Effects of Environmental Factors on the Physiology of two Species of Freshwater Cyanobacteria

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ABSTRACT

Anthropogenic activities have been responsible for the increased release of CO_2 into the atmosphere and resulting increases in global temperatures. The release of some anthropogenic pollutants to the atmosphere has also caused depletion of the stratospheric ozone layer, decreasing the screening of ultraviolet B radiation (UVB) by the atmosphere. In addition, ozone levels in the lower stratosphere have also been declining since 1998. Continued depletion of ozone in the stratosphere allows more ultraviolet radiation (UVR), specifically UVB, to reach the Earth's surface. As increased temperature is believed to favour cyanobacteria in general and with future climate change scenarios predicting that surface water temperature will increase, it is important to study the interactive effects of these environmental factors on cyanobacterial growth and physiology. In this study we therefore investigated the effects of light, temperature and UVB on the growth, survival, photosynthetic capacity and recovery from UVB exposure, of two cyanobacterial species, namely *Microcystis aeruginosa* and *Anabaena circinalis*. Toxic and non-toxic strains of both species were grown at different light intensities to determine the responses of growth to light. Toxic strains were also grown at two different temperatures and exposed to either an acute dose of UVB or longer-term exposures to low-, mid- and high-UVB in order to determine differences in cellular responses, in terms of photoinhibition and repair, both between species and within species (between strains).

Toxic strains of both species showed saturation of growth at a higher light intensity than the non-toxic strains, possibly reflecting the energetic costs of toxin production. There were also differences in physiological characteristics between species and strains. However, factors other than toxin production may be involved in the observed differences. Growth rates between species also varied, with relatively higher growth of

A. circinalis at higher temperature than *M. aeruginosa*. Variations in terms of levels of UV absorbing compounds between species was also observed. An observed increased repair rate of photosynthetic characteristics with increased temperature suggests an improved ability of cyanobacteria to overcome UV induced damage at higher temperature. However, there were also intra-strain variations in terms of survival and recovery at different temperatures and UVB interactions. This could be due to the strain specific photo-reactive mechanisms. Consequently, because of the existence of multiple strains with differing physiological characteristics, it is not easy to make any broad conclusions about changes to cyanobacterial populations with future climate change. It is suggested that further studies include more species/strains of cyanobacteria and other environmental factors (such as changes in atmospheric CO_2 and nutrient availability) in future. Future study should also consider testing how toxicity changes with these environmental variables.

Publications during enrolment:

Islam, M.A., Beardall, J. Growth and Photosynthetic Characteristics of Toxic and Non-Toxic Strains of the Cyanobacteria *Microcystis aeruginoa* and *Anabaena circinnalis* in Relation to Light. *Microorganisms* **2017**, *5*, 45. doi:10.3390/microorganisms5030045

Thesis including published works General Declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes one original paper published in peer reviewed journals and two unpublished manuscripts. The core theme of the thesis is to understand the effects of light, temperature and UVB on the growth and physiology of two species of freshwater cyanobacteria. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the School of Biological Sciences under the supervision of **Prof. John Beardall** and **Associate Prof. Perran Cook**.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

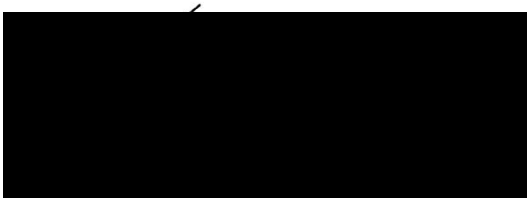
In the case of chapters number 2, 3 & 4 my contribution to the work involved the following:

Thesis chapter	Publication Title	Publication status*	Nature and % of student contribution	Co-authors name (s) Nature and % of co-author's contribution	Co-author Monash student (Y/N*)
2	Growth and photosynthetic characteristics of toxic and non-toxic strains of the cyanobacteria *Microcystis aeruginosa* and *Anabaena circinalis* in relation to light	Published	The candidate collected and interpreted most of the data (input 85%)	John Beardall reviewed the draft manuscript, Input 15%	N
3	Effects of temperature on the UVB sensitivity of the toxic cyanobacteria *Microcystis aeruginosa* CS558 and *Anabaena circinalis* CS537	This chapter is currently under revision with the European Journal of Phycology	The candidate collected and interpreted most of the data (input 85%)	John Beardall reviewed the draft manuscript, Input 15%	N
4	Intra-strain variability in effects of temperature on UVB sensitivity of cyanobacteria	This chapter is currently under revision with the Photochemistry and Photobiology Journal	The candidate collected and interpreted most of the data (input 80%)	John Beardall and Perran Cook reviewed the draft manuscript, Input 20%	N

I have not renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature: **Date:** 26th March 2018

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.



Main Supervisor signature: **Date:** 24th March 2018

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I joined the Algal Physiology lab with very little knowledge on algae. I still remember the first person I met in the lab was Ly Dao. My lab members helped me to learn the operation of most of the equipments/techniques available in the lab. Our lab assistant Shirley Davy Perfumo introduced me with the lab and was always available in case of need. Thanks to Manoj Kamalanathan and Thomas Lines for helping me to prepare culture media and to use the microscope for counting cells. Thomas Lines also taught me the use of PhytoPAM. It was Mattia Pierangelini and Ly Dao who taught me how to use the oxygen electrode and analyse the data. I am grateful to Slobodanka Stojkovic who came over to teach me the use of SpectraSense for my UV experiments.

I enjoyed my stay at Monash and it wouldn't be possible to finish the study without the help of my past and present colleagues. Thanks a lot to Paulina Mikulic, Mattia Pierangelini, Manoj Kamalanathan, Ly Dao, Thomas Lines, Belal Bakheet, Prachi Varshney, Yussi Palacios Delgado, Melissa Wartman, Tamsyne Smith Harding, Pranali

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Lastly, thanks to the Monash University for a Dean's International Postgraduate Scholarship (DIPRS) to support my PhD study.

Dedication

I would like to dedicate this thesis to my parents and the person I admire most, Prof. Dr. Md Abdul Wahab

Contents

Chapter 1. GENERAL INTRODUCTION

1.1 What are cyanobacteria?

Cyanobacteria, an ancient group of prokaryotic organisms which evolved approximately 3.5 billion years ago (1), are found in almost every environment, ranging from deserts to hot springs. Cyanobacteria are major bloom-forming organisms in freshwater ecosystems all over the world, some of which are toxic and blooms cost millions of dollars annually in lost income and amenities (2). According to Connolly et al. (3), the predicted cost of nutrient-induced cyanobacterial blooms in just a single system in Australia, the Gippsland Lakes of Victoria, has been predicted to be $256 million over the next 20 years. Globally, these economic costs are predicted to increase over time, due to a warming climate increasing the incidence of algal blooms (4, 5). Cyanobacteria prefer lentic environments with high concentrations of nitrogen, phosphorus and carbon. The ability to fix atmospheric nitrogen gas (diazotrophy) is an important characteristic of some groups of cyanobacteria, though not all species possess this ability (e.g. the potentially toxic genus *Microcystis*).

1.2 Role of climate change on cyanobacterial growth and physiology

1.2.1 Temperature

After light, the single most important parameter controlling algal and cyanobacterial growth is temperature. It is expected that the global temperature will increase by between 0.3 and 4.8 ^{o}C by the end of the current century (6) and this will thus, directly or indirectly, influence algal growth. The majority of freshwater eukaryotic phytoplankton decrease or maintain growth when temperatures exceed ~20 ^{o}C, whereas many cyanobacteria show accelerated growth under such conditions (7, 8). Temperature

increases not only stimulate growth of cyanobacteria but also change some physical characteristics of the water in which they grow, including a decrease in surface water viscosity resulting in increased sinking rate of larger, non-motile phytoplankton, which eventually favours cyanobacteria, especially those species that can regulate buoyancy with gas vesicles. Moreover, nutrient depletion in the surface water due to enhancement of stratification associated with the temperature rise may benefit diazotrophic cyanobacteria and those that can regulate their buoyancy to obtain nutrients from deep water. In a nutshell, it has been suggested that increases in temperature will accelerate the growth, distribution and bloom formation of freshwater cyanobacteria (9, 10). However, the effect of temperature rise on the production of toxins by cyanobacteria has not yet been previously explored.

1.2.2 Carbon dioxide (CO_2)

By the end of this century atmospheric CO_2 levels are expected to increase to between 730 and 1010 ppm from its current level of just over 400 ppm (11). As phytoplankton are major consumers of atmospheric CO_2 significant changes in CO_2 will likely affect their ecological and physiological processes. Some phytoplankton's may benefit from increases in atmospheric, and consequently dissolved, CO_2 but many species will show minimal stimulation by elevated CO_2 (12) due to the presence of active transport systems for scavenging inorganic carbon (CO_2 Concentrating Mechanisms or CCMs). Although less is known about the effects of increased CO_2 on natural cyanobacteria species, it has been hypothesized, on the basis of studies performed, that the chance of cyanobacterial blooms occurring will be enhanced with future climate change (13, 9).

1.2.3 Ultraviolet radiation (UVR)

Since the onset of industrial development, the release of anthropogenic pollutants (chlorofluorocarbons, chlorocarbons, organobromides) has reduced the stratospheric ozone layer, leading to increases in the level of UVR radiation (specifically UVB) reaching Earth's surface. The level of UVR at the Earth's surface also depends on other factors such as cloud cover. A study performed in tropical Australia (from 1979-1999) showed a significant increase in UVR (10% per decade) associated with reduced ozone (1–2% per decade) and reduced cloud cover (15–30% per decade). On the other hand, in southern regions of Australia, UVR did not increase over time, partially due to increased cloud cover (14). However, at high latitudes the breakdown of ozone layer in the stratosphere leads to increases in the transmission of UVB to the Earth's surface (15). According to Ball et al. (16) ozone in the lower stratosphere (17-24 km) has continued to decline since 1998. Global warming also leads to further ozone depletion (17-19) which eventually allows more UVB to reach the surface. Thus, understanding how UVB impacts interact with other environmental effects is important.

UVB radiation has a serious deleterious effect on all living organisms. As one of the Earth's earliest photosynthetic prokaryotes, cyanobacteria may have faced high levels of solar UVB early in their evolution and might therefore be expected to show an enhanced ability to withstand UVB-induced damage. However, experiments show UVR negatively affects both the growth and survival of cyanobacteria. Effects of UVB include damage to PSII reaction centres (20), and the D1 protein in particular (21), damage to the CO_2 fixing enzyme Rubisco (22) and photobleaching of photosynthetic pigments (23). UVR is also known to reduce CO_2 uptake in cyanobacteria (24). Although many studies have been performed on the effects of UVB on cyanobacterial physiology, in

particular growth and survival (25-27), there are still other factors that have not been previously examined - for example, toxicity.

1.3 Interactive effects of temperature, CO_2 and UVB on cyanobacteria

Interactive effects of temperature and CO_2 and/or UVB and temperature are likely to have differential consequences on phytoplankton cell size, bloom formation, elemental stoichiometry, growth rates, community structure, and adaptive capacity (28). Despite this, most of the studies performed on the effects of climate change factors on cyanobacteria mainly deal with a single variable at a time. For example, Gao et al. (29) observed a reduction in the *Anabaena sp.* trichome length of about 49% (exposed to UVR) compared to control (PAR without UVR) at the incubation temperature of 20 ^oC. Giordanino et al. (30) found a reduction in size (measured as chain area (μm^2)) of *Nostoc sp.* and *Anabaena sp.* when cells were exposed to UVR at 18^oC. According to Wu et al. (31) phytoplankton are more resistant to UVR in summer even though UVR is highest at that time. Li et al. (32) found that increased temperature increase the repair rate of PSII either under ambient or elevated CO_2 levels in the presence of UVA or UVB diatom *Phaeodactylum tricornutum*. Wong et al. (33) found similar results working with *Chlorella* strains from polar, temperate and tropical environments in studies of temperature and UV interactions. On the other hand, Sobrino et al. (34) reported that the diatom *Thalassiosira pseudonana* shows increased sensitivity to UVB with increased CO_2. However, very little is known on the interactive effects in responses to climate change factors, including elevated temperature, CO_2 and UVB on different strains of the major toxic bloom species *Anabaena circinalis* and *Microcystis aeruginosa*.

1.4 Effects of climate change on cyanobacterial toxin production

Nodularia spumigena in Lake Alexandrina was the first species of cyanobacterium reported to be responsible for blooms in Australia (35), but the toxicity of the bloom was not identified. Since then, studies on cyanobacterial toxicity have grown enormously. Several genera of cyanobacteria are responsible for potent toxin production, of which most are bloom-forming. There is evidence of deleterious toxin effects on animals and human health due to cyanobacteria (36-39). The most widespread cyanobacterial toxins are hepatotoxins (microcystins, nodularins and cylindrospermopsins) followed by neurotoxins such as saxitoxin. *Anabaena* is the most prevalent toxic cyanobacterial genus throughout the world, some species of which produce microcystins (MCYs), anatoxin-a and anatoxin-a(S) or cylindrospermopsin (CYN), and others, mainly *Anabaena circinalis*, a saxitoxin (STX). While *Anabaena circinalis* produces saxitoxin, *Microcystis,* another widespread genus, mostly produces microcystin (MCY). Toxin production can represent a significant investment of energy and other resources by cells and could potentially have effects on cyanobacterial growth, especially under light-limiting conditions.

A range of experiments have been performed to understand the effects of elevated temperature and CO_2 on toxin production of *Microcystis.* These have shown increased growth of MCY cells (MYC+) at high temperature (40) whereas growth of MCY- cells decreased in high CO_2 (41). Moreover, recent studies (42, 43) on the freshwater cyanobacterium *Cylindrospermopsis raciborskii* have shown differential growth rates, morphologies and toxin production within different strains. However, similar experiments on *Anabaena circinalis* or *Microcystis aeruginosa* are limited.

As the use of CO_2 in freshwater ecosystem is complex and CO_2 levels in many freshwater lakes vary seasonally over much greater ranges than the changes predicted

for atmospheric CO_2 rises, this project focussed mainly on the effects of light, temperature and UVB on two species of cyanobacteria *M. aeruginosa* and *A. circinalis*.

1.5 Objectives of the thesis

Given the consequence of climate change on algae, investigations on the effects of global change-related environmental factors on cyanobacteria is very important. The result will enrich the literature as there are very limited studies performed on the interactive effects of climate change factors on cyanobacteria. The overall aim of this study was to understand the effects of light, temperature and UVB singly and interactively on the growth and physiology of toxic and non-toxic cyanobacteria. Specific objectives were:

- To assess effects of light on the growth and physiology of toxic and non-toxic cyanobacteria
- To assess the interactive effects of temperature and UVB on growth and physiology of toxic cyanobacteria
- To assess the interactive effects of temperature and UVB on intra-strain variability of cyanobacteria in terms of survival, photodamage and recovery.

1.6 Thesis outline

This thesis consists of three data chapters in the form of manuscripts. The first has already been published and the second and third are currently under review with the journals. To understand the background of the present research a comprehensive literature review and the research gaps are discussed on Chapter 1. A general conclusion chapter (chapter 5) after the data chapters summarises key results and recommendations for future research.

In order to determine the growth saturating light intensity, toxic and non-toxic strains of both species *A. circinalis* and *M. aeruginosa* were grown at six different light intensities ranging from 10 to 200 µmol photon m^{-2} s^{-1} followed by measurement of physiological characteristics at optimal light. Toxic strains of both species saturates growth at a higher light intensity than the non-toxic strains. This has been discussed in detail in Chapter 2 and already published in *Microorganisms*.

Chapter 3 aimed to investigate the effects of temperature and an acute dose of UVB on the growth and photosynthetic characteristics of toxic strains of both species. The increased repair rate of photosynthetic characteristics with increased temperature in case of both species suggests an improved ability of cyanobacteria to overcome UV induced damage. As there are intra-strain variations, Chapter 4 investigated the effects of temperature and different levels of UVB on the physiology of two toxic strains of *A. circinalis*. Both strains responded differently in terms of growth, survival and recovery under temperature and UVB interactions. Chapter 3 & 4 are currently under revision with European Journal of Phycology and Photochemistry and Photobiology, respectively.

References

1. Schopf, J. W. (1993) Microfossils of the early archean apex chert - new evidence of the antiquity of life. *Science* **260,** 640 - 646.
2. Hamilton, D. P., S. A. Wood, D. R. Dietrich and J. Puddick (2014) Costs of harmful blooms of freshwater cyanobacteria. In *Cyanobacteria*. pp. 245-256. John Wiley & Sons, Ltd.
3. Connolly, B., P. Hylands and P. Brain (2009) Economic impact of the 2008 blue green algal bloom on the Gippsland tourism industry report - April 2009. *Nexus Consulting, Australia*.
4. Carey, C. C., B. W. Ibelings and E. P. Hoffman (2012) Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. *Water Res.* **46,** 1394-1407.
5. Wagner, C. and R. Adrian (2009) Cyanobacteria dominance: Quantofying the effects of climate change. *Limnol. Oceanogr.* **54,** 2490-2468.
6. IPCC (2007) The Physical Science Basis, Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC climate change 2007.
7. Canale, R. P. and A. H. Vogel (1974) Effects of temperature on phytoplankton growth. *J. of Environ. Eng (American Society of Civil Engineers)* **100,** 229-241.
8. Peperzak, L. (2003) Climate change and harmful algal blooms in the North Sea. *Acta Oecol.* **24, Supplement 1,** 139-144.
9. Paerl, H. W. and J. Huisman (2009) Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environmental microbiology reports* **1,** 27-37.
10. Paul, V. J. (2008) Global warming and cyanobacterial harmful algal blooms. In: Hudnell, H.K. (Ed.), Cyanobacterial Harmful Algal Blooms: State of the Science

and Research Needs. . *Adcances in Experimental Medicine and Biology* **619,** 239-257.

11. Meehl, G. A., T. F. Stocker, W. D. Collins, P. Friedlingstein, A. T. Gaye, J. M. Gregory, A. Kitoh, R. Knutti, J. M. Murphy, A. Noda, S. C. B. Raper and W. I. G. (2007) Global Climate Projections. *In: Climate Change 2007: The Physocal Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor and H. L. Miller eds.)* pp. 749-828.
12. Beardall, J., S. Ihnkken and A. Quigg (2009) Gross and net primary productions: closing the gape between concepts and measurements. *Aquat. Microb. Ecol.* **56,** 113-122.
13. Low-DÉCarie, E., G. F. Fussmann and G. Bell (2011) The effect of elevated CO_2 on growth and competition in experimental phytoplankton communities. *Global Change Biol.* **17,** 2525-2535.
14. Udelhofen, P. M., P. Gies, C. Roy and W. J. Randel (1999) Surface UV radiation over Australia, 1979–1992: Effects of ozone and cloud cover changes on variations of UV radiation. *Journal of Geophysical Research* **104,**19135-19159.
15. Hegglin MI, S. T. (2009) Large climate-induced changes in ultraviolet index and stratospheric-to-troposphere ozone flux. *Nat Geosci* **2,** 687-691.
16. Ball, W. T., J. Alsing, D. J. Mortlock, J. Staehelin, J. D. Haigh, T. Peter, F. Tummon, R. Stubi, A. Stenke, J. Anderson, A. Bourassa, S. M. Davis, D. Degenstein, S. Frith, L. Froidevaux, C. Roth, V. Sofieva, R. Wang, J. Wild, P. F. Yu, J. R. Ziemke and E. V. Rozanov (2018) Evidence for a continuous decline in lower stratospheric ozone offsetting ozone layer recovery. *Atmospheric Chemistry and Physics* **18,** 1379-1394.

17. Hofmann, D. J. and T. Deshler (1991) Evidence from balloon measurements for chemical depletion of stratospheric ozone in the Arctic winter of 1989-1990. *Nature* **349,** 300-305.

18. Molina, M. J. and F. S. Rowland (1974) Stratospheric sink for chlorofluoromethanes - chlorine atom catalyzed destruction of ozone. *Bulletin of the American Meteorological Society* **55,** 491-491.

19. Whitehead, R. F., S. J. de Mora and S. Demers (2000) Enhanced UV radiation – a new problem for the marine environment. In: *The effects of UV radiation in the marine environment* (Ed. by S. de Mora, S. Demers & M. Vernet). *Cambridge University Press, Cambridge***,** pp.1-34.

20. Key, T., A. McCarthy, D. A. Campbell, C. Six, S. Roy and Z. V. Finkel (2010) Cell size trade-offs govern light exploitation strategies in marine phytoplankton. *Environ. Microbiol.* **12,** 95-104.

21. Greenberg, B. M., V. Gaba, O. Canaani, S. Malkin, A. K. Matoo and M. Edelman (1989) Separate photosensitizers mediate degradation of the 32 kDa photosystem II reaction centre protein in the visible and UV spectral regions. *Proc. Natl. Acad. Sci. USA.* **86,** 6617-6620.

22. Raven, J. A. (1991) Responses of aquatic photosynthetic organisms to increased solar UVB. *J. Photochem. Photobiol. B Biol.* **9,** 239-244.

23. Sinha, R. P., A. Kumar, M. B. Tyagi and D.-P. Hader (2005) Ultraviolet-B-induced destruction of phycobiliproteins in cyanobacteria. *Physiol. Mol. Biol. Plants* **11,** 313-319.

24. Sinha, R. P., R. P. Rastogi, N. K. Ambasht and D. P. Hader (2008b) Life of wetland cyanobacteria under enhancing solar UV-B radiation. *Proc. Natl. Acad. Sci. India* **B 78,** 53-65.

25. Sinha, R. P., H. D. Kumar, A. Kumar and D. P. Hader (1995a) Effects of UV-B irradiation on growth, survival, pigmentation and nitrogen metabolism enzymes in cyanobacteria *Acta Protozool.* **34,** 187-192.

26. Blackfield, M. K. and D. O. Harris (1994) Delay of cell differentiation in *Anabaena aequalis* caused by UV-B radiation and the role of photoreactivation and excision repair. *Photochem. Photobiol.* **59,** 204-208.

27. Tyagi, R., G. Srinivas, D. Vyas, A. Kumar and H. D. Kumar (1992) Differential effects of ultraviolet-B radiation on certain metabolic processes in a chromatically adapting *Nostoc*. *Photochem. Photobiol.* **55,** 401-407.

28. Finkel, Z. V., J. Beardall, K. J. Flynn, A. Quigg, R. T. A. V. and J. A. Raven (2010) Phytoplankton in a changing world: cell size and elemental stoichiometry. *J. Plankton Res.* **32,** 119-137.

29. Gao, K., H. Yu and M. T. Brown (2007) Solar PAR and UV radiation affects the physiology and morphology of the cyanobacterium *Anabaena* sp. PCC 7120. *J. Photochem. Photobiol. B Biol.* **89,** 117-124.

30. Giordanino, M. V. F., S. M. Strauch, V. E. Villafañe and E. W. Helbling (2011) Influence of temperature and UVR on photosynthesis and morphology of four species of cyanobacteria. *J. Photochem. Photobiol. B: Biol.* **103,** 68-77.

31. Wu, Y. P., K. S. Gao, G. Li and E. W. Helbling (2010a) Seasonal Impacts of Solar UV Radiation on Photosynthesis of Phytoplankton Assemblages in the Coastal Waters of the South China Sea. *Photochem. Photobiol.* **86,** 586-592.

32. Li, Y., K. Gao, V. E. Villafane and E. W. Helbling (2012) Ocean acidification mediates photosynthetic response to UV radiation and temperature increase in the diatom Phaeodactylum tricornutum. *Biogeosciences* **9,** 3931-3942.

33. Wong, C. Y., M. L. Teoh, S. M. Phang, P. E. Lim and J. Beardall (2015) Interactive effects of temperature and UV radiation on photosynthesis of *Chlorella* strains from polar, temperate and tropical environments: differential impacts on damage and repair. *Plos One* **10** (10):e0139469. doi:10.1371/journal.pone.0139469.

34. Sobrino, C., M. L. Ward and P. J. Neale (2008) Acclimation to elevated carbon dioxide and ultraviolet radiation in the diatom *Thalassiosira pseudonana*: effects on growth, photosynthesis, and spectral sensitivity of photoinhibition. *Limonol. Oceanogr.* **53,** 494-505.

35. Francis, G. (1878) Poisonous Australian Lake. *Nature* **18,** 2-11.

36. Bourne, D. G., R. L. Blakeley, P. Riddles and G. J. Jones (2006) Biodegradation of the cyanobacterial toxin microcystin LR in natural water and biologically active slow sand filters. *Water Res.* **40,** 1294-1302.

37. Griffiths, D. J. and M. L. Saker (2003) The Palm Island mystery disease 20 years on: A review of research on the cyanotoxin cylindrospermopsin. *Environ. Toxicol.* **18,** 78-93.

38. Gugger, M., S. Lenoir, C. Berger, A. Ledreux, J. C. Druart, J. F. Humbert, C. Guette and C. Bernard (2005) First report in a river in France of the benthic cyanobacterium Phormidium favosum producing anatoxin-a associated with dog neurotoxicosis. *Toxicon* **45,** 919-28.

39. Ibelings, B. W. and I. Chorus (2007) Accumulation of cyanobacterial toxins in freshwater "seafood" and its consequences for public health: A review. *Environ. Pollut.* **150,** 177-192.

40. Davis, T. W., D. L. Berry, G. L. Boyer and C. J. Gobler (2009) The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of Microcystis during cyanobacteria blooms. *Harmful Algae* **8,** 715-725.

41. Van de Waal, D. B., J. M. H. Verspagen, J. F. Finke, V. Vournazou, A. K. Immers, E. A. Kardinaal, L. Tonk, S. Becker, E. Van Donk, P. M. Visser and J. Huisman (2011) Reversal in competitive dominance of a toxic veersus non-toxic cyanobacterium in response to rising CO_2. *ISME J.***,** pp.1-13.

42. Davis, T. W., P. T. Orr, G. L. Boyer and M. A. Burford (2014) Investigating the production and release of cylindrospermopsin and deoxy-cylindrospermopsin by Cylindrospermopsis raciborskii over a natural growth cycle. *Harmful Algae* **31,** 18-25.

43. Pierangelini, M., S. Stojkovic, P. T. Orr and J. Beardall (2014a) Photosynthetic characteristics of two *Cylindrospermopsis raciborskii* stains differing in their toxicity. *J. Phycol.* **50,** 292-302.

Declaration for Thesis Chapter

Declaration for Thesis Chapter 2

Declaration by candidate

In case of chapter 2, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
The experimental design, lab work, data analysis and writing of the manuscript were my responsibility.	85

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	**Nature of contribution**	**Extent of contribution (%)**
John Beardall	The idea for the experiment, interpretation of results, assistance with writing the manuscript, feedback on thesis.	

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's signature	[redacted]	Date 26th March 2018
Main Supervisor's signature	[redacted]	Date 24th March 2018

*Note: Where the responsible author is not the candidate's main supervisor, the main supervisor should consult with the responsible author to agree on the respective contributions of the authors.

Chapter 2 (Presented as published paper). Growth and Photosynthetic Characteristics of Toxic and Non-Toxic Strains of the Cyanobacteria Microcystis aeruginosa and Anabaena circinalis in Relation to Light

This chapter is currently published online in the journal *Microorganisms*

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Growth and Photosynthetic Characteristics of Toxic and Non-Toxic Strains of the Cyanobacteria *Microcystis aeruginosa* and *Anabaena circinalis* in Relation to Light

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Abstract: Cyanobacteria are major bloom-forming organisms in freshwater ecosystems and many strains are known to produce toxins. Toxin production requires an investment in energy and resources. As light is one of the most important factors for cyanobacterial growth, any changes in light climate might affect cyanobacterial toxin production as well as their growth and physiology. To evaluate the effects of light on the growth and physiological parameters of both toxic and non-toxic strains of *Microcystis aeruginosa* and *Anabaena circinalis*, cultures were grown at a range of light intensities (10, 25, 50, 100, 150 and 200 μmol m^{-2} s^{-1}). The study revealed that the toxic strains of both species (CS558 for *M. aeruginosa* and CS537 and CS541 for *A. circinalis*) showed growth (μ) saturation at a higher light intensity compared to the non-toxic strains (CS338 for *M. aeruginosa* and CS534 for *A. circinalis*). Both species showed differences in chlorophyll *a*, carotenoid, allophycocyanin (APC) and phycoerythrin (PE) content between strains. There were also differences in dark respiration (R_d), light saturated oxygen evolution rates (P_{max}) and efficiency of light harvesting (α) between strains. All other physiological parameters showed no statistically significant differences between strains. This study suggest that the different strains respond differently to different light habitats. Thus, changes in light availability may affect bloom intensity of toxic and nontoxic strains of cyanobacteria by changing the dominance and succession patterns.

Keywords: cyanobacteria; growth; physiology; toxin; energy investment; strain

1. Introduction

Cyanobacteria play significant roles in the nitrogen, carbon and oxygen dynamics of many aquatic ecosystems. They are major bloom-forming organisms in freshwater ecosystems all over the world and blooms cost millions of dollars annually in lost income and amenities [1]. According to Connolly et al. [2], the predicted cost of nutrient-induced cyanobacterial blooms in just a single system in Australia, the Gippsland Lakes of Victoria, has been estimated to be $256 million over the next 20 years. Globally, these economic costs are predicted to increase over time, due to a warming climate increasing the incidence of algal blooms [3,4]. A number of factors are responsible for the expansion of harmful cyanobacterial blooms including nutrient enrichment, often associated with agricultural activity, and increased surface water temperature due to global climate change [5–7]. Nutrient, especially phosphorus (P), enrichment in freshwaters sometimes causes some cyanobacteria to dominate phytoplankton populations [5,8]. Since cyanobacteria have the ability to fix nitrogen, phosphorus (P) enrichment in freshwater is sometimes a major driver for cyanobacterial dominance [8]. However, many cyanobacteria do not fix nitrogen gas and their bloom formation may be limited by the availability of fixed nitrogen in the water column [9,10]. Several genera of cyanobacteria are

responsible for potent toxin production, of which most are bloom forming. The most widespread cyanobacterial toxins are hepatotoxins [11] followed by neurotoxins such as saxitoxin. *Anabaena* is the most prevalent toxic cyanobacterial genus throughout the world, some genera of which produce microcystins (MCYs), anatoxin-a and anatoxin-a(S) or cylindrospermopsin (CYN), while others, mainly strains of *Anabaena circinalis*, produce a saxitoxin (STX). While *Anabaena circinalis* produces saxitoxin, *Microcystis*, another widespread genus, mostly produces microcystin (MCY).

Perhaps the most significant factor controlling algal growth is light. Given that toxin production requires an investment in energy and resources, changes in light climate might affect cyanobacterial toxin production as well as their growth and physiology. Light also plays a role in the transition of toxic to non-toxic strains during blooms [12]. In some cyanobacterial genera, growth-saturating light intensities are also those in which intracellular toxin concentrations are highest [13], whereas for other genera maximum cyanotoxin production occurs at irradiances greater [14,15] or lower [16] than those required for maximum growth. Clearly, the effect of light on toxin production is species-specific. A number of studies [17–21] have been performed on cyanobacterial growth and physiology in terms of light climate, but there have been few investigations on the effects of light conditions on toxin production or indeed the consequences of toxin production to energetics and light use efficiency of cyanobacteria.

Therefore, in this paper we present the results of investigations of growth and physiological characteristics of both toxic and non-toxic strains of the cyanobacteria *Anabaena circinalis* and *Microcystis aeruginosa* in relation to light environment.

2. Materials and Methods

2.1. Strains and Culture Conditions

A. circinalis and *M. aeruginosa* strains were obtained from the CSIRO Marine and Atmospheric Research, Hobart, Australia (Australian National Algae Culture Collection). Strains CS338 (non-toxic) and CS558 (toxic) were originally isolated from Burrinjuck Dam, New South Wales and Shepparton, Victoria respectively, while strains CS534 (non-toxic), CS537 (toxic) and CS541 (toxic) were isolated, respectively, from Fitzroy River, Rockhampton, Queensland, Mount Bold Reservoir, South Australia and Tullaroop Reservoir, Victoria. Subcultures of the isolates were maintained in 250 mL Erlenmeyer flasks containing 120 mL MLA medium [22] in a controlled environment room (25 °C) under a 12:12 h dark:light cycle with a photon flux of photosynthetically active radiation (PAR) of between 40–50 µmol photons m^{-2} s^{-1}. The cell concentration at inoculation was 1×10^5 cell mL^{-1}. The flasks were shaken gently several times every day to ensure homogenous exposure of cells to the light environment.

To characterise the response of growth to light conditions, strains were grown at six photon flux levels (10, 25, 50, 100, 150 and 200 µmol photons m^{-2} s^{-1}). Light was supplied from cool-white fluorescent tubes (Philips, TLD 36W, Amsterdam, The Netherlands) and different intensities maintained by neutral density filters (shade cloth). Cells were grown in 30 mL Nalgene bottles containing MLA medium. The culture bottles were shaken gently daily to resuspend cells. Chlorophyll fluorescence was used as a proxy of biomass and measured directly by putting the culture bottles into the chamber of a fluorometer (Hitachi F-7000, Fluorescence spectrophotometer, Tokyo, Japan) at specified excitation and emission wavelengths of 525 and 680 nm respectively. The change in fluorescence values over time were then used to calculate the specific growth rates.

To compare physiological characteristics, strains were exposed to their growth-saturating light intensities in 250 mL conical flasks. After reaching the log phase, cells were inoculated in to each batch culture and the desired conditions were maintained. Sampling was performed between 9.00 a.m.–10.00 a.m. each time to avoid effects related to the light/dark cycle.

2.2. Cell Morphology and Trichome Length

Cell concentrations of *M. aeruginosa* were estimated by counting in a Neubauer Haemocytometer at 40× magnification on a Zeiss Axio Scope.A1, using samples fixed with Lugol's iodine.

For filamentous strains, the number of trichomes mL^{-1} were determined from trichome length per mL and the mean number of cells per trichome according to Pierangelini et al. [23], using an improved Neubauer hemocytometer. Cell biovolume was calculated from at least 20 measurements on each of three independent biological replicates under each condition using the equation for a sphere: $V = \pi/6 \times d^3$, where d = diameter [24].

2.3. Pigment Analysis

For chl *a* determinations, cells were collected by centrifugation at 3400× *g* for 15 min, resuspended in 100% methanol and kept in the dark at 4 °C overnight. The suspension was centrifuged again and the supernatant was used to measure the absorbance at 632, 652, 654 and 696 nm using a Cary-50 UV-V spectrophotometer. Chl *a* concentration was then calculated using the equation of Ritchie [25]. Carotenoid concentrations were estimated using the same extract but with absorbance measured at 480 nm [26]. For estimation of phycobilins (phycocyanin (PC), allophycocyanin (APC) and phycoerythrin (PE)), cells were centrifuged at 3400× *g* for 15 min, resuspended in 0.05 M phosphate buffer (pH 6.7) then ruptured using a probe sonicator (BRANSON Model 102). The extract was centrifuged at 3400× *g* for 15 min and the supernatant used to measure the absorbance at 562, 615 and 652 nm. The equations of Bennett and Bogorad [27] were used to calculate PC, APC and PE concentrations.

2.4. Photosynthetic Parameters

Relative electron transport rate (rETR) as a function of irradiance, maximum quantum yield (F_v/F_m), light harvesting efficiency (α) and the light saturation parameter I_k were measured using a PHYTO-PAM phytoplankton analyser (Heinz Walz GmbH, Effeltrich, Germany). For all experiments relating to photosynthetic parameters measurements, cells were grown to exponential phase and kept in the dark for 15 min before a sample (3.5 mL) was taken and put in the chamber of PHYTO-PAM to carry out measurements of F_v/F_m. Rapid Light Curves (RLCs) were measured with 30 s intervals at each actinic light level. Maximum rates of relative electron transport ($rETR_{max}$), α and I_k were calculated by using the instrument software for the cyanobacterial channel.

The PHYTO-PAM was also used to measure non-photochemical quenching (NPQ). Approximately 1×10^7 cells were collected by centrifugation and resuspended in 3.5 mL fresh culture medium then dark acclimated for 15 min before being exposed to a saturating pulse to estimate maximum fluorescence [4] and the maximum quantum yield of photosynthetic energy conversion of PSII (F_v/F_m). The sample was then illuminated for 5–10 min with actinic light of 480 µmol photons m^{-2} s^{-1} and a 60 s cycle of saturating red pulses was applied until a stable value of maximum fluorescence yield in the light, F_m' was obtained. The Stern-Volmer equation was used to calculate NPQ as $NPQ = (F_m/F_m') - 1$.

Light-saturated rates of photosynthetic oxygen evolution (P_{max}) and dark respiration (R_d) were measured using a Clark type O_2 electrode (Hansatech, Norfolk, UK). Approximately 25×10^6 cells were harvested from the culture, centrifuged (10 min, 3400× *g*) and the pellet resuspended in 2 mL of fresh HEPES-buffered (pH 6.3) medium containing 2 mM sodium bicarbonate. This suspension was then placed into an O_2 electrode chamber for measurement of oxygen exchange rates. Prior to R_d and P_{max} measurements, the O_2 concentration in the suspension was reduced to 20% of air saturation by bubbling with N_2. R_d measurements were taken after dark incubation of the cell suspension for 5 min. Maximum O_2 evolution rates of the culture were measured under a saturating light intensity previously determined from Phyto-PAM measurements.

2.5. Statistical Analysis

One way ANOVA followed by Tukey multiple comparison tests were performed to examine the statistical significance of variations among means between the strains. Comparison between two strains was tested by unpaired two tailed *t*-test with Welch's correction of data. All the analyses were carried out using the statistical software GraphPad Prism 6, with a significance level at $p < 0.05$.

3. Results

3.1. Growth and Acclimation of Cyanobacteria at Different Light Levels

The growth rate and final cell concentrations of both species were strongly affected by light conditions. It is evident from Figures 1 and 2 that the toxic strains of both species (CS558 for *M. aeruginosa* and CS537 and CS541 for *A. circinalis*) show saturation of growth (μ) at a higher light intensity compared to the non-toxic strains (CS338 for *M. aeruginosa* and CS534 for *A. circinalis*). For CS338, maximum μ was 0.55 day^{-1} at 25 μmol photon m^{-2} s^{-1} and for CS558 0.46 day^{-1} when grown at 50 μmol photon m^{-2} s^{-1}. The maximum μ of strain CS537 (0.44 day^{-1}) and CS541 (0.35 day^{-1}) was observed at 50 μmol photon m^{-2} s^{-1} whereas strain CS534 (0.33 day^{-1}) had a maximal growth rate at 25 μmol photon m^{-2} s^{-1}.

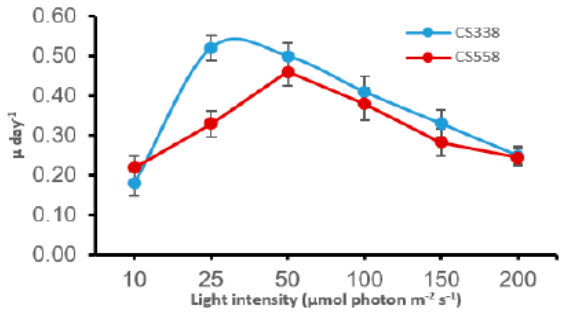


Figure 1. Effects of light intensity on specific growth rates (μ) of *Microcystis aeruginosa* strain CS338 and CS558. Red and blue symbols/lines are toxic and non-toxic strains respectively. Error bars represent the standard deviation of three replicates.

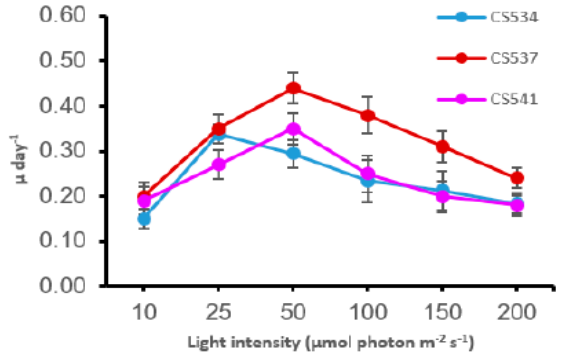


Figure 2. Effects of light intensity on specific growth rates (μ) of *Anabaena circinalis* strains CS534, CS537 and CS541. Red and purple symbols/line are toxic strains and blue is the non-toxic strain. Error bars represent the standard deviation of three replicates.

3.2. Cell Biovolume

M. aeruginosa strains showed significant differences (*t*-test, $p < 0.0001$) in cell biovolume, with values almost 1.5 times higher in CS558 (toxic) compared to CS338 (non-toxic) strains (Figure 3A).

On the other hand, strains of *A. circinalis* did not show any differences in biovolume (One-Way ANOVA: $F_{2,6} = 2.806$, $p = 0.138$) (Figure 3B).

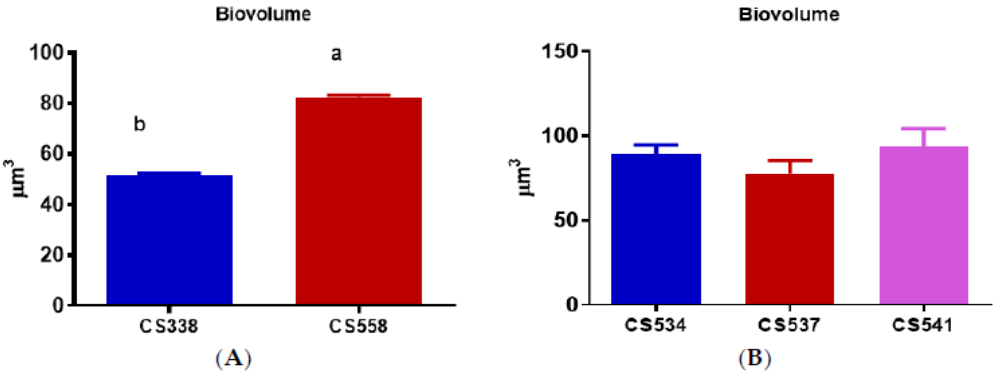


Figure 3. Cell biovolume of (**A**) *M. aeruginosa* strains CS338 and CS558 and (**B**) *A. circinalis* strains growing at their optimal light intensities (see text for details of these light levels). Samples were taken at exponential phase (day 5). Vertical bars indicate standard deviations of at least three replicates. Different letters above the bars indicate means that are significantly different at $p < 0.05$).

Despite the difference in biovolume between toxic and non-toxic strains of *M. aeruginosa*, the pigment contents and physiological parameters described below showed the same trend when expressed per cell or per volume so only the per cell data are presented below.

3.3. Pigments

In *M. aeruginosa*, cellular concentrations of chlorophyll *a* (*t*-test, $p = 0.0042$) and carotenoids (*t*-test, $p = 0.005$) were higher in CS558 (toxic) compared to CS338 (non-toxic) (Figure 4A,B). Other strain-specific differences were observed for APC (*t*-test, $p = 0.0198$; Figure 4D) and PE (*t*-test, $p = 0.0286$; Figure 4E) where strain CS558 (toxic) contained higher values than strain CS338. No strain-specific differences were observed for PC (*t*-test, $p = 0.1454$; Figure 4C).

In the case of *A. circinalis*, significant differences among the strains were observed in terms of cellular contents of chlorophyll *a* (One-Way ANOVA: $F_{2,6} = 18.7$, $p = 0.0026$, Figure 5A) and carotenoids (One-Way ANOVA: $F_{2,6} = 62.17$, $p < 0.0001$, Figure 5B). The chlorophyll *a* content was higher in CS534 (non-toxic) and CS541 (toxic) than in CS537 (toxic). The carotenoid content of the cells was significantly higher in strain CS534 (non-toxic) as compared to the other two strains. Other strain-specific differences were observed for APC (One-Way ANOVA: $F_{2,6} = 5.601$, $p = 0.0424$, Figure 5D) and PE (One-Way ANOVA: $F_{2,6} = 46.13$, $p = 0.0002$, Figure 5E) whereas PC showed no differences (One-Way ANOVA: $F_{2,6} = 1.497$, $p = 0.297$, Figure 5C) between the three strains.

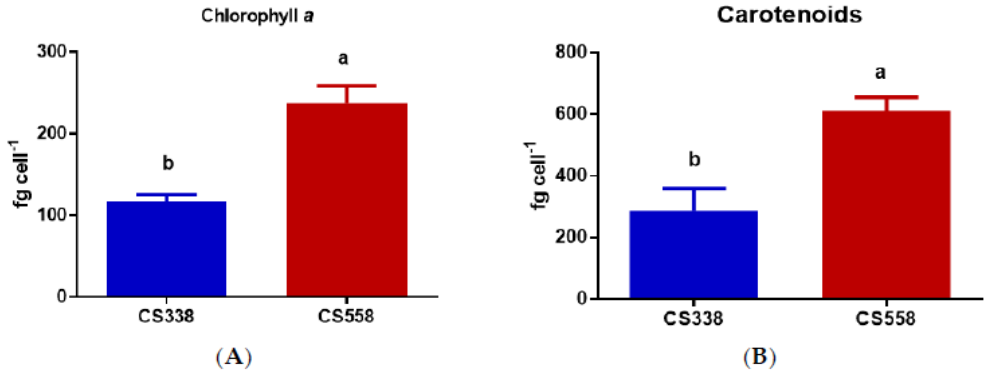


Figure 4. *Cont.*

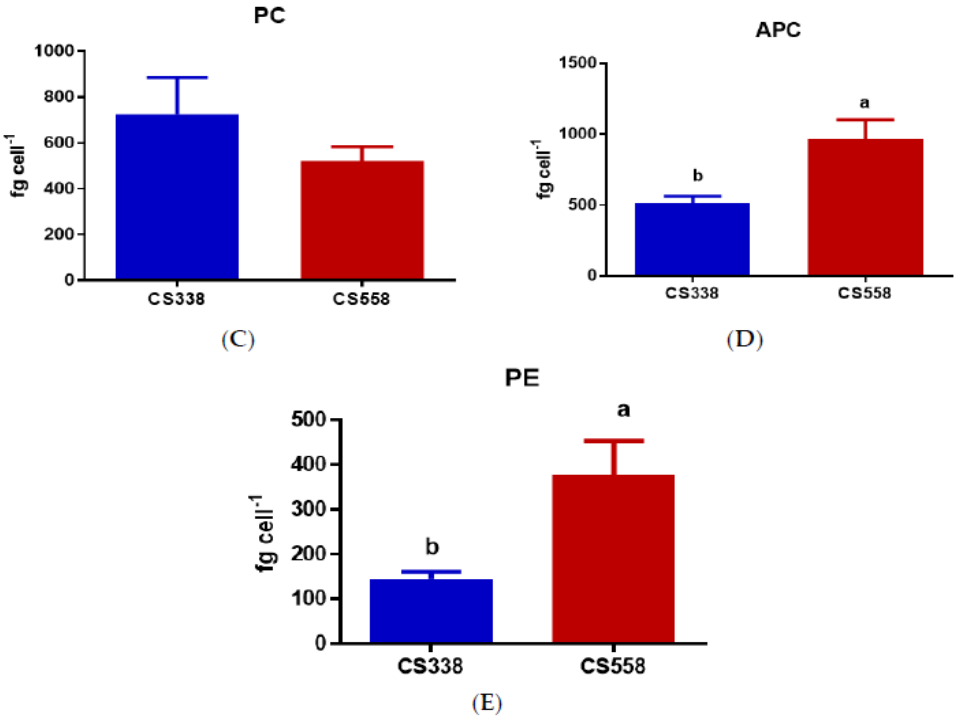


Figure 4. Cell concentrations of (A) chlorophyll *a*, (B) carotenoids, (C) phycocyanin, (D) allophycocyanin (APC), and (E) phycoerythrin (PE), in *M. aeruginosa* strains CS338 and CS558 growing in batch culture exposed at optimal light for growth. Samples were taken at exponential phase (day 5). Vertical bars indicate standard deviations of at least three replicates. Different letters above the bars indicate means that are significantly different at $p < 0.05$).

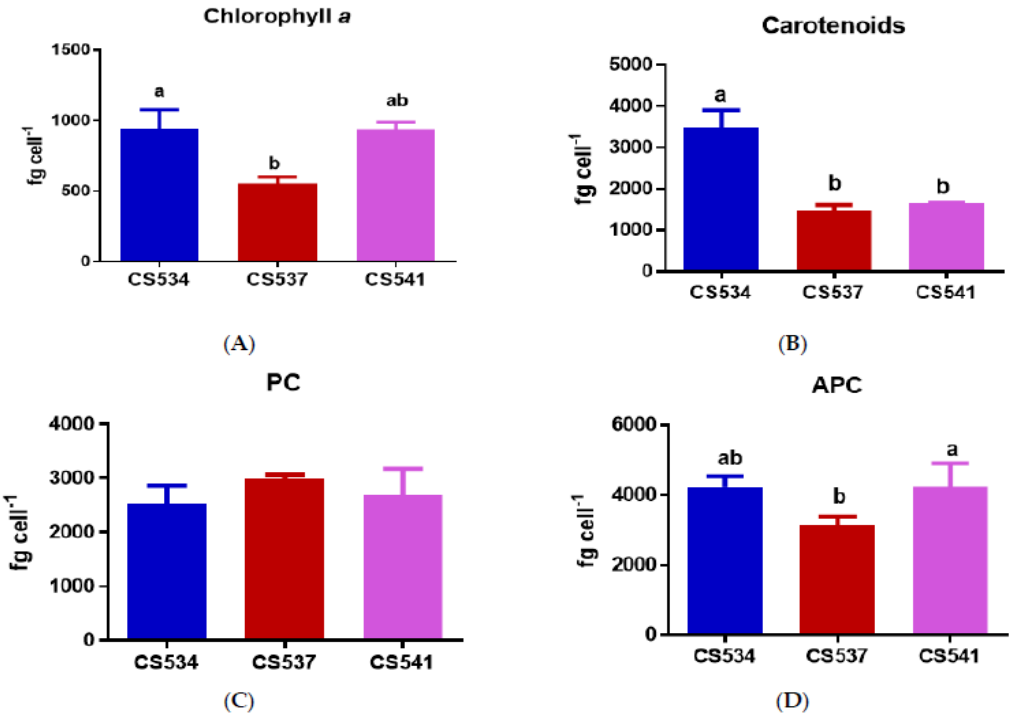
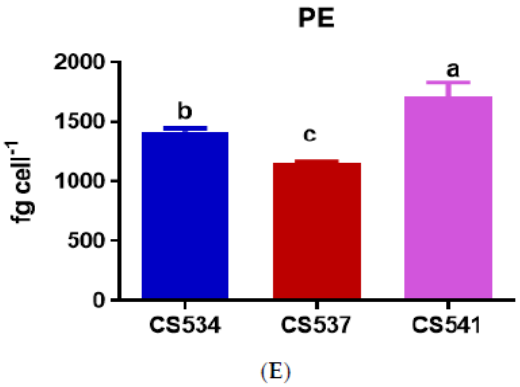


Figure 5. *Cont.*



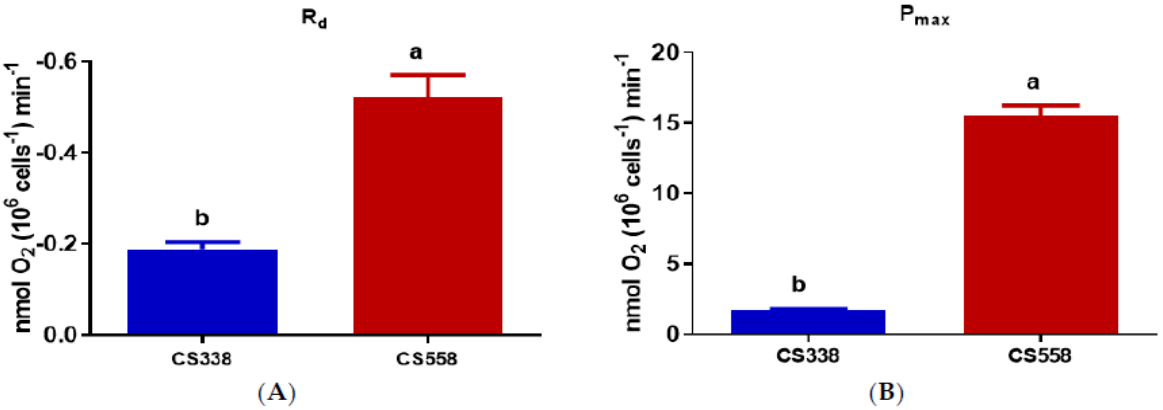
(E)

Figure 5. Cell concentrations of (**A**) chlorophyll *a*, (**B**) carotenoids, (**C**) phycocyanin, (**D**) allophycocyanin (APC), and (**E**) phycoerythrin (PE), in *A. circinalis* strains CS534, CS537 and CS541 growing in batch culture under optimal light for growth. Samples were taken at exponential phase (day 5). Vertical bars indicate standard deviations of at least three replicates. Different letters above the bars indicate means that are significantly different at $p < 0.05$).

3.4. Dark Respiration (Rd) and Photosynthesis

In case of *M. aeruginosa*, both R_d and P_{max} (maximum photosynthetic rate) showed significant differences (R_d: *t*-test, $p = 0.0037$; P_{max}: *t*-test, $p = 0.0002$) between strains (Figure 6A,B). Strain CS558 (toxic) showed lower R_d (0.522 nmol O_2 10^6 cells^{-1} min^{-1}) and higher P_{max} (15.530 nmol O_2 10^6 cells^{-1} min^{-1}) values than strain CS338 (R_d, 1.887 nmol O_2 10^6 cells^{-1} min^{-1}, P_{max}, 1.727 nmol O_2 10^6 cells^{-1} min^{-1}). These differences were not attributable to differences in biovolume as R_d and P_{max} expressed on a per μm^3 basis showed the same trend (data not shown).

The light harvesting efficiency (α) showed significant differences between strains (*t*-test, $p < 0.0001$, Figure 6C). However, $rETR_{max}$ (*t*-test, $p = 0.1967$, Figure 6D) and the light requirement for saturation of photosynthesis, I_k (*t*-test, $p = 0.1524$, Figure 6E) exhibited no significant differences between strains. Maximum quantum yield (F_v/F_m) (*t*-test, $p = 0.2511$, Figure 7A) and non-photochemical quenching (NPQ) also showed no variation across strains (*t*-test, $p = 0.9999$, Figure 7B).



(A) (B)

Figure 6. *Cont.*

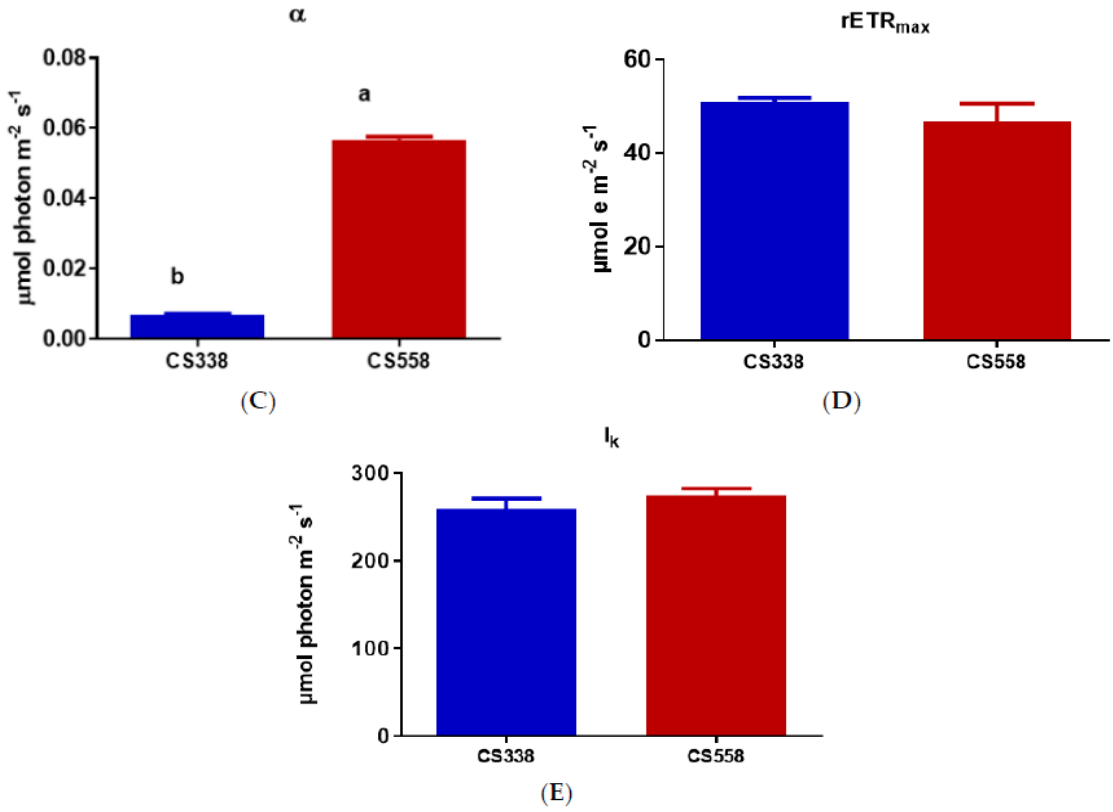


Figure 6. Comparison of dark respiration and photosynthesis in *M. aeruginosa* strains CS338 and CS558 growing in batch cultures at optimal light intensity. (**A**) dark respiration (R_d); (**B**) maximum photosynthetic rates (P_{max}); (**C**) light harvesting efficiency (α); (**D**) relative electron transport rate ($rEtr_{max}$); (**E**) Light saturation parameter. Samples were taken at exponential phase (day 5). Error bars represent standard deviation of three replicates. Different letters above the bars indicate means that are significantly different at $p < 0.05$).

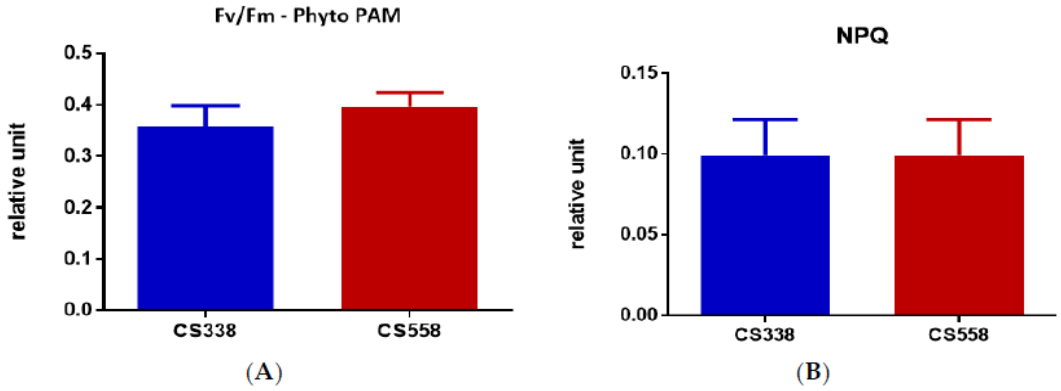


Figure 7. Comparison of both (**A**) maximum quantum yield (F_v/F_m) and (**B**) non-photochemical quenching (NPQ) of *M. aeruginosa* strains CS338 and CS558 growing in batch culture. Samples were taken at exponential phase (day 5). Error bars represent standard deviation of three replicates.

For *A. circinalis*, differences in both R_d and P_{max} (Figure 8A,B) were observed between the three strains of this species. The R_d of CS537 (2.675 nmol O_2 10^6 cells^{-1} min^{-1}) was significantly higher than that of CS534 (1.791 nmol O_2 10^6 cells^{-1} min^{-1}) and CS541 (1.764 nmol O_2 10^6 cells^{-1} min^{-1})

(One-Way ANOVA: $F_{2,6}$ = 13.03, p = 0.0066). The P_{max} values of the strains were also significantly different (One-Way ANOVA: $F_{2,6}$ = 17.19, p = 0.0059). The value was almost double in CS541 (57.180 µmol photon m^{-2} s^{-1}) compared to CS537 (33.86 nmol O_2 10^6 cells^{-1} min^{-1}) while in strain CS534 it was 28.97 nmol O_2 10^6 cells^{-1} min^{-1}.

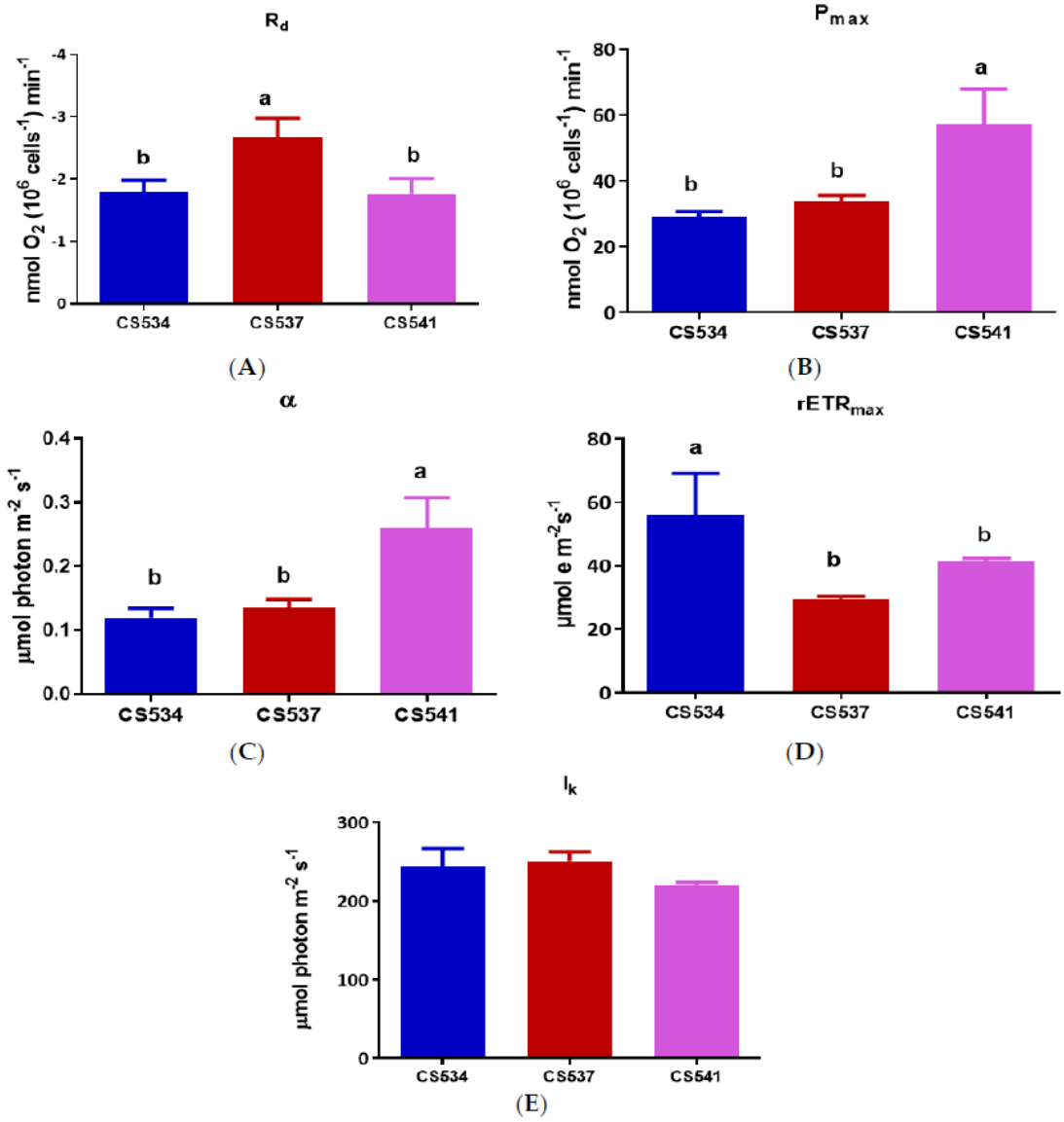


Figure 8. Comparison of both dark respiration and photosynthesis in *A. circinalis* strains CS534, CS537 and CS541 growing in batch culture exposed at the optimum light for growth. (**A**) dark respiration (R_d); (**B**) maximum photosynthetic rates (P_{max}); (**C**) light harvesting efficiency (α); (**D**) relative electron transport rate ($rETR_{max}$); (**E**) light saturation parameter (I_k). Samples were taken at exponential phase (day 5). Error bars represent standard deviation of three replicates. Different letters above the bars indicate means that are significantly different at $p < 0.05$).

The light harvesting efficiency (α) (Figure 8C) was significantly higher (One-Way ANOVA: $F_{2,6}$ = 20.05, p = 0.0022) in strain CS541 (0.2602) as compared to CS534 (0.1194) and CS537 (0.1354). However, the I_k (Figure 8E) values showed no significant differences (One-way ANOVA, p = 0.0952) between strains. The $rETR_{max}$ (Figure 8D) also varied between strains (One-Way ANOVA:

$F_{2,6}$ = 9.122, p = 0.0152). Values for CS534 were significantly higher than for CS537 but not CS541. There was a significant difference (One-Way ANOVA: $F_{2,6}$ = 71.87, $p < 0.0001$) in maximum quantum yield (F_v/F_m) (Figure 9A) between strains. The value for strain CS537 was lower compared to those of CS534 and CS541. Non-photochemical quenching (NPQ) also varied between strains with strain CS537 significantly lower than the other two strains (One-Way ANOVA: $F_{2,6}$ = 150.5, $p < 0.0001$, Figure 9B).

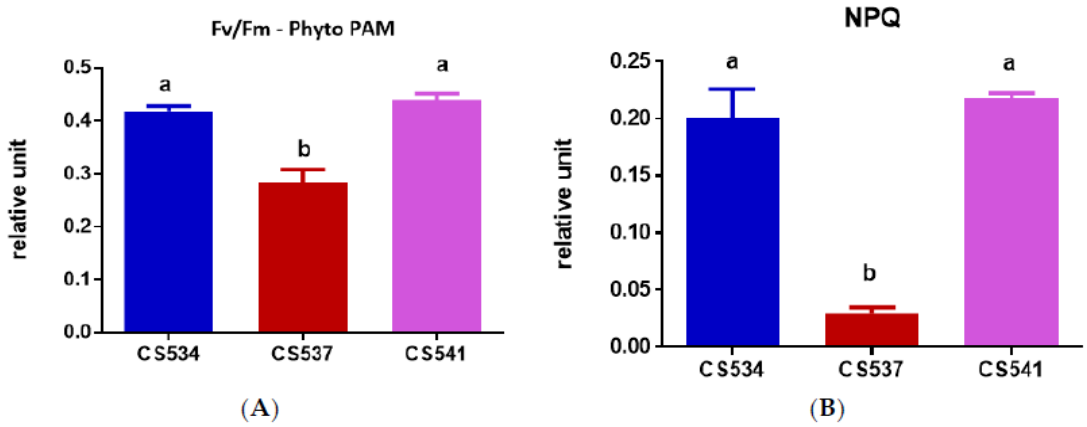


Figure 9. Comparison of both (**A**) maximum quantum yield (F_v/F_m) and (**B**) non-photochemical quenching (NPQ) of *A. circinalis* strains CS534, CS537 and CS541 growing in batch culture exposed at the optimum light intensity for growth. Samples were taken at exponential phase (day 5). Error bars represent standard deviation of three replicates. Different letters above the bars indicate means are significantly different at $p < 0.05$).

4. Discussion

The results presented in this study clearly indicate differences in growth and physiological characteristics of toxic and non-toxic strains of *M. aeruginosa* and *A. circinalis* in relation to light climate. Strains of both species are able to survive very low light intensity (10 µmol photons m^{-2} s^{-1}) to higher light intensity (200 µmol photons m^{-2} s^{-1}). The ability of the strains to survive across a range from low to high light intensity reflects their dominance in Australian freshwater systems characterized by variable light intensity [23,28]. In terms of specific growth rate, the toxic strains of both species required higher light intensities to reach saturation compared to non-toxic strains. However, at low light the toxic strains grew faster than the non-toxic strains suggesting that the energetic cost of toxin production is not enough to influence growth rates under energy-limited conditions and the differences between strains may be related to factors other than toxin production. Although light is a significant factor controlling algal growth, its role in toxin production is mostly species specific. There is some evidence, however, that species' toxin production varies between saturating, supra-saturating and limiting light intensities [13–16].

In the case of *M. aeruginosa*, cell biovolume was larger in strain CS558 (toxic) which might affect the light field. Big cells are more prone to self-shading with increasing cell concentration [29]. Due to their bigger cell size CS558 (toxic) contains higher Chl *a* and carotenoids compared to CS338 (non-toxic), though biovolume did not entirely account for this difference as similar trends were observed for chl *a* and carotenoids per unit cell volume. A similar result was found by Pierangelini et al. [23] for two strains of the cyanobacterium *Cylindrospermopsis raciborskii*. On the other hand, *A. circinalis* strains did not show any comparable differences based on cell biovolume. However, strain CS534 (non-toxic) contained relatively high chl *a* and carotenoid concentrations in cells relative to other strains. Among the other parameters APC and PE showed significant differences with the strain CS537 containing relatively low values than the other two strains.

The parameter α represents the light harvesting efficiency and the photosynthetic energy conversion efficiency [30] of cells. In some cases there are correlations found between the value of α and cellular pigment composition/content under different light intensities [31,32]. The lower α value of CS338 (non-toxic) as compared to CS558 (toxic) could be due to a lower Chl *a* to biovolume ratio, which suggests a poor ability to harvest light in low light conditions. Similar results were also found for *A. circinalis* strain CS537 (toxic).

In the case of *M. aeruginosa*, the higher values of both R_d and P_{max} in strain CS558 (toxic) could be partly due to the higher cell biovolume compared to CS338 (non-toxic) which results in higher respiratory consumption and photosynthetic production of oxygen, but similar trends between strains were observed in respiratory and photosynthetic rates per unit volume. However, the higher P_{max} value of CS541 (toxic) of *A. circinalis* cannot be explained in the same terms as there were not any comparable differences in cell biovolume between the strains.

F_v/F_m is a measure of the physiological status of PSII in algae/plants. The F_v/F_m values observed for strains of both species are within the range for unstressed cyanobacteria [33]. No differences were observed between strains in the case of *M. aeruginosa*, but *A. circinalis* showed significant differences between strains, with strain CS537 (toxic) having a lower F_v/F_m compared to the other two strains (one toxic, one non-toxic).

In cyanobacteria, NPQ is associated with dissipation of excess light energy as heat, i.e., it operates as a photoprotective mechanism [34,35] and can also reflect state transitions i.e., regulation of excitation energy transfer between phycobilisome/PSII and PSI [36,37]. The lower value of F_v/F_m in the case of *A. circinalis* strain CS537 (toxic) as compared to the other strains could be explained by the fact that this particular strain might be slightly stressed, but it is hard to conclude this with any certainty. If toxin production were high enough to act as a significant electron sink, using ATP/NADPH at a higher rate and thus recycling ADP/$NADP^+$ more rapidly, we would expect less stress on PSII and higher values of F_v/F_m and less need to up-regulate NPQ. However, toxicity of the strains did not appear to be related to the differences in F_v/F_m and NPQ. Thus, we propose that the NPQ measured in *M. aeruginosa* and *A. circinalis* is more likely to be driven by state transitions [37] rather than by heat dissipation, as described by Pierangelini et al. [23].

In conclusion, this study reveals differences in growth and physiology of strains within species and between species. The results suggest that different strains respond differently at different light intensities. Toxic strains tend to have required higher light for growth. Although some differences in physiological characteristics were observed between species and strains there were not any consistent trends in relation to toxicity, and we acknowledge that factors other than toxin production may also be involved in the observed strain differences. Changes in light availability may affect bloom intensity of toxic and non-toxic strains of cyanobacteria and also the amount of toxin produced. Further studies with a greater range of strains of differing toxicity would be helpful in elucidating this issue.

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References

1. Hamilton, D.P.; Wood, S.A.; Dietrich, D.R.; Puddick, J. Costs of harmful blooms of freshwater cyanobacteria. In *Cyanobacteria: An Economic Perspective*; Sharma, N.K., Rai, A.K., Stal, L.J., Eds.; John Wiley & Sons Ltd.: Chichester, UK, 2014; pp. 245–256.
2. Connolly, B.; Hylands, P.; Brain, P. *Economic Impact of the 2008 Blue Green Algal Bloom on the Gippsland Tourism Industry Report-April 2009*; Nexus Consulting Australia: Bairnsdale, Australia, 2009.

3. Wagner, C.; Adrian, R. Cyanobacteria dominance: Quantifying the effects of climate change. *Limnol. Oceanogr.* **2009**, *54*, 2460–2468. [CrossRef]
4. Carey, C.C.; Ibelings, B.W.; Hoffman, E.P. Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. *Water Res.* **2012**, *46*, 1394–1407. [CrossRef] [PubMed]
5. Paerl, H.W.; Huisman, J. Climate change: A catalyst for global expansion of harmful cyanobacterial blooms. *Environ. Microbiol. Rep.* **2009**, *1*, 27–37. [CrossRef] [PubMed]
6. Peperzak, L. Climate change and harmful algal blooms in the North Sea. *Acta Oecol.* **2003**, *24* (Suppl. 1), 139–144. [CrossRef]
7. Paul, V.J. Global warming and cyanobacterial harmful algal blooms. In *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*; Hudnell, H.K., Ed.; Springer Science: New York, NY, USA, 2008; Volume 619, pp. 239–257.
8. Watson, S.B.; McCauley, E.; Downing, J.A. Patterns in phytoplankton taxonomic composition across temperate lakes of differing nutrient status. *Limnol. Oceanogr.* **1997**, *42*, 487–495. [CrossRef]
9. Davis, T.W.; Harke, M.J.; Marcoval, M.A.; Goleski, J.; Orano-Dawson, C.; Berry, D.L.; Gobler, C.J. Effects of nitrogenous compounds and phosphorus on the growth of toxic and non-toxic strains of *Microcystis* during bloom events. *Aquat. Microb. Ecol.* **2010**, *61*, 149–162. [CrossRef]
10. Gobler, C.J.; Davis, T.W.; Coyne, K.J.; Boyer, G.L. The interactive influences of utrient loading and zooplankton grazing on the growth and toxicity of cyanobacteria blooms in a eutrophic lake. *Harmful Algae* **2007**, *6*, 119–131. [CrossRef]
11. Quiblier, C.; Wood, S.; Echenique-Subiabre, I.; Heath, M.; Villeneuve, A.; Humbert, J.F. A review of current knowledge on toxic benthic freshwater cyanobacteria-ecology, toxin production and risk management. *Water Res.* **2013**, *47*, 5464–5479.
12. Kardinaal, W.E.A.; Tonk, L.; Janse, I.; Hol, S.; Slot, P.; Huisman, J.; Visser, P.M. Competition for light between toxic and nontoxic strains of the harmful cyanobacterium microcystis. *Appl. Environ. Microbiol.* **2007**, *73*, 2939–2946. [CrossRef] [PubMed]
13. Rapala, J.; Sivonen, K.; Luukkainen, R.; Niemela, S.I. Anatoxin-a concentration in *Anabaena* and *Aphanizomenon* under different environmental-conditions and comparison of growth by toxic and nontoxic *Anabaena* strains—A laboratory study. *J. Appl. Phycol.* **1993**, *5*, 581–591. [CrossRef]
14. Watanabe, M.F.; Oishi, S. Effects of environmental-factors on toxicity of a cyanobacterium (*Microcystis aeruginosa*) under culture conditions. *Appl. Environ. Microbiol.* **1985**, *49*, 1342–1344. [PubMed]
15. Dyble, J.; Tester, P.A.; Litaker, R.W. Effects of light intensity on cylindrospermopsin production in the cyanobacterial hab species *Cylindrospermopsis raciborskii*. *Afr. J. Mar. Sci.* **2006**, *28*, 309–312. [CrossRef]
16. Sivonen, K.; Carmichael, W.W.; Namikoshi, M.; Rinehart, K.L.; Dahlem, A.M.; Niemela, S.I. Isolation and characterization of hepatotoxic microcystin homologs from the filamentous freshwater cyanobacterium *Nostoc* sp. strain-152. *Appl. Environ. Microbiol.* **1990**, *56*, 2650–2657. [PubMed]
17. Padisak, J. *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju, an expanding, highly adaptive cyanobacterium: Worldwide distribution and review of its ecology. *Arch. Hydrobiol. Suppl.* **1997**, *107*, 563–593.
18. Briand, J.F.; Leboulanger, C.; Humbert, J.F.; Bernard, C.; Dufour, P. *Cylindrospermopsis raciborskii* (Cyanobacteria) invasion at mid-latitudes: Selection, wide tolerance or global warming? *J. Phycol.* **2004**, *40*, 231–238. [CrossRef]
19. Vidal, L.; Kruck, C. *Cylindrospermopsis raciborskii* (Cyanobacteria) extends its distribution to latitude 34°53′s: Taxonomical and ecological features in uruguayan eutrophic lakes. *Pan-Am. J. Aquat. Sci.* **2008**, *3*, 142–151.
20. Piccini, C.; Aubriot, L.; Fabre, A.; Amaral, V.; Gonzalez-Piana, M.; Giani, A.; Figueredo, C.C.; Vidal, L.; Kruk, C.; Bonilla, S. Genetic and eco-physiological differences of south american *Cylindrospermopsis raciborskii* isolates support the hypothesis of multiple ecotypes. *Harmful Algae* **2011**, *10*, 644–653. [CrossRef]
21. Mehnert, G.; Rucker, J.; Nicklisch, A.; Leunert, F.; Wiedner, C. Effects of thermal acclimation and photoacclimation on lipophilic pigments in an invasive and a native cyanobacterium of temperate regions. *Eur. J. Phycol.* **2012**, *47*, 182–192. [CrossRef]
22. Bolch, C.J.S.; Blackburn, S.I. Isolation and purification of Australian isolates of the toxic cyanobacterium *Microcystis aeruginosa* Kutz. *J. Appl. Phycol.* **1996**, *8*, 5–13. [CrossRef]
23. Pierangelini, M.; Stojkovic, S.; Orr, P.T.; Beardall, J. Photosynthetic characteristics of two *Cylindrospermopsis raciborskii* stains differing in their toxicity. *J. Phycol.* **2014**, *50*, 292–302. [CrossRef] [PubMed]

24. Hillebrand, H.; Durselen, C.D.; Kirschtel, D.; Pollingher, U.; Zohary, T. Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.* **1999**, *35*, 403–424. [CrossRef]
25. Ritchie, R.J. Universal chlorophyll equations for estimating chlorophylls *a*, *b*, *c*, and *d* and total chlorophylls in natural assemblages of photosynthetic organisms using acetone, methanol, or ethanol solvents. *Photosynthetica* **2008**, *46*, 115–126. [CrossRef]
26. Jenssen, A. Chlorophyll and carotenoids. In *Handbook of Phycological Methods, Physiological and Biochemical Methods*; Hellebust, J.A., Craigie, J.S., Eds.; Cambridge University Press: London, UK, 1978; Volume 2, pp. 59–70.
27. Bennett, A.; Bogorad, L. Complementary chromatic adaptation in a filamentous blue-green alga. *J. Cell Biol.* **1973**, *58*, 419–435. [CrossRef] [PubMed]
28. Antenucci, J.P.; Ghadouani, A.; Burford, M.A.; Romero, J.R. The long-term effect of artificial destratification on phytoplankton species composition in a subtropical reservoir. *Freshw. Biol.* **2005**, *50*, 1081–1093. [CrossRef]
29. Raven, J.A.; Finke, Z.V.; Irwin, A.J. Picophytoplankton: Bottom-up and top-down controls on ecology and evolution. *Vie Milieu* **2005**, *55*, 209–215.
30. Henley, W.J. On the measurement and interpretation of photosynthetic light-response curves in algae in the context of photoinhibition and diel changes. *J. Phycol.* **1993**, *29*, 729–739. [CrossRef]
31. Richardson, K.; Beardall, J.; Raven, J.A. Adaptation of unicellular algae to irradiance: An analysis of strategies. *New Phytol.* **1983**, *93*, 157–191. [CrossRef]
32. MacIntyre, H.L.; Kana, T.M.; Anning, T.; Geider, R.J. Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria. *J. Phycol.* **2002**, *38*, 17–38. [CrossRef]
33. Gao, K.; Yu, H.; Brown, M.T. Solar PAR and UV radiation affects the physiology and morphology of the cyanobacterium *Anabaena* sp. Pcc 7120. *J. Photochem. Photobiol. B Biol.* **2007**, *89*, 117–124. [CrossRef] [PubMed]
34. Kirilovsky, D. Photoprotection in cyanobacteria: The orange carotenoid protein (ocp)-related non-photochemicalquenching mechanism. *Photosynth. Res.* **2007**, *93*, 7–16. [CrossRef] [PubMed]
35. Bailey, S.; Grossman, A. Photoprotection in cyanobacteria: Regulation of light harvesting. *Photochem. Photobiol.* **2008**, *84*, 1410–1420. [CrossRef] [PubMed]
36. Campbell, D.; Hurry, V.; Clarke, A.K.; Gustafsson, P.; Gunnar, O. Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. *Microbiol. Mol. Biol. Rev.* **1998**, *62*, 667–683. [PubMed]
37. Campbell, D.; Oquist, G. Predicting light acclimation in cyanobacteria from non-photochemical quenching of psii fluorescence, which reflects state transitions in these organisms. *Plant Physiol.* **1996**, *111*, 1293–1298. [CrossRef] [PubMed]

Declaration for Thesis Chapter

Declaration for Thesis Chapter 3

Declaration by candidate

In case of chapter 3, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
The experimental design, lab work, data analysis and writing of the manuscript were my responsibility.	85

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	**Nature of contribution**	**Extent of contribution (%)**
John Beardall	The idea for the experiment, interpretation of results, assistance with writing the manuscript, feedback on thesis.	

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's signature	[redacted]	Date 26th March 2018
Main Supervisor's signature	[redacted]	Date 24th March 2018

*Note: Where the responsible author is not the candidate's main supervisor, the main supervisor should consult with the responsible author to agree on the respective contributions of the authors.

Chapter 3. Effects of temperature on the UVB sensitivity of the toxic cyanobacteria Microcystis aeruginosa CS558 and Anabaena circinalis CS537

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ABSTRACT

The effects of temperature and UVB on the photosynthetic response of algae and cyanobacteria is an important issue due to their role at the base of food chains and the potential of these organisms to cause economically damaging blooms. Rising global temperatures have been suggested to favour growth of cyanobacteria over eukaryotic algae, but UVB fluxes are also predicted to remain high and may interact with temperature to affect algal growth. To understand the interactive effects of temperature and UVB radiation, cultures of *Microcystis aeruginosa* and *Anabaena circinalis* were grown at either 25 °C or 30 ^{o}C and then exposed to an acute dose of UVB (1.4 W m^{-2}). Rates of damage (*k*) and repair (*r*) were calculated from the kinetics of change in effective quantum yield, F_v'/F_m'. *A. circinalis* (CS537) showed higher growth rates at 30 ^{o}C whereas growth rates of *M. aeruginosa* (CS558) were higher at 25 ^{o}C. Analysis of the estimates of *r* and *k* shows that *M. aeruginosa* exhibited relatively high values for both parameters, compared to *A. circinalis,* at both growth temperatures. By measuring absorption spectra of cell extracts, we found *M. aeruginosa* mainly absorbs in the UVA region whereas *A. circinalis* absorbs in both the UVA and the UVB regions. This could explain why the damage rate of *M. aeruginosa* was higher than that of *A. circinalis*. In both species, repair rates were higher at 30 ^{o}C than at 25^{o}C but in *A. circinalis* damage was also greater at the higher temperature. In contrast, *M. aeruginosa* showed a lower damage rate at the higher temperature. For both species the ratio of *r:k* was higher at the higher temperature. However, the percent inhibition of effective quantum yield by UVB was greater in *A. circinalis* than in *M. aeruginosa* as the *r:k* was lower *A. circinalis*. Therefore, it could be concluded that temperature may influence growth and bloom formation of cyanobacteria and different species may respond differently to UVB and temperature interactions.

Keywords: Temperature; UVB; Effective quantum yield; Repair rate; damage rate; UV absorbing compounds

3.1 INTRODUCTION

Climate change is known to affect aquatic ecosystems directly and indirectly (1). Temperature and solar radiation are two major environmental factors that have been extensively studied to examine their impacts on diverse groups of primary producers. It is projected that global average surface water temperature by the year 2060 will increase by between 0.2 and 2.0 ^{o}C (2), and this will, directly or indirectly, influence algal growth. Ultraviolet radiation at the Earth's surface ranges from 280 to 400 nm (1). Due to the depletion of the stratospheric ozone layer as a consequence of the release of anthropogenic pollutants, the transmission of UV radiation, in particular UVB radiation (280-315 nm), through the atmosphere to the Earth's surface has been increasing for many decades (3). This is likely to continue into the future as global warming of the troposphere will lead to colder conditions in the stratosphere, a situation that promotes the various ozone depleting reactions, and warming also affects cloud cover and the transmission of UVB to the Earth's surface (4, 5). A recent study (6) concluded that ozone in the lower stratosphere (17-24 km) between 60^{o} S to 60^{o} N has continued to decline since 1998.Thus, studies on future climate change still need to consider UVB as a factor. The interactive effects of temperature and UVB radiation on algae, especially the surface bloom forming cyanobacteria, are therefore of considerable interest.

UVB radiation has the potential to cause serious deleterious effects on all living organisms and UVR negatively affects growth and survival of cyanobacteria (7-9). Effects of UVB include damage to PSII reaction centres (10) and especially the D1 protein (11), damage to the CO_2 fixing enzyme Rubisco (12) and photo-bleaching of photosynthetic pigments (13). The effect of UVB on the photosynthetic response of algae and cyanobacteria is an important issue due to their role at the base of food chains

and the potential economic costs of blooms (14). The response to UVB might vary depending on either UVB dose rate or cumulative dose, and can be explained by a model developed by Kok (15) to explain photoinhibition by photosynthetically active radiation (16). The Kok model is based on the balance between damage and recovery processes operating during photoinhibitory exposure.

According to the Kok model, photosynthetic rate (P) is proportional to the concentration of a light sensitive component U (i.e. P α U). The decrease in this component proceeds according to the concentration of U and a light-dependent specific damage rate, “k”. On the other hand, the rate of repair (used in a general sense to include all processes that counteract the inhibitory effect of UV: resynthesis, reactivation, etc.) is considered as the product of a specific repair rate (“r”) and the concentration of inactivated component. Under these assumptions, the time course of photosynthesis is predicted as:

$$\frac{P}{P_{initial}} = \frac{r}{k+r} + \frac{k}{k+r} e^{-(k+r)t} \qquad (1)$$

where, k and r are the rate constants for damage and repair. Thus, net photoinhibition is a balance between damage and repair (17, 18)**.** The consequence of this is that photosynthetic rate (or F_v'/F_m' as determined here) will decrease exponentially with time, gradually levelling off to an asymptote where repair capacity equals damage. This process can been represented by the PSII repair cycle shown below (redrawn after (17)).

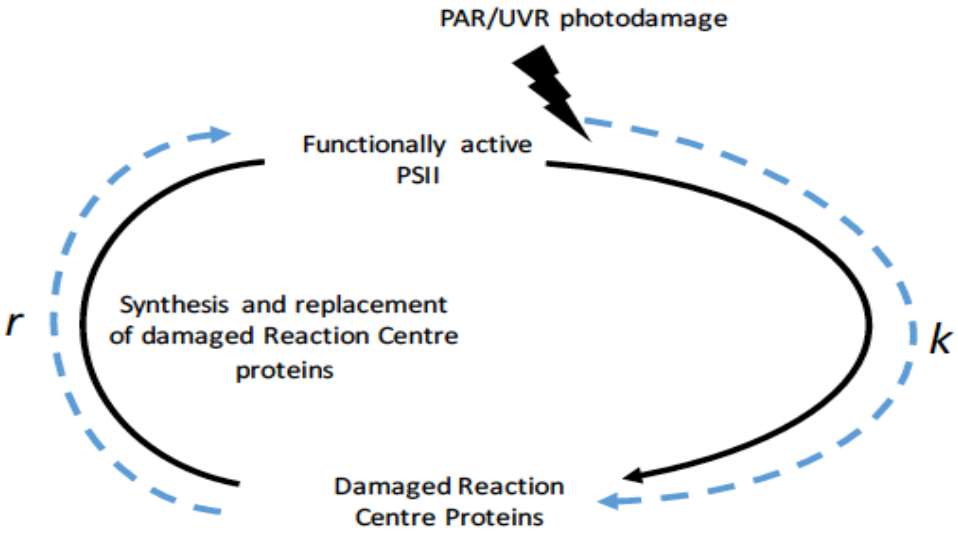

Figure 1. Simplified schematic diagram of the PSII damage and repair cycle (adapted from Bouchard et al. (17))

To avoid the harmful effects of UVB, algae and cyanobacteria have developed a number of protective mechanisms. These include DNA repair mechanisms, synthesis of protective pigments, such as the mycosporine-like amino acids (MAAs) and scytonemin (mostly in cyanobacteria) (19-25), which act as UV absorbing compounds (UVACs) and reduce damage. According to Jeffrey et al. (26) a number of marine bloom-forming microalgae have a marked ability to produce UVACs, which might contribute to their success. As cyanobacteria are one of the earliest photosynthetic prokaryotes and may have faced high levels of solar UVB during their evolution, they might therefore be expected to show an enhanced ability to withstand UVB-induced damage.

Changes in temperature can alter stratification which in turn will influence the nutrient status of water bodies and eventually impact the UVR sensitivity of algae (27). There are, however, many studies that have shown direct impacts of temperature on the effects of UVR on the physiological performance of algae (28-31). Given that damage is

a photochemical process and thus temperature independent, but repair, being enzymically driven, will be promoted (within limits) by temperature, it might be expected that temperature would influence the repair and damage cycle (described above) and alter the overall response of algae and cyanobacteria to UVB. In this study we used *Microcystis aeruginosa* and *Anabaena circinalis*, which are two of the most important species responsible for harmful cyanobacterial blooms in temperate freshwater ecosystems, not only of Australia but also across the world.

Therefore, this study aimed:

1) to determine the effects of growth temperature on the sensitivity of *M. aeruginosa* and *A. circinalis* to UVB.
2) to examine the effects of temperature on damage and repair processes in relation to UVB sensitivity of the two cyanobacteria.
3) to investigate the levels of UV absorbing compounds (UVACs) in *M. aeruginosa* and *A. circinalis*

3.2 MATERIALS AND METHODS

3.2.1 Microalgal culture

Microcystis aeruginosa (CS558) and *Anabaena circinalis* (CS537) were obtained from the Australian National Algae Culture Collection at CSIRO Marine and Atmospheric Research, Hobart, Australia. Subcultures were maintained in 250 mL Erlenmeyer flasks with 100 mL MLA medium (32) in controlled environment rooms (set to either 25 °C or 30 ^{o}C) under 12/12 h dark/light under a photon flux of 50 µmol photons m^{-2} s^{-1} supplied from cool-white fluorescent tubes (Philips, TLD 36W). The photon flux density of photosynthetically active radiation (PAR, 400–700 nm) was determined using a Li-

Cor (Nebraska, USA) LI-188 Integrating Quantum Radiometer-Photometer.

3.2.2 Cell morphology and trichome length

Cell concentrations of *M. aeruginosa* were estimated by counting in a Neubauer Haemocytometer at 40 × magnification on a Zeiss Axio Scope.A1, using samples fixed with Lugol's iodine.

For *A. circinalis*, the number of trichomes mL^{-1} were determined from trichome length per mL and the mean number of cells per trichome according to Pierangelini et al. (33) using an improved Neubauer hemocytometer.

3.2.3 Growth

The growth of the strains was followed by both cell enumeration and measurement of the optical density (OD) of the algal suspension at 750 nm. The batch cultures were inoculated with an initial OD < 0.01 to avoid cell self-shading. This equated to a cell concentration of $<1 \times 10^5$ cells mL^{-1}. OD was converted to cell concentrations to calculate the growth rates. After log transformation, values corresponding to the exponential phase in the growth curve were used to calculate the specific growth rate from the slope of log (cells mL^{-1}) vs time.

3.2.4 Ultraviolet radiation exposure

To understand the effects of an acute dose of UVB and temperature on the physiological characteristics of *Anabaena circinalis* and *Microcystis aeruginosa,* cultures were exposed to an acute dose of UVB (1.4 W m^{-2}) until the effective quantum yield reached an asymptote. UVB was supplied by a Xenon-arc lamp (Ernst Leitz GMBH Wetzlar, Germany, 315-280 nm), directly onto a 500 mL Quartz conical flask, with the sample being constantly stirred with a magnetic stirrer (refer to supplementary material for UV spectrum). Controls were run with UVB excluded using polymethylmethacrylate sheet (opaque to wavelengths <360 nm) with PAR of 50 µmol photons m^{-2} s^{-1} supplied from

the LED array of PhytoPAM pulse amplitude modulated (PAM) fluorometer (Heinz Walz GmbH, Effeltrich, Germany).

3.2.5 Chlorophyll Fluorescence measurements

For UV exposure experiments, sub-samples of cultures (3 mL) were placed in a quartz cuvette and effective quantum yield (F_v' /F_m') of PSII was measured every 5 minutes using a PhytoPAM. The data were applied to the Kok model (15) in GraphPad Prism 7 software and used to calculate the photoinhibition (*k*) and repair (*r*) rate under UVB.

The F_v' /F_m' data were normalised to the initial value at t=0 and used to calculate the rate constants for repair (*r*) and damage (*k*) according to equation 1.

The non-linear regression fit of the data always gave R^2 values of >0.9, indicating a strong fit to the Kok equation. We also ran curve fitting using a constant repair model (34) which gave an R^2>0.9, so had an equivalent fit to the Kok model.

3.2.6 Extraction of UV absorbing compounds

For determinations of UV absorbing compounds (UVACs), cells were collected by centrifugation at 3400 x *g* for 15 min, and resuspended in 1 ml cold (5 ^oC) tetrahydrofuran:methanol (20:80, v/v), a solvent mixture which provided maximum extraction efficiency for both UVACs and lipophilic pigments (20, 35). Cells were kept on ice during extraction and sonicated for 5 min. The solvent mixture extracted both UVACs and photosynthetic pigments, leaving a colourless pellet. The suspension was centrifuged again, and the absorption spectrum of the supernatant was measured from 200 to 700 nm using a Cary-50 UV-Vis spectrophotometer. The baseline was previously set to zero with a solvent blank. The relative proportion of UV absorbance to Chl-*a* was calculated as the ratio of absorbance intensity at the UV absorbance maximum between 280 to 390 nm to that of Chl-*a* at 665 nm.

3.2.7 Statistical analysis

To compare the growth rates, unpaired two tailed t-test with Welch's correction of data was performed using GraphPad Prism 7. The Kok model was used in GraphPad Prism 7.2 software to determine the rate constants for repair (*r*) and damage (*k*). Two-way ANOVA followed by Sidak's multiple comparison test was performed to check if there were any differences in *r* and *k* values both within and between species at the two temperature regimes.

3.3 RESULTS

3.3.1 Growth

Differences in cell growth curves were found in strains growing under the two temperature regimes. *A. circinalis* (Strain CS537) showed a similar growth pattern at both temperatures, but with higher growth rates (0.38 ± 0.01 day^{-1}) at 30 ^{o}C than at 25 ^{o}C (0.33 ± 0.01 day^{-1}) (t-test, $P = 0.0233$). However, final cell concentrations and growth rates of *M. aeruginosa* (Strain CS558) were higher at 25 ^{o}C (0.41 ± 0.02 day^{-1}) than at 30 ^{o}C (0.20 ± 0.02 day^{-1}) (t-test, $P = 0.0001$).

3.3.2 Inhibition of effective quantum yield (Fv'/Fm') during UVBR exposure

The values of F_v'/F_m' (Fig. 2 a,b) decreased monotonically with time to an asymptote. The pattern of decrease was similar in strains grown at different temperatures although the initial quantum yield of *M. aeruginosa* at 30 ^{o}C was lower than at 25 ^{o}C. The magnitude of *k* was higher than *r* (Fig. 3) at both temperatures in case of both species, leading to a net decline in effective quantum yield over time. The magnitude of *r* was higher at 30 ^{o}C than at 25 ^{o}C in both species, and *k* was slightly higher in *A. circinalis,* but slightly lower in *M. aeruginosa*, at the higher temperature. These differences were statistically significant (Two-way ANOVA, $P = 0.0001$) between the two temperature

regimes. Between species, *M. aeruginosa* exhibited relatively higher repair (*r*) and damage (*k*) rates compared to *A. circinalis* at both growth temperatures (Two-way ANOVA, $P = 0.0001$). Control values for F_v'/F_m' in case of *A. circinalis* in the absence of UVBR (Fig. 2b) showed a small decrease over time with a linear slope of <0.0013 min^{-1}.

Fig. 2

Fig. 3

In both species the ratio of *r* to *k* (Fig. 4) appeared to be higher at 30 ^oC as compared to 25 ^oC. While the difference in this ratio was statistically different (Two-way ANOVA, $P = 0.0043$) in the case of *M. aeruginosa*, there was not any statistical difference (Two-way ANOVA, $P = 0.1777$) in the case of *A. circinalis*. Between species *A. circinalis* exhibited a greater percent inhibition of F_v'/F_m' than *M. aeruginosa* at both temperatures. Although inhibition of F_v'/F_m' in *M. aeruginosa* did not exhibit any statistical difference (Two-way ANOVA, $P = 0.4316$, Fig. 4) between temperatures, there was significant difference (Two-way ANOVA, $P = 0.0366$, Fig. 4) between temperatures for *A. circinalis*.

Fig. 4

3.3.3 UV absorbing compounds

Both species had the ability to absorb UVA (Fig. 5 a, b). However, they differed in the extent to which they were able to absorb UV radiation. The relative proportion of UVA and UVB absorbing compounds compared to chlorophyll *a* (665 nm) is shown in Table 1. *M. aeruginosa* mainly absorbs in the UVA region (400-315 nm) and does not appear to contain any compounds that absorb UVB, whereas *A. circinalis* absorbs in both the UVA (400-315 nm) and the UVB (315-280 nm) regions.

Fig. 5

3.4 DISCUSSION

This study shows differences in growth rates within both species at both temperature regimes. The lower growth rate of *M. aeruginosa* that was observed at 30 ^{0}C could reflect the fact that this species generally does not prefer higher temperatures (36), although it is hard to make firm conclusions as different strains respond differently and the growth rate depends on many other factors.

The photosynthetic performance, as determined by effective quantum yield, of both species at both temperatures declined with UVBR exposure. The exposure response curves (ERCs) showed a similar pattern to that presented by Lesser et al. (37) and Heraud and Beardall (18), with an initial phase (time dependent), a transition state and a time-independent asymptote state. As both species reached the asymptote state relatively quickly, the photosynthetic apparatus was not damaged completely. This suggests that the level of UVBR fluence rate used in this study was not enough to fully inhibit photosynthetic activity but was sufficient to reduce the F_v'/F_m' to at least half within the timeframe of the exposure. In the absence of UVBR, F_v'/F_m' showed a small decrease over time, with a linear slope of <0.0013 min^{-1}. This decline is unlikely to be due to PAR photoinhibition, as the PAR intensity in the control was set at 50 µmol photons m^{-2} s^{-1}, which is the optimum light intensity for growth (38), but may reflect an artefact of continued stirring in the PAM cuvette.

The rate constant for repair (*r*) increased with temperature in both species. Thus the enzymatic driven processes for repair of reaction center proteins and Rubisco turnover increases with increasing temperature, thereby reducing photochemical damage (39-41). Although not carried out here, experiments in which repair is inhibited by the protein synthesis inhibitor lincomycin can provide estimates of damage that are more robust than the approach using the Kok model applied here, though Heraud and Beardall (17)

found good agreement between damage rates estimated using both methods. The apparent decrease of damage rate in *M. aeruginosa* with increased temperature is unexpected, but could result from a stimulation of synthesis of UVB screening compounds under elevated temperature. The rate constant for damage (*k*) is higher in *A. circinalis*, the repair constant (*r*) and ratio of *r:k* also increased with temperature which suggests that the damage caused by UVR was repaired faster at higher temperature. Wong et al. (27) found similar results working with *Chlorella* sp. from tropical waters. As we see a higher damage constant (*k*) than repair constant (*r*) in both species, the net effect was a decrease in the effective quantum yield of PSII and net damage by UVR (42, 43). The lower percent inhibition of F_v'/F_m' and higher *r:k* value at 30 ^oC support the findings of Gao et al. (44). This is also supported by observations that higher temperatures reduce the overall sensitivity of a number of algae to UVR (45, 27). The increase in repair rates and overall reduction in net damage to PSII at higher temperature in both cyanobacterial species examined suggests that these organisms, and indeed other algae and cyanobacteria, might be less affected by, or at least be able to recover quicker from, UVR exposure under future climate change scenarios (24, 41). However, as cyanobacteria and other microalgae live in very diverse habitats, including diverse temperatures, their response to global temperature changes can be quite contrasting and there is a need for more detailed study on many more species before it is possible to reach general conclusions about UVB effects in a future world.

Many algae have mechanisms to minimise UVR damage. These include synthesis of antioxidative stress compounds as well as production of UV absorbing MAAs and scytonemin (mostly in cyanobacteria) and carotenoids. In this study, the proportion of UVA and UVB compounds in relation to chlorophyll *a* (665 nm) shows that both species absorb UVA to some extent. There is evidence that cyanobacteria increase synthesis of

UV absorbing extracellular pigment after exposure to UVA and/or UVB radiation (46). The ability of *A. circinalis* to absorb in both UVA and UVB regions explains the observed higher damage rate of *M. aeruginosa* after UVBR exposure, though *A. circinalis* appeared to show the greater overall inhibition of PSII function. However, in this study we did not grow the algae under UVB, so UVAC levels many not reflect those that might be found in nature (47, 24, 46, 25). Although we see variation in UV absorbing compounds between species, there are also strain variations found (26), so future study needs to include more strains/species of cyanobacteria. Despite the higher UVACs in *A. circinalis*, *M. aeruginosa* is less affected overall. So, future study could also consider the competition between species under global climate change.

This study shows differences in growth and photosynthetic performances of *M. aeruginosa* and *A. circinalis* to UVB and temperature interactions. Although our results show a positive relationship between repair rate and temperature, the interactive effects of temperature and UVR with other parameters such as nutrient availability, atmospheric CO_2 level, and high/low PAR level also need to be considered (41). Inclusion of these parameters in future studies will provide a better understanding of how cyanobacteria will respond to further changes in global climate.

3.5 ACKNOWLEDGMENTS

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REFERENCES

1. Giordanino, M. V. F., S. M. Strauch, V. E. Villafañe and E. W. Helbling (2011) Influence of temperature and UVR on photosynthesis and morphology of four species of cyanobacteria. *J. Photochem. Photobiol. B: Biol.* **103,** 68-77.
2. Kirtman, B., S.B. Power, J.A. Adedoyin, G.J. Boer, R. Bojariu, I. Camilloni, F.J. Doblas-Reyes, A.M. Fiore, M. Kimoto, G.A. Meehl, M. Prather, A. Sarr, C. Schär, R. Sutton, G.J. van Oldenborgh, G. Vecchi and H.J. Wang (2013) Near-term Climate Change: Projections and Predictability. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
3. Day, T. A. and P. J. Neale (2002) Effects of UV-B radiation on terrestrial and aquatic primary producers. *Annu. Rev. Ecol. Syst.* **33,** 371-396.
4. Hartmann, D. L., J. M. Wallace, V. Limpasuvan, D. W. J. Thompson and J. R. Holton (2000) Can ozone depletion and global warming interact to produce rapid climate change? *Proceedings of the National Academy of Sciences of the United States of America* **97,** 1412-1417.
5. Williamson, C. E., R. G. Zepp, R. M. Lucas, S. Madronich, A. T. Austin, C. L. Ballare, M. Norval, B. Sulzberger, A. F. Bais, R. L. McKenzie, S. A. Robinson, D. P. Hader, N. D. Paul and J. F. Bornman (2014) Solar ultraviolet radiation in a changing climate. *Nature Climate Change* **4,** 434-441.
6. Ball, W. T., J. Alsing, D. J. Mortlock, J. Staehelin, J. D. Haigh, T. Peter, F. Tummon, R. Stubi, A. Stenke, J. Anderson, A. Bourassa, S. M. Davis, D. Degenstein, S.

Frith, L. Froidevaux, C. Roth, V. Sofieva, R. Wang, J. Wild, P. F. Yu, J. R. Ziemke and E. V. Rozanov (2018) Evidence for a continuous decline in lower stratospheric ozone offsetting ozone layer recovery. *Atmospheric Chemistry and Physics* **18,** 1379-1394.

7. Blackfield, M. K. and D. O. Harris (1994) Delay of cell differentiation in *Anabaena aequalis* caused by UV-B radiation and the role of photoreactivation and excision repair. *Photochem. Photobiol.* **59,** 204-208.

8. Sinha, R. P., H. D. Kumar, A. Kumar and D. P. Hader (1995a) Effects of UV-B irradiation on growth, survival, pigmentation and nitrogen metabolism enzymes in cyanobacteria *Acta Protozool.* **34,** 187-192.

9. Tyagi, R., G. Srinivas, D. Vyas, A. Kumar and H. D. Kumar (1992) Differential effects of ultraviolet-B radiation on certain metabolic processes in a chromatically adapting *Nostoc*. *Photochem. Photobiol.* **55,** 401-407.

10. Key, T., A. McCarthy, D. A. Campbell, C. Six, S. Roy and Z. V. Finkel (2010) Cell size trade-offs govern light exploitation strategies in marine phytoplankton. *Environ. Microbiol.* **12,** 95-104.

11. Greenberg, B. M., V. Gaba, O. Canaani, S. Malkin, A. K. Matoo and M. Edelman (1989) Separate photosensitizers mediate degradation of the 32 kDa photosystem II reaction centre protein in the visible and UV spectral regions. *Proc. Natl. Acad. Sci. USA.* **86,** 6617-6620.

12. Raven, J. A. (1991) Responses of aquatic photosynthetic organisms to increased solar UVB. *J. Photochem. Photobiol. B Biol.* **9,** 239-244.

13. Sinha, R. P., A. Kumar, M. B. Tyagi and D.-P. Hader (2005) Ultraviolet-B-induced destruction of phycobiliproteins in cyanobacteria. *Physiol. Mol. Biol. Plants* **11,** 313-319.

14. Hamilton, D. P., S. A. Wood, D. R. Dietrich and J. Puddick (2014) Costs of harmful blooms of freshwater cyanobacteria. In *Cyanobacteria*. pp. 245-256. John Wiley & Sons, Ltd.

15. Kok, B. (1956) On the inhibition of photosynthesis by intense light. *Biochim. Biophys. Acta* **21,** 234–244.

16. Thapar, R., A. K. Srivastava, P. Bhargava, Y. Mishra and L. C. Rai (2008) Impact of different abiotic stresses on growth, photosynthetic electron transport chain, nutrient uptake and enzyme activities of Cu-acclimated Anabaena doliolum. *J. Plant Physiol.* **165,** 306-316.

17. Bouchard, J. N., S. Roy and D. A. Campbell (2006) UVB effects on the photosystem II-D1 protein of phytoplankton and natural phytoplankton communities. *Photochem. Photobiol.* **82,** 936-951.

18. Hearud, P. and J. Beardall (2000) Changes in chlorophyll fluorescence during exposure of *Dunaliella tertiolecta* to UV radiation indicate a dynamic interaction between damage and repair processes. *Photosynthesis Res.* **63,** 123-134.

19. Buma, A. G. J., E. J. van Hannen, L. Roza, M. J. W. Veldhuis and W. W. C. Gieskes (1995) Monitoring ultraviolet-B induced DNA damage in individual diatom cells by immunofluorescent thymine dimer detection *J. Phycol.* **31,** 314-321.

20. Dunlap, W. C., B. E. Chalker and J. K. Oliver (1986) Bathymetric adaptations of reef-building corals at Davies Reef, Great Barrier Reef, Australia. 111. 'UV-B absorbing compounds. *J. Exp. Biol.* **104,** 239-248.

21. Nakamura, H., J. Kobayashi and Y. Hirata (1982) Separation of mycosporine-like amino acids in marine organisms using reversed-phase high-performance liquid chromatography. *J. Chromatogr.* **250,** 113-118.

22. Sancar, A. and G. B. Sancar (1988) DNA repair enzymes. *Annu. Rev. Biochem.* **57,** 29-67.

23. Xiong, F., J. Komenda, J. Kopecky and L. Nedbal (1997) Strategies of ultraviolet-B protection in microscopic algae. *Physiol. Plant.* **100,** 378-388.

24. Garcia-Pichel, F. and R. W. Castenholz (1991) Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. In *J. Phycol.*, Vol. 27. pp. 395-409.

25. Karsten, U. and F. Garcia-Pichel (1996) Carotenoids and mycosporine-like amino acid compounds in members of the genus *Microcoleus* (cyanobacteria): a chemosystemaic study. *Syst. Appl. Microbiol.* **19,** 285-294.

26. Jeffery, S. W., H. S. MacTavish, W. C. Dunlap, M. Vesk and K. Groenewoud (1999) Occurrence of UVA- and UVB-absorbing compounds in 152 species (206 strains) of marine microalgae. *Mar. Ecol. Prog. Ser.* **189,** 35-51.

27. Wong, C. Y., M. L. Teoh, S. M. Phang, P. E. Lim and J. Beardall (2015) Interactive effects of temperature and UV radiation on photosynthesis of *Chlorella* strains from polar, temperate and tropical environments: differential impacts on damage and repair. *Plos One* **10**(10):e0139469. doi:10.1371/journal.pone.0139469.

28. Franklin, L. A. and R. M. Forster (1997) The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. *Eur. J. Phycol.* **32,** 207-232.

29. Roleda, M. Y., C. Wiencke, D. Hanelt and K. Bischof (2007) Sensitivity of the early life stages of macroalgae from the northern hemisphere to ultraviolet radiation. *Photochem. Photobiol.* **83,** 851-862.

30. Teoh, M. L., W. L. Chu, H. Marchant and S. M. Phang (2004) Influence of culture temperature on the growth, biochemical composition and fatty acid profiles of six Antarctic microalgae. *J. Appl. Phycol.* **16,** 421-430.

31. Wiencke, C., U. H. Luder and M. Y. Roleda (2007) Impact of ultraviolet radiation on physiology and development of zoospores of the brown alga *Alaria esculenta* from Spitsbergen. *Physiol. Plant.* **130,** 601-612.

32. Bolch, C. J. S. and S. I. Blackburn (1996) Isolation and purification of Australian isolates of the toxic cyanobacterium *Microcystis aeruginosa* Kutz. *J. Appl. Phycol.* **8,** 5-13.

33. Pierangelini, M., S. Stojkovic, P. T. Orr and J. Beardall (2014a) Photosynthetic characteristics of two *Cylindrospermopsis raciborskii* stains differing in their toxicity. *J. Phycol.* **50,** 292-302.

34. Sobrino, C., P. J. Neale and L. M. Lubian (2005) Interaction of UV radiation and inorganic carbon supply in the inhibition of photosynthesis: Spectral and temporal responses of two marine picoplankton. *Photochem. Photobiol.* **81,** 384-393.

35. Chalker, B. E. and W. C. Dunlap (1982) Extraction and quantitation of endosymbiotic algal pigments from reef-building corals. In: Gomez ID et al. (eds) Proc 4th Int Coral Reefs Symp. Vol 2. Marine Sciences Center, University of the Phhpines,Manila pp. 45-50.

36. Lurlling, M., F. Eshetu, E. J. Faassen, S. Kosten and V. L. M. Huszar (2013) Comparison of cyanobacterial and green algal growth rates at different temperatures. *Freshwat. Biol.* **58,** 552-559.

37. Lesser, M. P., J. J. Cullen and P. J. Neale (1994) Carbon uptake in a marine diatom during acute exposure to ultraviolet B radiation: Relative importance of damage and repair. *J. Phycol.* **30,** 183-192.

38. Islam, M. A. and J. Beardall (2017) Growth and Photosynthetic Characteristics of Toxic and Non-Toxic Strains of the Cyanobacteria *Microcystis aeruginosa* and *Anabaena circinalis* in Relation to Light. *Microorganims* **5** (45): doi.10.3390/microorganims5030045..

39. Greer, D. H., J. A. Berry and O. Bjorkman (1986) Photoinhibition of photosynthesisin intact bean-leaves - Role of light and temperature, and requirement for chloroplast-protein synthesis during recovery. *Planta* **168,** 253-260.

40. Henley, W. J. and J. Ramus (1989) Photoacclimation of *Ulva rotundata* (Chlorophyta) under natural irradience. *Mar. Biol.* **103,** 261-266.

41. Hitchcock, G. L. (1980) Influence of temperature on the growth rate of *Skeletonema costatum* in response to variations in daily light intensity. *Mar. Biol.* **57,** 261-269.

42. Bischof, K., D. Hanelt and C. Wiencke (2000) Effects of ultraviolet radiation on photosynthesis and related enzyme reactions of marine macroalgae. *Planta* **211,** 555-562.

43. Bischof, K., G. Krabs, C. Wiencke and D. Hanelt (2002) Solar ultraviolet radiation affects the activity of ribulose-1, 5-bisphosphate carboxylase-oxygenase and the composition of photosynthetic and xanthophyll cycle pigments in the intertidal green alga *Ulva lactuca* L. *Planta* **215,** 502-509.

44. Gao, K. S., P. Li, T. R. Watanabe and E. W. Helbling (2008) Combined effects of ultraviolet radiation and temperature on morphology, photosynthesis, and DNA of *Arthrospira* (*Spirulina*) *platensis* (Cyanophyta). *J. Phycol.* **44,** 777-786.

45. Beardall, J., S. Stojkovic and K. S. Gao (2014) Interactive effects of nutrient supply and other environmental factors on the sensitivity of marine primary producers to ultraviolet radiation: implications for the impacts of global change. *Aquatic Biology* **22,** 5-23.

46. Garcia-Pichel, F. and R. W. Castenholz (1993) Occurrence of UVabsorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity. *Appl. Environ. Microbiol.* **59,** 163-169.

47. Oren, A. (1997) Mycosporine-like amino acids as osmotic solutes in a community of halophilic cyanobacteria. *Geomicrobiol. J.* **14,** 231-240.

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Table 1. Ratios of UV absorption to Chl-*a* absorption characteristics of two cyanobacterial species grown at 25 ^{o}C.

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Fig. 2: Time course of changes in the fluorescence parameter F_v'/F_m' in *M. aeruginosa* CS558 (a) and *A. circinalis* CS537 (b) exposed to an acute dose of UVBR (1.4 W m^{-2}) or exposed to PAR only (controls). The error bars represent the standard deviation from three independent replicate cultures.

Fig. 3: Repair (*r*) and damage (*k)* constant (min^{-1}) for *M. aeruginosa* and *A. circinalis* as a function of temperature. The error bar represents the standard deviation of three replicates.

Fig. 4: Ratio of *r* to *k* (*r:k*) (min^{-1}) and % inhibition of F_v'/F_m' in *M. aeruginosa* CS558 and *A. circinalis* CS537 after exposed to an acute dose of UVBR (1.4 W m^{-2}). The error bar represents the standard deviation of three replicates.

Fig. 5: UV-visible absorption spectra of tetrahydrofuran:methanol (20:80, v/v) extracts of selected microalgae (a) *Anabena circinalis* CS537 and (b) *Microcystis aeruginosa* CS558 maintained at 25 ^{0}C. The dark and light grey bars represent UVB (315-280 nm) and UVA (315-340 nm) regions respectively.

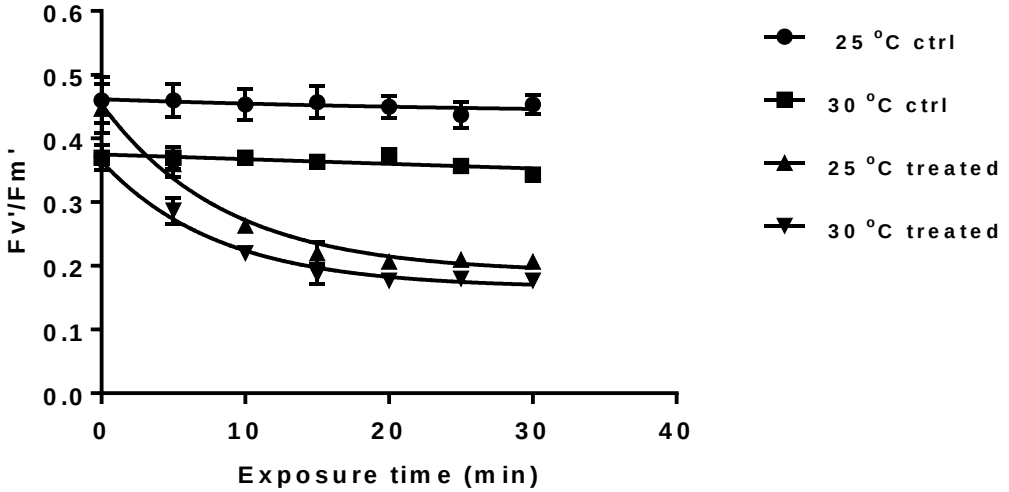
Table 1

Strain	Species	Temperature **(ºC)**	Abs. ratio of major UV peaks : Chl *a* (665 nm)		
			315-280 nm	315-340 nm	340-390 nm
CS558	*M. aeruginosa*	25	-	1.33	-
CS537	*A. circinalis*	25	0.4201	0.4631	0.7580

List of Supplementary Figures with legends

Supp. Fig.1: UV spectrum of the Xenon Arc Lamp used in UVB exposure experiments. The spectrum was measured using a spectroradiometer (Acton Research Corporation SpectroPro® 300i).

Figure 2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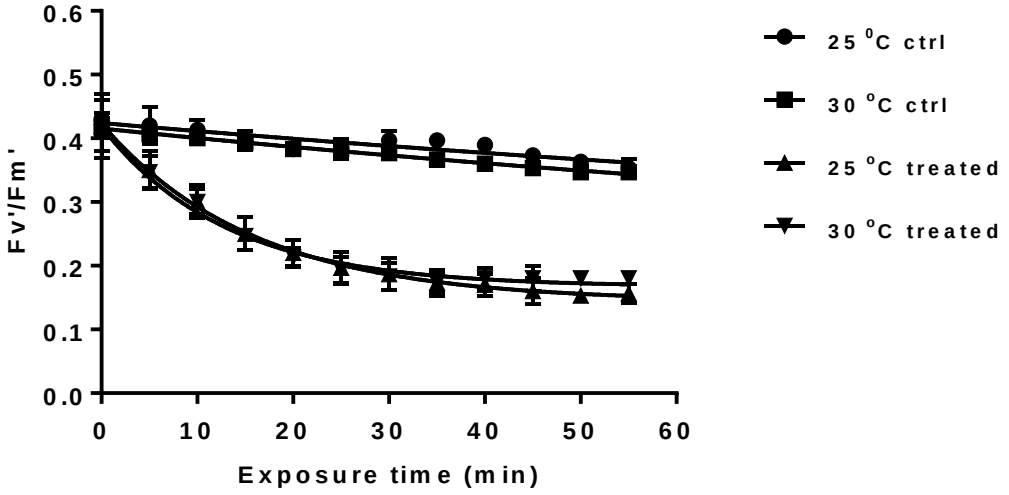
0.5
0.4
0.3
0.2
0.1
0.0
Fv'/Fm'
0
10
20
30
40
Exposure time (min)
25 °C ctrl
30 °C ctrl
25 °C treated
30 °C treated

a


0.6
0.5
0.4
0.3
0.2
0.1
0.0
Fv'/Fm'
0
10
20
30
40
50
60
Exposure time (min)
25 °C ctrl
30 °C ctrl
25 °C treated
30 °C treated

b

Figure 3

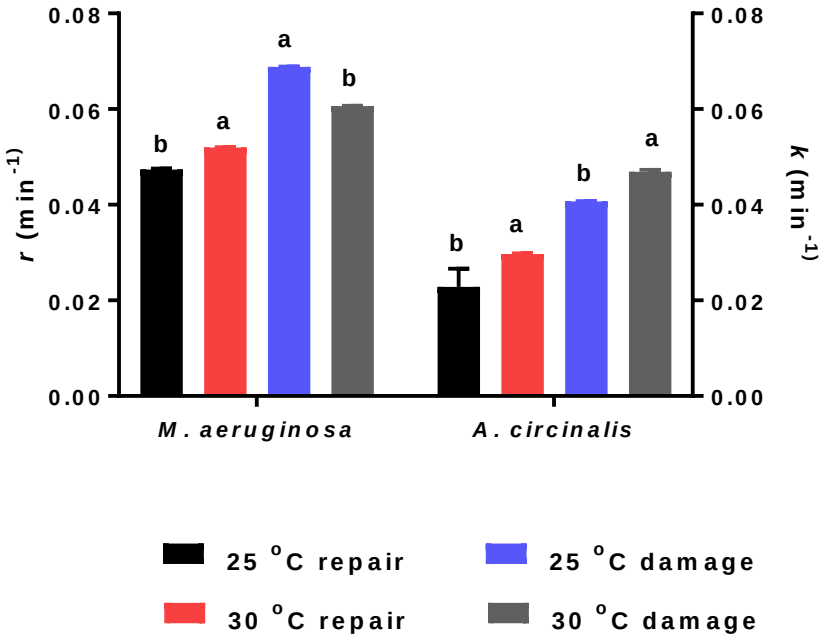

0.08
0.06
0.04
0.02
0.00
r (min-1)
k (min-1)
b
a
a
b
b
a
b
a
M. aeruginosa
A. circinalis
25 °C repair
30 °C repair
25 °C damage
30 °C damage

Figure 4

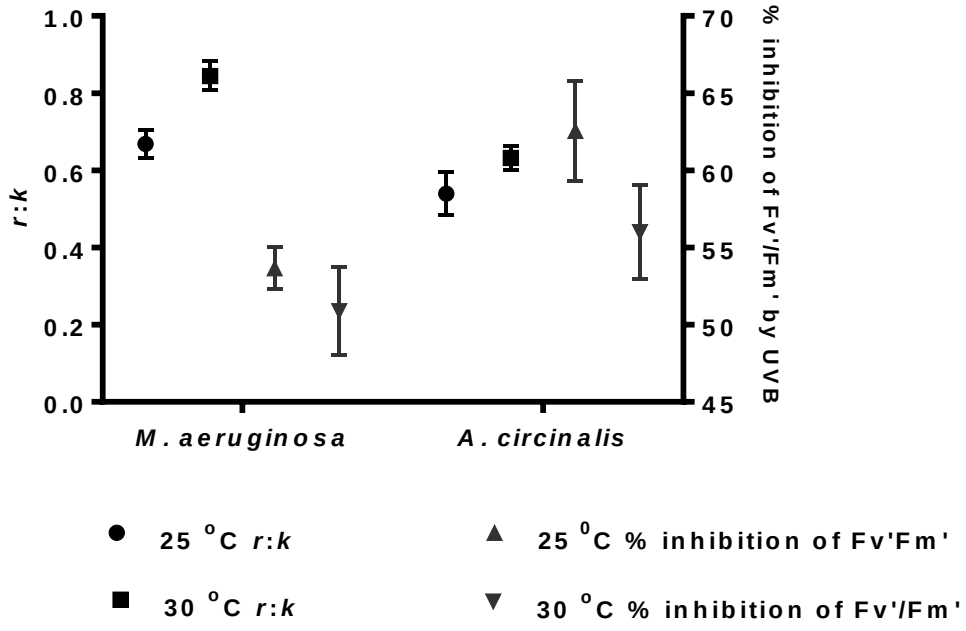
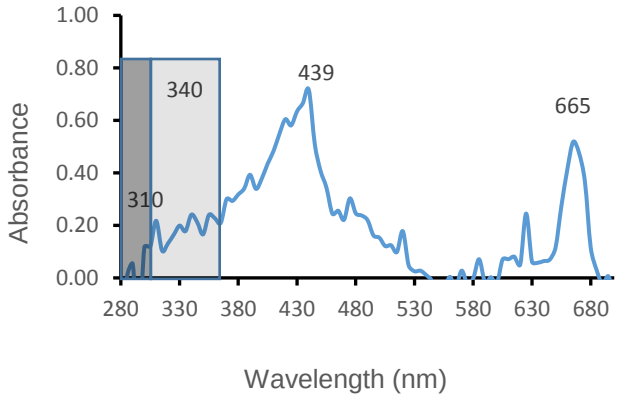

1.0
0.8
0.6
0.4
0.2
0.0
r:k
70
65
60
55
50
45
% inhibition of Fv'/Fm' by UVB
M. aeruginosa
A. circinalis
25 °C r:k
30 °C r:k
25 °C % inhibition of Fv'Fm'
30 °C % inhibition of Fv'/Fm'

Figure 5


1.00
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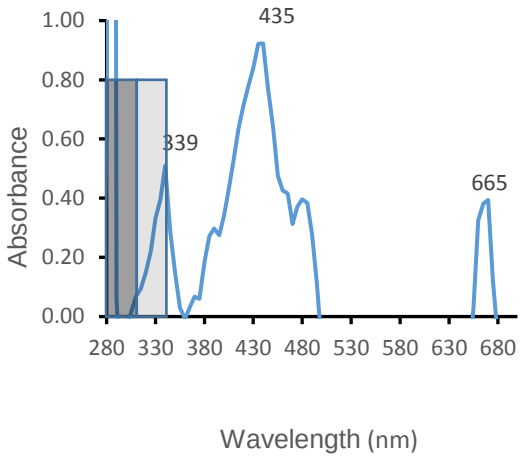
Absorbance
340
310
439
665
280 330 380 430 480 530 580 630 680
Wavelength (nm)

a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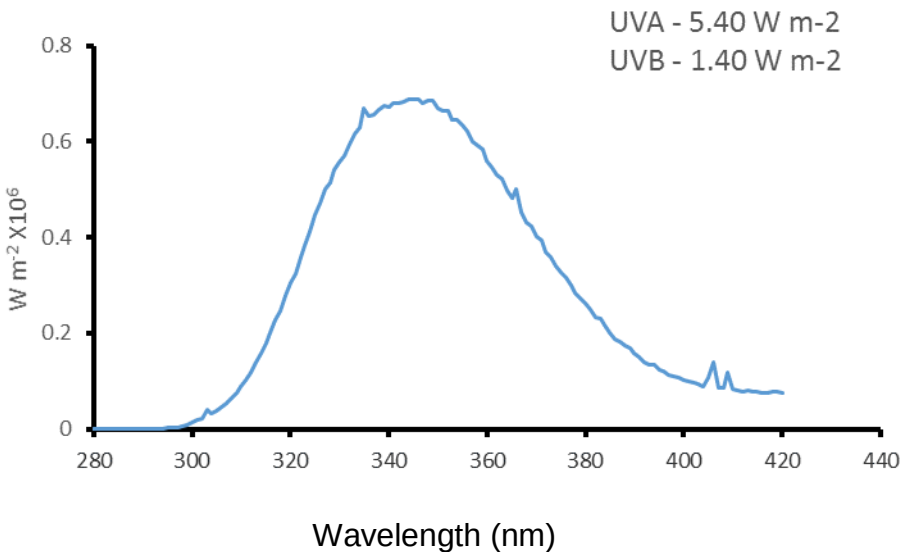
339
665
280 330 380 430 480 530 580 630 680
Wavelength (nm)

b

Supp. Figure 1



Declaration for Thesis Chapter

Declaration for Thesis Chapter 4

Declaration by candidate

In case of chapter 4, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
The experimental design, lab work, data analysis and writing of the manuscript were my responsibility.	80

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	**Nature of contribution**	**Extent of contribution (%)**
John Beardall	The idea for the experiment, interpretation of results, assistance with writing the manuscript, feedback on thesis.	
Perran Cook	Improved the manuscript	

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's signature		Date 26th March 2018
Main Supervisor's signature		Date 24th March 2018

*Note: Where the responsible author is not the candidate's main supervisor, the main supervisor should consult with the responsible author to agree on the respective contributions of the authors.

Chapter 4. Intra-strain variability in effects of temperature on UVB sensitivity of cyanobacteria

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This chapter is currently under revision with Photochemistry and Photobiology

ABSTRACT

Stratospheric ozone depletion (sometimes referred to as an ozone hole) is mostly marked over the Antarctic and to a lesser extent over the Arctic, though recent reports have revealed that this also occurs at lower latitudes. Continued depletion of ozone in the lower stratosphere allows more UVR to reach the Earth's surface. Furthermore, it is projected that surface water temperatures will increase by between 0.2 and 2.0 ^{o}C by the year 2060 and this will directly or indirectly influence algal growth. The interactions between environmental factors is complicated by the existence of different strains (ecotypes) of the same species that may respond differently. To understand the interactive effects of temperature and UVB on two strain of *Anabaena circinalis*, we investigated the damaging effects of UVB on cell numbers and photosynthetic characteristics and also examined the effect of temperature on the capacity of cells to recover from such stress. Both strains of *A. circinalis* responded differently in terms of survival, photosynthetic characteristics and recovery with interactions between temperature and UVB. This could be due to the variations in strain-specific photo-reactive mechanisms. This needs to be explored further including more strains and species before definitive conclusions can be reached about effects of global change on cyanobacteria generally.

Keywords: Ozone, Stratosphere, Temperature, UVB, Photoinhibition, Survival, Recovery

4.1 INTRODUCTION

The release of anthropogenic pollutants (chlorofluorocarbons, chlorocarbons, organobromides) to the atmosphere due to human activity have caused reductions in the stratospheric ozone layer from the second half of the 20th century onwards (1, 2), leading to increases in the level of UVR radiation (specifically UVB) reaching the Earth's surface. Stratospheric ozone depletion (sometimes referred to as an ozone hole) is mostly marked over the Antarctic and to a lesser extent over the Arctic, but recent reports have revealed that this also occurs at lower latitudes (3-5).

Since the banning of some of the substances responsible for ozone depletion after the Montreal Protocol in 1997, the decline in total column ozone had stopped at almost all non-polar latitudes (6) but a recovery in global mean stratospheric ozone has yet to be reported (7). Recently Solomon et al. (8) reported the recovery of total column ozone in Antarctica but the non-polar total column ozone has remained stable since 2000 (7). Although there is a lack of clear evidence for total ozone recovery, ozone concentrations in the upper stratosphere (>10 hPa) have been reported to be recovering significantly (9-14). However, the ozone in the stratosphere as a whole is not necessarily recovering and a decline in lower stratospheric ozone has been reported (15-19). More recently Ball et al. (1) have shown that the ozone in the lower stratosphere between 60 ^{o}S and 60 ^{o}N has been continuously declining since 1998. Continued depletion of ozone in the lower stratosphere allows more UVR (specifically UVB) to reach the Earth's surface. However, the level of total UVR at the Earth's surface also depends on other factors (cloud cover, nutrient availability). A study performed in tropical Australia (from 1979-1999) showed a significant increase in UVR (10% per decade) associated with reduced ozone (1–2% per decade) and reduced cloud cover (15–30% per decade). On the other

hand, in southern regions of Australia, UVR did not increase over time, partially due to increased cloud cover (20). Global warming also leads to further ozone depletion (2, 21, 22) which eventually allows more UV radiation to reach the surface. Therefore, understanding how UVR impacts interact with other environmental factors is important.

UVC does not penetrate the stratosphere, since ozone absorbs wavelengths below 280 nm. As UVA is known to have less damaging effects on biota compared to UVB, major concerns focus on UVB radiation and its impact. UVB radiation has a serious deleterious effect on all living organisms. However, as one of the Earth's earliest photosynthetic prokaryotes, cyanobacteria may have faced high levels of solar UVB early in their evolution (23) and might therefore be expected to show an enhanced ability to withstand UVB-induced damage. Known effects of UVB include damage to PSII reaction centres (24), and the D1 protein in particular (25), damage to the CO_2 fixing enzyme Rubisco (26), DNA degradation (27, 28) and photobleaching of photosynthetic pigments (29). However, cyanobacteria have evolved a range of mechanisms to avoid or ameliorate UV-induced damage. These include vertical migration (30) or synthesis of protective UV screening compounds such as carotenoids, mycosporine-like aminoacids (MAAs) or scytonemins (31) or proteins (32), as well as induction of enzymes (33) capable of repairing damage.

Temperature is the single most important factor after light that controls algal and cyanobacterial growth. It is projected that global average surface water temperature by the year 2060 will increase by between 0.2 and 2.0 ^{o}C (34) and this will directly or indirectly influence algal growth. Though a majority of freshwater eukaryotic phytoplankton decrease or maintain growth above 20 ^{o}C, many cyanobacteria show accelerated growth under such conditions (35-39). Moreover, it has been found that

increased temperature favoured the dominance of some cyanobacteria (40) in mixed culture.

Interactive effects of temperature and UVB are likely to have differential consequences on phytoplankton cell size, bloom formation, elemental stoichiometry, growth rates, community structure, and adaptive capacity (41). Despite this, most of the studies performed on the effects of climate change factors on cyanobacteria deal with a single variable at a time (42, 43) and there is very little known (44, 45) on the interactive effects of temperature and UVB on algae generally (46-48) and especially on major toxic bloom species of cyanobacteria.

The interactions between environmental factors is complicated by the existence of different strains (ecotypes) of the same species that may respond differently (35, 37,49--61). We tested this possibility by examining different strains of the toxic cyanobacterium *Anabaena circinalis* under two different temperatures. Specifically, we investigated the damaging effects of UVB on cell numbers and population declines and impacts of UVB on quantum yield of PSII at these two temperatures and also examined the effect of temperature on the capacity of cells to recover from such stress.

4.2 MATERIAL AND METHODS

4.2.1 Microalgal culture

Anabaena circinalis strains (CS537 and CS541) were obtained from the CSIRO Marine and Atmospheric Research, Hobart, Australia (Australian National Algae Culture Collection). Subcultures of the isolates were maintained in 250 mL Erlenmeyer flasks with 100 mL MLA medium (62) in controlled environment rooms (set to either 25 °C or

30 ^{o}C) under a 12/12 h dark/light cycle with a photon flux (photosynthetically active radiation (PAR, 400–700 nm)) of 50 µmol photons m^{-2} s^{-1}. Light was supplied from cool-white fluorescent tubes (Philips, TLD 36W). The photon flux density was determined using a Li-Cor (Nebraska, USA) LI-188 Integrating Quantum Radiometer-Photometer.

4.2.2 Cell morphology and trichome length

Cell concentrations were estimated using a Neubauer haemocytometer at 40 X magnification on a Zeiss Axio Scope.A1 microscope, using samples fixed with Lugol's iodine. Given the filamentous nature of the *Anabaena* strains used here, the number of trichomes mL^{-1} was counted microscopically and the number of cells mL^{-1} was obtained from the total length of the trichomes divided by the average cellular length.

4.2.3 Growth

The growth of the strains was monitored by both cell enumeration as detailed above, and by measurement of the optical density (OD) of the algal suspension at 750 nm. The batch cultures were inoculated with an initial OD < 0.01 to avoid cell self-shading. This equated to a cell concentration of <1 X 10^5 cells mL^{-1}. OD was converted to cell concentrations to calculate the growth rates. After log transformation, values corresponding to the exponential phase in the growth curve were used to calculate the specific growth rate from the slope of log (cells mL^{-1}) vs time.

4.2.4 Ultraviolet radiation exposure

To understand the effects of different levels of UVB and temperature on the physiological characteristics of *Anabaena circinalis,* strains were exposed to 0.8 (low), 1.8 (medium) and 2.8 W m^{-2} (high) fluxes of UVB. UVR was provided by two fluorescent tubes (Phillips TL20 W/12, operated for 200 h prior to use) directly onto 500 mL Quartz conical flask. PAR was provided concurrently by cool-white fluorescent tubes (Philips, TLD 36W, Amsterdam, The Netherlands). UVBR exposure, as measured by a

SpectraSense™ (Acton Research Ver. 4) spectroradiometer, was provided at three different levels by placing the flasks at 45, 30 and 20 cm from the UV light source. Samples were covered with new cellulose acetate film (Kodacel; acetate film base, Kodak Eastman Company, New York; opaque to wavelengths <280 nm), constituting the UV treatment and 6 mm polymethyacrylamide sheet, constituting a control treatment that only allowed PAR transmission. The level of UVB measured was compared with other published work (63-66) to give biologically effective fluence-rates. The lamps were allowed to warm up for 10 min before use in exposure of the cells.

4.2.5 Recovery

After the strains were treated with UVB, their recovery was investigated by exposing cells to PAR fluxes of 50 µmol photons m^{-2} s^{-1} provided by cool-white fluorescent tubes (Philips, TLD 36W) until the quantum yield reached the normal (pre-UVB exposure) value.

4.2.6 Fluorescence measurement

For both UV exposure and recovery experiments, algal samples (3 mL) were placed in a quartz cuvette and effective quantum yield (Fv'/Fm') of PSII was measured every 5 minutes using a AquaPen (AP100). The data were applied to the Kok model (67) in GraphPad Prism 7 software and used to calculate the photoinhibition (*k*) and repair (*r*) rate under UVB and recovery after UVB exposure.

The F_v' /F_m' data were normalised to the initial value at t=0 and used to calculate the rate constants for repair (*r*) and damage (*k*) according to

$$\frac{P}{P_{initial}} = \frac{r}{k+r} + \frac{k}{k+r} e^{-(k+r)t} \qquad (1)$$

The non-linear regression fit of the data always gave R^2 values of >0.9, indicating a strong fit to the Kok equation. We also ran curve fitting using a constant repair model (68) which gave an R^2>0.9, so had an equivalent fit to the Kok model.

4.2.7 Statistical analysis

Two-way ANOVA followed by Tukey's multiple comparison test were performed to check if there are any differences of *r* and *k* values within the strain at two temperature regimes under three UVB levels. Recovery rates were calculated for low and mid UVB levels and two-way ANOVA followed by Tukey's multiple comparison test were performed to check if there are any differences between the strains at two temperature regimes under two UVB levels.

4.3 RESULTS

4.3.1 Survival of *A. circinalis* strains under UVB exposure

The strains showed different responses (Fig.1 a,b,c,d) to medium term duration (up to 16 h) UV exposure. In the case of strain CS537, cell numbers started to decline after two hours of exposure to UVB at 25 ^{o}C whereas at 30 ^{o}C the strain remained quite robust. In contrast, the cell numbers of the strain CS541 at 25 ^{o}C were variable up to 6 hours followed by a steady decline. But at 30 ^{o}C CS541 showed a sharp decline in cell concentrations.

Fig. 1

4.3.2 Inhibition of effective quantum yield (F_v'/F_m') during UVBR exposure

In shorter term (up to 1 h) exposures, the values of F_v'/F_m' (Fig. 2 a,b,c,d) decreased monotonically with time. The pattern of decrease was similar in both strains at both temperatures.

Fits to the Kok model (see Methods) showed that the magnitude of the damage constant *k* was higher than that for repair *r* at both temperatures in both strains, leading to a net decline in effective quantum yield over time. Overall the magnitude of *k* was higher (Fig. 3b) at 30 ^{o}C in the case of strain CS541. In contrast the value was lower in strain CS537 at higher temperatures. In the case of strain CS541, the photoinhibition rate was significantly higher at 30 ^{o}C ($P<0.05$) with high-UVB levels as compared to the low- and mid-UVB levels. There were no statistical differences in terms of damage rate under all UVB levels for strain CS541 at 25 ^{o}C. In contrast, the damage rate was significantly higher ($P<0.05$) at high-UVB levels at both temperatures (Fig. 3a) in the case of strain CS537. However, there were no statistical differences observed between effects at low- and mid-UVB levels at both temperatures.

Differences in photoinhibition rate between temperature treatments (Fig. 3 a,b) were tested for individual strains. Strain CS537 showed no statistical differences between temperatures whereas the values in strain CS541 were statistically different at the different temperatures. For mid- and high-UVB levels damage rates were significantly higher ($P<0.05$) at 30 ^{o}C in comparison to 25 ^{o}C but for low-UVB exposure in this strain the effects were statistically insignificant between temperatures.

Fig. 2

Fig. 3

4.3.3 Recovery of photosynthetic performance following cessation of UVBR exposure

Recovery of the effective quantum yield of PSII, F_v'/F_m' (Fig. 4 a,b,c,d) was observed in both strains following exposure to UVBR. Both strains in both temperatures following exposure to the highest UVB level showed little or no signs of recovery. Strain CS537 showed relatively rapid recovery at 30 ^oC as compared to 25 ^oC in both mid- and low-UVB levels. On the other hand, strain CS541 took a longer time to recover at both temperatures as compared to strain CS537. At 30 ^oC, strain CS541 only recovered after the low UVB treatment whereas it recovered with both low- and mid-UVB levels at 25 ^oC which suggests that this strain is more sensitive to UVB at higher temperature.

Fig. 4

The recovery rates for F_v'/F_m' at both low- and mid-UVB levels were calculated at both temperatures (Fig 5 a,b). Strain CS537 showed a significantly higher recovery rate ($P< 0.032$) at 25 ^oC at low-UVB treatment as compared to the mid-UVB treatment. Between temperatures, the recovery rate following the mid-UVB treatment was significantly lower at 25 ^oC ($P<0.05$) as compared to 30 ^oC. In the case of strain CS541, there was no statistical difference between temperatures under the low-UVB treatment, but under the mid-UVB treatment the recovery rate was significantly lower ($P<0.05$) at 30 ^oC. All other interactions were significantly different ($P<0.05$).

Fig. 5

4.4 DISCUSSION

This study shows the interactions between increased temperature and sensitivity of algae to UVB in two strains of the cyanobacterium *A. circinalis*. There have been limited studies on the interactions of temperature and UVR on algae. In past work on a range of species, it has been found that increased temperature can reduce photoinhibition (69), facilitate repair of UV induced damage (70, 48) or have little or no effect on photosynthetic reaction centres (71). Therefore, we hypothesized that a rise in temperature might help cyanobacteria to recover from UV induced damage.

The two strains of *A. circinalis* responded differently in terms of survival, photosynthetic characteristics and recovery at temperature and UVB interactions. Elevated temperature is known to benefit some cyanobacteria in terms of increased growth, enhanced abundance and dominance in natural ecosystem (72). Increasing temperature with elevated UVR is known to reduce DNA damage in cyanobacteria (44). There is also evidence of beneficial effects of elevated temperature and UVB interactions on other algal species such as the observation that increasing temperature reduced UVR photoinhibition in the marine diatom *Thalasiossira pseudodona* (73).

Growth measurements are important to estimate changes in productivity. Negative effects of UVB on growth and biomass of algae have been documented by most previous studies (74-79). Some species of cyanobacteria are known to possess active photoreactive mechanisms to mitigate UVB related damage that allows survival under natural conditions (80, 81). In our study, survival of strain CS537 at 30 ^{o}C for an extended period of exposure to UVB could be due to such photoreactive mechanisms. In contrast, the sharp decline of cell number in strain CS541 at elevated temperature could be related to the accumulative effects of temperature and UVB.

In our study both *A. circinalis* strains recovered faster at elevated temperature after exposure to low-UVB. Gao et al. (44) found similar results with *Arthrospira platensis*. Steady recovery of strain CS537 after 25 ^{o}C at low- and mid-UVB treatments could reflect the possibility that that these levels of UVB are not high enough to destroy the cells completely. The faster recovery of strain CS537 at elevated temperature supports the idea of the temperature dependence of repair process as temperature increase enzymatic performance (46, 82). Giordanino et al. (82) also found higher recovery rates of the freshwater cyanobacterium *Anabaena sp.* with elevated temperature. There are also observations that higher temperatures reduce the overall sensitivity of a number of algae to UVR, primarily by an increased capacity for repair (46, 48). Recovery of strain CS541 at low UVB only at elevated temperature could be due to a threshold limit of UVB and temperature interactions. In our study the inability of both strains to recover from high-UVB treatment in both temperatures could be due to irreversible damage to membrane components, photosynthetic pigments, DNA, nitrogen transport systems, and the photosynthetic apparatus, particularly the reaction center of PS II and ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCo at high-UVB) (83).

The differences observed in sensitivity to damage between the strains could also be explained by the efficacy of different protective mechanisms used by different strains/species (82). Photoprotection is a mechanism by which algae reduce UV related damage to the photosynthetic apparatus. Many algae are known to produce UV absorbing MAAs and scytonemin (mostly in cyanobacteria) and carotenoids. There is evidence that cyanobacteria increase synthesis of UV absorbing extracellular pigments after exposure to UVA and/or UVB radiation (84). In this study the decrease of damage rate in strain CS537 with increased temperature could result from a stimulation of

synthesis of UVB screening compounds. But this kind of response could be species/strain specific as we see in this study that damage rates of strain CS541 were significantly higher at elevated temperature. Jeffery et al. (84) also reported strain variations in terms of producing UV absorbing compounds (UVACs). So, the concept of elevated temperatures in tropical environments increasing the tolerance of UV induced damage (44, 48) partially supports our findings.

Although temperature increase benefited *Anabaena sp*. significantly by improved recovery of photosynthetic performance (82), our study found contrasting results between the two strains. This might be due to genetic variations within the cells. As different strains behave differently, it is hard to make any clear statements about the effects of climate change on cyanobacteria as a whole, so future study should include more strains/species of cyanobacteria.

Increased carbon dioxide in the atmosphere due to burning fossil fuels is associated with temperature increases and, together with the activity of chlorofluorocarbons, depletion of stratospheric ozone layer which eventually results in increasing UVB flux to the Earth's surface (85). These factors affect aquatic organisms differently either by causing species extinction, changing population dynamics or favouring some organisms over others (86-88). However, here we see that the responses to climate change variables are strain specific.

In conclusion, increasing UVB radiation due to ozone depletion might have negative effects on cyanobacteria; however, the increasing global temperature due to human activities certainly influences the effects of UVB and might counteract some of the negative effects of UVB for some strains of cyanobacteria.

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REFERENCES

1. Ball, W. T., J. Alsing, D. J. Mortlock, J. Staehelin, J. D. Haigh, T. Peter, F. Tummon, R. Stubi, A. Stenke, J. Anderson, A. Bourassa, S. M. Davis, D. Degenstein, S. Frith, L. Froidevaux, C. Roth, V. Sofieva, R. Wang, J. Wild, P. F. Yu, J. R. Ziemke

and E. V. Rozanov (2018) Evidence for a continuous decline in lower stratospheric ozone offsetting ozone layer recovery. *Atmospheric Chemistry and Physics* **18,** 1379-1394.

2. Molina, M. J. and F. S. Rowland (1974) Stratospheric sink for chlorofluoromethanes - chlorine atom catalyzed destruction of ozone. *Bulletin of the American Meteorological Society* **55,** 491-491.

3. Farman, J. C., B. G. Gardiner and J. D. Shanklin (1985) Large losses of total ozone in antarctica reveal seasonal clox/nox interaction. *Nature* **315,** 207-210.

4. WMO (2011) Scientific Assessment of Ozone Depletion: 2010, Global Ozone Research and Monitoring Project-Report No. 52, 516 pp., Geneva, Switzerland, 2011.

5. WMO/NASA (1988) International Ozone Trends Panel Report, https://www.esrl.noaa.gov/csd/assessments/ozone/1988/report.

6. WMO (2007) Scientific Assessment of Ozone Depletion: 2006, Global Ozone Research and Monitoring Project – Report No. 50, 572 pp., Geneva, 2007.

7. WMO (2014) Scientific Assessment of Ozone Depletion: Global Ozone Research and Monitoring Project Report, World Meteorological Organization,Geneva, Switzerland, p. 416, 2014.

8. Solomon, S., D. J. Ivy, D. Kinnison, M. J. Mills, R. R. Neely and A. Schmidt (2016) Emergence of healing in the Antarctic ozone layer. *Science* **353,** 269-274.

9. Ball, W. T., J. Alsing, D. J. Mortlock, E. V. Rozanov, F. Tummon and J. D. Haigh (2017) Reconciling differences in stratospheric ozone composites. *Atmospheric Chemistry and Physics* **17,** 12269-12302.

10. Bourassa, A. E., C. Z. Roth, D. J. Zawada, L. A. Rieger, C. A. McLinden and D. A. Degenstein (2018) Drift-corrected Odin-OSIRIS ozone product: algorithm and

updated stratospheric ozone trends. *Atmospheric Measurement Techniques* **11,** 489-498.

11. Frith, S. M., R. S. Stolarski, N. A. Kramarova and R. D. McPeters (2017) Estimating uncertainties in the SBUV Version 8.6 merged profile ozone data set. *Atmospheric Chemistry and Physics* **17,** 14695-14707.
12. Harris, N. R. P., B. Hassler, F. Tummon, G. E. Bodeker, D. Hubert, I. Petropavlovskikh, W. Steinbrecht, J. Anderson, P. K. Bhartia, C. D. Boone, A. Bourassa, S. M. Davis, D. Degenstein, A. Delcloo, S. M. Frith, L. Froidevaux, S. Godin-Beekmann, N. Jones, M. J. Kurylo, E. Kyrola, M. Laine, S. T. Leblanc, J. C. Lambert, B. Liley, E. Mahieu, A. Maycock, M. de Maziere, A. Parrish, R. Querel, K. H. Rosenlof, C. Roth, C. Sioris, J. Staehelin, R. S. Stolarski, R. Stubi, J. Tamminen, C. Vigouroux, K. A. Walker, H. J. Wang, J. Wild and J. M. Zawodny (2015) Past changes in the vertical distribution of ozone - Part 3: Analysis and interpretation of trends. *Atmospheric Chemistry and Physics* **15,** 9965-9982.
13. Steinbrecht, W., L. Froidevaux, R. Fuller, R. Wang, J. Anderson, C. Roth, A. Bourassa, D. Degenstein, R. Damadeo, J. Zawodny, S. Frith, R. McPeters, P. Bhartia, J. Wild, C. Long, S. Davis, K. Rosenlof, V. Sofieva, K. Walker, N. Rahpoe, A. Rozanov, M. Weber, A. Laeng, T. von Clarmann, G. Stiller, N. Kramarova, S. Godin-Beekmann, T. Leblanc, R. Querel, D. Swart, I. Boyd, K. Hocke, N. Kampfer, E. M. Barras, L. Moreira, G. Nedoluha, C. Vigouroux, T. Blumenstock, M. Schneider, O. Garcia, N. Jones, E. Mahieu, D. Smale, M. Kotkamp, J. Robinson, I. Petropavlovskikh, N. Harris, B. Hassler, D. Hubert and F. Tummon (2017) An update on ozone profile trends for the period 2000 to 2016. *Atmospheric Chemistry and Physics* **17,** 10675-10690.

14. Tummon, F., B. Hassler, N. R. P. Harris, J. Staehelin, W. Steinbrecht, J. Anderson, G. E. Bodeker, A. Bourassa, S. M. Davis, D. Degenstein, S. M. Frith, L. Froidevaux, E. Kyrola, M. Laine, C. Long, A. A. Penckwitt, C. E. Sioris, K. H. Rosenlof, C. Roth, H. J. Wang and J. Wild (2015) Intercomparison of vertically resolved merged satellite ozone data sets: interannual variability and long-term trends. *Atmospheric Chemistry and Physics* **15,** 3021-3043.

15. Gebhardt, C., A. Rozanov, R. Hommel, M. Weber, H. Bovensmann, J. P. Burrows, D. Degenstein, L. Froidevaux and A. M. Thompson (2014) Stratospheric ozone trends and variability as seen by SCIAMACHY from 2002 to 2012. *Atmospheric Chemistry and Physics* **14,** 831-846.

16. Kyrola, E., M. Laine, V. Sofieva, J. Tamminen, S. M. Paivarinta, S. Tukiainen, J. Zawodny and L. Thomason (2013) Combined SAGE II-GOMOS ozone profile data set for 1984-2011 and trend analysis of the vertical distribution of ozone. *Atmospheric Chemistry and Physics* **13,** 10645-10658.

17. Nair, P. J., L. Froidevaux, J. Kuttippurath, J. M. Zawodny, J. M. Russell, W. Steinbrecht, H. Claude, T. Leblanc, J. A. E. van Gijsel, B. Johnson, D. P. J. Swart, A. Thomas, R. Querel, R. Wang and J. Anderson (2015) Subtropical and midlatitude ozone trends in the stratosphere: Implications for recovery. *Journal of Geophysical Research-Atmospheres* **120,** 7247-7257.

18. Sioris, C. E., C. A. McLinden, V. E. Fioletov, C. Adams, J. M. Zawodny, A. E. Bourassa, C. Z. Roth and D. A. Degenstein (2014) Trend and variability in ozone in the tropical lower stratosphere over 2.5 solar cycles observed by SAGE II and OSIRIS. *Atmospheric Chemistry and Physics* **14,** 3479-3496.

19. Vigouroux, C., T. Blumenstock, M. Coffey, Q. Errera, O. Garcia, N. B. Jones, J. W. Hannigan, F. Hase, B. Liley, E. Mahieu, J. Mellqvist, J. Notholt, M. Palm, G.

Persson, M. Schneider, C. Servais, D. Smale, L. Tholix and M. De Maziere (2015) Trends of ozone total columns and vertical distribution from FTIR observations at eight NDACC stations around the globe. *Atmospheric Chemistry and Physics* **15,** 2915-2933.

20. Udelhofen, P. M., P. Gies, C. Roy and W. J. Randel (1999) Surface UV radiation over Australia, 1979–1992: Effects of ozone and cloud cover changes on variations of UV radiation. *Journal of Geophysical Research* **104,** 19135-19159.
21. Hofmann, D. J. and T. Deshler (1991) Evidence from balloon measurements for chemical depletion of stratospheric ozone in the Arctic winter of 1989-1990. *Nature* **349,** 300-305.
22. Whitehead, R. F., S. J. de Mora and S. Demers (2000) Enhanced UV radiation – a new problem for the marine environment. In: *The effects of UV radiation in the marine environment* (Ed. by S. de Mora, S. Demers & M. Vernet). *Cambridge University Press, Cambridge***,** pp.1-34.
23. Beardall, J. and J. A. Raven (2004) The potential effects of global climate change on microalgal photosynthesis, growth and ecology. *Phycologia* **43,** 26-40.
24. Key, T., A. McCarthy, D. A. Campbell, C. Six, S. Roy and Z. V. Finkel (2010) Cell size trade-offs govern light exploitation strategies in marine phytoplankton. *Environ. Microbiol.* **12,** 95-104.
25. Greenberg, B. M., V. Gaba, O. Canaani, S. Malkin, A. K. Matoo and M. Edelman (1989) Separate photosensitizers mediate degradation of the 32 kDa photosystem II reaction centre protein in the visible and UV spectral regions. *Proc. Natl. Acad. Sci. USA.* **86,** 6617-6620.
26. Raven, J. A. (1991) Responses of aquatic photosynthetic organisms to increased solar UVB. *J. Photochem. Photobiol. B Biol.* **9,** 239-244.

27. Obrien, P. A. and J. A. Houghton (1982) UV-induced DNA degradation in the cyanobacterium *Synechocystis* PCC 6308. *Photochem. Photobiol.* **36,** 417-422.

28. Rastogi, R. P., S. P. Singh, D. P. Hader and R. P. Sinha (2011) Ultraviolet-B induced DNA damage and photorepair in the cyanobacterium *Anabaena variabilis* PCC 7937. *Environ. Exp. Bot.* **74,** 280-288.

29. Sinha, R. P., A. Kumar, M. B. Tyagi and D.-P. Hader (2005) Ultraviolet-B-induced destruction of phycobiliproteins in cyanobacteria. *Physiol. Mol. Biol. Plants* **11,** 313-319.

30. Bebout, B. M. and F. Garciapichel (1995) UVB-induced vertical migrations of cyanobacteria in a microbial mat. *Appl. Environ. Microbiol.* **61,** 4215-4222.

31. Sinha, R. P., M. Klisch, E. W. Helbling and D. P. Hader (2001) Induction of mycosporine-like amino acids (MAAS) in cyanobacteria by solar ultraviolet-B radiation. *Journal of Photochemistry and Photobiology B-Biology* **60,** 129-135.

32. Campbell, D., V. Hurry, A. K. Clarke, P. Gustafsson and O. Gunnar (1998) Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. *Microbiol. Mol. Biol. Rev.* **62,** 667-83.

33. Sinha, R. P. and D. P. Hader (2002) UV-induced DNA damage and repair: a review. *Photochemical & Photobiological Sciences* **1,** 225-236.

34. Kirtman, B., S.B. Power, J.A. Adedoyin, G.J. Boer, R. Bojariu, I. Camilloni, F.J. Doblas-Reyes, A.M. Fiore, M. Kimoto, G.A. Meehl, M. Prather, A. Sarr, C. Schär, R. Sutton, G.J. van Oldenborgh, G. Vecchi and H.J. Wang (2013) Near-term Climate Change: Projections and Predictability. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and

P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

35. Briand, J. F., C. Leboulanger, J. F. Humbert, C. Bernard and P. Dufour (2004) *Cylindrospermopsis raciborskii* (Cyanobacteria) invasion at mid-latitudes: selection, wide tolerance or global warming? *J. Phycol.* **40,** 231-38.

36. Canale, R. P. and A. H. Vogel (1974) Effects of temperature on phytoplankton growth. *J. of Environ. Eng (American Society of Civil Engineers)* **100,** 229-241.

37. Everson, S., L. Fabbro, S. Kinnear and P. Wright (2011) Extreme differences in akinete, heterocyte and cylindrospermopsin concentrations with depth in a successive bloom involving *Aphanizomenon ovalisporum* (Forti) and *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju. *Harmful Algae* **10,** 265-276.

38. Peperzak, L. (2003) Climate change and harmful algal blooms in the North Sea. *Acta Oecol.* **24, Supplement 1,** 139-144.

39. Soares, M. C. S., M. Lurling and V. L. M. Huszar (2013) Growth and temperature-related phenotypic plasticity in the cyanobacterium *Cylindrospermopsis raciborskii*. *Phycol. Res.* **61,** 61-67.

40. Paerl, H. W. and J. Huisman (2009) Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environmental microbiology reports* **1,** 27-37.

41. Finkel, Z. V., J. Beardall, K. J. Flynn, A. Quigg, R. T. A. V. and J. A. Raven (2010) Phytoplankton in a changing world: cell size and elemental stoichiometry. *J. Plankton Res.* **32,** 119-137.

42. Gao, K., H. Yu and M. T. Brown (2007) Solar PAR and UV radiation affects the physiology and morphology of the cyanobacterium *Anabaena* sp. PCC 7120. *J. Photochem. Photobiol. B Biol.* **89,** 117-124.

43. Sobrino, C., M. L. Ward and P. J. Neale (2008) Acclimation to elevated carbon dioxide and ultraviolet radiation in the diatom *Thalassiosira pseudonana*: effects on growth, photosynthesis, and spectral sensitivity of photoinhibition. *Limonol. Oceanogr.* **53,** 494-505.

44. Gao, K. S., P. Li, T. R. Watanabe and E. W. Helbling (2008) Combined effects of ultraviolet radiation and temperature on morphology, photosynthesis, and DNA of *Arthrospira* (*Spirulina*) *platensis* (Cyanophyta). *J. Phycol.* **44,** 777-786.

45. Roos, J. C. and W. F. Vincent (1998) Temperature dependence of UV radiation effects on Antarctic cyanobacteria. *J. Phycol.* **34,** 118-125.

46. Beardall, J., S. Stojkovic and K. S. Gao (2014) Interactive effects of nutrient supply and other environmental factors on the sensitivity of marine primary producers to ultraviolet radiation: implications for the impacts of global change. *Aquatic Biology* **22,** 5-23.

47. Hader, D. P. and K. Gao (2015) Interactions of anthropogenic stress factors on marine phytoplankton. *Frontiers in Environmental Science* **3**.

48. Wong, C. Y., M. L. Teoh, S. M. Phang, P. E. Lim and J. Beardall (2015) Interactive effects of temperature and UV radiation on photosynthesis of *Chlorella* strains from polar, temperate and tropical environments: differential impacts on damage and repair. *Plos One* **10** (10):e0139469. doi:10.1371/journal.pone.0139469.

49. Ammar, M., K. Comte, T. D. C. Tran and M. El Bourl (2014) Initial growth phases of two bloom-forming cyanobacteria (*Cylindrospermopsis raciborskii* and *Planktothrix agardhii*) in monocultures and mixed cultures depending on light and nutrient conditions. *Annales De Limnologie-International Journal of Limnology* **50,** 231-240.

50. Burford, M. A., K. L. McNeale and F. J. McKenzie-Smith (2006) The role of nitrogen in promoting the toxic cyanophyte *Cylindrospermopsis raciborskii* in a subtropical water reservoir. *Freshwat. Biol.* **51,** 2143-2153.

51. Carneiro, R. L., M. E. V. dos Santos, A. B. F. Pacheco and S. Azevedo (2009) Effects of light intensity and light quality on growth and circadian rhythm of saxitoxins production in *Cylindrospermopsis raciborskii* (Cyanobacteria). *J. Plankton Res.* **31,** 481-488.

52. Dyble, J., P. A. Tester and R. W. Litaker (2006) Effects of light intensity on cylindrospermopsin production in the cyanobacterial HAB species *Cylindrospermopsis raciborskii*. *Afr. J. Mar. Sci.* **28,** 309-312.

53. Kehoe, M., K. R. O'Brien, A. Grinham and M. A. Burford (2015) Primary production of lake phytoplankton, dominated by the cyanobacterium *Cylindrospermopsis racibroskii* in response to irradiance and temperature. *Inland Waters* **5,** 93-100.

54. Pierangelini, M., S. Stojkovic, P. T. Orr and J. Beardall (2014b) Elevated CO_2 causes changes in the photosynthetic apparatus of a toxic cyanobacterium, *Cylindrospermopsis raciborskii*. *J. Plant Physiol.* **171,** 1091-1098.

55. Prentice, M. J., K. R. O'Brien, D. P. Hamilton and M. A. Burford (2015) High- and low-affinity phosphate uptake and its effect on phytoplankton dominance in a phosphate-depauperate lake. *Aquat. Microb. Ecol.* **75,** 139-153.

56. Recknagel, F., P. T. Orr and H. Q. Cao (2014) Inductive reasoning and forecasting of population dynamics of *Cylindrospermopsis raciborskii* in three sub-tropical reservoirs by evolutionary computation. *Harmful Algae* **31,** 26-34.

57. Saker, M. L. and B. A. Neilan (2001) Varied diazotrophies, morphologies, and toxicities of genetically similar isolates of *Cylindrospermopsis raciborskii*

(Nostocales, Cyanophyceae) from northern Australia. *Appl. Environ. Microbiol.* **67,** 1839-1845.

58. Stucken, K., U. John, A. Cembella, K. Soto-Liebe and M. Vasquez (2014) Impact of nitrogen sources on gene expression and toxin production in the diazotroph *Cylindrospermopsis raciborskii* CS-505 and non-diazotroph *Raphidiopsis brookii* D9. *Toxins* **6,** 1896-1915.

59. Wu, Z. X., J. Q. Shi and R. H. Li (2009) Comparative studies on photosynthesis and phosphate metabolism of *Cylindrospermopsis raciborskii* with *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*. *Harmful Algae* **8,** 910-915.

60. Wu, Z. X., B. Zeng, R. H. Li and L. R. Song (2012a) Combined effects of carbon and phosphorus levels on the invasive cyanobacterium, *Cylindrospermopsis raciborskii*. *Phycologia* **51,** 144-150.

61. Wu, Z. X., B. Zeng, R. H. Li and L. R. Song (2012b) Physiological regulation of *Cylindrospermopsis raciborskii* (Nostocales, Cyanobacteria) in response to inorganic phosphorus limitation. *Harmful Algae* **15,** 53-58.

62. Bolch, C. J. S. and S. I. Blackburn (1996) Isolation and purification of Australian isolates of the toxic cyanobacterium *Microcystis aeruginosa* Kutz. *J. Appl. Phycol.* **8,** 5-13.

63. Behrenfeld, M. J., J. W. Chapman, J. T. Hardy and H. Lee (1993) Is there a common response to ultraviolet-b radiation by marine-phytoplankton. *Mar. Ecol. Prog. Ser.* **102,** 59-68.

64. Hearud, P. and J. Beardall (2000) Changes in chlorophyll fluorescence during exposure of *Dunaliella tertiolecta* to UV radiation indicate a dynamic interaction between damage and repair processes. *Photosynthesis Res.* **63,** 123-134.

65. Jones, L. W. and B. Kok (1966) Photoinhibition of chloroplast reactions. I. Kinetics and action spectra. *Plant Physiol.* **41,** 1037-1043.

66. Quaite, F. E., B. M. Sutherland and J. C. Sutherland (1992) Action spectrum for DNA damage in alfalfa lowers predicted impact of ozone depletion. *Nature* **358,** 576-578.

67. Kok, B. (1956) On the inhibition of photosynthesis by intense light. *Biochim. Biophys. Acta* **21,** 234–244.

68. Sobrino, C., P. J. Neale and L. M. Lubian (2005) Interaction of UV radiation and inorganic carbon supply in the inhibition of photosynthesis: Spectral and temporal responses of two marine picoplankton. *Photochem. Photobiol.* **81,** 384-393.

69. Gomez, I., F. L. Figueroa, I. Sousa-Pinto, B. Vinegla, E. Perez-Rodriguez, C. Maestre, S. Coelho, A. Felga and R. Pereira (2001) Effects of UV radiation and temperature on photosynthesis as measured by PAM fluorescence in the red alga *Gelidium pulchellum* (Turner) Kutzing. *Bot. Mar.* **44,** 9-16.

70. van de Poll, W. H., A. Eggert, A. G. J. Buma and A. M. Breeman (2002) Temperature dependence of UV radiation effects in Arctic and temperate isolates of three red macrophytes. *Eur. J. Phycol.* **37,** 59-68.

71. Rautenberger, R. and K. Bischof (2006) Impact of temperature on UV-susceptibility of two *Ulva* (Chlorophyta) species from Antarctic and Subantarctic regions. *Polar Biol.* **29,** 988-996.

72. Elliott, J. A. (2010) The seasonal sensitivity of Cyanobacteria and other phytoplankton to changes in flushing rate and water temperature. *Global Change Biol.* **16,** 864-876.

73. Sobrino, C. and P. J. Neale (2007) Short-term and long-term effects of temperature on photosynthesis in the diatom *Thalassiosira pseudonana* under UVR exposures. *J. Phycol.* **43,** 426-436.

74. Aguilera, J., U. Karsten, H. Lippert, B. Vogele, E. Philipp, D. Hanelt and C. Wiencke (1999b) Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the Arctic. *Mar. Ecol. Prog. Ser.* **191,** 109-119.

75. Altamirano, M., A. Flores-Moya and F. L. Figueroa (2000b) Long-term effects of natural sunlight under various ultraviolet radiation conditions on growth and photosynthesis of intertidal Ulva rigida (Chlorophyceae) cultivated in situ. *Bot. Mar.* **43,** 119-126.

76. Dring, M. J., V. Makarov, E. Schoschina, M. Lorenz and K. Luning (1996a) Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of Laminaria (phaeophyta). *Mar. Biol.* **126,** 183-191.

77. Grobe, C. W. and T. M. Murphy (1997) Artificial ultraviolet-B radiation and cell expansion in the intertidal alga *Ulva expansa* (Setch.) S. and G. (Chlorophyta). *J. Exp. Mar. Biol. Ecol.* **217,** 209-223.

78. Pang, S. J., I. Gomez and K. Luning (2001) The red macroalga Delesseria sanguinea as a UVB-sensitive model organism: selective growth reduction by UVB in outdoor experiments and rapid recording of growth rate during and after UV pulses. *Eur. J. Phycol.* **36,** 207-216.

79. Van de Poll, W. H., A. Eggert, A. G. J. Buma and A. M. Breeman (2001) Effects of UVB-induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: Habitat-related differences in UV-B tolerance. *J. Phycol.* **37,** 30-37.

80. Kumar, A., M. B. Tyagi, N. Singh, R. Tyagi, P. N. Jha, R. P. Sinha and D. P. Häder (2003) Role of white light in reversing UV-B-mediated effects in the N2-fixing cyanobacterium *Anabaena* BT2. *Journal of photochemistry and photobiology. B, Biology* **71,** 35-42.

81. Xue, L. G., Y. Zhang, T. G. Zhang, L. Z. An and X. L. Wang (2005) Effects of enhanced ultraviolet-B radiation on algae and cyanobacteria. *Crit. Rev. Microbiol.* **31,** 79-89.

82. Giordanino, M. V. F., S. M. Strauch, V. E. Villafañe and E. W. Helbling (2011) Influence of temperature and UVR on photosynthesis and morphology of four species of cyanobacteria. *J. Photochem. Photobiol. B: Biol.* **103,** 68-77.

83. Quesada, A. and W. F. Vincent (1997) Strategies of adaptation by Antarctic cyanobacteria to ultraviolet radiation. *Eur. J. Phycol.* **32,** 335-342.

84. Jeffery, S. W., H. S. MacTavish, W. C. Dunlap, M. Vesk and K. Groenewoud (1999) Occurrence of UVA- and UVB-absorbing compounds in 152 species (206 strains) of marine microalgae. *Mar. Ecol. Prog. Ser.* **189,** 35-51.

85. McKenzie, R. L., P. J. Aucamp, A. F. Bais, L. O. Bjorn and M. Ilyas (2007) Changes in biologically-active ultraviolet radiation reaching the Earth's surface. *Photochemical & Photobiological Sciences* **6,** 218-231.

86. Thomas, C. D., A. Cameron, R. E. Green, M. Bakkenes, L. J. Beaumont, Y. C. Collingham, B. F. N. Erasmus, M. F. de Siqueira, A. Grainger, L. Hannah, L. Hughes, B. Huntley, A. S. van Jaarsveld, G. F. Midgley, L. Miles, M. A. Ortega-Huerta, A. T. Peterson, O. L. Phillips and S. E. Williams (2004) Extinction risk from climate change. *Nature* **427,** 145-148.

87. Molinero, J. C., F. Ibanez, S. Souissi, E. Buecher, S. Dallot and P. Nival (2008) Climate control on the long-term anomalous changes of zooplankton communities in the Northwestern Mediterranean. *Global Change Biol.* **14,** 11-26.

88. Paerl, H. W. and J. Huisman (2008) Climate - Blooms like it hot. *Science* **320,** 57-58.

List of Figures with legends

Fig. 1: Effects of UVB and temperature on cell concentrations in cultures of of *Anabaena circinalis* strains (a) CS537 25 ^{o}C (b) CS537 30 ^{o}C (c) CS541 25 ^{o}C and (d) CS541 30 ^{o}C.

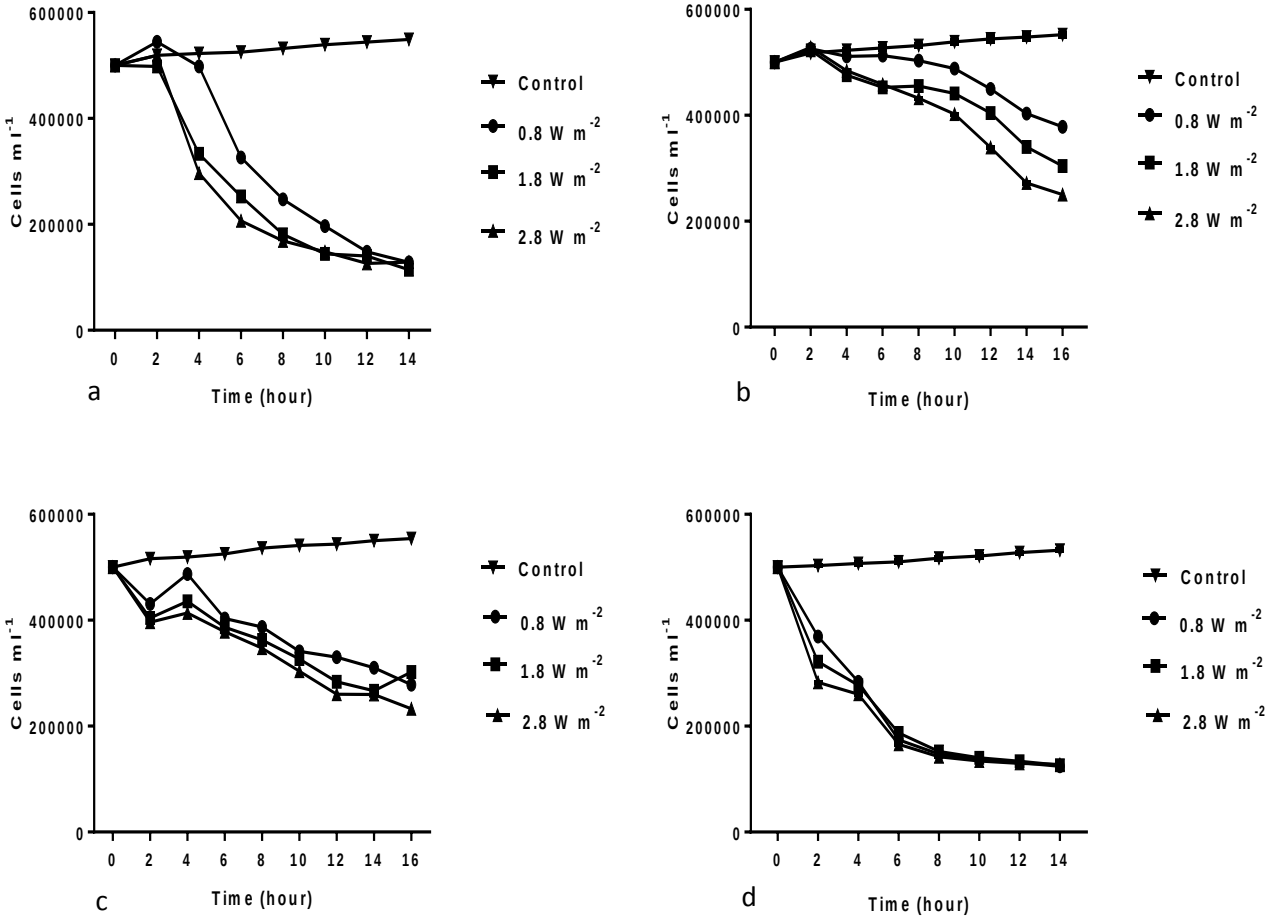
Fig. 2: Time course of changes in the fluorescence parameter F_v'/F_m' in *A. circinalis* strains (a) CS537 25 ^oC (b) CS537 30 ^oC (c) CS541 25 ^oC and (d) CS541 30 ^oC. The error bars represent the standard deviation from three independent replicate cultures.

Fig. 3: Photoinhibition rate (*k*) of *A. circinalis* strains CS537 (a) and CS541 (b) at 25 and 30 ^oC. The error bars represent the standard deviation from three independent replicate cultures. Different letters above the bars indicate means that are significantly different at $P<0.05$.

Fig. 4: Recovery of fluorescence parameters F_v'/F_m' after UVBR exposure in *A. circinalis* strains (a) CS537 25 ^oC (b) CS537 30 ^oC (c) CS541 25 ^oC and (d) CS541 30 ^oC. The error bars represent the standard deviation from three independent replicate cultures.

Fig. 5: Recovery rate of fluorescence parameters F_v'/F_m' after UVBR exposure in *A. circinalis* strains (a) CS537 and (b) CS541 at 25 and 30 ^oC. The error bars represent the standard deviation of three independent replicate cultures. Different letters above the bars indicate means that are significantly different at $P<0.05$.

Fig. 1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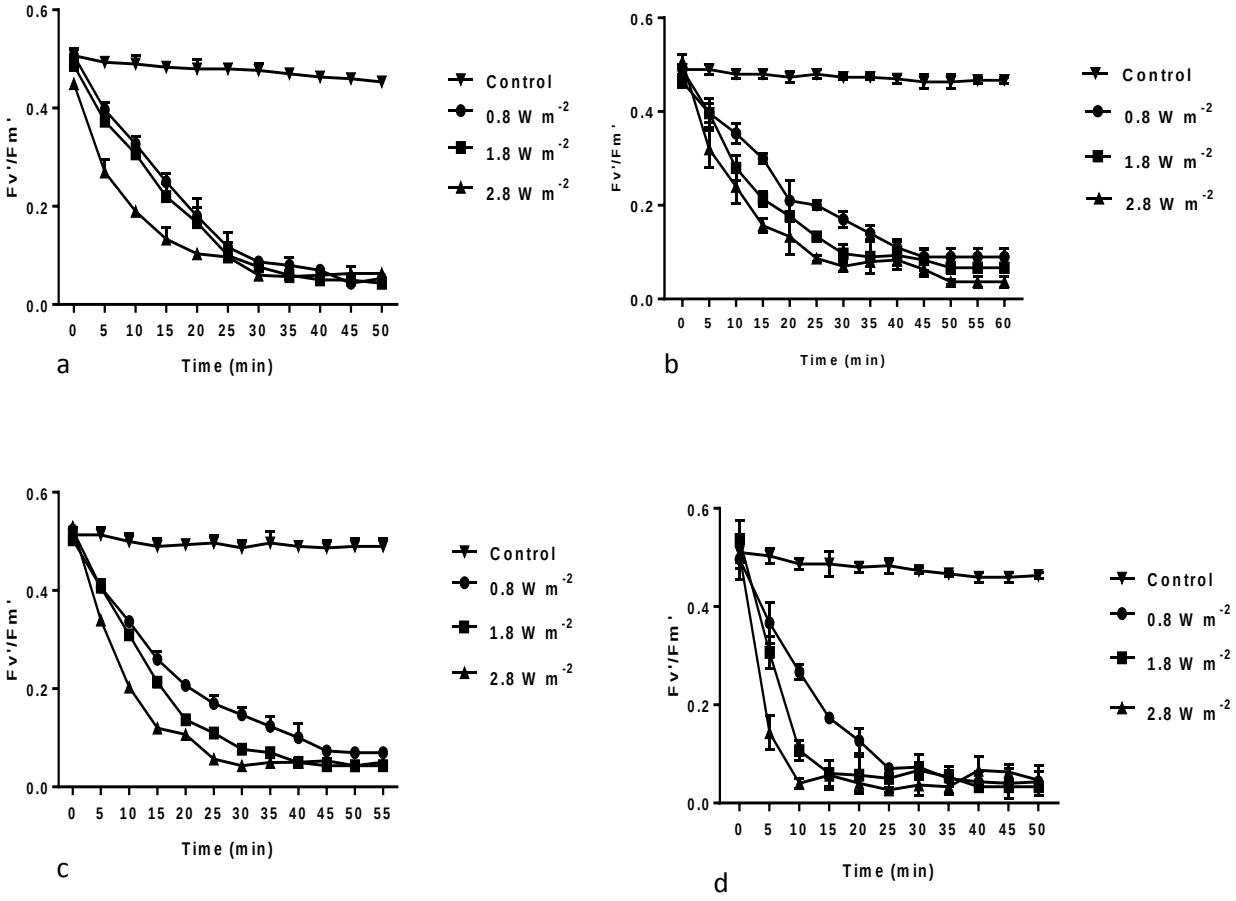

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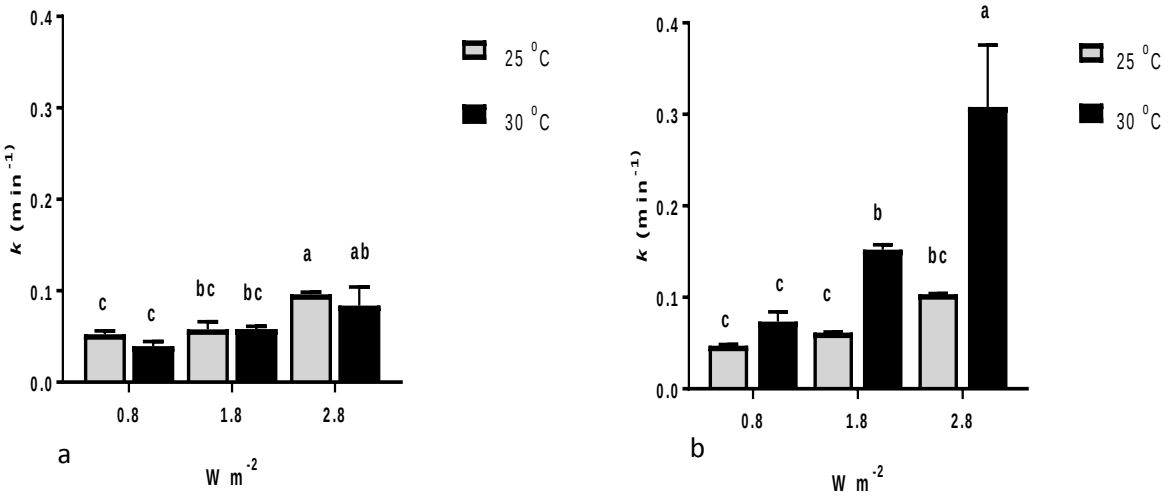
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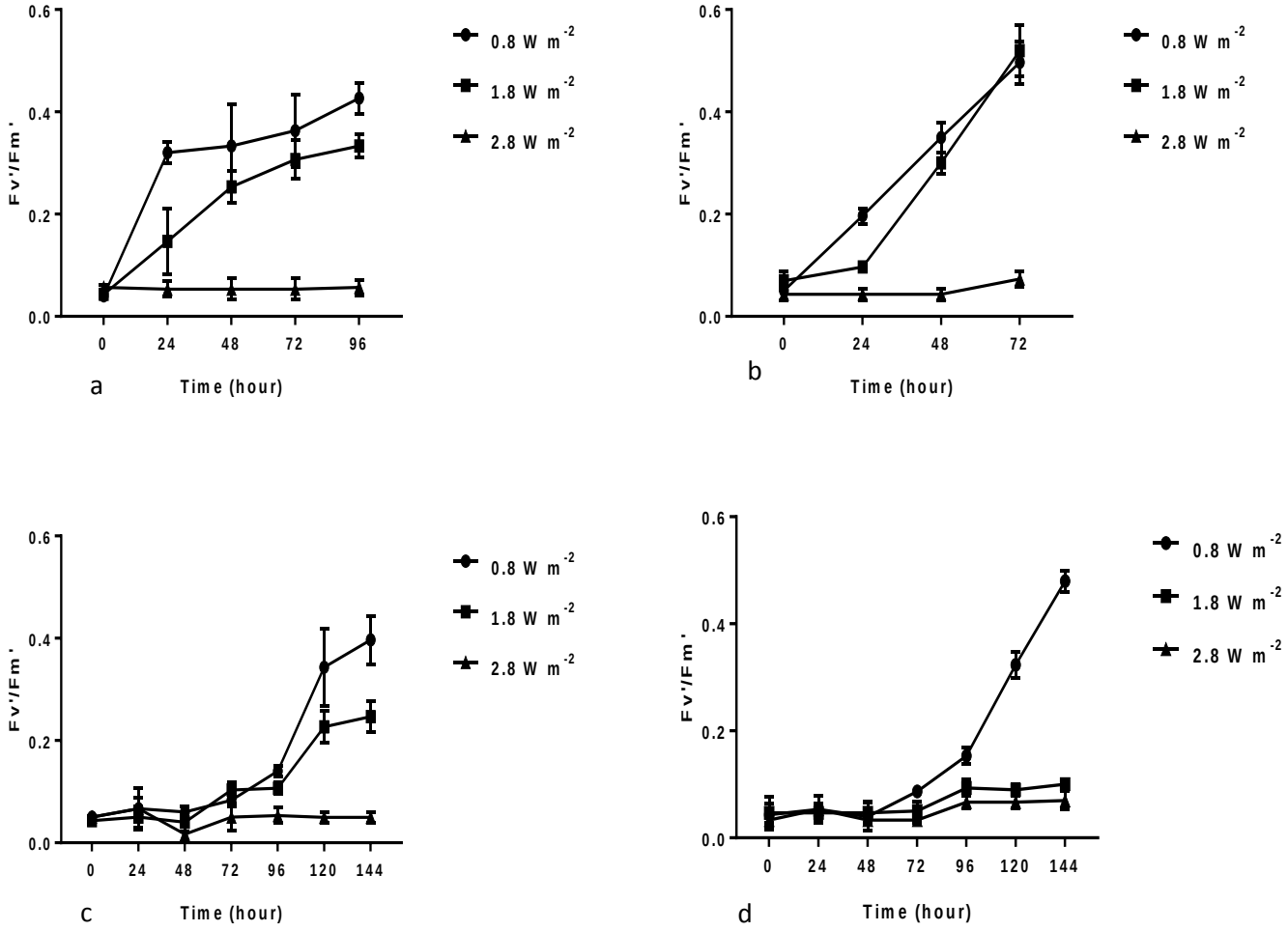
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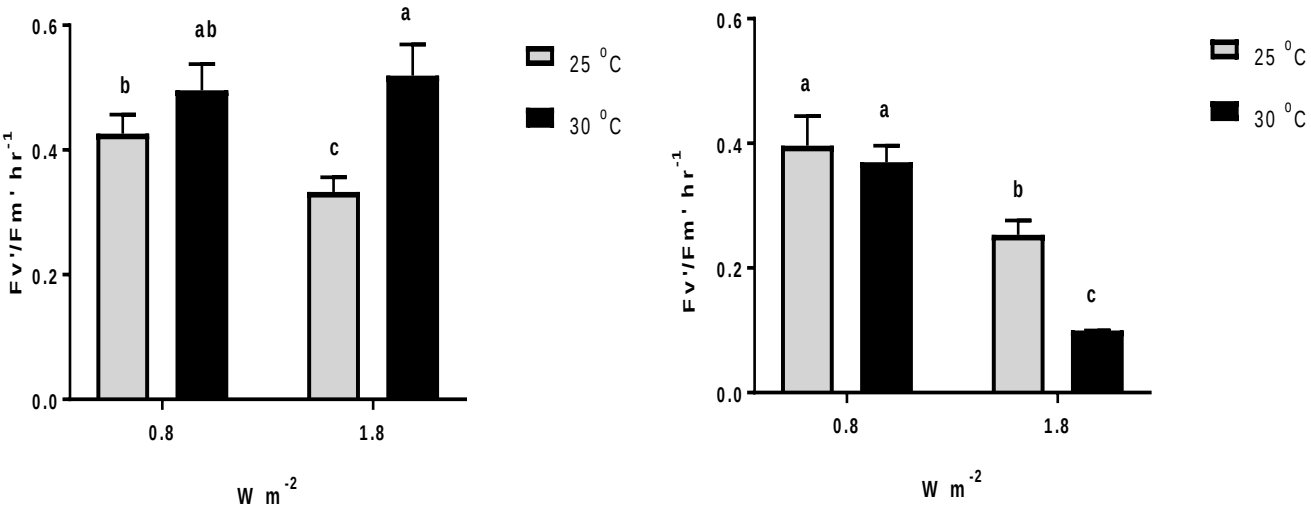
Fig. 5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Chapter 5. SUMMARY AND CONCLUSIONS

With the predicted changes to future climate, this study aimed to investigate the effects of some key environmental factors such as light and temperature on the growth and photosynthetic characteristics of toxic and non-toxic strains of two species of freshwater cyanobacteria. We also tested the susceptibility of photosynthesis to short-term UVB exposure at two growth temperatures and also studied the influence of temperature on survival and recovery of cells during and after medium term (up to 16 h) UVB exposure.

Differences in the growth and physiological characteristics of toxic and non-toxic strains of both species of cyanobacteria were observed. We found that the strains were able to survive at very low to very high light intensity which reflects on their ability to their dominate in Australian freshwater ecosystems (1, 2). In terms of growth rates, the toxic strains of both species showed saturation of growth at higher light intensities than the non-toxic strains (Chapter 2, Fig. 1). Although light is the most important factor for algal growth its role in toxin production might be species specific as toxin production has been shown in some species to vary between saturating, supra-saturating and limiting light intensities (3-6). We also found differences in physiological characteristics between species and strains but there were no consistent trend in relation to toxicity. Thus, the differences observed might be strain/species specific.

The results of studies on the sensitivity of cyanobacteria to temperature and acute UVB exposure, together with measurements of photoinhibition (*k*) and repair (*r*) constants and the cells' capacity to absorb UVB are presented and discussed in Chapter 3. There were differences in growth rates observed between both species under both temperature regimes. *A. circinalis* (Strain CS537) showed significantly higher growth rates (0.38 ± 0.01 day^{-1}) at 30 ^{o}C than at 25 ^{o}C (0.33 ± 0.01 day^{-1}) (t-test, $P = 0.0233$). In contrast the growth rates of *M. aeruginosa* (Strain CS558) were higher at 25 ^{o}C (0.41

± 0.02 day^{-1}) than at 30 ^{o}C (0.20 ± 0.02 day^{-1}) (t-test, *P* = 0.0001) suggesting that this species has a preference for the lower temperature.

We found that the magnitude of damage, *k* ,was higher than repair, *r,* (Chapter 3, Fig. 2) at both temperatures in the case of both species, leading to a net decline in effective quantum yield over time. However, the magnitude of *r* was higher at 30 ^{o}C than at 25 ^{o}C in both species (Two-way ANOVA, *P* = 0.0001). This is associated with the fact that elevated temperature increases the rate of enzymatic driven processes, which eventually increase the rate of repair of reaction centre proteins and Rubisco turnover and thereby reduce overall photochemical damage. We also found that the ratio of *r:k* (Chapter 3, Fig. 3) increased with temperature which is also consistent with the suggestion that the damage caused by UVR was repaired faster at higher temperature. The increase in repair rates and overall reduction in net damage to PSII at higher temperature in both cyanobacterial species examined suggests that these organisms, and indeed other algae and cyanobacteria, might be less affected by, or at least be able to recover quicker from, UVR exposure under future climate change scenarios (7, 8).

Many algae and cyanobacteria have the ability to produce UV absorbing compounds. In this study (Chapter 3) we found that both species absorbs UVA to some extent but the better ability of *A. circinalis* to absorb both UVA and UVB may be a reason for the observed lower damage rate of this species, compared to *M. aeruginosa,* after UVBR exposure.

Interactions between environmental factors in natural populations could be complicated due to the existence of different strains of the same species that might respond differently. To understand the intra-strain variability of *A. circinalis* strains CS537 and CS541, we investigated the damaging effects of UVB on cell numbers and population declines and impacts on quantum yield of PSII at two temperatures. We also examined

the effect of temperature on the capacity of cells to recover from such stress. Strain CS537 survived under UVB for an extended period at higher temperature compared to strain CS541 (Chapter 4, Fig. 1). This could be due to the ability to the presence of active photoreactive mechanisms to mitigate UVB related damage (9, 10). Overall, the magnitude of photoinhibition *k* was higher at 30 ^{o}C in the case of strain CS541. On the other hand, it was lower at 30 ^{o}C in the case of strain CS537 (Chapter 4, Fig. 3). We also found variations between temperature treatments for individual strains. Although damage in strain CS537 showed no statistical differences between temperatures, strain CS541 showed significantly different rates of damage at the different temperatures. The value of *k* for mid- and high-UVB levels were significantly higher ($P<0.05$) at 30 ^{o}C in comparison to those at 25 ^{o}C but for low-UVB exposure there were no statistical differences in *k* between temperatures.

In terms of recovery of the effective quantum yield of Photosystem II, F_v'/F_m', after UVB exposure, both strains showed little or no signs of recovery after exposure at high-UVB levels. After exposures to medium and low UVB, Strain CS537 recovered faster than strain CS541 at both temperatures. The recovery rate for both low- and mid-UVB levels was higher at 30 ^{o}C in the case of strain CS537 whereas it was higher at 25 ^{o}C in the case of strain CS541. The faster recovery of strain CS537 at elevated temperature supports the idea of the temperature dependence of repair process (11, 9). However, the lower recovery of strain CS541 at the higher temperature could be due to cumulative negative effects of temperature and UVB.

This project provides some insight into the effects of environmental factors on the response of cyanobacteria with future climate change. Previous work has suggested that increased temperatures in future might favour cyanobacterial growth (12). However, the differences in growth and physiological characteristics both within and between

strains/species makes it hard to draw any firm general conclusions about responses of cyanobacteria to global change. The relationship between light and toxicity also appears to be species/strain specific. Consequently, future studies should include a greater range of strains from more species.

This study also presented differences in growth and photosynthetic performances of *M. aeruginosa* and *A. circinalis* to UVB and temperature interactions, both between and within species. Although our results show, a positive relationship between repair rate and temperature, implying cells might be able to repair UVB-induced damage faster at higher temperatures, the interactive effects of temperature and UVR with other parameters such as nutrient availability, atmospheric CO_2 level, and high/low PAR level also need to be considered (13). Inclusion of these parameters in future studies will provide a better understanding of how cyanobacteria will respond to further changes in global climate. Testing of changes in cellular toxicity with predicted climate change would also provide insights into the toxicity of cyanobacterial blooms in future climates.

Further studies could also consider the competitive capacity of cyanobacteria and other algal species in the natural environment, both individually and together. Measurements of all possible physiological parameters would provide a better understanding of impacts of the future climate change on the dominance of algae/cyanobacteria in natural environment. Although competition experiments focussing on nutrients, temperature and the presence of organic and inorganic compounds have been carried out (14-17), more factors such as CO_2, high and low level of PAR should also be considered in future studies.

REFERENCES

1. Antenucci, J. P., A. Ghadouani, M. A. Burford and J. R. Romero (2005) The long-term effect of artificial destratification on phytoplankton species composition in a subtropical reservoir. *Freshwat. Biol.* **50,** 1081-93.

2. Pierangelini, M., S. Stojkovic, P. T. Orr and J. Beardall (2014a) Photosynthetic characteristics of two *Cylindrospermopsis raciborskii* stains differing in their toxicity. *J. Phycol.* **50,** 292-302.

3. Dyble, J., P. A. Tester and R. W. Litaker (2006) Effects of light intensity on cylindrospermopsin production in the cyanobacterial HAB species *Cylindrospermopsis raciborskii*. *Afr. J. Mar. Sci.* **28,** 309-312.

4. Rapala, J., K. Sivonen, R. Luukkainen and S. I. Niemela (1993) Anatoxin-a concentration in anabaena and aphanizomenon under different environmental-conditions and comparison of growth by toxic and nontoxic anabaena-strains - a laboratory study. *J. Appl. Phycol.* **5,** 581-591.

5. Sivonen, K., W. W. Carmichael, M. Namikoshi, K. L. Rinehart, A. M. Dahlem and S. I. Niemela (1990) Isolation and characterization of hepatotoxic microcystin homologs from the filamentous fresh-water cyanobacterium *Nostoc sp* strain-152. *Appl. Environ. Microbiol.* **56,** 2650-2657.

6. Watanabe, M. F. and S. Oishi (1985) Effects of environmental-factors on toxicity of a cyanobacterium (*Microcystis aeruginosa*) under culture conditions. *Appl. Environ. Microbiol.* **49,** 1342-1344.

7. Henley, W. J. (1993) On the measurement and interpretation of photosynthetic light-response curves in algae in the context of photoinhibition and diel changes. *J. Phycol.* **29,** 729-39.

8. Sancar, A. and G. B. Sancar (1988) DNA repair enzymes. *Annu. Rev. Biochem.* **57,** 29-67.

9. Giordanino, M. V. F., S. M. Strauch, V. E. Villafañe and E. W. Helbling (2011) Influence of temperature and UVR on photosynthesis and morphology of four species of cyanobacteria. *J. Photochem. Photobiol. B: Biol.* **103,** 68-77.

10. Xue, L. G., Y. Zhang, T. G. Zhang, L. Z. An and X. L. Wang (2005) Effects of enhanced ultraviolet-B radiation on algae and cyanobacteria. *Crit. Rev. Microbiol.* **31,** 79-89.

11. Beardall, J., S. Stojkovic and K. S. Gao (2014) Interactive effects of nutrient supply and other environmental factors on the sensitivity of marine primary producers to ultraviolet radiation: implications for the impacts of global change. *Aquatic Biology* **22,** 5-23.

12. Paerl, H. W. and J. Huisman (2009) Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environmental Microbiology reports* **1,** 27-37.

13. Henley, W. J. and J. Ramus (1989) Photoacclimation of *Ulva rotundata* (Chlorophyta) under natural irradience. *Mar. Biol.* **103,** 261-266.

14. Cai, H. J., X. X. Tang and P. Y. Zhang (2005) The effect of initial cell density on the interspecific competition between three species of red tide microalgae. *Acta Ecologica Sinica* **25,** 1331-1336.

15. Hu, S. H. and D. Y. Zhang (1993) The effects of initial population density on the competition for limiting nutrients in two freshwater algae. *Oecologia* **96,** 569-574.

16. Zhai, C., S. Song, S. Zou, C. Liu and Y. Xue (2013) The mechanism of competition between two bloom-forming *Microcystis* species. *Freshwat. Biol.* **58,** 1831-1839.

17. Zhang, P., C. M. Zhai, X. X. Wang, C. H. Liu, J. H. Jiang and Y. R. Xue (2013) Growth competition between *Microcystis aeruginosa* and *Quadrigula chodatii* under controlled conditions. *J. Appl. Phycol.* **25,** 555-565.