Effects of rising CO₂ on the harmful cyanobacterium Microcystis

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List of frequently used abbreviations, symbols and genes
2PG  2-phosphoglycerate
3PG  3-phosphoglycerate
ATP  adenosine triphosphate
BCT1 ATP-dependent bicarbonate uptake system (high affinity, low flux)
bicA  gene encoding sodium-dependent bicarbonate uptake system BicA
BicA  sodium-dependent bicarbonate uptake system (low affinity, high flux)
CA  carbonic anhydrase
CCM  CO₂-concentrating mechanism
ccmR  gene encoding transcriptional regulator CcmR
ccmR2  gene encoding transcriptional regulator CcmR2
cDNA  complementary DNA, synthesized from messenger RNA
Ci  inorganic carbon
Chl a  chlorophyll a (pigment)
chpY (=cupA)  gene encoding CO₂ hydration subunit of CO₂ uptake system NDH-I₃
chpX (=cupB)  gene encoding CO₂ hydration subunit of CO₂ uptake system NDH-I₄
cmpA  gene encoding bicarbonate-binding subunit of uptake system BCT1
CO₂(aq)  carbon dioxide dissolved in water
CO₃²⁻  carbonate
DIC  dissolved inorganic carbon
DOC  dissolved organic carbon
HCO₃⁻  bicarbonate
gCO₂  CO₂ gas influx
gDNA  genomic DNA
ftv1-4  genes encoding flavodiiron proteins, involved in oxidative stress protection
I / Iᵢᵣ / Iᵢᵗᵣᵢᵣ  irradiance / incident irradiance / irradiance penetrating through vessel
isiA  gene encoding iron stress-induced protein
K₀.₅  half-saturation constant
Kₛ  solubility constant of CO₂ gas in water
MC  microcystin (hepatotoxin)
mcyB  microcystin synthetase gene
NADPH  nicotinamide adenine dinucleotide phosphate
NDH-I₃  CO₂ uptake system (high affinity, low flux)
NDH-I₄  CO₂ uptake system (low affinity, high flux)
nhaS1-6  genes encoding sodium/proton antiporters
PAR  photosynthetically active radiation, spectral range used for photosynthesis
pCO₂  partial pressure of CO₂
List of abbreviations, symbols and genes

PKS  polyketide synthetase
ppm  parts per million
PSI  photosystem I
PSII photosystem II
rbcX  gene encoding RuBisCO chaperone
ROS  reactive oxygen species, chemically reactive molecules containing oxygen
RT-qPCR reverse transcription quantitative polymerase chain reaction
RuBisCO ribulose-1,5-bisphosphate carboxylase/oxygenase
sbtA  gene encoding bicarbonate uptake system SbtA (high affinity, low flux)
SbtA sodium-dependent bicarbonate uptake system (high affinity, low flux)
sbtB  gene found downstream of sbtA, associated with sbtA
v  gas transfer velocity (piston velocity) across the air-water interface
Effects of rising CO₂ on the harmful cyanobacterium *Microcystis*

Harmful cyanobacteria (‘blue-green algae’) are notorious for causing worldwide ecological and economical problems in eutrophic lakes and reservoirs, where they can produce dense and often toxic blooms. Climate change is foreseen to have large effects on these photosynthetic microorganisms. Yet, while several studies have investigated effects of global warming on harmful cyanobacteria, the implications of rising CO₂ have received relatively little attention. Cyanobacteria are often assumed to be favored at low inorganic carbon conditions, because of the presence of an effective CO₂-concentrating mechanism (CCM) to fix CO₂. But how will they perform at elevated CO₂ levels? This thesis investigates the impact of elevated CO₂ on various strains of the ubiquitous harmful cyanobacterium *Microcystis aeruginosa*.

The following questions are addressed:

1) How variable are the CCMs within the genus *Microcystis*? (*Chapter 2*)
2) What are the adaptations of *Microcystis* to elevated CO₂? (*Chapter 3*)
3) What are the similarities and differences in CCM gene expression (1) among *Microcystis* strains, and (2) between *Microcystis* and other cyanobacteria? (*Chapters 2 and 4*)
4) How are the *Microcystis* CCM genes regulated *in situ*? (*Chapter 6*)
5) Can genetic variability of the *Microcystis* CCM cause strain-specific differences in growth rate at elevated CO₂ concentrations? (*Chapters 2 and 4*)
6) Does rising CO₂ affect the competition between different *Microcystis* strains, and if so, which strains will benefit most? (*Chapter 5*)
7) What are the effects of rising CO₂ on other harmful cyanobacteria? (*Chapter 7*)
8) Will rising CO₂ concentrations stimulate cyanobacterial blooms and make them more toxic? (*Chapters 2, 3, 5 and 7*)
9) Is potassium ion addition an effective method to combat harmful cyanobacterial blooms? (*Chapter 8*)

To investigate the first question, 20 *Microcystis* strains from different continents were studied at the gene level. Cyanobacteria often use a combination of CO₂ and bicarbonate uptake systems to import inorganic carbon (Cᵢ). Genes encoding the two CO₂ uptake systems, the ATP-dependent bicarbonate transporter BCT1, the CO₂-fixing enzyme RuBisCO and carboxysomes (compartments containing RuBisCO) were detected in all 20 *Microcystis* strains. Eight of the analyzed strains also contain the genes *bicA* and *sbtA*, encoding the sodium-dependent bicarbonate uptake systems BicA and SbtA, respectively. BicA has a low affinity for bicarbonate
and high flux rate, whereas SbtA has a high affinity and low flux rate. Affinity refers to the effectiveness of bicarbonate uptake at low bicarbonate concentrations, whereas the flux rate refers to the bicarbonate uptake rate at high bicarbonate concentrations. A unique feature of these *Microcystis* strains is that the genes *bicA* and *sbtA* are present in one operon and are co-transcribed. In contrast to these C$_i$ uptake generalists, 12 of the 20 analyzed *Microcystis* strains lack either the *bicA* or *sbtA* gene. The results show that *Microcystis* strains have adapted differently to the wide natural variation in CO$_2$ concentrations.

The second question was answered in chemostat experiments with *Microcystis* PCC 7806. Changes in the transcriptome (expression of all genes in the genome) were monitored from 45 minutes up to 2 weeks after increasing the CO$_2$ concentration. Surprisingly, elevated CO$_2$ affected the expression of only a small number of genes. The bicarbonate uptake genes were downregulated at elevated CO$_2$. Other regulated genes were involved in the stress response of the cells, control of the cellular C/N ratio, and the production of two weakly characterized polyketides. Expression of genes encoding the CO$_2$ uptake systems, carboxysome, RuBisCO, photosystems, C metabolism and microcystin synthetases did not respond significantly to elevated CO$_2$.

The third question was answered by exposing batch cultures of six different *Microcystis* strains to elevated CO$_2$. The high-affinity gene *cmpA* (encoding a subunit of the bicarbonate uptake system BCT1) was downregulated at elevated CO$_2$ in all strains. Most strains also downregulated *bicA* and *sbtA* at elevated CO$_2$, but two strains showed constitutive expression of these bicarbonate uptake genes. The high-flux BicA uptake system remained active at high CO$_2$ levels in all strains containing the *bicA* gene. Interestingly, expression of the high- and low-affinity CO$_2$ uptake genes of *Microcystis* was not affected by elevated CO$_2$, which deviates from most other cyanobacterial species that downregulate the high-affinity CO$_2$ uptake genes. The carboxysome and RuBisCO genes were also constitutively expressed in all *Microcystis* strains. We discovered a new CCM transcriptional regulator gene (*ccmR2*), located upstream of the *bicA*-sbtA operon. Both *ccmR2* and the *bicA*-sbtA operon are so far unique for *Microcystis*.

The fourth question was answered with an *in situ* study at Lake Kennemermeer (the Netherlands) of a cyanobacterial bloom that contained *Microcystis*. The lake showed large diel fluctuations in bicarbonate, pH and dissolved oxygen as a consequence of the photosynthetic activity of the bloom. Expression of the bicarbonate uptake genes of *Microcystis* was tuned to the diel variation in bicarbonate concentration. In contrast, expression of the CO$_2$ uptake genes was constitutive, and expression of the RuBisCO and carboxysomal genes was slightly increased during nighttime.
To address the fifth question, a series of laboratory experiments were carried out in batch culture at different CO₂ concentrations. The results showed that strains with the high-affinity gene *sbtA* perform better at low Cᵢ concentrations, strains with the high-flux gene *bicA* perform better at high Cᵢ concentrations, and *bicA+sbtA* strains containing both genes perform well across the entire range of Cᵢ conditions investigated.

The sixth question was addressed by investigating mixtures with multiple *Microcystis* strains in laboratory competition experiments and a lake study. The competition experiments and lake study both showed that strains with the high-flux gene *bicA* have a selective advantage at elevated Cᵢ levels. These results provide laboratory and field evidence that changes in Cᵢ availability induce rapid adaptive changes in the genotype composition of harmful cyanobacterial blooms. Hence, future cyanobacterial blooms may have a genetic composition that differs from contemporary blooms.

The seventh question was answered by analyzing recently sequenced genomes of other harmful cyanobacteria, including *Anabaena*, *Aphanizomenon* and *Planktothrix* strains. These cyanobacteria also showed intraspecific variation in the presence of the *bicA* and *sbtA* genes, similar to *Microcystis*, suggesting that they are well adapted to a wide range of CO₂ conditions. However, in *Anabaena*, *Aphanizomenon* and *Planktothrix*, *bicA* and *sbtA* are not organized in one operon, and in some strains both *bicA* and *sbtA* are absent. Presumably, these harmful cyanobacteria display a similar phenotypic variation as *Microcystis*, with a selective advantage for strains with the high-affinity uptake systems SbtA and/or Bct1 at low Cᵢ conditions and a selective advantage for strains with the high-flux uptake system BicA at high Cᵢ conditions.

To address the eighth question, it was shown that there was no direct connection between the presence of the Cᵢ uptake genes *bicA* or *sbtA* and the microcystin synthetase genes. In chemostat experiments with *Microcystis* PCC 7806, elevated CO₂ levels led to a shift from carbon- to light-limited conditions. The strain contained ~2.5 times more unbound microcystins per cell at elevated CO₂, indicating that the cells can become more toxic at elevated CO₂ levels. Biomass of this strain increased strongly at elevated CO₂, suggesting that cyanobacterial blooms will intensify in eutrophic lakes. Furthermore, the dry weight of the cells was reduced twofold, indicating that elevated CO₂ can promote buoyancy of the cells, and thus scum layer formation in lakes. Hence, elevated CO₂ is foreseen to worsen the problems with *Microcystis* blooms in eutrophic lakes.

The ninth question was answered by investigating the potassium ion sensitivity of selected laboratory *Microcystis* strains. Strain PCC 7806, originating from brackish water, was not affected by the increased potassium ion concentration, while the growth of two freshwater *Microcystis* strains was strongly reduced. The potassium ion sensitivity of the freshwater strains was linked to the absence of specific salt tolerance genes. These results show that the salt
tolerance and potassium sensitivity of *Microcystis* differ between strains. Hence, on the short run, potassium ion addition might be a successful remediation strategy to combat *Microcystis* blooms in freshwater lakes, but over time more tolerant *Microcystis* strains or other cyanobacteria are likely to become dominant.

This thesis contributes to a better understanding of how harmful cyanobacteria respond to climate change. The results show how cyanobacteria subtly adjust their cells at the molecular and physiological level to changes in $C_i$ availability. Furthermore, the results demonstrate how genetic diversity in the $C_i$ uptake systems provide cyanobacteria with the potential for rapid microevolutionary adaptation to changes in $CO_2$ conditions, with a selective advantage for strains with the high-flux bicarbonate uptake gene *bicA* at elevated $CO_2$ levels. Hence, one of the key lessons of this work is that future studies of climate change effects should keep in mind the large genetic and physiological variation within species. In total, the results indicate that a further rise of the atmospheric $CO_2$ concentration is likely to increase the frequency and intensity of cyanobacterial blooms in eutrophic waters, and possibly may also increase their toxicity. The predicted intensification of cyanobacterial blooms should be countered by the reduction of $CO_2$ emissions and the development of effective methods to combat and prevent harmful cyanobacterial blooms.
Samenvatting
Effecten van stijgend CO₂ op de schadelijke cyanobacterie *Microcystis*

Schadelijke cyanobacteriën (‘blauwalgen’) zijn bekende veroorzakers van wereldwijde ecologische en economische problemen in oppervlaktewateren zoals voedselrijke meren, waar ze dichte en vaak toxische bloeien kunnen vormen. Het is voorzien dat klimaatverandering grote effecten zal hebben op deze fotosynthetische microorganismen. Terwijl verschillende studies de effecten van stijgende temperaturen hebben onderzocht, hebben de mogelijke gevolgen van stijgende CO₂-concentraties tot nog toe weinig aandacht gekregen. Vaak wordt aangenomen dat cyanobacteriën voordeel hebben bij lage CO₂-condities, door de aanwezigheid van een effectief CO₂-concentreermechanisme (CCM) om CO₂ te fixeren. Maar hoe presteren ze bij verhoogde CO₂-niveaus? Dit proefschrift onderzoekt de effecten van stijgende CO₂-concentraties op verschillende stammen van de wijdverspreide schadelijke cyanobacterie *Microcystis aeruginosa*.

De volgende vragen worden behandeld:

1) Hoe variabel zijn de CCM’s binnen het genus *Microcystis*? (Hoofdstuk 2)

2) Wat zijn de aanpassingen van *Microcystis* bij verhoogde CO₂-concentraties? (Hoofdstuk 3)

3) Wat zijn de overeenkomsten en verschillen in CCM-genexpressie (1) tussen *Microcystis* stammen onderling, en (2) tussen *Microcystis* en andere cyanobacteriën? (Hoofdstukken 2 en 4)

4) Hoe worden de CCM-genen van *Microcystis* gereguleerd? (Hoofdstuk 6)

5) Kan genetische variatie in de CCM’s leiden tot verschillen in groeisnelheid van *Microcystis* stammen bij verhoogde CO₂-concentraties? (Hoofdstukken 2 en 4)

6) Heeft stijgende CO₂ invloed op de competitie tussen verschillende *Microcystis* stammen, en zo ja, welke stammen hebben het meeste voordeel? (Hoofdstuk 5)

7) Wat zijn de effecten van stijgend CO₂ op andere schadelijke cyanobacteriën? (Hoofdstuk 7)

8) Zullen stijgende CO₂-concentraties bloeien van schadelijke cyanobacteriën stimuleren en hun toxiciteit verhogen? (Hoofdstukken 2, 3, 5 en 7)

9) Is toevoeging van kaliumionen een effectieve methode om schadelijke cyanobacteriën te bestrijden? (Hoofdstuk 8)

Om de eerste vraag te onderzoeken zijn 20 *Microcystis* stammen uit verschillende delen van de wereld op genniveau onderzocht. Cyanobacteriën gebruiken vaak een combinatie van CO₂- en bicarbonaat-opnamesystemen om anorganische koolstof (Cₐ) te importeren in hun cellen. Alle
20 stammen bevatten genen die coderen voor de twee CO₂-opnamesystemen, de ATP-afhankelijke bicarbonaattransporter BCT1, het CO₂-fixatie-enzym RuBisCO en carboxyzomen (compartimenten die RuBisCO bevatten). Acht van de onderzochte stammen hadden ook de genen bicA en sbtA, die coderen voor de natrium-afhankelijke bicarbonaatopnamesystemen BicA en SbtA. BicA heeft een lage affiniteit voor bicarbonaat en een hoge opnamesnelheid, terwijl SbtA een hoge affiniteit heeft en een lage opnamesnelheid. Met affiniteit wordt hier bedoeld de effectiviteit van bicarbonaatopname bij lage bicarbonaatconcentraties, terwijl de opnamesnelheid refereert naar de bicarbonaatopname bij hoge bicarbonaatconcentraties. Een unieke eigenschap van deze Microcystis stammen is dat de genen bicA en sbtA zich bevinden op hetzelfde operon en dus gezamenlijk worden getranscribeerd (co-transcriptie). In tegenstelling tot deze Ci-opnamegeneralisten, misten 12 van de 20 Microcystis stammen het bicA of sbtA gen. De resultaten laten zien dat Microcystis stammen zich op verschillende wijze hebben aangepast aan de natuurlijke variatie in CO₂-concentraties.

De tweede vraag is beantwoord met behulp van chemostaatexperimenten met Microcystis PCC 7806. Veranderingen in het transcriptoom (expressie van alle genen in het genoom) van deze stam werden bekeken van 45 minuten tot 2 weken na het verhogen van de CO₂-concentratie. De verhoogde CO₂-concentratie had slechts effect op de expressie van een verrassend klein aantal genen. De bicarbonaatopnamesystemen waren omlaag gereguleerd bij verhoogde CO₂. Andere gereguleerde genen waren betrokken bij de stress respons van cellen, controle van de cellulaire C/N ratio en de productie van twee nader te karakteriseren polyketides. De verhoogde CO₂-concentratie leidde niet tot significante veranderingen in genexpressie van de CO₂-opnamesystemen, het carboxysoom, RuBisCO, de fotosystemen, het C metabolisme en de microcystine synthetases.

De vierde vraag is beantwoord met een veldstudie in het Kennemermeer (Nederland) van een cyanobacteriële bloei met *Microcystis*. Het meer vertoonde grote dagelijkse schommelingen in bicarbonaat, pH en opgelost zuurstof als een gevolg van de fotosynthese-activiteit van de bloei. Expressie van de bicarbonaatopnamegenen van *Microcystis* was afgestemd op de dagelijkse variatie in de bicarbonaatconcentratie. Expressie van de CO₂-opnamegenen vertoonde echter geen dagelijkse variatie en expressie van de RuBisCO- en carboxysoomgenen was iets verhoogd tijdens de nacht.

Om de vijfde vraag te onderzoeken zijn een serie van laboratoriumexperimenten uitgevoerd in batch-culturen bij verschillende CO₂-concentraties. De resultaten laten zien dat stammen met het hoge-affiniteit-gen *sbtA* beter presteren bij lage Cᵣ-concentraties, stammen met het hogenesnelheids-gen *bicA* beter presteren bij hoge Cᵣ-concentraties, terwijl stammen die beschikken over beide genen goed presteren over het hele bereik van onderzochte Cᵣ-condities.

De zesde vraag is onderzocht door mengsels van verschillende *Microcystis* stammen te bestuderen in laboratorium competitie-experimenten en in een veldstudie van een cyanobacteriële bloei in een eutroof meer. De competitie-experimenten en de veldstudie laten beide zien dat stammen met het hogenesnelheids-gen *bicA* een selectief voordeel hebben bij verhoogde Cᵣ-concentraties. De resultaten leveren zowel experimenteel als observationeel bewijs dat veranderingen in de Cᵣ beschikbaarheid leiden tot snelle adaptieve veranderingen in de genotypesamenstelling van schadelijke cyanobacteriën. Dit suggereert dat cyanobacteriële bloeien in de toekomst waarschijnlijk een genetische samenstelling zullen hebben die verschilt van de huidige bloeien.


De achtste vraag is beantwoord door aan te tonen dat er geen direct verband is tussen de aanwezigheid van de Cᵣ-opnamegenen *bicA* of *sbtA* en de microcystine-synthetase-genen. In chemostaatexperimenten met *Microcystis* PCC 7806 leidde verhoogde CO₂-concentraties tot een verschuiving van koolstof-gelimiteerde naar licht-gelimiteerde condities. De stam bevatte ~2.5 keer meer ongebonden microcystine per cel bij verhoogde CO₂-concentraties, wat
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aangeeft dat de cellen toxischer kunnen worden bij stijging van het CO₂-niveau. De biomassa van de stam was sterk toegenomen bij verhoogde CO₂-concentraties, wat suggereert dat cyanobacteriële bloeien in eutrofe meren intenser worden. Daarnaast was het drooggewicht van de cellen gehalveerd, wat doet vermoeden dat verhoogde CO₂-concentraties het drijfvermogen van cellen kan bevorderen en daarmee de vorming van drijflagen in meren kan stimuleren. Deze resultaten geven aan dat stijgende CO₂-concentraties de problemen met Microcystis in eutrofe meren waarschijnlijk zullen verergeren.

De negende vraag is beantwoord door onderzoek naar de kaliumgevoeligheid van geselecteerde Microcystis stammen. Stam PCC 7806, die oorspronkelijk is geïsoleerd uit brak water, werd niet beïnvloed door verhoogde kalium concentraties, terwijl de groei van twee zoetwater Microcystis stammen sterk gereduceerd werd. De kaliumgevoeligheid van de zoetwater stammen lijkt samen te hangen met de afwezigheid van specifieke genen betrokken bij zoutwatertolerantie. Dit laat zien dat Microcystis stammen verschillen in hun zoutwatertolerantie en kaliumgevoeligheid. Het gevolg is dat kaliumtoevoeging op korte termijn een succesvolle beheersmethode kan zijn om Microcystis bloeien in zoet water te bestrijden, maar dat op de langere termijn waarschijnlijk minder kaliumgevoelige Microcystis stammen of andere tolerante cyanobacteriën de dominantie zullen overnemen.

Dit proefschrift draagt bij aan een beter begrip van hoe schadelijke cyanobacteriën reageren op klimaatverandering. De resultaten laten zien hoe cyanobacteriën hun cel op moleculair en fysiologisch niveau op subtiele wijze kunnen aanpassen aan de C₄ beschikbaarheid. Ook laten de resultaten zien dat de genetische diversiteit in C₄-opnamesystemen van cyanobacteriën kan leiden tot een snelle micro-evolutie, waarbij de genotype samenstelling van de bloei zich aanpast aan veranderingen in de CO₂-concentratie. Hierbij hebben stammen met het hoge snelheids-bicarbonaatopnamegen bicA een selectief voordeel bij hoge C₄-concentraties. Een van de belangrijkste lessen van dit werk is daarom dat toekomstige studies naar de effecten van klimaatsverandering attent moeten zijn op de genetische en fysiologische variatie binnen soorten. Alles samenvattend geven de resultaten aan dat verdere stijging van de CO₂-concentratie waarschijnlijk zal leiden tot een toename van de frequentie en intensiteit van cyanobacteriële bloeien in eutrofe wateren, en mogelijk ook de toxiciteit van deze bloeien zal verhogen. De voorspelde toename van cyanobacteriële bloeien zou kunnen worden beperkt door reductie van de CO₂-uitstoot en door de verdere ontwikkeling van effectieve methoden om schadelijke cyanobacteriën te bestrijden.
Acknowledgements
Luctor et Emergo
Ik worstel en kom boven

After several years of hard work it is finally done! Time went by very fast and so much changed since I started working here at the Science Park of Amsterdam in February 2011. I really enjoyed being part of the Aquatic Microbiology (AMB) group and I learned a lot. Yet, I could not have done the work presented in my thesis without the help of several other people. Also many people made my stay at the University of Amsterdam more pleasant, or supported me outside working hours.

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Curriculum Vitae
Giovanni Sandrini was born on the 16th of June 1986, in Terneuzen, the Netherlands. After completing secondary school at the Zeldenrust-Steelant College in Terneuzen in 2004, he completed the bachelor and master program of Life Science & Technology organized by a collaboration between Leiden University and Delft University of Technology. During his bachelor internship he worked on the isolation and genetic analysis of novel streptomycetes from soil samples, in the Microbial Development group of prof. dr. Gilles van Wezel at Leiden University. During his masters he did his industrial internship at the Netherlands Organisation for Applied Scientific Research (TNO) in Delft, in the group of dr. Harald J. Ruijssenaars, where he used genetic engineering on the solvent tolerant soil bacterium *Pseudomonas putida* S12 to allow it to grow on lignocellulose hydrolysate. He did his master research internship at the nidrovirus group of prof. dr. Eric J. Snijder, at the Leiden University Medical Center (LUMC), under supervision of dr. Clara C. Poshuma and dr. Sjoerd van den Worm, where he investigated the presence of an RNA proofreading mechanism in coronaviruses. He graduated in 2010, with the major Functional Genomics. Early 2011, he started his PhD in the group of Aquatic Microbiology of prof. dr. Jef Huisman at the University of Amsterdam, under supervision of dr. Hans C.P. Matthijs. The work of his PhD is described in this thesis and resulted in several publications. He also presented the results of this thesis at national and international conferences.
Publications


Sandrini G, Ji X, Verspagen JMH, Tann RP, Slot PC, Luimstra VM, Schuurmans JM, Matthijs HCP, Huisman J. Rapid microevolutionary adaptation of harmful cyanobacteria to changes in CO₂ availability. (submitted manuscript)

Sandrini G, Tann RP, Schuurmans JM, Van Beusekom SAM, Matthijs HCP, Huisman J. Diel variation of gene expression of the CO₂-concentrating mechanism during a harmful cyanobacterial bloom. (submitted manuscript)


Attended conferences

ESF-EMBO symposium on Molecular Bioenergetics of Cyanobacteria: From Cells to Community. Sant Feliu de Guixols, Spain, April 2011.
9th European Workshop on the Molecular Biology of Cyanobacteria. Texel, the Netherlands, September 2014.
15th International Symposium on Phototrophic Prokaryotes. Tübingen, Germany, August 2015.