppm)
Esopus Trib (PCC 7813)		
surface	13.3	9.1
mid	14.9	6.6
deep	15.8	11.6
Stockport Flats (PCC7806)		
surface	10.8	14.9
mid	10.8	14.9
deep	7.5	19.1

Table 2. July and August chamber microcystin concentrations determined using an ELISA-based assay.

Low concentrations of microcystin toxin were detected in river water collected using the Van Dorn samplers on two of the sampling dates, in July at the Stockport Flats

Van Dorn	JULY Final Mcyst (ppt)	AUGUST Final Mcyst (ppt)
Esopus Trib		
surface	ND	4.6
mid	ND	6.6
deep	ND	9.1
Stockport Flats		
surface	8.3	ND
mid	5.8	ND
deep	7.5	ND

Table 3. July and August Van Dorn microcystin concentrations determined using an ELISA-based assay.

site and in August at the Esopus Creek site. There is no apparent difference in microcystin concentrations with depth of incubation measured at Stockport Flats, where

the values ranged from 5.8-8.3 ppt (Table 3). There is a slight increase in toxin concentration with depth at the Esopus Creek site (4.6-9.1ppt) but increased sample size and replicate measures of toxin concentrations would provide more statistically valid numbers (Table 3).

*mcy*B mRNA transcript levels

Differences in transcript levels of *mcy*B from cells incubated in diffusion chambers varied according to location and culture. The relative levels of transcripts increased with increased light intensity at Stockport Flats, where the *M. aeruginosa* PCC 7806 culture was used to inoculate chambers (Figure 4). At the Stockport Flats, there was nearly a 5-fold increases in transcription abundance in July and around a 15-fold increase in August (Figure 4). However, at the Saugerties Creek site, where *M. aeruginosa* PCC 7813 was used, the differences in transcript abundance were negligible (Figure 4). The largest relative increase appeared to be isolated to those cells incubated near the surface, while cells incubated midway between the Secchi Depth and the surface demonstrated only minor variation.

The Lightcycler® failed to amplify RNA extracted from the river water collected in Van Dorn samplers. Either the extremely low concentrations of RNA isolated, or PCR inhibitors commonly found in environmental samples, would likely account for the failed amplification.

In addition, single peak melting curves were obtained for all reactions, verifying the lack of non-specific amplification. The melting temperature (T_m) of the reaction products was approximately 81 °C.

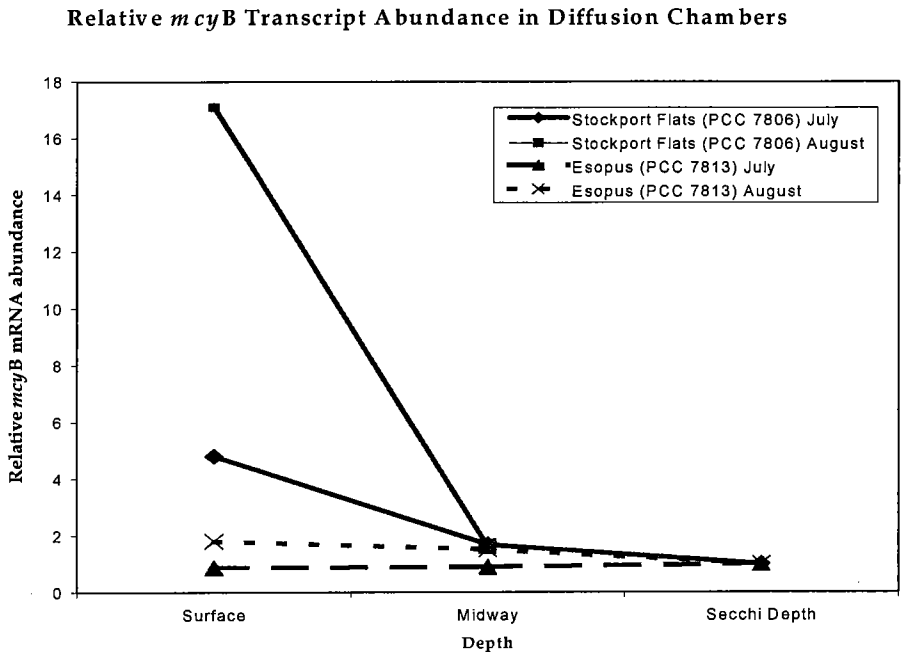

Figure 4. Relative *mcy*B abundance in diffusion chambers suspended at different depths in the Hudson River Estuary at Stockport Flats and the Esopus Tributary. The mRNA transcript abundance is presented as fold increases relative to the dark incubated culture. Calculations of relative *mcy*B mRNA amounts are based on computer-generated concentrations derived from the standard curve using Lighcycler® software.

DISCUSSION

The findings from this study suggest that the microcystin synthetase gene transcription is under the influence of light intensity. Increased transcript levels were evident in *Microcystis aeruginosa* strain PCC 7806 exposed to the high light intensities near the surface of a natural water body, the Hudson River, while contained in a diffusion chamber. This suggests that the transcription of these genes may be mediated by a light

receptor or that the high levels of photosynthetically active radiation (PAR) may enhance some other metabolic activity. While intensity and quality were not examined in the time course, the cells were also shown to respond to light. Cells exposed to limited light, as in the case of those incubated at or below the Secchi depth or those incubated in the foil wrapped tubes failed to exhibit enhanced transcript levels.

While other factors influencing this response can not be entirely ruled out, the rapidity of increase in *mcy*B transcript levels indicates the response is disconnected from cell division. Cell division in *M. aeruginosa* occurs less than once a day, whilst transcript level increases enfold in under an hour. The lack of perceptible microcystin toxin increase may also be the result of the time scale used in this study. Light has been shown to have a positive effect on microcystin production and content (Wiedner et al. 2003). This is generally accepted as a result of high rates of cell growth under these circumstances. However, transcription initiation and enhancement would be immediate in the cells' metabolic time scale while translation, organization of the enzyme complex and toxin synthesis would be far less so. The stability of the mRNA detected in this assay may also be a limiting factor preventing the consummation of the microcystin enzyme complex synthesis. Similarly, consistent toxin concentrations observed among cells exposed to different light conditions make sense if the cells maintain a fixed number of active microcystin synthetase complexes during the course of the experiment.

The failure of *M. aeruginosa* strain PCC 7813 to demonstrate the same response may be explained by the strain itself. The experimental design may not have been suited to this strain. For example, these cells may have a different light intensity threshold or require a longer transcription upregulation phase than conditions used in this study. Also,

the cells may have more active mRNA degradative enzymes. The susceptibility of this strain to oxidative damage associated with the high light intensities near the water's surface could be a factor as well. Furthermore, the weather conditions during the sampling days may have influenced the results of this study. Partly cloudy conditions were prevalent for the duration of incubation at the Saugerties Creek with *M. aeruginosa* PCC 7813. Conversely, at Stockport Flats, where *M. aeruginosa* strain PCC 7806 was used, sampling was done on one date, under a cloudless sky and the other, under consistently overcast skies. The intermittent nature of the light may influence regulation of *mcy*B gene transcription. This phenomenon would have to be explored further for confirmation.

The very low concentrations of total RNA isolated from all *M. aeruginosa* cells used in this study must also be considered. The ng/µl amounts of total RNA suggest extremely low concentrations of *mcy*B mRNA. As always with PCR, the reactions are highly sensitive, with extraordinarily low limits of detection, yet, have high odds for error and distortion.

When compared to the previous lab-based study of light-induced upregulation of *mcy*B gene transcripts by Kaebernick et al. (2000), the results of this research are compatible. At least in the case of strain PCC 7806, light was seen to have a positive effect on the *mcy*B transcription. Also, no discernable differences in toxin content were detected in cells exposed to different light conditions. In this study however, RT-PCR products were always present, even in cells that remained completely in the dark for over two hours. The products were manifest in both the fluorometric detection system of the Lightcycler® and in ethidium bromide stained gels. This may be expected when

considering the relative sensitivities of Real-Time PCR using the Lightcycler® versus the RNase protection assay used in the Kaebernick study (Kaebernick et al. 2000).

From these results, we conclude that the upregulation of the microcystin synthetase enzyme operon *mcy*B is stimulated by higher light intensities. Natural underwater illumination and all the physical effects of this type of light environment did not appear to negate this response in *M. aeruginosa* strain PCC 7806. Thus, the possibility that populations of buoyant *M. aeruginosa* cells, such as in a toxic microcystin bloom, could augment toxin production by means of a light-induced transcriptional response should not be overlooked.

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