African Journal of Marine Science 2006, 28(2): 309–312
Printed in South Africa — All rights reserved

Copyright © NISC Pty Ltd
AFRICAN JOURNAL OF
MARINE SCIENCE
ISSN 1874–232X

# Effects of light intensity on cylindrospermopsin production in the cyanobacterial HAB species *Cylindrospermopsis raciborskii*

**J Dyble[1,2]*, PA Tester[1] and RW Litaker[1]**

[1] *Center for Coastal Fisheries and Habitat Research, NOAA, 101 Pivers Island Road, Beaufort, NC 28516, USA*
[2] *Current address: Great Lakes Environmental Research Laboratory, NOAA, 2205 Commonwealth Boulevard, Ann Arbor, MI 48105, USA*
* *Corresponding author, e-mail: juli.dyble@noaa.gov*

**The role of light intensity on growth and the production of the hepatotoxin cylindrospermopsin (CYN) in the cyanobacterial harmful algal bloom species *Cylindrospermopsis raciborskii* was investigated using cultured isolates grown in N-free media under a series of neutral density screens. Maximum growth as indicated by chlorophyll *a* concentrations was measured at 75µmol photons m$^{-2}$ s$^{-1}$, whereas maximum intracellular and extracellular CYN concentrations occurred in cultures grown under the highest light intensity (140µmol photons m$^{-2}$ s$^{-1}$). During exponential growth phase, N-limited *C. raciborskii* cultures grown under light intensities of 18–75µmol photons m$^{-2}$ s$^{-1}$ exhibited a strong linear relationship between light intensity and both intracellular and extracellular CYN concentrations. Extracellular CYN concentrations increased significantly as the culture moved from log to stationary growth phase. These results indicate that the highest intracellular toxin concentrations in the field are likely to occur in *C. raciborskii* populations that have been actively growing at light intensities of 75–150µmol photons m$^{-2}$ s$^{-1}$ for more than two weeks, and that peak soluble toxin levels will be found at the end of a bloom.**

*Keywords*: cyanobacteria, cylindrospermopsin, *Cylindrospermopsis raciborskii*, HAB, light

## Introduction

*Cylindrospermopsis raciborskii* is a filamentous cyanobacterial harmful algal bloom (cyanoHAB) species that is becoming increasingly prevalent in lakes, reservoirs and other potential drinking and recreational water supplies worldwide. Some *C. raciborskii* strains can produce the hepatotoxin cylindrospermopsin (CYN) and/or the neurotoxins anatoxin-a and saxitoxin, resulting in detrimental health effects in both humans and animals (Hawkins *et al.* 1985). The mere presence of a *C. raciborskii* bloom, however, does not necessarily imply a health hazard because cyanobacterial blooms vary greatly in their ability to produce toxins. It is not known if these differences are the result of specific environmental factors that induce toxin production, an alteration in bloom composition between genetically toxic and non-toxic strains, or some combination of the two.

Certain strains of *C. raciborskii* lack the genetic machinery necessary for toxin production and therefore are incapable of producing toxin (JD, unpublished data). This means the toxicity of a *C. raciborskii* bloom in the field must be influenced by the relative proportion of toxic and non-toxic strains. Environmental factors, such as nitrogen (N) availability and temperature, have also been shown to regulate toxin production in genetically capable strains. CYN production, for example, was two times greater in *C. raciborskii* cultures grown in N-free media than in those grow with $NH_4$ (Saker and Neilan 2001). Maximum CYN production was also found to occur at 20°C (Saker and Griffiths 2000), whereas maximum growth rates occurred between 29°C and 35°C (Briand *et al.* 2004). These data would indicate that, at least with respect to temperature effects, there is no direct linear relationship between growth and toxin production, as has been reported for some other cyanoHAB species (Sivonen 1996, Orr and Jones 1998).

Light intensity represents another potential environmentally important regulator of growth and toxin production, which has yet to be fully investigated in *C. raciborskii*. *C. raciborskii* has been hypothesised to be a low-light adapted species. Briand *et al.* (2004) measured growth rates in 10 strains of *C. raciborskii* under different light intensities and found positive growth rates over a wide range of light intensities (30–400µmol photons m$^{-2}$ s$^{-1}$), with maximum growth rates at 80µmol photons m$^{-2}$ s$^{-1}$. Another study showed the maximum growth rates in a Lake Balaton strain to be under 120µmol photons m$^{-2}$ s$^{-1}$ (Padisak 1997). The $I_k$ values (the light intensity approximating the onset of light saturation) for each of these strains were around 20µmol photons m$^{-2}$ s$^{-1}$, which is similar to other low-light adapted species like *Planktothrix agardhii* and *Phormidium luridum* (Padisak 1997, Litchman 2000). In some cyanobacterial genera, the light intensities in which growth rates were optimum were also those in which intracellular toxin concentrations were the highest (Rapala *et al.* 1993), whereas for other genera,

**Table 1:** Percentage light attenuation and total light exposure received by *C. raciborskii* culture SDS under different layers of neutral density screening and cylindrospermopsin (CYN) production rates over the course of the 16-day experiment in the intracellular and extracellular fractions

| Number of screens | Light attenuation (%) | Light exposure (μmol photons $m^{-2}$ $s^{-1}$) | CYN production rate (μg CYN $l^{-1}$ $day^{-1}$) | |
|---|---|---|---|---|
| | | | Intracellular | Extracellular |
| 0 | 100 | 139.4 | 192.0 | 9.8 |
| 1 | 54 | 75.3 | 169.3 | 8.9 |
| 2 | 38 | 53.0 | 78.8 | 4.2 |
| 3 | 25 | 34.9 | 27.3 | −0.02 |
| 6 | 13 | 18.1 | 0 | −0.3 |

maximum cyanotoxin production was at irradiances greater (Watanabe and Oishi 1985) or lower (Sivonen 1990) than those required for maximum growth. Clearly, the effect of light on toxin production is species-specific. Therefore, this study was undertaken to determine how light intensity regulates CYN production in *C. raciborskii*.

## Material and Methods

The *C. raciborskii* cultures used in these experiments were originally isolated from Solomon Dam, Queensland, Australia (by M Saker, Centro de Investigacão Marinha e Ambiental, Portugal) and have consistently produced the hepatotoxin cylindrospermopsin in culture. Strain SDS has a straight-filament morphology and strain SDC had a coiled form when first isolated, but transformed into a straight form during the course of culturing. These cultures were maintained in N-free Z8 media (Kotai 1972) in a constant temperature incubator set at 25°C. Illumination was provided by two Philips F20T12 cool white bulbs (20W each) in a 15:9h light: dark cycle and light intensities were measured using a $4\pi$ light sensor (Biospherical Instruments, QSL-100, San Diego, California, USA).

For the first experiment, light intensity was altered using different numbers of layers of neutral density screening and placement position in the incubator to give a range of light exposures from 18–140μmol photons $m^{-2}$ $s^{-1}$ (Table 1). There were two replicate 300ml flasks for each of the five light intensities, each consisting of 175ml of Z8 media inoculated with 1ml of SDS *C. raciborskii* culture in early log phase. These temperature and light regimes reflect those that would be expected in places where *C. raciborskii* is found in the natural environment. Samples were collected every 48 hours over a 16-day period for chlorophyll *a* (Chl *a*) and CYN analysis. In a second experiment, both *C. raciborskii* cultures SDS and SDC were incubated under 140μmol photons $m^{-2}$ $s^{-1}$ for 30 days to determine the concentrations of CYN in the extracellular fraction over the course of logarithmic and stationary growth phases. In this experiment, samples were collected at 3 days, 18 days and 30 days for CYN analysis.

Relative fluorescence was used as a proxy for Chl *a* (Briand *et al.* 2004). Calibration of fluorescence data against Chl *a* concentrations was established by comparing fluorescence readings using a fluorometer (Turner Designs, 10-000R, Sunnyvale, California) against extracted chlorophyll *a* (90% acetone extraction according to Parsons *et al.* 1984). Intra- and extracellular CYN concentrations were measured by high-performance liquid chromatography (HPLC) using an Alltima C18, 5μm column (Alltech, Deerfield, Illinois, USA). Cells were concentrated on a 0.6μm filter and frozen at –20°C until toxin extraction. To extract the intracellular CYN, cells were sonicated in 100% methanol containing 0.5M acetic acid and then concentrated on an Oasis HLB reversed-phase SPE column (Waters, Milford, Massachusetts, USA). Both the load and 30% methanol elution fractions were analysed for CYN. Extracellular CYN in the cell free media (<0.6μm) was concentrated onto Waters HLB columns and both the load and 30% methanol elution fraction were analysed for CYN. Concentrations of CYN were calculated based on a cylindrospermopsin standard curve (standard kindly provided by W Carmichael, Wright State University, USA). Statistical analysis of the response of CYN concentration to light intensity was performed using a one-way analysis of variance (ANOVA), with a *post hoc* analysis using a Tukey multiple comparison of means, once the data were shown to satisfy the normality assumption (SAS software, Version 8.2, Copyright 2001, SAS Institute Inc., Cary, North Carolina, USA).

## Results and Discussion

### *Growth*

Chlorophyll *a* values were calculated from fluorescence readings based on a calibration curve generated by measuring 10 samples over a range of cell densities by both methods ($r^2$ = 0.94). Increases in Chl *a* were highest at 75μmol photons $m^{-2}$ $s^{-1}$, closely followed by cultures grown at 140μmol photons $m^{-2}$ $s^{-1}$ and 53μmol photons $m^{-2}$ $s^{-1}$ for *C. raciborskii* strain SDS (Figure 1). Growth was slower in cultures maintained at 35μmol photons $m^{-2}$ $s^{-1}$ and there was no net increase in Chl *a* at 18μmol photons $m^{-2}$ $s^{-1}$ over the course of the 16-day experiment (Figure 1). Thus, light intensities of 18μmol photons $m^{-2}$ $s^{-1}$ and less were insufficient to support growth. In other studies, maximum growth rates were achieved at light intensities ranging from 50μmol photons $m^{-2}$ $s^{-1}$ to 100μmol photons $m^{-2}$ $s^{-1}$ (Briand *et al.* 2004). In natural settings, light attenuation with depth is dependent upon factors such as the amount of dissolved humics and other suspended particulates in the water column. Irradiance values ranging from 15μmol photons $m^{-2}$ $s^{-1}$ to 200μmol photons $m^{-2}$ $s^{-1}$ are typical of depths at which *C. raciborskii* is abundant (Havens *et al.* 1998, Bouvy *et al.* 1999).

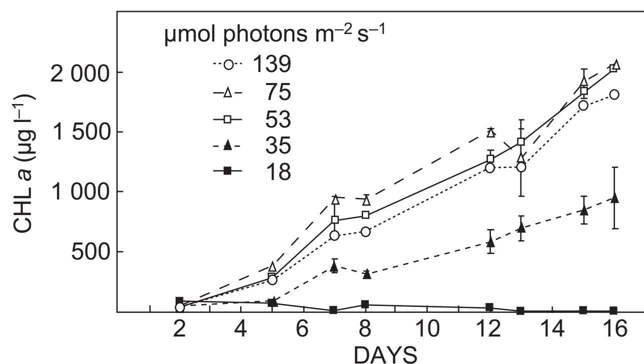


**Figure 1:** Chlorophyll *a* concentrations over the course of 16 days in *C. raciborskii* culture SDS grown under a range of light intensities. Error bars denote ±1SD

### *Toxin production*

Intracellular CYN concentrations were normalised to Chl *a* to account for variability in number of cells between light treatments. Even accounting for the increased Chl *a*, there was an increase in both intra- and extracellular CYN concentrations over the course of the 16-day experiment in all but the lowest light intensity treatment. Rates of CYN production (μg CYN $l^{-1}$ $day^{-1}$, Table 1) and total CYN concentration (μg CYN μg Chl $a^{-1}$, Figure 2) were highest at the highest light intensities for both the intracellular and extracellular fractions and decreased with decreasing light intensity. Data are presented for CYN concentrations in *C. raciborskii* strain SDS on Day 8 and Day 16 (Figure 2). Intracellular CYN concentrations at Day 16 were significantly higher ($p < 0.05$) than those at Day 8 for all light intensities except 18μmol photons $m^{-2}$ $s^{-1}$. At the end of 16 days, intracellular CYN concentrations normalised to Chl *a* were 1.39 ± 0.08μg CYN μg Chl $a^{-1}$ under 140μmol photons $m^{-2}$ $s^{-1}$ and decreased to undetectable concentrations in cultures grown under the lowest light intensity of 18μmol photons $m^{-2}$ $s^{-1}$. Whereas intracellular CYN concentrations between the 140μmol photons $m^{-2}$ $s^{-1}$ and 75μmol photons $m^{-2}$ $s^{-1}$ treatments were not significantly different, CYN concentrations were significantly lower ($p < 0.05$) at each subsequently lower light intensity. Extracellular CYN concentrations followed the same pattern. After 16 days, extracellular CYN concentrations in cultures grown under the highest light intensity were 140 ± 14μg $l^{-1}$, dropping to 6.9 ± 2μg $l^{-1}$ at 18μmol photons $m^{-2}$ $s^{-1}$. The extracellular CYN concentrations were not significantly different between the two highest light intensities (140μmol photons $m^{-2}$ $s^{-1}$ and 75μmol photons $m^{-2}$ $s^{-1}$) on Day 8 or Day 16, but did decrease in lower light intensity treatments, particularly at Day 16 (Figure 2b). At the four lowest light intensities, there was a strong linear relationship between light intensity and both intracellular and extracellular CYN concentrations ($r^2$ = 0.983 and 0.996 respectively). Whereas *C. raciborskii* cultures grown under the highest light intensity (140μmol photons $m^{-2}$ $s^{-1}$) still produced the highest CYN concentrations, it was less than what would be predicted from a linear relationship.

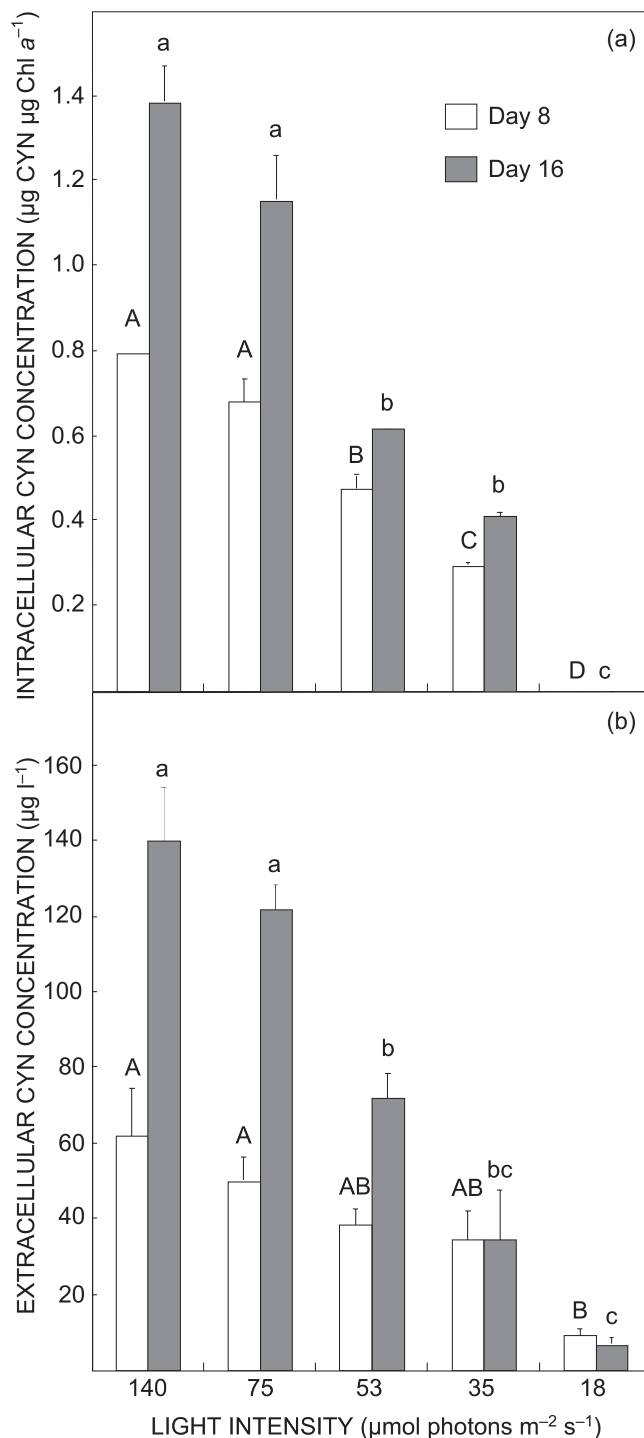


**Figure 2:** Cylindrospermopsin (CYN) concentrations in the (a) intracellular and (b) extracellular fractions of *C. raciborskii* cultures grown under a range of light intensities. Data are shown for Day 8 and Day 16. Bars are the mean of two replicates, error bars denote +1SD, and the upper case (for Day 8) or lower case (for Day 16) letters group the means according to a Tukey *post hoc* comparison

The preliminary data presented here suggests that CYN concentrations in N-limited *C. raciborskii* cultures are proportional to light intensity ranging from 18μmol photons

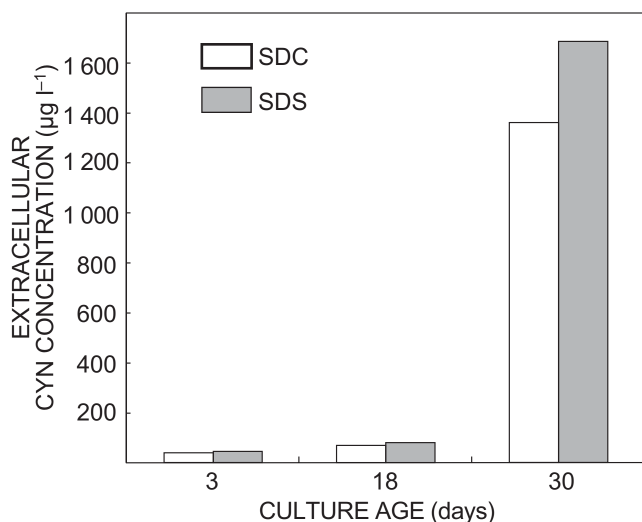


**Figure 3:** Extracellular cylindrospermopsin (CYN) concentrations (µg $l^{-1}$) in two strains (SDC and SDS) of *C. raciborskii* at 3 days, 18 days and 30 days after inoculation

$m^{-2}$ $s^{-1}$ to 75µmol photons $m^{-2}$ $s^{-1}$. In the strain SDS, the highest CYN concentrations were measured in the cultures maintained at the highest light intensities (140µmol photons $m^{-2}$ $s^{-1}$), but growth as measured by Chl *a* concentration was highest under lower light intensities (75µmol photons $m^{-2}$ $s^{-1}$ and 53µmol photons $m^{-2}$ $s^{-1}$). These data are consistent with the hypothesis that light regulation of CYN concentrations is not strictly a function of the effect of light on growth rate. However, additional experiments are needed to test whether this same pattern of response to light intensity is observed in other toxic strains.

Extracellular CYN concentrations were also measured in *C. raciborskii* strains SDS and SDC over a 30-day period to identify how the age of the culture affects the amount of CYN released into the media either through cell lysis or leakage of toxin through the cell membrane. Extracellular CYN remained relatively low while the culture was in logarithmic growth phase, but by 30 days the cells had progressed into stationary growth phase and the CYN concentration had increased significantly (Figure 3). Other studies also observed an increase in CYN per unit biomass as *C. raciborskii* cultures moved into stationary phase of growth and a concurrent increase in extracellular CYN from 15–20% of the total CYN to 50% (Saker and Griffiths 2000, Hawkins *et al.* 2001). This suggests that, during a bloom, the highest CYN (or cyanotoxin) concentrations may occur near the end of the bloom, likely due to cell lysis and accumulation of CYN over the course of the bloom (Hawkins *et al.* 2001).

In the future, assessing the public health risks posed by *C. raciborskii* will require determining the proportion of toxic strains present (Saker and Neilan 2001), as well as understanding how the prevailing conditions will stimulate toxin production in strains genetically capable of producing toxin. The results here clearly show that the light environment will influence toxin production and may prove a crucial factor in assessing bloom toxicity.

*Manuscript received January 2005; accepted January 2006*

*Acknowledgements* — This study was supported by a National Research Council Postdoctoral Fellowship to JD through NOAA, Center for Coastal Fisheries and Habitat Research. We thank Andrew Chapman for supplying the *C. raciborskii* cultures, and Mark Vandersea and two other reviewers for useful comments on the manuscript.

## References

Bouvy M, Molica R, Oliveira SD, Marinho MM, Becker B (1999) Dynamics of a toxic cyanobacterial bloom *Cylindrospermopsis raciborskii* in a shallow reservoir in the semi-arid region of northeast Brazil. *Aquatic Microbial Ecology* **20**: 285–297

Briand JF, Leboulanger C, Humbert JF, Bernard C, Dufour P (2004) *Cylindrospermopsis raciborskii* (Cyanobacteria) invasion at mid-latitudes: selection, wide physiological tolerance, or global warming. *Journal of Phycology* **40**: 231–238

Havens KE, Philips EJ, Cichra MF, Li BL (1998) Light availability as a possible regulator of cyanobacterial species composition in a shallow subtropical lake. *Freshwater Biology* **39**: 547–556

Hawkins PR, Putt E, Falconer I, Humpage A (2001) Phenotypical variation in a toxic strain of the phytoplankter, *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) during batch culture. *Environmental Toxicology* **16**: 460–467

Hawkins PR, Runnegar MTC, Jackson ARB, Falconer IR (1985) Severe heptatoxicity caused by the tropical cyanobacterium (blue-green alga) *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya and Subba Raju isolated from a domestic water supply reservoir. *Applied and Environmental Microbiology* **50**: 1292–1295

Kotai J (1972) Instruction for preparation of modified nutrient solution Z8 for algae. Publication **B-11/69**. Norwegian Institute for Water Research, Blindern, Oslo, 5pp.

Litchman E (2000) Growth rates of phytoplankton under fluctuating light. *Freshwater Biology* **44**: 223–235

Orr PT, Jones GJ (1998) Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. *Limnology and Oceanography* **43**: 1604–1614

Padisak J (1997) *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju, an expanding, highly adaptive cyanobacterium: worldwide distribution and review of its ecology. *Archiv für Hydrobiologie Supplement* **107**: 563–593

Parsons TR, Maita Y, Lalli CM (1984) *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon Press, Oxford, 173pp

Rapala J, Sivonen K, Luukkainen R, Niemela SI (1993) Anatoxin-a concentration in *Anabaena* and *Aphanizomenon* under different environmental conditions and comparison of growth by toxic and non-toxic *Anabaena*-strains – a laboratory study. *Journal of Applied Phycology* **5**: 581–591

Saker ML, Griffiths DJ (2000) The effect of temperature on growth and cylindrospermopsin content of seven isolates of *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) from water bodies in northern Australia. *Phycologia* **39**: 349–354

Saker ML, Neilan BA (2001) Varied diazotrophies, morphologies, and toxicities of genetically similar isolates of *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) from northern Australia. *Applied and Environmental Microbiology* **67**: 1839–1845

Sivonen K (1990) Effects of light, temperature, nitrate, orthophosphate and bacteria on growth of and heptatoxin production by *Oscillatoria agardhii* strains. *Applied and Environmental Microbiology* **56**: 2658–2666

Sivonen K (1996) Cyanobacteria toxins and toxin production. *Phycologia* **35**: 12–24

Watanabe MF, Oishi S (1985) Effects of environmental factors on toxicity of a cyanobacterium (*Microcystis aeruginosa*) under culture conditions. *Applied Environmental Microbiology* **49**: 1342–1344