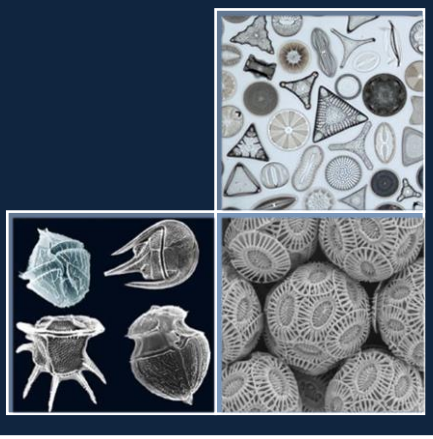
The effect of differences in light and nitrogen availability on the composition of phytoplankton communities

A graduation paper for the study applied biology at the CAH-Vilentum Almere



The effect of differences in light and nitrogen availability on the composition of phytoplankton communities

A graduation paper for the study applied biology at the CAH-Vilentum Almere

Name: Maria Meijer
Student number: 3009143
Education and class: Applied biology/4TB
Major: Animal ecology
Graduation supervisor CAH-Vilentum Almere: Maaike Cox
Written under the supervision of Maayke Stomp &
Jolanda Verspagen (UvA, IBED-AMB)

Source cover picture of the pike at the vinkeveense plassen, Netherlands: http://wonenaandevinkeveenseplas.nl foto duiken

Preface

Herewith I present to you my graduation paper for the study applied biology. This report forms a combination of information gathered from fundamental research and applied research. The reason for this combination is because during my study applied biology my interest in fundamental research increased. This with special focus on how fundamental principles play a role in ecosystems and how they can be used in applied biology. Therefore, this report starts with the function and characterizations of pigments. Thereafter the role and dynamics of pigments in ecosystems is discussed and how this influences the distribution of phytoplankton. But also, the role of pigments in for example biofuel and healthcare products is being discussed. This report, therefore, forms an overall source of fundamental and applied information on the effect of nitrogen and light on pigments and phytoplankton.

During the writing of this report and the last year of my study, I have spent my time at IBED-AMB under the supervision of Maayke Stomp and Jolanda Verspagen. It is also thanks to Maayke Stomp and Jolanda Verspagen who made the writing of this report possible. I would especially like to thank Jolanda Verspagen for reading and checking all these pages. But also would I like to thank her for always being there whenever I had a question, even questions not directly concerning this report. I am very thankful for your help and guidance. I also would like to thank Maayke Stomp. It is Maayke Stomp by whom I got inspired most. Furthermore, I would like to thank you for all your help, time and guidance during the last year and I hope to follow another internship under your supervision in the future. Furthermore, I would like to thank Veerle Luimstra and Hans Matthijs for their help and time while explaining about their work of field and about applied (phytoplankton) research.

I especially would like to thank Maaike Cox for willing to supervise me during my graduation year. Where Maayke Stomp inspired me to continue my study at the UvA, you where the one that inspired me to apply for the study applied biology. For the last years I have learned a lot from you as my teacher in animal ecology but certainly also as my mentor. Thank you for the past four years! Furthermore, I would like to thank Roos van Maanen. Being my mentor for the past two years you have always been more than helpful. Thank you for your involvement and guidance. Finally I would like to thank the following people that were not directly involved during my graduation year: Coby van Dooremalen, Naresh Devarajan, John Poté and all the people who had helped me during my education and from whom I learned the things necessary to write this graduation paper.

Abstract

Phytoplankton, known by many for its surprisingly high biodiversity, provides important resources for the planet and its human inhabitants. Distributed throughout the globe they inhabit many different aquatic environments. These environments differ in their resource availability, such as light and nitrogen. But also within one water body the light and nitrogen availability differs over depth often with opposite availability. Phytoplankton uses pigments to capture photons from the light spectrum. To assimilate these pigments, nitrogen is used. Interestingly, the costs of these pigments in terms of nitrogen and the benefits in terms of photon absorption, differs per pigment group. Furthermore, the pigment composition varies between phytoplankton species, resulting in differences in wavelength absorption and nitrogen requirements. In addition, phytoplankton species are dynamic organisms, capable of changing the composition and quantity of pigments through complementary chromatic adaption and photoacclimation.

So far, many studies have been conducted on the effect of light or nitrogen on pigment composition, phytoplankton growth or phytoplankton community assembly. However, few studies have been conducted on the combined effect of light and nitrogen availability. In addition, these factors could have an effect on the large-scale distribution patterns of phytoplankton. The effect of nitrogen and light is of great importance for phytoplankton cultivation used in several applied sciences for new sustainable products. Therefore, this graduation paper forms a literature review focusing on the overall effect of light and nitrogen availability on the composition of pigments, phytoplankton communities and distribution. Furthermore, there is a special focus on the findings can be used within applied sciences. Prior to writing and during writing literature has been collected, data on the N-content within pigment-protein complexes has been retrieved from the NCBI data bank. Global data of phytoplankton distribution, nitrogen and light availability was collected using Giovanni system utilizing data from the MODIS mission (NASA).

Phytoplankton is a group of photosynthetic prokaryotic and eukaryotic microorganisms. Within the prokaryotes *Cyanophyta* are the only organisms considered as phytoplankton belonging to the gram negative bacteria. Common eukaryotic divisions are: *Heterokontophyta*, *Dinophyceae* (Dinoflagellates), *Rhodophyta* (red algae), *Chlorophyta* (the green algae and *Prasinophyte*) and *Haptophyte* (coccolithophores). The habitat of phytoplankton differs greatly. Overall, clear waters have a low nitrogen content where blue light is able to penetrate the deeper layers. In contrast, waters rich in nutrients tend to absorb light in the blue part of the spectrum causing a deeper penetration of green or red light. While light is abundant in the top layers, the availability of NO_3^- increases with depth.

All phytoplankton species synthesize pigments that bind to protein complexes to absorb light energy in different parts of the light spectrum. Once a photon is captured by a pigment, the energy is transferred to the reaction center within the photosystem where light energy is being converted to chemical energy. Within phytoplankton, three main pigments are known, chlorophylls, carotenoids and phycobilins. Chlorophylls absorb light in the blue and red part of the spectrum. Furthermore, since the centered pigment (the so called primary donor) in the reaction center is a chlorophyll*a*, all species of phytoplankton synthesize chlorophyll*a.* Carotenoids may have a photosynthetic function by strongly absorbing light in the blue part of the spectrum or a photoprotective function by quenching singlet

oxygen caused pigments exposed to excess energy. Most chlorophylls and carotenoids are built in light harvesting complexes (protein-structures) surrounding the photosystem within the membrane. Phycobilins absorb light in the green and red part of the light spectrum. Phycobilins are built in protein-structures called phycobilisomes that are attached to the membrane of cyanobacteria and red algae. Depending on the size of the pigment-protein structures, sufficient nitrogen is required for synthesis. Most nitrogen is invested in large protein structures while less nitrogen is required for small structures. Expensive pigment-protein structures are phycobilisomes and require a fourfold larger amount of nitrogen than structures such as chlorophyll *a*/*b* complex (synthesized by e.g. green algae).

To obtain energy, photosynthetic organisms such as phytoplankton are in need of light energy. However, too much light, blue light or ultra violet can become damaging to cells while too little light might not be enough to stimulate growth. If light is limited or only red light is available, cells synthesize more thylakoid membrane, pigment-protein complexes and photosynthetic pigments such as phycobilins, phycobilisomes (especially phycocyanin) Chl*b* and Chl*d,* while photoprotective pigments decrease. Also the photosystem changes by synthesizing more reaction centers. Cells under low light are efficient in transferring energy, due to the increase of photosynthetic pigments. However, the efficiency per chromophore is low due to the low amount of photons absorbed. This results in an overall lower growth rate. In contrast, species (such as species from cyanobacteria) that synthesize pigments which are efficient in harvesting light during low light availability, have a higher growth rate at a relative low light irradiance.

At high light pigment-protein complexes become saturated. Hence, synthesizing large pigment-protein complexes becomes redundant. Too much light or ultra violet results in a decrease of chlorophyll*b*, chlorophyll*d*, phycocyanin, the highly light sensitive PCB light-harvesting protein and photosystems. In contrast, during high light exposure more photoprotective pigments, chlorophyll*c* and phycourobilin (relative to phycoerythrobilin) is synthesized. A fast response to protect the cell of light inhibition is the xanthophyll cycle where excess light is being discard as heat. Phytoplankton within the classes *Bacillariophyceae*, *Xanthophyceae*, *Haptophyceae*, and *Dinophyceae* have a xanthophyll cycle and are therefore well protected against intermittent high light exposure. Furthermore, light harvesting complexes stress-related proteins bind a large amount of photoprotective pigments. Therefore, classes such as diatoms that assemble these complexes are better protected than classes such as cyanobacteria that do not synthesize light harvesting complexes stress-related proteins. During high light, interaction between pigments is suppressed due to the decoupling of pigments or photoprotective pigments. As a result, the efficiency of energy transport and the photosynthetic efficiency of the cell is decreased but less reaction centers become damaged. Due to the lower photosynthetic efficiency, of, for example photoprotective pigments, high light adapted classes require more light for growth.

When nitrogen becomes limited more carotenoids, chlorophyll*c* and phycourobilin (relative to phycoerythrobilin) are synthesized while chlorophyll*b*, phycobilisomes and reaction centers decrease. Furthermore, light sensitive pigments require indirectly more nitrogen to repair the damaged structures which also costs more energy that cannot be invested in growth. Depending on the species or strain different chemical forms of nitrogen are used to synthesize pigment-protein complexes. In general diatoms or strains living in deeper parts of the water column prefer NO_3^- while cyanobacteria,

cryptomonads and dinoflagellates or species inhabiting surface waters prefer NH_4^+, urea, dissolved free amino acids and adenine. And because these species synthesize expensive pigment-protein complexes they bloom when reduced nitrogen is highly available. In contrast diatoms bloom when the water column is well mixed and NO_3^- is available and whereas coccolithophores bloom when nutrients are often too low for other species.

By reviewing literature novel trends such as the trend between light absorption and pigment-protein complex costs have been found that are useful for the applied field of research. Knowing the costs of pigment-complexes of different phytoplankton species helps to culture phytoplankton or to prevent harmful algae blooms. Therefore, if carotenoids or lipids (bind to the cheaper pigment-protein structure or lipids) are of interest, phytoplankton species such as diatoms and green algae should be exposed to high light while NO_3^- should be slightly N-limited. In contrast, if expensive pigment-protein structures such as phycocyanin are of interest cyanobacteria should not experience light stress and preferable grown under light near the red part of the spectrum while having sufficient NH_4^+ available.

Most cases of harmful algae blooms are due to cyanobacteria and dinoflagellates. While extensive blooms are often a result of eutrophication, cyanobacteria but also dinoflagellates are favored by stable stratified environments. Stimulating water mixture such as applied in het Nieuwe Meer in Amsterdam causes light stress to these species but favors species which adapt fast to changes such as diatoms and green algae. Therefore, cyanobacteria and dinoflagellates have to repair their pigment-protein structure that might at a certain moment cost too much energy which cannot be used for growth. However, the ultimate goal would be to treat the cause of the blooms. Because nitrogen is often a trigger for phytoplankton with expensive pigment-protein complexes to bloom, nitrogen run-offs have to be limited. Cyanobacteria are well capable of depleting water bodies of nitrogen, because they use high concentration of nitrogen for the synthesis of protein structures. Therefore, cyanobacteria can be used in sustainable waste water treatment plants to limit the N-flow into the environment. Because stratification and N-deposit increases due to increasing temperature and increased rainfall respectively. If, in addition, anthropogenic N-deposit will not be limited, light will become the limiting factor that favors species with expensive pigments that will outcompete other species. While the diversity of phytoplankton is the highest when resources are limited.

In conclusion high light adapted pigment-protein complexes absorb strong in the blue part of the spectrum and require low amounts of nitrogen. Species synthesizing these complexes are often well adapted to high light and produce high amounts of carotenoids and lipids. Pigment-protein complexes adapted to low light or red light, are expensive in terms of nitrogen. Species synthesizing these complexes are often good competitors over light and may be used in waste water treatment plants to depleted waters of nitrogen. Still little is known on the additive effects of light and nitrogen. Therefore, investigating these effects is highly recommended.

Samenvatting

Fytoplankton staat niet alleen bekend om hun verassend hoge biodiversiteit, maar is ook vanwege zijn vele toepassingen een belangrijke bron van verschillende producten en diensten voor de samenleving en de planeet. Verspreid over de aardbol zijn fytoplankton cellen in vele aquatische habitatten te vinden. Ieder van deze habitatten verschilt in nutriënt en licht beschikbaarheid. Deze verschillen zijn er niet alleen per gebied maar ook over de diepte van één gebied. Zo is er bijvoorbeeld genoeg licht beschikbaar aan de oppervlakte van een water kolom terwijl daar juist minder stikstof beschikbaar is. Nutriënten als stikstof zijn belangrijk voor het opbouwen van pigmenten. Deze pigmenten worden gebruikt om licht energie op te vangen en zo de cel van energie te voorzien. Maar de hoeveelheid stikstof nodig voor het opbouwen van pigmentcomplexen en de lichtabsorbtie van de pigmenten verschilt per klasse en soort. Bovendien kunnen fytoplankton cellen ook de compositie van hun pigmenten en de hoeveelheid pigmenten veranderen door middel van complementaire chromatische aanpassing en lichtacclimatisatie.

Tot nu toe zijn er veel onderzoeken uitgevoerd omtrent het effect van licht of stikstof op pigmenten, groei en de samenstelling van fytoplankton soorten. Er zijn echter maar weinig studies uitgevoerd betreffende het gecombineerde effect van stikstof en licht op fytoplankton, terwijl deze combinatie wel grote effecten kan hebben op de verspreiding, maar ook het kweken, van fytoplankton. Om deze reden vormt dit afstudeerwerstuk een literatuur review waarin de effecten van licht en stikstof op pigmenten, fytoplankton gemeenschappen en de verspreiding van fytoplankton wordt behandeld. Ook is er een speciale focus gelegd op hoe de resultaten toegepast kunnen worden binnen het watermanagement of algenkwekerijen. Voor en gedurende het schrijven van dit afstudeerwerkstuk is er literatuur verzameld, de gegevens over de kosten van pigment complexen zijn opgevraagd bij de NCBI data bank. Globale data betreffende de fytoplankton verspreiding, licht en stikstofbeschikbaarheid is verzameld door middel van het Giovanni programma van de MODIS missie uitgevoerd door NASA.

Fytoplankton is een groep fotosynthetiserende prokaryotische en eukaryotische organismen. Binnen de prokaryoten zijn de *Cyanophyta* de enige organismen die vallen binnen de groep fytoplankton en behoren tot de gramnegatieve bacteriën. Veel voorkomende eukaryoten zijn: *Heterokontophyta*, *Dinophyceae* (Dinoflagellaten), *Rhodophyta* (roodalgen), *Chlorophyta* (de groenalgen en *Prasinophyte*) en *Haptophyta* (coccolithophores). Het leefgebied van fytoplankton verschilt sterk; over het algemeen, heeft helder water een laag stikstofgehalte, waar blauw licht doorschijnt tot de diepere lagen van het kolom. Daarentegen eutrofe wateren absorberen het licht in het blauwe deel van het spectrum waardoor er relatief meer groen of rood licht beschikbaar is. En terwijl de licht beschikbaarheid afneemt met toenemende diepte, neemt de beschikbaarheid van NO_3^- juist toe met de diepte.

Alle fytoplanktonsoorten synthetiseren pigmenten die binden aan eiwitcomplexen om lichtenergie te absorberen in verschillende delen van het lichtspectrum. Wanneer een foton wordt opgevangen door een pigment, wordt de energie overgedragen aan het reactiecentrum dat is gelokaliseerd in het centrum van het fotosysteem. In het reactiecentrum wordt lichtenergie omgezet in chemische energie. Binnen fytoplankton zijn er drie belangrijke pigmenten bekend, chlorofyl, carotenoïden en fycobilinen. Chlorofyllen absorberen licht in het blauwe en rode deel van het lichtspectrum. Aangezien de gecentreerde pigment (de zogenaamde primaire donor) in het reactiecentrum een chlorofyl*a* molecuul is, synthetiseren alle soorten fytoplankton chlorofyl*a*. Fotosynthetische carotenoïden absorberen licht

voornamelijk in het blauwe deel van het lichtspectrum. Cel beschermende carotenoïden doven singletzuurstof moleculen, die worden gecreëerd wanneer pigmenten worden blootgesteld aan te veel lichtenergie. De meeste chlorofyl en carotenoïden zijn gebonden aan eiwit complexen (genoemd LHC) rondom het fotosysteem in het membraan. De laatste veel voorkomende pigmenten zijn fycobilinen en worden gesynthetiseerd door cyanobacteriën, rood algen en cryptomonads. Fycobilinen absorberen licht in de groene en rode deel van het lichtspectrum. Fycobilinen zijn ingebouwd in eiwit-structuren genaamd fycobilosomen. Fycobilosomen zijn in tegenstelling tot LHCs bevestigd aan het membraan. Hoeveel stikstof nodig is voor de synthese van deze pigment-eiwitstructuren is afhankelijk van de grootte. Grote hoeveelheden stikstof worden geïnvesteerd in grote eiwitstructuren, terwijl minder stikstof nodig is voor kleine structuren. Stikstof rijke pigment-proteïnestructuren zijn fycobilosomen en vereisen een 4 keer meer stikstof dan structuren zoals chlorofyl *a*/*b* complex (gesynthetiseerd door bijvoorbeeld groenalgen).

Fotosynthetiserende organismen zoals fytoplankton hebben licht-energie nodig voor synthese en dus groei. Echter, te veel licht, blauw licht of ultra violet, kan schadelijk zijn voor de cellen. Terwijl als er weinig licht beschikbaar is, er niet genoeg energie beschikbaar is voor groei. Als er weinig licht of alleen rood licht beschikbaar is, synthetiseren cellen meer thylakoidmembranen, pigment-eiwitcomplexen en fotosynthetische pigmenten zoals fycobilinen, fycobilosomen (vooral fycocyanine), chlorofyl*b* en chlorofyl*d*, terwijl fotobeschermende pigmenten afnemen. Ook de fotosystemen veranderen door de synthese van meer reactiecentra. Door de toename van fotosynthetische pigmenten zijn cellen onder weinig licht efficiënt in het overbrengen van energie. Maar de efficiëntie per chromosfeer is laag door het lage aantal beschikbare fotonen dat resulteert in een totale lagere groei en lagere biomassa. Echter soorten (zoals cyanobacteriën) die pigmenten synthetiseren welke efficiënt zijn in het absorberen van licht hebben een hogere groei snelheid bij een relatief lage lichtinstraling.

Bij hoge licht beschikbaarheid raken pigment-eiwitcomplexen verzadigd. Hierdoor is het synthetiseren van grote pigment-eiwitcomplexen overbodig. Te veel licht of ultra violet resulteert in een daling van chlorofyl*b*, chlorofyl*d*, fycocyaninen, de lichtgevoelige PCB eiwitten en fotosystemen. Echter de hoeveelheid van lichtbeschermende pigmenten, chlorofyl*c* en fycourobilin (ten opzichte van fycoerytrobilin) neemt toe. Een snelle reactie om de cel te beschermen tegen licht is de xanthofyl cyclus, waarbij overtollig lichtenergie wordt uitgestoten als warmte. Fytoplankton binnen de klassen *Bacillariophyceae*, *Xanthophyceae*, *Haptophyceae* en *Dinophyceae* hebben een xanthophyl cyclus en zijn dus goed beschermd tegen afwisselende (hoge) blootstelling aan licht. Bovendien LHC stresgerelateerde eiwitten binden een grote hoeveelheid lichtbeschermende pigmenten. Daarom zijn klassen zoals diatomeeën die deze complexen synthetiseren beter beschermd dan klassen zoals cyanobacteriën die deze complexen niet synthetiseren. Tijdens blootstelling aan veel licht worden pigmenten ontkoppelt van de reactiecentra of extra lichtbeschermende pigmenten worden gesynthetiseerd. Hierdoor wordt de interactie tussen pigmenten onderdrukt. Waardoor de efficiëntie van het energie transport en fotosynthese daalt, hierdoor raken echter ook minder reactiecentra beschadigd. Door de lagere fotosynthetische efficiëntie van beschermende pigmenten hebben klassen die voorkomen in wateren met een hoge licht beschikbaarheid ook meer licht nodig voor groei in vergelijking met klassen die niet zijn blootgesteld aan veel licht.

Wanneer stikstof beperkt beschikbaar is, worden meer carotenoïden, chlorofyl*c* en fycourobilin (ten opzichte van fycoerythrobilin) gesynthetiseerd, terwijl de hoeveelheid chlorofyl*b*, fycobilosomen en reactiecentra afneemt. Bovendien, lichtgevoelige pigmenten vereisen indirect meer stikstof omdat licht beschadigde structuren moeten worden gerepareerd. Deze reparaties vereisen stikstof en energie wat vervolgens niet geïnvesteerd kan worden in de groei. Afhankelijk van de soort of stam kunnen voor het synthetiseren van pigment-eiwitcomplexen verschillende chemische stikstofvormen worden gebruikt. In het algemeen hebben diatomeeën of stammen die leven in diepere delen van de waterkolom een voorkeur voor NO_3^-, terwijl cyanobacteriën, cryptomonads en dinoflagellaten of soorten die leven in het oppervlaktewater een voorkeur hebben voor NH_4^+, ureum, opgelost vrije aminozuren en adenine. Omdat deze laatst genoemde soorten stikstof rijke pigment-eiwitcomplexen synthetiseren, bloeien ze wanneer gereduceerd stikstof ruim beschikbaar is. Dit is in tegenstelling tot diatomeeën die bloeien wanneer de waterkolom goed gemengd en NO_3^- beschikbaar is en coccolithoforen juist vaak bloeien wanneer de beschikbaarheid van voedingsstoffen te laag is voor andere soorten.

Door het verzamelen van literatuur zijn er nieuwe trends gevonden omtrent de beschikbaarheid van licht en stikstof. Zoals de trend tussen de licht absorptie en de stikstof kosten van pigment-eiwitcomplexen; waarbij stikstof goedkope pigment-eiwitcomplexen meer licht absorberen in het blauwe deel van het lichtspectrum, terwijl stikstof rijke pigment-eiwitcomplexen sterk absorberen in het rode deel van het lichtspectrum. Het kennen van de kosten van de pigment-complexen helpt om fytoplanktonculturen te optimaliseren of om een schadelijke algenbloei te voorkomen. Bijvoorbeeld als carotenoïden of lipiden (carotenoïden binden aan de goedkopere pigment-eiwitstructuur of lipiden) van belang zijn, moeten fytoplankton soorten zoals diatomeeën en groenalgen worden blootgesteld aan veel licht, terwijl NO_3^- beperkt moet worden. Daarentegen voor de productie van stikstof rijke pigment-eiwitstructuren zoals fycocyanin is het van belang dat cyanobacteriën niet worden blootgesteld aan veel licht en dat er voldoende NH_4^+ beschikbaar is.

De meeste gevallen van schadelijke algenbloei zijn te wijten aan cyanobacteriën en dinoflagellaten, welke vaak het gevolg zijn van eutrofiëring. Bovendien bloeien cyanobacteriën maar ook dinoflagellaten optimaal in stabiele gestratificeerde wateren. Het mixen van de waterkolom is een goede oplossing tegen schadelijke algenbloei. Een voorbeeld hiervan is het mixen van de water kolom in het Nieuwe Meer in Amsterdam. Dit veroorzaakt licht stress bij cyanobacteriën terwijl de groei van diatomeeën en groenalgen wordt bevorderd. Echter het uiteindelijke doel is om de oorzaak van het probleem op te lossen en niet de symptomen te behandelen. Omdat stikstof vaak een trigger is voor een algenbloei, vooral soorten met stikstof kostbare pigment-eiwitcomplexen, moet de afvoer van stikstof in wateren worden beperkt. Juist omdat cyanobacteriën veel stikstof nodig hebben voor de synthese van hun eiwitstructuren, nemen ze veel stikstof op in een korte tijd. Ze nemen zelfs zoveel stikstof op dat de stikstof beschikbaarheid gelimiteerd wordt. Daarom kunnen cyanobacteriën gebruikt worden in duurzame waterzuiveringsinstallaties, om zo de afvoer van stikstof in het milieu te beperken. De beschikbaarheid van stikstof neemt niet alleen toe door de directe gevolgen van de samenleving. Door toenemende temperaturen en meer regenbuien zal de stratificatie van wateren en de stikstof neerslag toenemen. Als daarbij de antropogene stikstof afvoer niet wordt beperkt, zullen de soorten met dure

pigmenten wateren gaan domineren. Terwijl ook in aquatische milieus de diversiteit (van het fytoplankton) het hoogst is wanneer nutriënten beperkt zijn.

Kortom, pigment-eiwitcomplexen geadapteerd aan een hoge licht beschikbaarheid, absorberen sterk in het blauwe deel van het spectrum en gebruiken lage hoeveelheden stikstof voor het synthetiseren van de complexen. Deze soorten produceren grote hoeveelheden van carotenoïden en lipiden. Pigment-eiwitcomplexen geadapteerd aan weinig licht of rood licht, zijn kostbaar in termen van stikstof. Soorten die deze complexen synthetiseren zijn vaak goede concurrenten om licht en kunnen worden gebruikt in waterzuiveringsinstallaties om wateren te ontdoen van stikstof. Toch is er nog steeds weinig bekend over de additieve effecten van licht en stikstof terwijl er verbanden zijn tussen het effect van stikstof en licht. Verder onderzoek omtrent de combinaties van licht en stikstof is daarom zeer aan te raden.

Contents

Figures and tables

Figures

Tables

Abbrevations and symbols

AND	atmospheric N deposited
APC	allophycocyanin
Car	carotenoids
Chl	chlorophyll
CP	cyanophycin
DCM	deep chlorophyll maxima
DD	diadinoxanthin
DNA	Desoxyribonucleïnezuur
DON	dissolved organic nitrogen
DT	diatoxanthin
DV	divinyl
FCP	fucoxanthin chlorophyll a/c2proteins
HABs	Harmful Algal Blooms
IPCC	Intergovernmental Panel on Climate Change
LED	light-emitting diode
LHC	light harvesting complex
LHCsR	light harvesting complex stress response
MAA	mycosporine like amino acids
NASA	National Aeronautics and Space Administration
NOBM	National Aeronautics and Space Administration Ocean Biochemical Model
NPQ	non-photochemical quencher
OCP	orange carotenoid protein
PAR	photosynthetic active radiation (400-700nm)
PB	phycobilins
PBP	phycobiliproteins
PC	phycocyanin
PCB	phycocyanobilin
Pcb	Chlorophyll a/b-binding proteins
PCP	peridinin-chlorophyll complexes
PE	phycoerythrin
PEB	phycoerythrobilin
P_{max}	maximum photosynthetic rate
PS	photosystem
PUB	phycourobilin
RCP	red carotenoid protein
SRES	Special Report on Emissions Scenarios
UV	ultraviolet radiation
β-car	Beta-carotene
ε	molar extinction coefficient

Glossary

Chromophore	The molecule responsible for light absorption
Cyanophycin	An amino acid functioning as nitrogen storage in cyanobacteria
Donor chlorophyll	The last chlorophyll to except the electron within a photosystem
Gilvin	A yellow substance composed of dissolved organic matter absorbing blue light
Light quality	Differences in wavelentgh irradiance
Light quantity	Differences in quantities of irradiance
Ornithine-urea cycle	An effective manner used by diatoms to redistribute nitrogen
Photoacclimation	Adjusting pigment composition in response to changes in light
Photoinhibition	Inhibition of the activity of photosystem II caused by high light irradience
Pigment bleaching	High energy causes the release from an electron in the atom, changing the propeties of the atom
Pyrrole	A chemical structure formed by a five-membered ring with the formula C4H4NH
Quenching pigment	Absorb light and discard the light from the cell as heat
Reaction center	The core of the photosystem responsible for the primary energy conversion
Singlet oxygen	Unstable oxygen molecules causing damage to proteins and DNA
Tripton	Inanimate particulate matter that absorbs light in the blue part of the spectrum
π bonds	Molecular bonds with a low energy cap absorbing photons with low energy
σ bonds	Molecular bonds with a high energy cap absorbing photons with high energy

1. Introduction

Phytoplankton account for less than 1% of earth's photosynthetic biomass, yet they contribute to almost half (namely, 45-50 billion tones inorganic carbon uptake in their cells) of earth's total primary production (Falkowski, 2012). In addition, phytoplankton plays a role in the climate feedback loop (Charlson *et al.,* 1987; Monastersky, 1987), provides our air with oxygen, their remains provide our cars with fuel and is used in healthcare products and as fertilizers (Häubner *et al.,* 2014; Snoeijs & Häubner, 2014; Steinhoff *et al.,* 2014). Furthermore, phytoplankton does not only provide crucial resources, but they are also known for their surprising high biodiversity. Within the Baltic Sea only, an estimated more than 1700 species exist (Ojaveer *et al.,* 2010).

Phytoplankton species are found in both fresh- and salt water bodies, as well as in extreme habitats like extremely acidic lakes (pH 2.6; Kamjunke *et al.,* 2004) and nutrient poor mountain lakes (Catalan *et al.,* 2006). Light and nitrogen are considered critical resources for the growth of phytoplankton (Colijn & Cadée, 2003; Ryther & Dunstan, 1971). Both nitrogen and light availability show a great variety within aquatic ecosystems, ranging from clear oligotrophic oceans towards turbid eutrophic lakes. The amount of nitrogen and light also varies within the same water body over depth (and time). Furthermore, light and nitrogen have opposite gradients. Light availability is high in the top layers of the water column but decreases with depth (Cullen & Horrigan, 1981). Nitrogen availability is low in the top of the water column but increases in the lower layers of the water column (Kerimoglu *et al,* 2012). Furthermore, different water bodies absorb photons with different wavelengths, due to different concentrations of dissolved substance (gilvin) and inorganic particulate matter (tripton) (Kirk, 1994). In clear lakes or oceans, containing little gilvin and tripton, light absorption at the blue side of the spectrum is low. However, in peat lakes containing high levels of gilvin and tripton, photons at the blue side of the spectrum are absorbed, letting red light penetrate the water column (Stomp *et al.,* 2007a). Often, nutrient availability and gilvin concentration are correlated, e.g. oligotrophic waters often have a low gilvin content, whereas eutrophic waters generally have a high gilvin content.

Not only their habitat, but also, their characteristics such as size and shape (Montoya *et al.,* 2004; Paerl, 1990) and composition of phytoplankton species (Kilham & Hecky, 1988) differ greatly. Common groups of phytoplankton are: cyanobacteria, diatoms, dinoflagellates, green algae and coccolithophores. Phytoplankton carry up to three major classes of pigment, namely: chlorophylls, carotenoids and phycobiliproteins (Greg-Mitchell, 1988; Rowan, 1989; Sathyendranath *et al*, 1987; Wright, 2005). These pigments are crucial for the phytoplankton's survival, as they harvest light used for photosynthesis. The pigment composition differs greatly between species (Colyer *et al.,* 2005; Green & Parson, 2003; Kutser, 2004; Paerl, 1984). Furthermore, pigments absorb photons of different wavelengths within the light spectrum (Hunter *et al.,* 2008; Rowan, 1989; Wright, 2005). Hence, differences in pigment composition between species allow partitioning of the light spectrum which may promote phytoplankton diversity (Stomp *et al.* 2004; 2007b). For example, two related types of picocyanobacteria , one with the pigment phycoerythrin absorbing photons in the green-yellow part (560-570nm) of the spectrum and one with the pigment phycocyanin absorbing photons in the orange-red part (620-630nm) of the spectrum, could coexist when exposed to a full spectrum of light (Stomp *et al.,*2004).

However, the amount of pigments in a cell is restricted to the availability of nutrients like nitrogen. The production of chlorophyll *a* is assumed to be coupled to nitrogen assimilation within phytoplankton cells (Geider *et al.,* 1998).Therefore, it seems likely that the assimilation of pigments by a phytoplankton cell requires nitrogen. It differs per pigment class how "expensive'' the costs of assimilation are in terms of nitrogen. The pigments within the group phycobiliproteins need more nitrogen to assimilate than

chlorophylls and carotenoids (Raven, 1984). However, phycobiliproteins are capable of absorbing more photons in shaded aquatic environments compared to other pigments (Raven, 1984).

Phytoplankton species are capable of changing their physiology in response to changing environments. Some species are capable of complementary chromatic adaptation (Bogorad, 1975). With complementary chromatic adaption, species are able to adjust their pigment composition in response to changes in the spectral composition of light (De Marsac *et al.,* 1988). Furthermore, some species within phytoplankton are capable of photoacclimation, adjusting the pigment chlorophyll *a* to higher concentrations when exposed to lower light intensity (Dubinsky, 2009; Macintyre, 2002). However, when phytoplankton cultures in a stationary-phase are exposed to both nitrogen and light limitation the ability to photoacclimate to the lower light intensity is lost (Prézelin, & Matlick 1983). But, the rate at which they lose this ability and how phytoplankton species deal with a depletion of nitrogen and light is diverse among species, with the greatest difference between cyanobacteria and eukaryotic algae (Kromkamp, 1987). However, most studies have focused only on the effect of light or nutrients on phytoplankton growth and physiology (Ficek *et al.,* 2004; Six *et al.,* 2007; Stomp *et al.,* 2004, 2007a, 2007b; Young & Beardall, 2003). Here the aim is to investigate the combined effect of light and nitrogen on phytoplankton growth and pigment composition.

Considering the interest in phytoplankton cultivation for biofuels, fertilizers, and health products, an overview of the effect of nitrogen and light is not only important information for ecologists and biologist but also for companies manufacturing products using phytoplankton (Häubner *et al.,* 2014; Snoeijs & Häubner, 2014; Steinhoff *et al.,* 2014). Recent years, scientists are searching for sustainable products as a replacement for current fuel, fertilizers, but also food and medicine (Aourahoun *et al.,* 2014; Burton *et al.,* 2014; Morar *et al.,* 2008). An important aspect of this process is the cultivation of phytoplankton and to enhance products of interest within these cells (Figure 1-1). Because all phytoplankton species use pigments as their 'energy converters' it is of great importance to understand the effect of factors involved in the pigments formation, composition and therefore phytoplankton composition. Furthermore, often are the pigments of phytoplankton itself the products of interest (Li *et al.,* 2014; Pallela, 2014).

Figure 1-1 Sapphire Energy's phytoplankton cultivation tanks in Columbus, NM USA. Source: http://biofuelsdigest.com/bdigest/wp-content/uploads/2010/06/sapphireenergy.jpg

In addition, within applied research, knowledge on nutrient and light requirements of individual species is of major importance for water managers in order to predict and prevent the occurrence of phytoplankton blooms. In 1984 Tilman presented the resource-ratio hypothesis. The resource-ratio hypothesis suggests that if species A is superior competitor for resource X and species B is superior competitor for resource Y, species A will dominate and win the competition at a low X:Y ratio. Whereas species B will dominate and win the competition at a high X:Y ratio. Traditionally, the ratio of nitrogen and phosphorus loading (N:P ratio) has been used as predictor for phytoplankton community composition, for example for the presence of toxic algal blooms (Dignum *et al.,* 2004; Elser, *et al.,* 1990). However, phytoplankton growth is limited by more resources than N and P. Recent work indicates that with increasing nutrient load, the system switches from nutrient to light limitation due to shading

effects, called the Nutrient-Load hypothesis (Brauer *et al.,* 2012). Hence, the combined effects of nutrients and light availability play an important role in phytoplankton communities. Therefore, a new approach for lake managers to predict the composition of phytoplankton communities would be to take the nitrogen-costs of the photosynthetic apparatus into account. For instance, the occurrence of dense blooms of blue-green cyanobacteria in eutrophic lakes can be explained by the high nitrogen requirements of their expensive pigments (Raven, 1984; Yang & Jin, 2008).

Figure 1-2 a dinoflagellates bloom called "a red tide" because of the red appearance. Source: Woods Hole Oceanographic Institution, www.whoi.edu.

Not only lake managers deal with toxic blooms of phytoplankton: the oceans and coastal waters can also experience toxic blooms (Figure 1-2). An example is the toxic red tide of dinoflagellates responsible for "neurotoxic shellfish poisoning" (Baden & Mende, 1982; Lindholm & Nummelin, 1999). To prevent or to predict phytoplankton blooms, oceanographers build models and distribution maps. An important tool used to estimate phytoplankton distribution is remote sensing, where satellite observations are used to quantify chlorophyll concentrations. However, chlorophyll concentrations differ per phytoplankton group and are depending on the environmental resources available (Bautista & Necchi-Júnior, 2008; Glover *et al.,* 1987; Partensky, *et al.,* 1997; Schagerl & Müller, 2006). Other technics applied for the estimation of phytoplankton distribution is Differential Optica Absorption Spectroscopy used in the NASA Ocean Biochemical Model (NOBM). This technics allows to analyze the distribution of different phytoplankton classes such as cyanobacteria and diatoms based on the different optical characteristics of phytoplankton classes. Based on the absorption spectra, the density of phytoplankton cells is estimated (Bracher *et al.,* 2009). Despite the fact that NOBM is the most accurate estimation available, it does not take the changes of the pigment composition per cell and thus the dynamic light absorption of a phytoplankton cell in account. Furthermore, models are available on the global light irradiance and nitrate availably. However, considering only nitrate as a resource for nitrogen would not be accurate since the preference differs per phytoplankton group and therefore if they use nitrate, nitrite, ammonium, urea or nitrogen gas as a source of nitrogen (Lomas, 2004; Maldonado & Price, 1996; Waser *et al.,* 1999).

1.1 Objective

Within the Institute for Biodiversity and Ecosystem Dynamics (IBED) of the University of Amsterdam, biologist of the department aquatic microbiology (IBED-AMB) work on the effect of abiotic and biotic factors on the molecular, physiological and ecological characteristics of phytoplankton and phytoplankton communities. Most of the research conducted has a fundamental focus that often results in conclusions used for applied science. Therefore, this bridge between fundamental and applied science often leads to new innovations. An example is the technique developed to mix water through an aeration system that prevent stratification or the application of H2O2 reducing the photosynthetic capability of cyanobacteria and therefore reducing cyanobacteria blooms in lakes during the summer time (Huisman *et al.,* 2004; Matthijs *et al.,* 2012; Visser *et al.,* 1996). Furthermore, associated workers at

IBED-AMB conducting fundamental research on topics like energy efficiency of photosynthesis, use the results for further research within applied science such as the optimization of phytoplankton growth for commercial purposes (Matthijs *et al.,* 1996).

To continue on the subject of physiological and ecological characteristics of phytoplankton the knowledge on requirements of individual species in terms of nutrients and light as provided in this review is of major importance in order to understand and predict the resources needed by different species, the distribution of phytoplankton and the occurrence of nuisance phytoplankton blooms in lakes and oceans. It will therefore help to optimize water management strategies for preventing and predicting nuisance phytoplankton blooms, optimizing plankton growth or pigment composition for e.g. biofuel industries or health care products.

Therefore, the goal of the graduation paper is to write a literature review on the effect of light and nitrogen availability on the pigment composition, photosynthesis and growth of different phytoplankton groups. Therefore, the following questions will be asked:

1. What is the effect of light- and nitrogen availability on the composition of pigments from different phytoplankton species?
2. How dynamic are phytoplankton species relative to the availability of light- and nitrogen changes in their environment?
3. How does light- and nitrogen availability affect the distribution patterns of pigments and therefore phytoplankton?
4. How can the new knowledge of nitrogen and light be applied in the working field of applied science?

Furthermore, it will be discussed if there are any trade-offs such that, some species are superior competitors for light but have high nitrogen demands, whereas others are worse competitors for light but with lower nitrogen demands? Moreover, the gradients of nitrogen and light availability over depth in aquatic ecosystems, ranging from eutrophic and turbid lakes towards the clear oligotrophic oceans are taken into account.

A broad range of information from different fields of ecology and biology has been gathered. And because this is the first review describing the effect of nitrogen and light on the pigment composition of phytoplankton in combination with the distribution of phytoplankton, this graduation paper will be useful for a broad range of fields covering science, applied science, but it is also useful for organizations dealing with water quality such as municipalities, water boards, or organization such as Wetsus dealing with sustainable water technology. It adds information to the already well-studied effect of phosphate and temperature on phytoplankton blooms. Furthermore, this study will provide an answer whether nitrogen and light availability could be implicated in the cultivation of phytoplankton and prevention of nuisance phytoplankton blooms. To make sure the information will be useful the report will be sent to the employees of the University of Amsterdam who are associated or working together with water boards, municipalities and Wetsus.

The report consists of 8 chapters discussing the effects of nitrogen, light and the application of these results. To give a good overview of the different phytoplankton classes chapter 2 describes the different phytoplankton classes and their habitat. Chapter 3 gives an overview of what pigments are, how expensive the pigments are in terms of nitrogen and what the pigment composition of different phytoplankton classes are. Chapter 4 discusses the effect of light on the pigment composition of phytoplankton and the distribution of phytoplankton. The effects of nitrogen availability on the pigment

composition and the distribution of phytoplankton is discussed in chapter 5. The effect of the combination of light and nitrogen on pigments and phytoplankton classes is discussed in chapter 6. Chapter 7 gives an overview of the possibilities in which the results can be used, subjects such as harmful algae blooms and biofuel are discussed. Finally, in chapter 8 the conclusion of this report is given.

1.2 Methods

Prior and during the writing of this report literature has been collected and read. The nitrogen costs of the protein-pigment complexes has been calculated according the calculation of Raven, 1984. To do so the following values had to be known: the molecular mass of the protein, the number of chromophore within the protein, the percentage of N per protein and the molecular weight of N (all values are attached in appendix I). An algal protein complex consists on average of 1.16 mol of N. The molecular weight of N is 14, resulting in 16.24 g of N per mol protein making up 16.32% of the proteins compounds (Appendix IA). The nitrogen costs of the protein-pigment complexes expressed as mol N/mol chromophore, had been calculated according the following formula:

$$\text{molN/mol chromophore} = \frac{mol\ P}{Nr\ chr} 0.1632/14$$

Where *mol P* is the molecular weight of the pigment-protein complex and *Nr chr* the number of chromophores within the protein-pigment complex. The percentage of N is expressed as a fraction 0.1632 and 14 is the molecular weight of N (all results are in attached in appendix IB). Molecular weights of the pigment-proteins were found using NCBI-pdb and NCBI-structure search. Further analysis and picture of protein-pigment complexes have been extracted using the software. Correlation coefficient of metadata has been calculating with Spearman's rank using Microsoft Excel 2010.

Cn3D 4.3.1 macromolecular structure viewer (NCBI). Analysis and graphs created of global phytoplankton distribution in relationship to nitrogen and light availability was done using Giovanni system utilizing data from the MODIS mission (NASA).

2. Phytoplankton and their habitats

Phytoplankton, literally translated form Greek, means *phytos*: plant *planktos*: wandering and consists mostly of single celled photosynthetic organisms drifting in the currents of fresh- or salt water. Phytoplankton extend from the prokaryotic cyanobacteria to the eukaryotic picoplankton (< 2µm) (Falkowski *et al.*, 2004), inhabiting aquatic environments from the deep aphotic zone (250–3,000 m deep) in the Antarctic's (López-García *et al.*, 2001) to the peat pog pools of Argentina (Quiroga *et al.*, 2013). To present an overview of the variety of phytoplankton chapter 2 and 3 will discuss the different groups of phytoplankton, their habitats and the pigments used for photosynthesis.

2.1 Habitat

The habitat of phytoplankton ranges from the open oceans to freshwater ponds and the frozen water of the Arctic (Thomas & Dieckmann, 2002) as well as wetlands (Rojo *et al.*, 2010), streams and rivers (de Domitrovic *et al.*, 2014), estuaries (Jiang *et al.*, 2014), intertidal zones such as lagoons (Lafabrie *et al.*, 2013) and the ocean pelagic zone (Brotas *et al.*, 2013). However, there is a significant difference between these waters in terms of light availability and nitrogen availability.

In the habitats of phytoplankton, light is an important factor creating niches throughout the water body and among water bodies. Light is an electromagnetic radiation consisting of photons with wavelengths (the frequency of the wave) ranging from 400 to 700 nm; the smaller the wavelength of the emitted photon the higher is its energy level. Water itself absorbs light mostly in the red region of the light spectrum. In pure distilled water, photons with a wavelength of ~415 nm have the lowest absorption coefficient, causing the blue color to penetrate deepest and thus causing the blue color of the clear oceans (Figure 2-1A). However, dissolved substances in water absorb photons at other wavelengths. Most important of these dissolved substances are gilvin and tripton. Gilvin is a yellow substance composed of dissolved organic matter (Kirk, 1994), and absorbs light in the violet and blue part of the spectrum with an average absorption peak at 440nm (Davies-Colley & Vant, 1987). In addition, Tripton, or inanimate particulate matter, also absorbs light in the violet and blue part of the spectrum (Kirk, 1994). Gilvin and tripton absorb mostly violet and blue light, and in combination

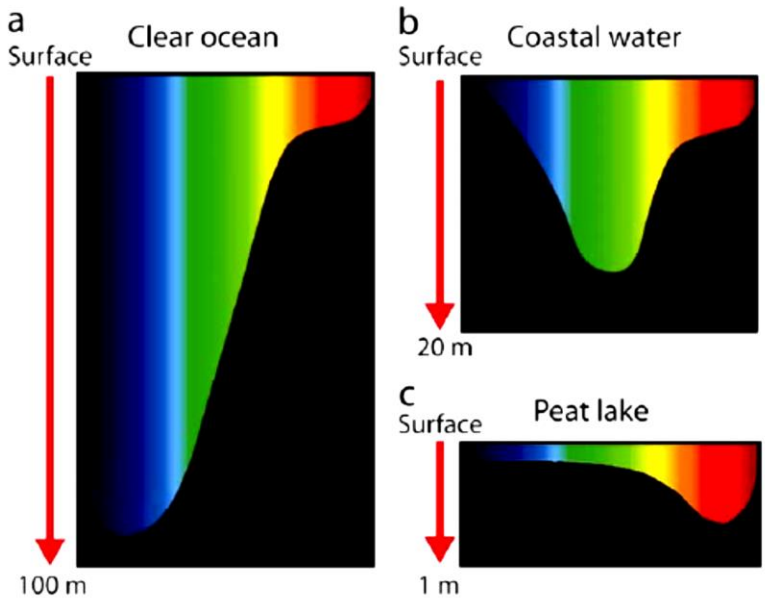


Figure 2-1 A conceptual figure of the light irradiance in different water bodies. In clear oceans the blue light penetrate deepest, while in coastal waters blue light is being absorbed by dissovled organic matter. The high concentration of organic matter in peat lakes causes a strong absorption in the blue-green part of the spectrum, resulting in the deepest penetration of red light (Stomp *et al.*, 2007a).

with the light absorption of water in the red part of the spectrum, in waters with moderate amounts of gilvin and tripton, green light has the lowest absorption coefficient and penetrates the deepest (Figure 2-1B). If high amounts of organic matter are dissolved in water, most photons are being absorbed in the blue part of the spectrum, shifting the remaining light into the red part of the spectrum (Figure 2-1C). And once photons in an area of the spectrum are absorbed by any substance, these are not anymore available for phytoplankton. Therefore, it depends on the characteristics of the water body, which part of the spectrum is available for phytoplankton photosynthesis (Stomp *et al.*, 2007a).

Lakes that contain high levels of gilvin and thus lower light availability, more nutrients are available compared with the clear oceans (Hecky *et al.*, 1993). In addition, nitrogen and light have opposite gradients, with more light available in the top layers compared to the lower layers (Cullen & Horrigan, 1981), while nitrogen concentrations are higher in the lower layers of the column (Kerimoglu *et al,* 2012). However, different forms of nitrogen may have different vertical distributions. Nitrate (NO_3^-) is usually more abundant than ammonium (NH_4^+). NH_4^+ is, for phytoplankton, easier to assimilate. However, when NH_4^+ reacts to NH_3 at high pH, it easily diffuses out of the water (Raven, 1984). While NH_4 is more or less equal distributed throughout the water column, NO_3^- is more abounded in the lower layer of the water column (Figure 2-2) (Findlay *et al.*, 2014).

During the summer time lakes may become stratified. The warmer, less dense surface water becomes separated from the colder denser bottom layer. This causes the nutrient rich water to stay in the lower layers of the water. Therefore, in the northern hemisphere, during months of more sunshine, nutrient levels decreases until September. In addition, form approximately October till March, when less sunlight is available, nutrient concentrations in the water column are relative high, and peak during seasonal turnovers (Fujita *et al.*, 1989).

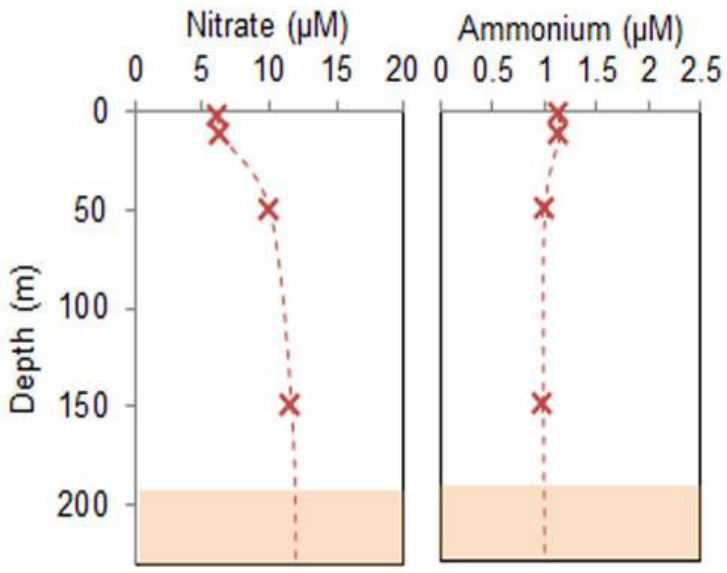


Figure 2-2 The nitrate and ammonium concentration at the Rockall bank in the Atlantic Ocean. The availability of NO_3^- decreases with depth while the less abundant NH_4^+ remains relative constant over depth (Findlay *et al.*, 2014)

2.2 Common phytoplankton classes

In this section a selection is made of most common classes of phytoplankton (Jeffrey *et al.*, 2011; Falkowski & Raven, 2013). The classes, with commons names are presented in Table 2-1 together with the known estimated number of species and the division of species habiting fresh- and marine water.

2.2.1 Division of *Cyanophyta*

Cyanobacteria are the only prokaryotic organisms within phytoplankton, and belong to the gram negative bacteria. Cyanobacteria are found in most temperate and tropical habitats, with a great number

of species found in freshwater (Kilham & Hecky, 1988), in a unicellular or filamentous form (Roy *et al.*, 2011). They often dominate eutrophic freshwaters, often causing, harmful blooms for other organisms (Paerl *et al.*, 2001; Steffen *et al.*, 2014). One of the distinguishable features of this class is the capability to morphologically change in a reaction to their environment (Carr & Whitton 1982). In a reaction to combined nitrogen starvation, filamentous cyanobacteria are capable of developing heterocysts (Adams, 2000), allowing them to fix atmospheric N_2 (Flores & Herrero, 2009; Wolk *et al.*, 2004) and thus having an advantage over other organisms that are not capable of fixing atmospheric N_2. Another important differentiation, triggered by several environmental factors such as limitation of light and nutrition, is the development of hormogonia filaments (Campbell & Meeks, 1989; Carr & Whitton 1982; de Marsac, 1994). Hormogonia cells distinguish them self from their parental cell by the property of gliding motility (Campbell & Meeks, 1989). Many species of cyanobacteria possess gas vacuoles which give cells buoyancy and the advantage to float up to the water surface where light availability is high (Carr & Whitton 1982). In contrast to some phytoplankton species cyanobacteria lack flagella, which, as mentioned earlier, do not necessarily make cyanobacteria immobile (Waterbury *et al.*, 1985). Furthermore, some species of cyanobacteria are found in the deep layers of euphotic zones (Stramski & Morel, 1990), where they are abundant in the lowest layer of the water column were chlorophyll α can still be found, namely the deep chlorophyll maxima (DCM) (Camacho, 2006).

Table 2-1 classification of the largest phytoplankton classes with the approximated number of known species and the distribution over fresh- and marine water (Adapted from: Falkowski & Raven, (2013) and Roy *et al.*, (2011))

Division	Class	Common name	Known species *(marine/freshwater)*	
Prokaryote				
Cyanophyta	*Cyanophyceae*	Cyanobacteria	1500	*(150/1350)*
Eukaryote				
Heterokontophyta	*Bacillariophyceae*	Diatoms	10.000	*(5000/5000)*
	Chrysophyceae	Golden-brown algae	1000	*(800/200)*
	Pelagophyceae	Pelagophyte	-[1]	
	Raphidophyceae	Raphidophyte	-[1]	
	Synurophyceae	Synurophyte	250	*(0/250)*
	Xanthophyceae	Xanthophyte	600	*(50/550)*
Rhodophyta	*Rhodophyceae*	Red algae	6000[2]	*(5880/120)*
Chlorophyta	*Chlorophyceae*	Green algae	2500	*(100/2400)*
	Prasinophyceae	Prasinophyte	120	*(100/20)*
Dinophyta	*Dinophyceae*	Dinoflagellate	2000	*(1800/200)*
Euglenophyta	*Euglenophyceae*	Euglenophyte	1050	*(30/1020)*
Haptophyta	*Prymnesiophyceae*	Coccolithophorid	500	*(100/400)*
Cryptophyta	*Cryptophyceae*	Cryptomonad	200	*(100/100)*

[1] **Missing number of species, but taken into account because the tides these species cause (Imai *et al.*, 2001; Lomas *et al.*, 2001)**
[2] **Approximated count includes as well +/- 4000 macrophytes species**

2.2.2 Division of *Heterokontophyta*

With an estimated 10.000 species, the diatoms form the larges class within the *Heterokontophyta* (Falkowski & Raven, 2013). Most diatoms are unicellular and although there are some species that form colonies, there is little difference between colonial and unicellular species (Round *et al.*, 1990). In addition diatoms are lacking flagella. Diatoms are broadly distributed over fresh- and marine waters and even found in sea-ice (Horner *et al.*, 1992; Roy *et al.*, 2011; Thomas & Dieckmann, 2002). Some of the most common diatom species form an endosymbiosis relation with unicellular cyanobacteria, allowing some diatom species to live in environments with relative low available fixed nitrogen (Fiore *et al.*, 2010; Rai *et al.*, 2002; Usher *et al.*, 2007).

Furthermore, diatoms are distinguished by their silicon shell and therefore, in contrast to most phytoplankton, require sufficient free available silicon in their environment for growth (Armbrecht *et al.*, 2014; Werner, 1977). The silicon shells provide diatoms a number of benefits. The shell provides a mechanical protection again predators (Hamm *et al.*, 2003; Pondaven *et al.*, 2007). Furthermore, based on the properties of silica gel, it has been suggested that diatoms are more effective in absorbing low concentrated nutrients in comparison to other phytoplankton (Werner, 1977). In addition the polycondensation of $Si(OH)_4$ in silica walls of diatoms is energetic more economical than formation of cell walls such as e.g. cellulose or chitin (Raven & Waite, 2004; Werner, 1977). However, increased silification in diatoms increases the sinking rate of the cells. Higher densities of silica walls in diatoms –relative to the density of water- are induced by increased levels of SiO2 and reduced levels of iron or nitrogen in the water. Once the density of the cells increases, the cells will sink to lower levels of the column where more nutrients are available (reviewed in: Raven & Waite, 2004).

Not only diatoms have silica shells. Some species within the class of golden-brown algae (Chrysophyceae and Synurophyceae) also obtain a silica shell (Hansen, 1996; McGrory & Leadbeater, 1981; Sandgren *et al.*, 1996). The golden-brown algae are distributed worldwide in both marine- and freshwaters, but most species are found in freshwater (Croome & Tyler, 1985; Hoffmann *et al.*, 2000). Also species of *Synurophytes* are widely distributed, ranging from the polar seas to tropical freshwater. A unique trait of species within the golden-brown algae and *Synurophyte*, is the capability to form stomatocysts, when going into a resting stage. Stomatocysts are found often in the sediments of water bodies (Jordan & Iwataki, 2012).

Species within the class *Xanthophyceae* are as well widespread, although most species are found in freshwater (Falkowski & Raven, 2013). Both *Pelagophytes* and *Raphidophytes* do not count a great number of species in the division *Heterokontophyta*. However, they are capable of forming harmful blooms (Gobler *et al.*, 2005; Jeong *et al.*, 2013). *Pelagophytes* consist mostly of pico- and nanoplankton (0.2-20 µm), and can cause brown tides in lagoons (Gobler *et al.*, 2013; Roy *et al.*, 2011). *Raphidophytes* are mostly found in oligotrophic acidic freshwater (Menezes & Bicudo, 2010; Roy *et al.*, 2011), but are also capable of forming harmful blooms in coastal waters (Kim *et al.*, 2013).

2.2.3 Division of *Dinophyta*

Species within the class *Dinophyceae* (Dinoflagellates) are considered along with diatoms major contributors to primary production (Kirk, 1994). Dinoflagellates consist of photosynthetic- as well as heterotrophic plankton (Brown *et al.*, 2004; Schnepf & Elbrächter, 1992). Dinoflagellates are widely distributed over many waters, with most species living in marine waters. Dinoflagellate blooms often occur near coastal regions (Roy *et al.*, 2011); preferring overall well-illuminated water surfaces (Paerl *et al.*, 2001). Dinoflagellates have two distinguishable organelles, the pusule and extrusomes. The pusule might function in the uptake of macromolecules, osmoregulation and or secretion (Dodge, 1972; Klut *et al.*, 1987). Several kinds of extrusomes may exist in dinoflagellates, which also play a role in the secretion of material (Hausmann, 1978; Rosati & Modeo, 2003). Beside phototrophic and heterotrophic, some dinoflagellates are mixotrophic, which makes them capable of photosynthesis and engulfing particulate organic matter. This gives mixotrophic dinoflagellates an advantage over purely phototrophic dinoflagellates in nitrogen limited environments (Bockstahler & Coats, 1993; Falkowski *et al.*, 2004; Schnepf & Elbrächter, 1992), and waters with low light availability dinoflagellates (Jones, 2000).

Furthermore, dinoflagellates can have a symbiotic relation with cyanobacteria (Carpenter & Foster, 2002; Usher *et al.*, 2007). This increases the abundance of dinoflagellates in the summer months when nitrogen is often low (Fiore *et al.*, 2010). In addition, heterotrophic dinoflagellates form a symbiotic relation with cyanobacteria causing original unpigmented dinoflagellates to show color (Carpenter & Foster, 2002). There is no evidence, however, that the cyanobacteria provide energy to these dinoflagellates by photosynthesis (Raven, 2002).

2.2.4 Division of *Rhodophyta* and *Chlorophyta*

Within the division *Rhodophyta,* the class red algae contain both aquatic plants and phytoplankton (Falkowski & Raven, 2013; Gómez Garreta *et al.*, 2001). Red algae are found in both fresh- and marine water (Falkowski & Raven, 2013; Meyer, 1969). Furthermore, within the class of the red algae there is still a great uncertainty concerning the taxonomy and classification (Broadwater & Scott, 1994). Among the division *Chlorophyta* two classes of phytoplankton are abundant, namely: the green algae and *Prasinophytes*. Green algae are widely distributed in freshwater bodies (Roy *et al.*, 2011). In contrast, species of the class *Prasinophyte* are found mainly in marine waters and are often well presented in deep chlorophyll communities (Falkowski *et al.*, 2004). There are places where also the distribution of green algae increases with increasing depth (Riegman & Kraay, 2001).

2.2.5 Remaining divisions

Many species of coccolithophores (*Haptophyte*) inhabit freshwaters (Falkowski & Raven, 2013), but overall dominate the marine waters (Kilham & Hecky, 1988) and are well abundant in the euphotic layer of the oceans (Honjo, 1976) where the blooms are often seen form space. Coccolithophores assimilate surface scales named coccoliths consisting of calcium carbonate crystals (Paasche, 1968). Thus, coccolithophores need to grow in environment rich with calcite, often in the top layers of the oceans (Paasche, 1968). Another reason for the strong blooms of coccolithophores in the top layers of the oceans is that the calcification of coccoliths is strongly light depended (Paasche, 2001); making coccolithophores in general more light depended. However, some species, such as that *Emiliania huxleyi* can grow well at environments with low light availability (Zondervan, 2007).

Cryptomonads are ubiquitous in freshwater, marine water and brackish water as well as groundwater and snow (Felip *et al.*, 1995; Loquay, *et al.*, 2009; Roy *et al.*, 2011; Taylor, & Lee 1971). Some species within the cryptomonad class are mixotrophic, and preferably ingest organisms such as cyanobacteria and small plankton (Roberts & Laybourn-Parry, 1999; Tranvik *et al.*, 1989). However, it seems that the bacterivory of cryptomonad cells does not contribute to the carbon content of the cells, but is more likely to an important source of inorganic nutrients (Tranvik *et al.*, 1989). Furthermore, mixotrophic strategies could allow species of the class *Cryptophyta* to live in environments such as snow and groundwater with no availability of photosynthetic active radiation (Marshall & Laybourn-Parry, 2002).

Euglenophyta are considered a taxonomic puzzle because of their diversity, unrelatedness and yet their resemblance to other phytoplankton (Kivic & Walne, 1984). Most found in freshwater habitats with high levels of decaying organic matter (Amengual-Morro *et al.*, 2012; Caljon, 1987), but are also found in marine- and brackish waters (Roy et la., 2011).

3 Pigments

The different colors of pigments depend on the absorption and reflection of photons. Pigments absorb suitable photons and reflect the photons, and therefore the color, they cannot absorb. The absorption of photons depends on the structure and the bonds between atoms in the chromophore, which functions as the light capturing molecule (Table 3-1; Prathapan *et al.*, 1993). Molecule groups with double and triple bonds contain conjugated π bonds. In these π bonds, the difference between the ground state and the exited state of electrons is lower than in stable covalent bonds (the σ bonds). Because the energy gap is lower in π bonds, less energy is required to excite an electron (Beaujuge *et al.*, 2010; Valeur & Berberan-Santos, 2012). In other words, the energy carried by the absorbed photon is equal to the difference between the exited state and the resting state of the chromophore (Croce & van Amerongen, 2014; Falkowski & Raven, 2013). Therefore, π bonds absorb photons with less energy, longer wavelength, yielding light absorption closer to the red part of the light spectrum. The probability and the amount of light absorption (ε= extinction coefficient) is depending on the atomic bonds and increases when bonds are less stable or contain less energy in the bonds (Beaujuge *et al.*, 2010; Falkowski & Raven, 2013). However, the extinction coefficient and wavelength absorption of the pigments in an organism depends on many more factors than the molecular composition of the pigment (Adeloye & Ajibade, 2011).

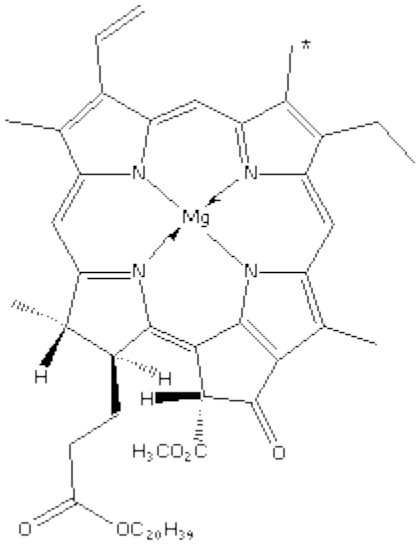


Figure 3-1 The structure of chlorophyll *a*, a pigment found in all phytoplankton

The light harvesting complex (LHC) is assembled of chromophores and proteins and is responsible for transferring energy tot the photosystems (PS) which is responsible for the conversion of light energy to chemical energy. Within the LHC, pigments are conformation dependent (Scholes *et al.*, 2011), where the efficiency and the spectral absorption of the chromophore depends on the lattice structure within the LHC (Croce & van Amerongen, 2014; Noy *et al.*, 2006). However, it should be noted that these structures are built in a cell, and thus the reflection of the cell wall and the cell size (light absorption decreases as the cell size increases) have also a major influence on the spectral absorption of pigments (Rabinowitch & Govindjee, 1969; Kirk, 1994).

In phytoplankton, three main pigments are known, chlorophylls, carotenoids and phycobilins (Table 3-1).There is a significant difference between the architecture and absorption of these pigments. In addition, there is also a distinction between the pigment chlorophyll *a* and other pigments, the so called accessory pigments. In the following five sub-sections the characteristics of these pigments are discussed.

3.1 Chlorophylls

Chlorophyll is assembled of four pyrroles, which is a five-membered ring (C_4H_4NH) consisting of four carbons and one nitrogen atom (Figure 3-1). The nitrogen atoms are coordinated towards a

Table 3-1 light harvesting pigments the absorption spectra maxima may vary significantly depending on the protein environment (Grossman *et al.*, 1993).

Pigment	Chromophore	formula	λ^A, nm	λ^F, nm	reference	ε= extinction coefficient	reference
Chlorophylls	Chlorophyll *a*	$C_{55}H_{72}MgN_4O_5$	430[1], 662^	666	1	$1{,}14 \times 10^4$ (428nm); $8{,}8 \times 10^3$ (661 nm)#	12
	Chlorophyll *b*	$C_{55}H_{70}MgN_4O_6$	453[1], 642#	646	2	$1{,}58 \times 10^4$ (453 nm); $5{,}5 \times 10^3$ (643 nm)#	12
	Chlorophyll c_1	$C_{35}H_{30}MgN_4O_5$	446[1], 629^	633	3	$2{,}14 \times 10^4$ (446 nm); $2{,}4 \times 10^3$ (629 nm)#	12
Carotenoids	β,β-Carotene	$C_{40}H_{56}$	454[1], 480^	-	4	$1{,}25 \times 10^5$ (462nm)+	13
	β,ε-Carotene	$C_{40}H_{56}$	448[1], 476^	-	4	-	
	fucoxanthin	$C_{42}H_{58}O_6$	444[1], 467^	-	5	1×10^4 (449nm)-	12
	lutein	$C_{40}H_{56}O_2$	454[1], 480^	-	6	$1{,}27 \times 10^5$ (458nm)+	13
	violaxanthin	$C_{40}H_{56}O_4$	415, 438[1], 467	-	7	-	
	zeaxanthin	$C_{40}H_{56}O_2$	454[1], 481^	-	6	$1{,}32 \times 10^5$ (452nm)+	13
	diadinoxanthin	$C_{40}H_{54}O_3$	449[1], 479	-	8	-	
	diatoxanthin	$C_{40}H_{54}O_2$	430, 453[1], 480	-	9	$1{,}3 \times 10^3$ (445nm)^	14
phycobilins	Phycourobilin (PUB)	$C_{33}H_{42}N_4O_6$	491	573	10	$3{,}79 \times 10^3$ (690 nm)	12
	Phycoerythrobilin (PEB)	$C_{33}H_{38}N_4O_6$	553[1], 615	563, 646[1]	10	$3{,}55 \times 10^3$ (663 nm)	12
	Phycoviolobilin (PVB)	$C_{33}H_{42}N_4O_6S$	568	-	11	-	
	Phycocyanobilin (PCB)	$C_{33}H_{38}N_4O_6$	642	658	10	$4{,}3 \times 10^3$ (555 nm)	12

[1] maximal absorption of the absorption spectrum
^ Absorbtion in aceton solution.
# Absorbtion diethyl ether solution
+ Absorbtion benzene solution
- petrolium ether

Reference: 1 de Paula et al. (1995)^ ; 2 Seager et al. (2005)#; 3 Jeffrey (1972) ^; 4 Hiyama et al. (1969)^; 5 Haugan et al. (1992)^; 6 Jeffrey et al. (1997) ^; 7 Haugan & Liaaen-Jensen (1994a); 8 Bjørnland & Tangen (1979); 9 Haugan & Liaaen-Jensen (1994b); 10 Falkowski & Raven (2011); 11 Grossman et al. (1993); 12 reviewed in Raven (1984); 13 IARC (2003); 14 Jeffrey (1997)

magnesium atom, forming a ring around the centered magnesium atom. Based on their molecular structure, chlorophylls are distinguished in two groups, the chlorin and porphyrin derived structures. Chlorins, Chl*a* and Chl*b*, have in the fourth ring a statured bond, whereas porphyrin derived structures (Chl*c*) have an unsaturated bond. This double bond in the fourth ring of Chl*c* causes an absorption shift towards the red part of the light spectrum (Falkowski & Raven, 2013). Chlorophyll *a* (Chl*a*) is a group that is present in all photosynthetic organisms, with the exception of photosynthetic bacteria that use the related bacterial chlorophyll a (BChl*a*).

All chlorophylls have two major absorption bands in the blue part and the red part of the spectrum. In the red part of the spectrum, Chl*a* has a narrow but strong absorption band (the so called Q_y band). This band causes a high probability of fluorescence emission (685nm), giving Chl*a* the advantage of transferring energy (Falkowski & Raven, 2013; Scholes *et al.*, 2011). In addition, light energy harvested by accessory pigments is always being transferred to a primary donor consisting of Chl*a* (e.g. Chl*a* P680) (Glazer, 1985). Hence, Chl*a* is the only pigment which is capable of directly converting photo energy into chemical energy (Motten, 2004).

3.2 Carotenoids

Carotenoids (Car) exist of two main groups, namely the carotenoids and xanthophylls, forming a large group of more than 750 different pigments (Britton *et al.*, 2004). Carotenoids consist of an open chain structure with 9-13 conjugated double bonds ending with ionone rings (Rabinowitch & Govindjee, 1969; Vershinin, 1999). Carotenoids absorb light near the blue part of the light spectrum, reflecting orange red light. Carotenoids are extremely hydrophobic, and thus nested into the membrane of the cell (Gruszecki & Strzałka, 2005).

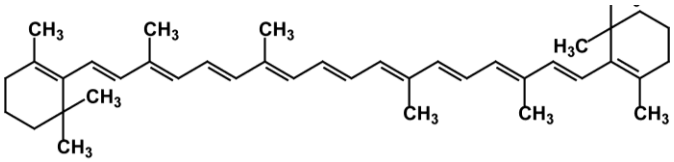


Figure 3-2 The structure of B-carotene, a carotenoids common found in phytoplankton species.

Except for harvesting light, carotenoids function as antioxidants in the feedback de-excitation process of oxygen molecules (Vershinin, 1999). Due to the high energy level of Chl*a*, in high illuminated environments Chl*a* can react with oxygen forming singlet oxygen. Singlet oxygen molecules are unstable molecules causing damage to proteins, DNA and lipids (Di Mascio *et al.*, 1990). Thus, carotenoids are capable of quenching singlet oxygen, making them no longer harmful for the cell (Krinsky, 1989). When plant cells are exposed to high light illumination more carotenoids are synthesized. Especially, high light activates the formation of zeaxanthin from violaxanthin via the xanthophyll cycle (Holt *et al.*, 2005), thus changing the Chl/Car ratio (as further described in 3.6 “Photo inhibition”).

3.3 Phycobilins

Phycobilisomes are pigment complexes shaped by the open-chain tetrapyrroles named phycobilins and linker proteins, absorbing light shifted to the red light of the light spectrum. Phycobilisomes consist of two main parts: the peripheral rods and a core (Figure 3-3 A). The antenna-like peripheral rods are composed phycobiliproteins (PBP), which bind phycobilins (PB): phycourobilin (PUB), phycoerythrobilin (PEB) and phycocyanobilin (PCB) that act as chromophores (Beale, 1994; Glazer, 1985; Ke, 2003; Sidler, 2004). Where the PBs are assembled as a lattice structure attached by pigment-bearing proteins, which serve to keep the PBs in a precise and a more absorbent active position (Fetisova *et al.*, 1988). Centered by phycobiliproteins, lays the core. The core is mostly assembled of three rods consisting of the phycobiliprotein allophycocyanin (Glazer, 1985).

Unlike other pigment complexes, phycobilisomes (PBS's) are attached to the surface of the photosynthetic membrane, with the PBs acting like light harvesting antenna (Sidler, 2004). PBs occur in two or three homologous α, β or γ subunits, which differ in molecular mass (De Marsac, 2003; Gant, 1981). Three α β subunits form a trimer ($(\alpha\beta)_3$) each consisting of six to fifteen PB's. A double trimer forms a hexamer ($(\alpha\beta)_6\gamma$), where also the heavier 'γ' subunit may be assembled (Chang *et al.*, 1996; Glazer, 1985; Sidler, 2004). Furthermore, to connect the trimers and the hexamers, linker polypeptides are assembled between the trimers and the hexamers to form the rods (Ke, 2003). Both the composition and the construction of trimers and hexamers as well as the linker polypeptides have an influence on the absorption wavelength (Ke, 2003; Sidler, 2004). The composition of phycobiliproteins and their wavelength absorption is presented in Table 3-2.

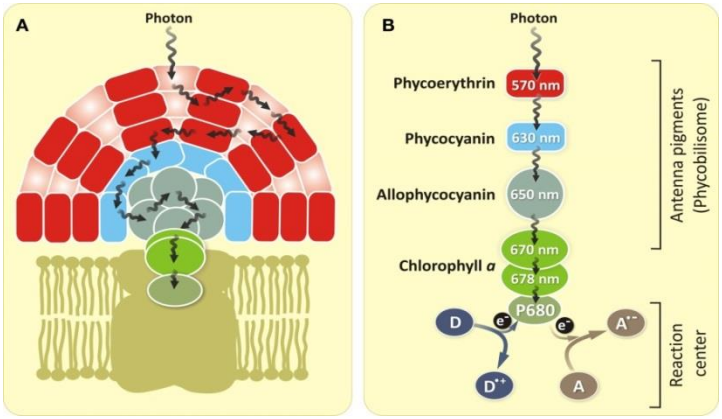


Figure 3-3 A: The architecture of a phycobilosome. In this example the peripheral rods are formed by phycoerytrin and phycocyanin, surrounding the the core assembled of allophycocyanin. B: the structure and electron transfer with the antenna of the phycobilosome (Govindjee, 2011).

Because of the different architecture and composition of PBPs, each kind of PBP has different wavelength absorptions. However, the distal PBPs in PBS absorb photons from the environment, emitting exited electrons with a greater wavelength, which then get absorbed by the other PBPs (Grossman *et al.*, 1993). Each time, there is a transfer in energy in the PBP, energy gets lost. That is why electrons emitted have a greater wavelength than when they were absorbed.

Table 3-2 The composition and the spectral characteristics of phycobiliproteins. The architecture of phycobiliproteins (PBP) occurs in two forms: a trimer assembled from three α and β subunits ($(\alpha\beta)_3$) and a hexamer assembled from two trimers ($(\alpha\beta)_6\gamma$) with an additional heavier subunit γ. Each trimer or hexamer bind phycobilins: phycourobilin (PUB), phycoerythrobilin (PEB) and phycocyanobilin (Table assembled from Sidler (2004) and Glazer (1985)).

Phycobiliproteins (PBP)	Architecture [6,7]	Phycobilins content per unit $(\alpha\beta)_{3;6}$	λ^A, nm[1]	λ^F, nm[1]	Reference
R -Phycoerythrin (R-PE)	$(\alpha\beta)_6\gamma$	α 2 PEB; β 2 PEB; β PUB; γ 1 PUB; 3 γ PEB	564, 536	576	2
B-Phycoerythrin (B-PE)	$(\alpha\beta)_6\gamma$	α 2 PEB; β 3 PEB; γ 2 PEB; γ 2 PUB	562, 543	576	3
C-Phycoerythrin (C-PE)	$(\alpha\beta)_3$	α 2 PEB; β 3 PEB	564	577	4
Phycoerythrocyanin (PEC)	$(\alpha\beta)_3$	α PVB; β 2 PCB	568	607	4
R-Phycocyanin (RPC)	$(\alpha\beta)_3$	α PCB; β PCB; β PEB	553, 618	642	4
C-Phycocyanin (CPC)	$(\alpha\beta)_3$	α PCB; β 2PCB	620	648	3
allophycocyanin (APC)	$(\alpha\beta)_3$	α PCB; β PCB	620, 650	660	5
allophycocyanin B (APC-B)	$(\alpha\beta)_3$	α PCB; β PCB	618, 673	680	1

Reference: 1 Gantt (1981); 2 D'Agnolo et al. (1994); 3 Glazer & Hixson (1977); 4 Glazer & Hixson (1975);
5 Cohen-Bazire et al. (1977); 6 Sidler (2004); 7 Glazer (1985)

For this reason, phycoerythrin, phycocyanin, which absorb photons with a lower wavelength, are located at the distal site of the PBS. Once a photon is absorbed by a phycoerythrin (λ^A_{max} ≈564nm) or a phycoerythrocyanin (λ^A_{max} ≈568nm), trough phycocyanin (λ^A_{max} ≈620nm), energy travels through the core PBPs allophycocyanin (λ^A_{max} ≈650nm) and Allophycocyanin B (λ^A_{max} ≈670nm) to the primary donor P680 (chlorophyll *a* λ^A_{max} ≈680nm) where it continuous the photosynthetic energy transfer chain (Croce & van Amerongen, 2014; Glazer, 1982; Glazer, 1985) (Figure 3-3 B).

3.4 Pigment composition in phytoplankton

The phytoplankton classes and the pigments they carry are presented in Table 3-3. Because Chl*a* is part of photosystem II, it is present in all photosystem complexes of phytoplankton. Chl*b* is found in fewer species and is mainly found in the light harvesting complex LHCsR. The LHCsR complex is a stress responsive complex found in brown and green algae. Furthermore, an LHCsR comparable complex, the Lhcx1 complex is found in diatoms (Bailleul *et al.*, 2010; Mou *et al.*, 2013; Zhu *et al.*, 2010). All classes within *Heterokontophyta* obtain, more or less, Chl*c*. The protein-pigment complex LHCaR found in diatoms bind beside Chl*c* also the carotenoids fucoxanthin and diadinoxanthin (Grabowski *et al.*, 2001). Another Chl*c* binding complex is fucoxanthin chlorophyll a/c2proteins (FCP) and is also found in diatoms and in addition found in coccolithophores (Premvardhan *et al.*, 2000). It binds just like LHCaR fucoxanthin but lacks diadinoxanthin. Present in most dinoflagellates is the peridinin-chlorophyll a-protein, binding mostly the carotenoid peridinin.

Phycobiliproteins are only obtained by a few species of phytoplankton. However, in cyanobacteria phycobilisomes are present in most species. The phycobilisomes in cyanobacteria, optional with additional zeaxanthin, build up their light harvesting antenna (Niyog & Truong, 2013). Red algae might additionally obtain a functional phycobilosome assembled to their LHCaR1 (Grabowski *et al.*, 2001; Green & Parson, 2003). Although dinoflagellates and cryptomonads do assemble phycoerythrin and phycocyanin they do not contain allophycocyanin, the core of the phycobilosome and cannot transfer the energy to the reaction center. This difference results in a different metabolism, where cyanobacteria and red algae store their energy as carbohydrates while in cryptomonads the assimilated carbon is directly used for assimilation for lipids and proteins (Kunath, *et al.*, 2012).

Table 3-3 The major pigments of the largest phytoplankton classes. Table assembled from (Kirk 1994; Roy *et al.*, 2011)

	Chlorophyll			Carotenoids									phycobiliproteins		
Pigments	Chl*a*	Chl*b*	Chl*c*	β,β-Carotene	β,ε-Carotene	fucoxanthin	violoxanthin	zeaxanthin	diadinoxanthin	diatoxanthin	lutein	remaining	Phycoerythrin	Phycocyanin	allophycocyanin
Phytoplankton class															
Cyanobacteria	+*			-				+					+*	+*	+*
Diatoms	+*		+	-		+	-	-	+	-					
Golden-brown algae	+*		+	-		+	+	+				+			
Pelagophyte	+*		+*	-		+		+	+	-		+			
Raphidophyte	+*		+	-		+	-	-				#			
Synurophyte	+*		+	-		+	+		-			-#			
Xanthophyte	+*		-	-					+	-		-			
Red algae	+*			+				+					+	-	-
Green algae	+*	+		+	-						+				
Prasinophyte	+*	+		+	-		+	-			+				
Dinoflagellate	+*	#	+	-	-	+			+	-		+	+[1]	+[1]	
Euglenophyte	+*	+		-	-#				+	-		-			
Coccolithophorid	+*		+	-		+			+	-		+			
Cryptomonad	+*		+	-	-							+	+[1]	+[1]	

+* found in all species
\+ pressent
\- pressent in less or variable quantities
\# found in only a few species
[1] Only present in thylakoid lumen (not found in a phycobilosome)

The only cyanobacteria not obtaining phycobilisomes are *Prochlorococcus*. Instead they form protein-pigment complexes from chlorophyll a/b-binding proteins. These chlorophyll a/b-binding proteins imbedded in PSII differ from other photosynthetic organisms because they are capable of binding divinyl-derived chlorophylls. Chlorophyll a/b-binding proteins (here after named as Pcb) are derived from the *Pcb* genes from for example in *Prochlorococcus* (Garczarek *et al.*, 2011). The number of genes expressed and therefore the number of different protein complexes differs per *Prochlorococcus* strains. *Prochlorococcus* SS120 the 'low light' strain expresses 8 *Pcb* genes (*Pcb*A-H), the 'moderate light' strain MIT9313 expresses 2 *Pcb* genes (*Pcb* A-B), while the 'high light' strain only expresses *Pcb*A (Bibby *et al.*, 2003).

3.5 Costs and benefits of pigments in term of nitrogen and light

When considering the molecule structures of the chromophores only small amounts of nitrogen are imbedded in the molecule (Table 3-2): phycobilins and chlorophylls both contain four nitrogen atoms. In addition, considering single chromophores, chlorophylls and carotenoids have a higher extinction coefficient and thus absorb more photons. However, it is not only the chromophores which determine the costs and benefits of photosynthesis. In addition, pigment-protein complexes make up most of the costs in terms of energy per synthesized unit (Manceau *et al.*, 2011). Furthermore, there is a difference in costs between different pigment-protein complexes. Raven (1984) concluded that the phycobiliprotein complex, allophycocyanin costs four times more in term of nitrogen compared to a chlorophyll *a*/*b* complex. This difference is caused by the overall composition of the pigment complexes. Relative 'cheap' pigment-protein structures like peridinin-chlorophyll complexes (PCP) carry more mol chromophores per mol protein comparison to an 'expensive' pigment like allophycyanins (APC) (Figure 3-4), and thus making them relative cheaper in terms of nitrogen. However, the extinction coefficient of APC is almost four times greater than of a PCP (Table 3-4), and is especially greater when compared relative to the number of chromophores (Zehetmayer *et al.*, 2004).

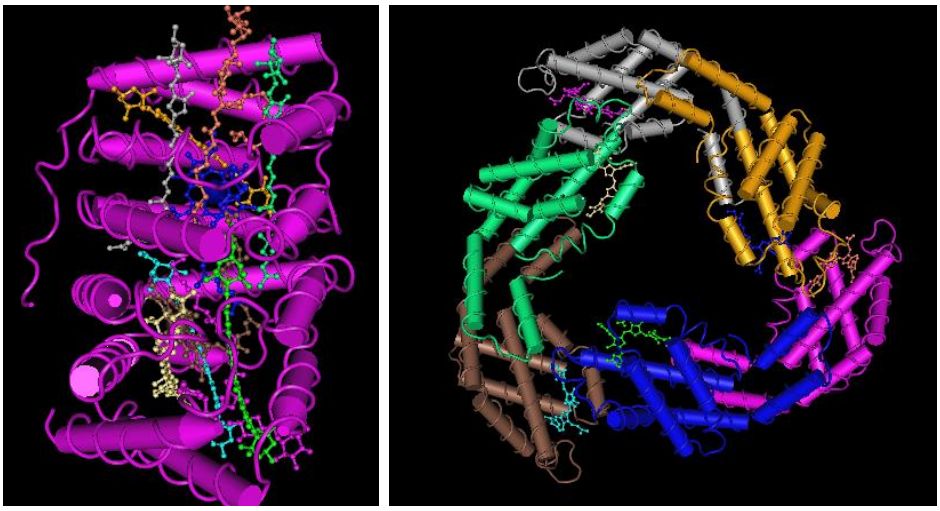


Figure 3-4 Two pigment-protein complexes, where the wormlike structures represent the proteins and the ball and stick structures the chromophores. The structure on the left hand site presents a peridinin-chlorophyll structure with 30 chromphores. The structure on the right hand site presents allophycocyanin with six proteins (three monomers) and six pigments.

Table 3-4 An overview of the pigment-protein complexes their composition, the costs in terms of mol nitrogen per mol chromophore and the benefits in terms of light absorption.

Pigment-protein complex	Composition	molN per mol chromophore	Reference	ε_m M^{-1} cm^{-1} (λ^A_{max})	Reference
peridinin-chlorophyll a-protein	6 Chl a; 24 peridinin	46,34	Haxo et al., (1976); Kamiya & Shen (2003)	8.44×10^4 (≈463nm)	Song et al., (1976)
fucoxanthin chlorophyll a/c2 complex (FCPa+b)	8 Chl-a; 8 Fuco; 2 Chl-c2	23,32	Premvardhan et al., (2000)	1.85×10^5 (553nm)	Ikeda et al., (2013)
LHCaR1 (Rhodophyceae)	8 Chl a; 4 Zea	20,69	Grabowski et al., (2001)		
LHCaR1 (Bacillariophyceae)	7 Chl a; 1 Chl c; 1 Fuco; 2 diadin	22,57	Grabowski et al., (2001)	-	
LHCsR3	6 Chl a; 1 Chl b; 3 Car; 1 Viox; 2 Lut	22,42	Bonente et al., (2011)	-	
PcbA gene (D1)	6 Chl *a* ; 2 pheophytin *a*	56,12	Bibby et al., (2013); Dekker & Boekema, (2005)	-	
PcbB gene (CP47)	16 Chl *a*	29,68	Bibby et al., (2013); Dekker & Boekema, (2005); Kamiya & Shen, (2003)	-	
PcbC gene (CP43)	14 Chl *a* ; βCar	33,42	Rocap et al., (2003); Kamiya & Shen, (2003)	-	
PcbD gene(D2)	6 Chl *a* ; 2 pheophytin *a* ; βCar	42,75	Bibby et al., (2013); Dekker & Boekema, (2005)	-	
R -Phycoerythrin (R-PE)	α 2 PEB; β 2 PEB; β PUB; γ 1 PUB; 3 γ PEB	89,15	Gantt (1981)	1.03×10^6 (565 nm)	D'Agnolo et al. (1994)
B-Phycoerythrin (B-PE)	α 2 PEB; β 3 PEB; γ 2 PEB; γ 2 PUB	89,15	Gantt (1981)	2.41×10^6 (545 nm)	Glazer & Hixson (1977)
C-Phycoerythrin (C-PE)	α 2 PEB; β 3 PEB	178,76	Gantt (1981)	4.88×10^5 (562 nm)	Glazer & Hixson (1975)
R-Phycocyanin (RPC)	α PCB; β PCB; β PEB	168,40	Gantt (1981)	1.51×10^5 (555 nm)	Glazer & Hixson (1975)
C-Phycocyanin (CPC)	α PCB; β 2PCB	155,44	Satyanarayana et al. (2011)	2.41×10^6 (545 nm)	Glazer & Hixson (1977)
allophycocyanin (APC)	α PCB; β PCB	204,02	Marx & Adir (2013)	6.96×10^5 (650 nm)	Cohen-Bazire et al. (1977)
Cr-PE555	6 PEB; 2DBV	65,08	Harrop et al., (2014)	-	
Cr-PC612 (cryptomonads)	6 PCB; 2DBV	86,72	Harrop et al., (2014)	-	

This difference between free phycobilins and bounded bilins is due to the lattice structure, where they are being 'stretched' and lose their symmetric structure and thus becoming more unstable (Fetisova *et al.*, 1988; Zehetmayer *et al.*, 2004). However, phycobilisomes which cost more in terms of nitrogen also cost more in terms of photons (Marosvölgyi & Gorkom, 2010; Raven, 1984), and thus need to have more light to assemble the pigments than other pigments need. This means that in the first time less energy is available for growth. Therefore, it would be expensive for a cell to assemble phycobilisomes only for a short period of time.

In contrast to phycobilins complexes, light harvesting complexes (LHCaR or LHCsR) are composed of many chromophores. LHCsR3 (Light harvesting complex stress response 3) consist of seven chlorophylls and five carotenoids, causing LHCsR3 to obtain a function which is more related to repelling light energy than harvesting light energy with the function to limit cell damage caused by light (Green & Parson, 2003).

Chlorophyll a/b-binding proteins are subunits imbedded in PSII and found in many photosynthetic organisms. However, *Prochlorococcus* differs in this complex by binding different amino acids and DV-Chl (Bibby *et al.*, 2003; Garczarek *et al.*, 2001; Kettler *et al.*, 2007). The amino acids in the complex protect 'high light' strains (SS120) against excessive light, whereas DV-Chl in low light strains (SS120) transfers energy more efficiently (Yokono *et al.*, 2012). However, once DV-Chl is exposed to high light environments it alters into a triplet-state making it non-reactive (Andrizhiyevskaya *et al.*, 2005; Mella-Flores *et al.*, 2012). Although the SS120 strain is efficient with harvesting light in environments with low light irradiance, *Pcb*'s are relative to LHC expensive in terms of nitrogen. Furthermore, it should be noted that SS120 expresses 8 *Pcb* genes (*Pcb*A-H) while the high light strain MED 4 expresses *Pcb*A only (Bibby 2003 *et al.*,) and thus acquiring less nitrogen. However, in general Pcb proteins have fewer costs in terms of nitrogen than phycobilisomes, these cyanobacteria might have an advantage over cyanobacteria which do assemble phycobilisomes in the eutrophic oceans.

In conclusion, theoretically, phytoplankton with cheaper pigment-protein structures assembled of Chls and carotenoids, pose the cell with the advantaged to be better protected in rich illumined environments (Falkowski, 1993). Hence, they are better capable of surviving in N limited environments, such as the aphotic-oligotrophic zone of the oceans. In contrast, phytoplankton containing phycobilisomes require more N but would be more efficient in harvesting light. And thus, might survive better in oligotrophic lakes which are in general environments containing light absorbing particles such as gilvin and tripton.

3.6 The adaption of pigment composition in response to changing light intensity

When phytoplankton cells (with exception of cyanobacteria, *Cryptophyta* and most *Rhodophyta*) are exposed to high light the pH in the thylakoid membrane drops, in response to this acidification a process called the xanthophyll cycle will be activated. Two xanthophyll cycles are known in phytoplankton: the violaxanthin cycle and the diadinoxanthin (Jahns *et al.*, 2009). Among others, pigments formed in this process are quenching pigments. These pigments quench the excess light energy in the cell (Holt *et al.*, 2005), by capturing the light energy and releasing it as heat. Therefore, excess energy cannot reach the reaction center in the cell and thus damage to the reaction center caused by excess light energy is limited (Müller *et al.*, 2001).

3.6.1 Mycosporine like amino acids

Additional photo protective molecules are the mycosporine like amino acids (MAAs). MAAs are small (<400Da) secondary metabolite molecules, with a still relative unknown biosynthesis pathway. MAAs absorb light in the UV part of the spectrum, mainly between 310-362nm (Singh *et al.*, 2008). In dinoflagellates and cyanobacteria MAA has a high absorption at 310nm, while in green algae the absorption in slightly shifted to the red part of the spectrum and has an absorption peek at 324nm (Gröniger & Häder, 2002; Klisch & Häder, 2002). Because, of the absorption near UV-B, MAAs mainly quench photons near the range of UV-B. Furthermore, the exact function of MAAs is still not clear and susceptibly they might have an additional function to UV protection (Carreto & Carignan, 2011; Singh *et al.*, 2008).

4. Effects of light

Light is the main energy source used by photosynthetic organisms such as phytoplankton. Therefore, without light phytoplankton growth would not be possible. However, light availability in different environments differs quantitatively (in total amount) and qualitatively (in amount per wavelength) and changes over time. It is therefore important that phytoplankton species can adapt to the available light climate. To understand the composition of pigments and phytoplankton in different light environments, this chapter will discuss: the effect of light availability on pigment composition of different phytoplankton species, how light availability affects the distribution patterns of pigments and phytoplankton, and the dynamics of phytoplankton species relative to the light changes in their environment.

4.1 Light limitation

Phytoplankton species adapted to low light synthesize more chlorophyll per cell and have a smaller cell size compared to high light adapted species (Harrison *et al.,* 1990). A smaller cell size means a larger surface-volume ratio that increases light absorbance per volume. Furthermore, synthesizing more chlorophyll per (a relative small) cell increases the chance of absorbing a photon, thus increasing the photosynthetic efficiency per biomass. This means that the few photons available are absorbed as efficiently as possible and can be invested in the synthesis of new pigment-protein complexes (Geider *et al.,* 1996). Photosynthetic efficiency per biomass is higher during light limitation. However, the photosynthetic efficiency per chlorophyll and the growth rate are lower during light limitation because energy is invested in synthesizing more pigments rather than the growth rate (Geider *et al.,* 1996; Zou & Gao, 2009). Therefore ultimately, under low light the amount of pigment per liter medium is lower than in high light (Schagerl & Müller, 2006) due to an overall lower growth rate and therefore a lower biomass during light limitation.

4.1.1 Pigment composition in phytoplankton adapted to low light availability

Phytoplankton classes that are adapted to low-light synthesize a different pigment composition compared to classes that are not adapted to low light (Falkowski, 1980). When light availability decreases the total photosynthetic pigment content increases, whereas photo protective pigment content decreases (Deblois *et al.,* 2013; Fujiki & Taguchi, 2002, Lavaud & Kroth, 2006; Partensky *et al.,* 1993). For example, green algae grown in low light synthesize up to four fold more chlorophyll (≈4 fold), but four fold less carotenoids compared to cells grown in an environment where light is sufficient (Bautista & Necchi-Júnior, 2008; Melis *et al.,* 1998). However, the increase in chlorophyll under light limitation is not evenly distributed among the different types of chlorophyll. For example, in low light up to three times more Chl*b* and Chl*d* compared to Chl*a* is synthesized (Gan *et al.,* 2014; Gloag *et al.,* 2007; Melis *et al.,* 1998; Partensky *et al.,* 1993; Partensky *et al.,* 1997).

Unlike many other classes can cyanobacteria often maintain growth in light limited environments and are thought to be superior competitors for light (Downing *et al.,* 2001). Indeed, cyanobacteria pose a number of adaptations to low light; cyanobacteria adjusted to low light environments contain more thylakoid membranes, which contain more PBSs, which increases the chance of a photon absorbance (Kana & Glibert, 1987). Furthermore, when cells of cyanobacteria are faced with low-light availability

they synthesize more PC compared to PE (De Marsac, 1977). Also cryptomonads faced with a light limitation assemble about four fold more PE and chlorophyll with a slightly increased PE/Chl ratio (from ≈3 to 4.2) (Akimoto *et al.,* 2012). However, it is unknown if in cryptomonads the PC/PE too increases, resembling the pattern seen in cyanobacteria.

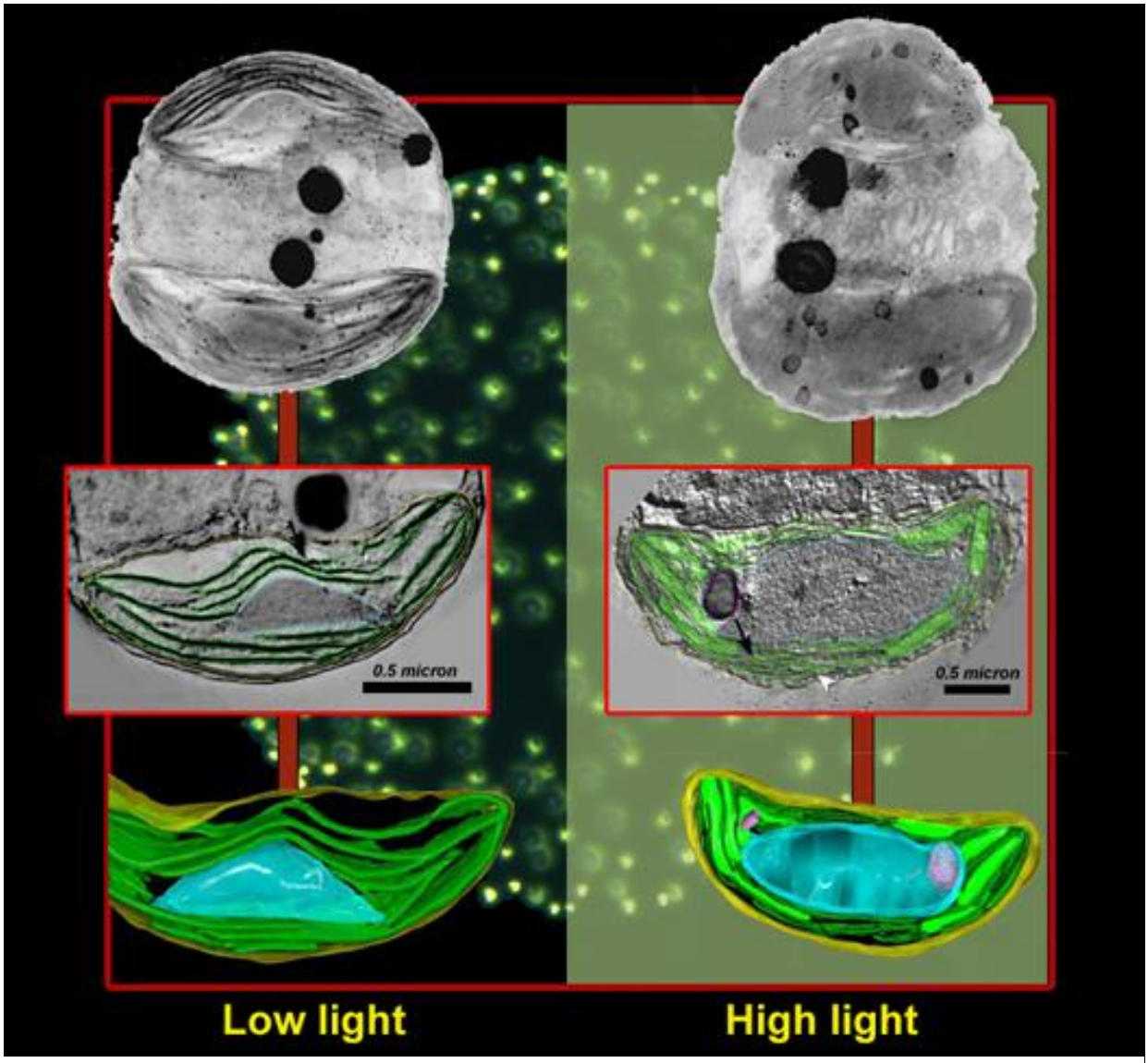


Figure 4-1 An electron tomography of a *Haptophyte* cell (*Phaeocystis*) grown in low light and high light. The cells show three distinct differences, the cell grown in low light has: 1 a smaller volume, 2 more and stacked thylakoid membranes (dark green structures), and 3 less starched stored in the cell (the light blue structure). Reference: Moisan *et al.*, (2006)

A good example of a species that is adapted to different light environments is the marine cyanobacterium *Prochlorococcus*. *Prochlorococcus* can live in the oceans up to 200 meters depth but is most abounded at 100 meters depth, and contributes the largest part of the deep chlorophyll maximum (DCM) (Agustí, 2004; Campbell and Vaulot 1993; Cordeiro *et al.,* 2013; Shibl *et al.,* 2014). This extreme difference in depth distribution results in a large range in light irradiance. Phytoplankton in the top layers

of the ocean are exposed to ≈1500 photons m^{-2} s^{-1}, while at great depth as little as 0.1 photon m^{-2} s^{-1} remains available for photosynthesis. There are three strains of *Prochlorococcus* described in the literature, namely: MED4 a high light adapted strain, MIT9313 a moderate light adapted strain and SS120 a low light adapted strain. While being exposed to relative high light (80 µmol photons m^{-2} s^{-1}) all strains reduced the number of D1 (part of the PS reaction center). However, the high light adapted strain MED4 reduced the concentration of D1 more strongly compared with the low light adapted strain SS120. This faster decrease in D1 found in MED4 might due to the rapid replacement of an alternate D1 protein ($D1_2$) or because of the degradation of D1 which might be replaced later on in the process during longer exposure to high light (Aro *et al.,* 1992; Clarke *et al.,* 1993; Garczarek *et al.,* 2008; Thiele *et al.,* 1996).

The high-light adapted strain MED4 not only has a stronger decrease in D1 when exposed to light, but also has fewer genes encoding for core protein-pigment complexes D1 and none for the second reaction center D2 (Rocap *et al.,* 2003). However, the protein-pigment complex CP43 (encoded by the *isi*A gene) occurring in the reaction center complex containing carotenoids is higher in MED4 compared to the low light adapted strain SS120 (Table 4-1). Furthermore, also in both the moderate and low light adapted strains CP43 increased with increasing light irradiance (Partensky *et al.,* 1993). Due to the carotenoids, CP43 protects PSII from photo-inhibition and therefore protecting high light adapted strains from excessive light irradiance (Cadoret *et al.*, 2004; Ivanov *et al.*, 2007). Furthermore, changes in D1 and CP43 are not only seen in *Prochlorococcus* but in more cyanobacteria (Ivanov *et al.*, 2007; Lohscheider *et al.,* 2011). However, it should be noted that other phytoplankton species do not assemble CP43 encoded by the *isi*A gene, but assemble a different CP43 encoded by the *psb*C gene which is lacking the quenching capability (Green & Durnford, 1996).

Table 4-1 The relative concentration of pigments and the pigment-protein complexes D1, D2 and CP43 of the strains *Prochlorococcus* MED4 and *Prochlorococcus* SS120. The complexes D1, D2 and CP43 are all located in PSII. In PSII CP43 passes energy to the reaction center complexes D1 and D2 that are located in the core of PSII. The complexes D1 and D2 are responsible for the energy conversion process.

Pigment complex or ratio	MED4 (high light adapted)	SS120 (low light adapted)
D1 reduction	High	Low
D2	None	Present
CP43	High	Low
DV-Chl a / zeaxanthin	Low	High
DV-Chl a/DV-Chl b	High	Low
Chl a/Chl d	High	Low

The differences between these strains not only concern the amount of pigment-complexes but also the pigment ratios. For example, MED4 has a higher DV-Chl a/DV-Chl b ratio and a lower DV-Chl a/zeaxanthin ratio. Thus, when cells are exposed to high light this leads to a pigment composition where zeaxanthin > DV-Chl a >> DV-Chl b. The low light adapted SS120 strain contains approximately a ten times lower DV-Chl a/DV-Chl b ratio and a five times higher DV-Chl a/zeaxanthin ratio (Partensky *et al.,* 1993, Partensky *et al.,* 1997).

4.1.2 Photosynthetic efficiency and growth of cells during low light availability

When pigments of phytoplankton are exposed to low light the pigments transfer the light energy as efficiently as possible, so that less energy is lost and a higher percentage of energy reaches the reaction center (Akimoto *et al.,* 2012). Despite the high efficiency of energy transfer, less light is available and therefore less carbon for growth can be fixed. Furthermore, most energy is invested in synthesizing more pigments rather than growth (Geider *et al.,* 1996; Zou & Gao, 2009). Because low light irradiance decreases the growth rate, the growth rate of cryptomonads plummeted until 20 hours after decreased light irradiance, thereafter the number of cells stays relative the same. Despite the fact that cells grown in low light have a slower growth rate, there is a difference in optimal light irradiance depending on the strain or species and the pigment they synthesize. Strains and species adapted to high light synthesize higher amounts of PE relative to PC, Chl*a* and carotenoids relative to Chl*b* and Chl*d* would, theoretically, have lower growth rates grown under low light environments than high light environments. For example, the high light adapted cyanobacterial strain MED4 has an optimal growth between a light irradiance of 15 and 80 µmol photons m^{-2} s^{-1}. In contrast, the optimal growth of the low light adapted strain SS120 occurs at a lower irradiance between 8 and 30 µmol photons m^{-2} s^{-1} (Partensky *et al.,* 1997).

4.2 Light inhibition

Light is an important source of energy for phytoplankton. However, high photosynthetic active radiation (PAR) and ultra violet (UV) irradiance causes photo oxidative damage to the photosystem that leads to photo inhibition (Falkowski, 1984; Murata *et al.,* 2007; Vass, 2012). Therefore, with increasing light, the cellular content of quenching pigments such as zeaxanthin or diadinoxanthin increases, while the cellular content of photosynthetic pigments such as Chl decreases (Deblois *et al.,* 2013; Fujiki & Taguchi, 2002, Lavaud & Kroth, 2006; Partensky *et al.,* 1993). The increase of the quenching pigments zeaxanthin and diadinoxanthin is regulated by the xanthophyll cycle. Phytoplankton within the classes *Bacillariophyceae*, *Xanthophyceae*, *Haptophyceae*, and *Dinophyceae* have a xanthophyll cycle (Goss & Jakob, 2010) (see subsection 3.6 for an introduction of the xanthophyll cycle). The quenching capability of the xanthophyll cycle gives excellent protection during high or intermittent light exposure (Lavaud *et al.,* 2002). Cells with a xanthophyll cycle do not transport the available photons to the reaction center when they are exposed to high light, but discard them from the cell as heat due to the quenching pigments (Geider *et al.,* 1996; Mostofa *et al.,* 2013). Hence, at high light, the pigment complexes become saturated, and large antenna pigments become redundant (Blankenship & Chen, 2013).

In reaction to light inhibition, the amount of functioning chlorophylls per phytoplankton cell decreases (Cruz & Serôdio, 2008; Partensky *et al.,* 1993; Zhu *et al.,* 2010). Such a reaction includes the detachment of chlorophyll from the photosystem which decreases light absorption and thus also decreases damage to the core of the photosystem caused by excess energy (Mostofa *et al.,* 2013). The observed decrease in chlorophyll might be also caused by the bleaching of chlorophyll caused by photo oxidative damage (Cruz & Serôdio, 2008) (see textbox). Another adaptation against damage of the photosystem, is by increasing the number of quenching pigments such as photo protective carotenoids. This results in an

Chlorophyll or other pigments can become bleached when a pigment absorbs a photon that carries enough energy not only to excite an electron but to free an electron from the atom. This causes changes the charge of the atom, making the pigment less efficient for absorbing light.

increase of the Car:Chl ratio, with increasing light irradiance (Lavaud & Kroth, 2006; Partensky *et al.,* 1993; Zhu *et al.,* 2010). However, the composition of pigments in reaction to light inhibition differs per class, species and even strain and often depends on their habitat.

4.2.1 The composition of pigments during high light availability

Diatom cells are most efficient in dealing with high light exposure, and can down-regulate their photosystem II by 90% during prolonged light inhibition. The down regulation of photosystems prevents photo inhibition and thus damage of the pigment complex caused by excess light energy (Brand & Guillard, 1981; Goss & Jakob, 2010; Lavaud *et al.,* 2002). Furthermore, an important feature of diatoms to cope with high light, are light harvesting centers (LHC) that attach large amounts of quenching pigments to the protein-pigment complex, such as fucoxanthin and diatoxanthin (Grabowski *et al.,* 2001; Premvardhan *et al.,* 2000). Diatoms are able to synthesize two LHCs that are specifically involved in photo protective mechanisms. The light harvesting complex stress-related proteins (LHCsR) and the fucoxanthin chlorophyll a/c2 complex (FCP) (Grabowski *et al.,* 2001; Premvardhan *et al.,* 2000). Diatoms, always contain maximum amounts of the LHCsR family pigment-protein complexes with high amounts of Car and are therefore always prepared for excess light irradiance (Abe & Gianesella-Galvão, 1991; Bailleul *et al.,* 2010; Falkowski, & Owens, 1980; Niyogi & Truong, 2013). This preventive protection causes a very efficient non photochemical quenching (NPQ) for diatoms. Compared to higher plants, the diatom's NPQ is three to five times higher (Ruban *et al.,* 2004). Thus, the high NPQ and therefore the possibility for diatoms to maintain the reaction center during high light makes diatom cells well-adjusted to high light conditions.

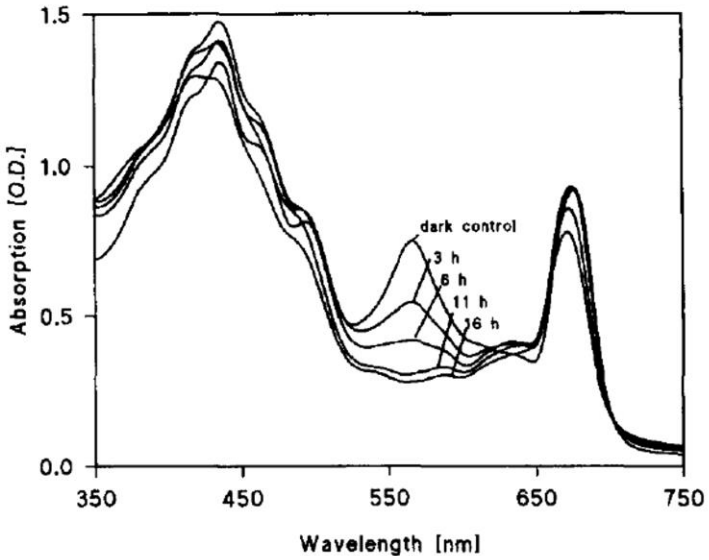


Figure 4-2 The absorption spectra of cryptomonas over increasing time to unfiltered solar radiation exposure. After 16 hours the light absorption of PE (≈560nm) is drastically decreased (Gerber & Häder, 1993)

In contrast to diatoms, green algae have to synthesize extra quenching pigments to protect the cell from damage caused by excess energy (Holt *et al.,* 2005). Cells grown in high light environments synthesize six times more zeaxanthin than cells grown in low light (Perrine *et al.,* 2012). In fact, under light inhibition, the pigment content of some species of green algae can consists of up to 59% of carotenoids (Qin *et al.,* 2008). Also dinoflagellates exposed to high light synthesize large amounts of quenching pigments and also increase the Chl*c* content (Berdalet *et al.,* 1992). Hence, even though their quenching pigments have to be induced, green algae and dinoflagellates are classes that are also well adapted to high light environments.

Cyanobacteria, too, increase their carotenoid content (Pattanaik *et al.,* 2012). Furthermore, cells exposed to high light synthesize less but larger PBPs, and less thylakoid membranes (Kana & Glibert, 1987; Pattanaik *et al.,* 2012). Within the PBPs the PE:PC ratio increases up to 26:1 (Aráoz & Häder, 1999). Within PE, the chromophores phycourobilin (PUB) and phycoerythrobilin (PEB) ratios change. If cells are exposed to bright light the PUB:PEB ratio increases (Küpper *et al.,* 2009). Aráoz & Häder (1999) suggest that PE might have an additional protective function, due to the high losses of absorbed energy as fluorescence. However, little evidence is available to support this hypothesis. Furthermore, in all species that synthesize PE, PE drastically decreases when cells are exposed to high light (Figure 4-2) (Gerber & Häder, 1993; Kana & Glibert, 1987). Although cyanobacteria decrease all PBP pigments, a strong decrease in chlorophyll has not been observed (Pattanaik *et al.,* 2012). When exposed to high light, cyanobacteria accumulate the CP43 complex (*Isi*A) (Table 4-1). If the *Isi*A operon is removed, pigments become bleached when exposed to high light. Therefore, *Isi*A has an important function in the protection against light inhibition (Cadoret *et al.,* 2004; Havaux *et al.,* 2005). In contrast, the DV-Chl *a* binding PCB light-harvesting protein complex of *Prochlorococcus* is highly sensitive to high light exposure and bleaches quickly once exposed to high light (Andrizhiyevskaya *et al.,* 2005; Mella-Flores *et al.,* 2012; Mimuro *et al.,* 2011). Consequently, *Prochlorococcus marines* must depend on the quenching function of zeaxanthin to limit protein damage (Partensky *et al.*, 1999).

When the high light adapted *Synechococcus* strain MED4 and the low light adapted strain SS120 strain are being exposed to high light (80 µmol photons m^{-2} s^{-1}) both strains reduce their D1 (reaction center) concentration. However, MED4 reduces the concentration of D1 more strongly compared with SS120. The decrease in D1 is part of the photo inhibition process and might be due to the rapid replacement of the alternate D1 protein ($D1_2$) or the degradation of D1 that might be replaced later on in the process or is not be replaced at all when irradiance stays high (Aro *et al.,* 1992; Clarke *et al.,* 1993; Garczarek *et al.,* 2008; Thiele *et al.,* 1996).

Another adaptation of cyanobacteria to high light is the orange carotenoid protein (OCP). OCP has a low quantum yield of about 0.03 (Punginelli *et al.,* 2009). Compared to CPC and B-PE with a quantum yield of 0.51 and 0.98, energy transfer by OCP is negligible and OCP probably only functions as a sensor for high blue light irradiance and as a non-photochemical quencher (NPQ) (Wilson *et al.,* 2008). A closely related protein complex is the red carotenoid protein (RCP) which is derived from OCP by removal of approximately 160 amino acids (Kerfeld, 2004a).This removal exposes the carotenoid molecule 3'-hydroxyechinenone (Chábera *et al.,* 2011). Therefore RCP has a higher quenching capability (Kerfeld, 2004b). However, at this day little is known about the adaptive capabilities and the distribution of OCPs and RCPs.

4.2.2 The efficiency of pigments during high light availability

Too much light energy may become damaging to pigment as well as protein-complexes such as reaction centers. To protect the reaction centers during high-light, the total number of photosynthetic pigments decreases or pigment-complexes can decouple from PSII, which decreases the interaction between pigments and suppresses the energy transfer (Akimoto *et al.,* 2012). Hence, less energy reaches the reaction center, therefore less damage to the reaction centers will occur (Nakajima & Ueda, 2000). As a result the photosynthetic productivity of phytoplankton with fewer interacting pigments increases

compared to cells that become light damage due to excess transfer of energy (Nakajima & Ueda, 2000). Yet, compared to low light, under high light the photosynthetic efficiency decreases and cells become light saturated faster and therefore have a lower maximum photosynthetic rate (P_{max}) (Falkowski, 1984). However, this only applies to the photosynthetic efficiency per phytoplankton biomass, the photosynthetic efficiency per chlorophyll is higher when light availability is higher (Zou & Gao, 2009).

How much the photosynthetic efficiency increases or decreases per biomass depends not only on the number of functional photosynthetic pigments but also on the number of quenching carotenoids (Zhu *et al.,* 2010). Because quenching pigments have a low photosynthetic efficiency, the 6.8 fold increase of quenching pigments in green algae might be the reason for the strong decrease in maximum photosynthetic rate during prolonged light inhibition (Perrine *et al.,* 2012). Similarly, the increase of diatoxanthin in diatoms also causes a significant decrease in the maximum quantum yield of photosynthesis (Domingues *et al.,* 2012). However, more damage occurs when fewer carotenoids are available (Sandmann *et al.,* 1993) which also results in a decrease of photosynthetic efficiency. Thus, species that are capable of coping with high light irradiance can, despite their lowered P_{max}, grow better when exposed to high light (Falkowski, & Owens, 1980). In example, many species of green algae do not experience growth inhibition while exposed to a constant high irradiance (Deblois *et al.,* 2013).

In *Prochlorococcus,* DV-Chl is prone to damage caused by singlet oxygen and becomes therefore non-reactive quickly in high-light environments (Andrizhiyevskaya *et al.,* 2005; Domingues *et al.,* 2012; Mella-Flores *et al.,* 2012; Mimuro *et al.,* 2011). In addition, cyanobacteria that contain high PC amounts also have low photosynthetic activity when exposed to high light (Nakajima & Ueda, 2000). Hence, their photosynthetic activity increased when cells were modified to synthesize fewer PCs (Nakajima, *et al.,* 1998). PE-rich strains are also sensitive to high light conditions and reached highest photosynthetic and growth rates at low light intensities (Moser *et al.,* 2009). It is suggested that cyanobacterial growth is inhibited under high light conditions (1000 µmol photons m^{-2}) (Deblois *et al.,* 2013), and that cyanobacteria therefore are superior light competitors in light limited waters (Downing *et al.,* 2001).

4.3 Temporal dynamics in pigment composition in reaction to light

Unlike most photosynthetic organisms, phytoplankton are not fixed within their environment, but drift along different light environments depending on the currents or the wind mixing the water body. Therefore, depending on the habitat, most phytoplankton can adapt to light changes. However, species like *Prochlorococcus* that live deep in the ocean and that are adapted to low light environments may face fewer changes in light environments than species living at the water surface, or in coastal waters that have, because of tides and waves, more fluctuation in light environments. Therefore, changes in pigment composition in response to light changes varies among phytoplankton classes (Falkowski, & Owens, 1980).

Diatoms that are suddenly exposed to high light showed little changes in their chlorophyll content and donor chlorophyll Chl p700 (located in the reaction center converting energy). This implies that the number of photosynthetic systems in the diatoms' thylakoid membrane stays the same when exposed to different light irradiances (Anning *et al.,* 2000; Falkowski, & Owens, 1980). However, when cells are exposed for a longer time to high light the chlorophyll level decreased by 2.5 fold. This adaptation was

reached after five days. Thereafter, when cells were exposed to low light it took three days to get back to the steady state (Anning *et al.,* 2000). Anning *et al.,* (2000) suggested that the lower quantity of chlorophyll during periods of high light exposure is not due to degradation of chlorophyll, but is due to a higher growth rate, while the chlorophyll synthesis rate lowers or remains the same (Post *et al.,* 1984). Nevertheless, chlorophyll exposed to high light produces singlet oxygen that harm the pigment-protein complex which could be an additional cause for the reduction of chlorophyll in high-light exposed cells.

While changes in Chl concentrations take places over days the xanthophyll cycle of diatoms is a fast process (Ruban *et al.,* 2004). Diatoxanthin (DT) concentration in diatom cells sharply rise within 10 minutes when exposed to high light and within 1 hour 62% of diadinoxanthin (DD) was converted to DT (Domingues *et al.,* 2012). Hence, the ratio of DD and DT differed significantly between high and low light irradiance. The fact that the total DD+DT in cells exposed to high-light and low-light stayed the same, confirmed that DTs are involved in the xanthophyll cycle (Domingues *et al.,* 2012). Furthermore, cells may synthesize additional diadinoxanthin when chlorophyll is damaged due to photo oxidative stress (Cruz & Serôdio, 2008). When diatom cells were relived from light stress DD fully recovered (non-photochemical quenching relaxation) within ≈20 minutes (Domingues *et al.,* 2012).

The fast changes in the xanthophyll cycle of diatoms when they are faced to high light pose an excellent protection during intermittent light exposure, and thus in turbulent waters (Lavaud *et al.,* 2002). The fast synthesis of LHCsR also in non-light stressed situations (Bailleul *et al.,* 2010; Niyogi & Truong, 2013), which maintains the photosynthetic reaction center (Falkowski, & Owens, 1980) together with the diatoms xanthophyll cycle, make diatoms well-adjusted to fast changing light environments.

In green algae, during exposure to high light, zeaxanthin is formed from violaxanthin to quench excess energy (Holt *et al.,* 2005). Non-photochemical quenching already sharply increased after 15 seconds (Bautista & Necchi-Júnior, 2008). However, when green algae are exposed to high light for a longer period of time (28 days) the maximum photosynthetic rate decreases significantly, whereas no difference is seen after four days high-light exposure (Bautista & Necchi-Júnior, 2008). Furthermore, in contrast to diatoms, in green algae the number of Chl p700 changes when adapting to light shaded environments (Falkowski, & Owens, 1980). Some green algae are well adapted to changing environments because of the synthesis of LHCsR which in green algae, consist of 7 chlorophylls and 6 carotenoids (Table 3-4) and are capable of activating the xanthophyll cycle (Bonente *et al.,* 2011; Mou *et al.,* 2013). However, unlike diatoms, green algae only express the genes encoding for LHCsR under light stressed situation (Dong *et al.,* 2012; Liguori *et al.,* 2013; Niyogi & Truong, 2013). Therefore, green algae are less efficient in dealing with short light saturating intensities than diatoms.

Cyanobacteria are capable of changing the composition of their PBSs and compared to *Prochlorococcus*, cyanobacteria that do synthesize PBS have an advantage in habitats where light may alternate between high and low (Six *et al.,* 2007a). Such adaptation is seen in PE, which can change the PUB:PEB ratio depending on the environment and strain. For example, PE under low light showed a 20 fold higher plasticity in adaptation compared to cells exposed to high light (Harrison *et al.,* 1990). Furthermore, strains with a low PUB:PEB ratio tend to adapt the PUB:PEB ratio more strongly in reaction to a changing environment (Palenik, 2001). Hence, high light adapted strains with fewer but larger PBS may already

contain the maximum number of PEs with a maximum PUB:PEB ratio which leaves little space left for further adaption.

When light drastically increases, PBS starts to decouple from the donor chlorophyll in the reaction center within ≈20 seconds and after within ≈5 minutes most PBPs are decoupled (Küpper *et al.,* 2009). However, how fast PBPs such as PE decrease after decoupling may depend on the strain. The higher light adapted strain *Synechococcus*, has a relatively fast response in reducing PE levels when exposed to high light. After five days PE levels have plummeted as a reaction to high irradiance. In contrast, the PE levels from the strain BO8808 adapted to lower light starts to decrease PE levels after five days and PE levels are dropped steadily only after 12 days (Postius *et al.,* 1998). Hence this slow reaction from the strain BO8808 could lead to significant damage to the photosystem when exposed to high light irradiance. Furthermore, not only cyanobacteria are capable of decoupling their PBS, also red algae are capable of decoupling their PBS from the PS (Stadnichuk *et al.,* 2011).

While, in cyanobacteria, PBS decreases in high light environments, levels of CP43 (expressed by the *isi*A gene) increase (Partensky *et al.,* 1997). In the presence of CP43 cells recover within 24 hours from light stress, whereas cells without CP43 did not recover to the original pigment content (Park *et al.,* 1999). In addition cyanobacteria respond quickly to UV-B; within 15 minutes the reaction center $D1_1$ gets replaced by an alternatively high light adapted $D1_2$ protein (Campbell 1998; Clarke *et al.,* 1993; WU *et al.,* 2011). However, in contrast to the decoupling process of D1, it takes 16 hours before the concentration of the original $D1_1$ is fully recovered (Lohscheider *et al.,* 2011). And although cyanobacteria are capable of adjusting the concentration of their photo protective chromophores they do not possess a xanthophyll cycle (Rascher *et al.,* 2003; Schagerl & Müller, 2006), therefore the synthesis of photo protective chromophores takes longer than in e.g. diatoms and green algae.

4.4 Light quality

Different classes of pigment absorb photons with different wavelengths (Figure 4-3). Therefore by changing the pigment composition cells can alter the light absorption, hence absorbing more light in the blue or the red part of the spectrum depending on the light availability. However, which pigments are synthesized and which light color triggers the synthesis of pigments differs per class and even per species.

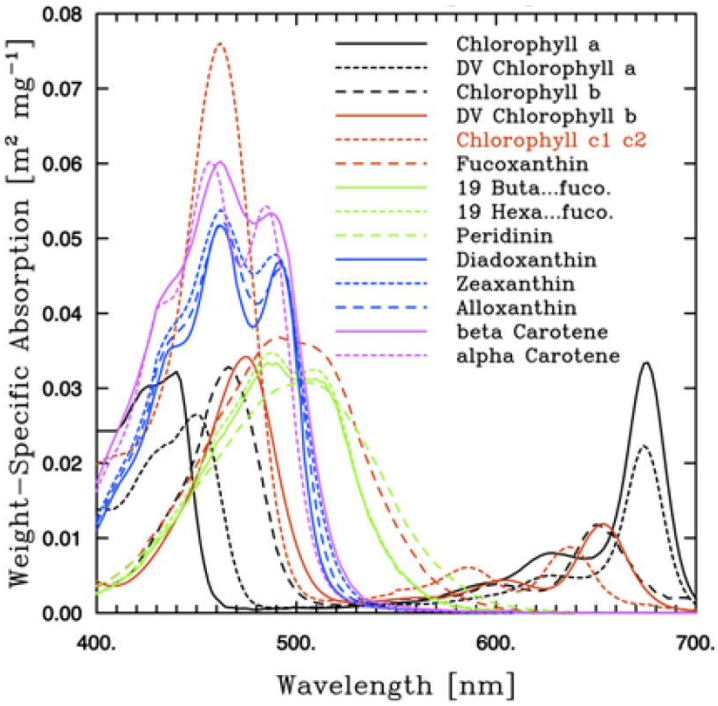


Figure 4-3 The *in vitro* weight specific absorption spectra of several photosynthetic and photoprotective pigments (Devred *et al.*, 2013)

4.4.1 Composition of pigments during red shifted light

During red shifted light, overall, more Chl*a* is synthesized as well as the yellow/red absorbing PBSs and the far-red absorbing Chl*d*. When diatoms are grown under red light Chl increases compared to cells grown under white light (Abe & Gianesella-Galvão, 1991; Aidar et al, 1994). However, the Chl*a*/Car ratio does not increase, corresponding to reports that diatoms in general assemble Car when Chl is assembled (Abe & Gianesella-Galvão, 1991). Cyanobacteria grown under red-light synthesize more PC compared to PE (De Marsac, 1977; Parmar *et al.,* 2013; Stomp *et al.,* 2008; Whitaker *et al.,* 2011; Whitaker *et al.,* 2009), also more APC is synthesized and therefore cells synthesize more but smaller PBSs (Whitaker *et al.,* 2011; Whitaker *et al.,* 2009). However, not always do cyanobacteria need to synthesize more PBS. If the freshwater cyanobacteria *Arthrospira platensis* is exposed to red light they synthesize lower quantities of PBSs compared to other colors. In addition cells of *A. platensis* did grow even more rapidly under red light (Akimoto *et al.,* 2012). Hence, cells of *A. platensis* grown under red light can harvest the light by using the donor Chl in the reaction center ChlF760 and therefore directly invest the energy in growth rather than in pigment content (Akimoto *et al.,* 2012).

Due to the high efficiency of PBSs, energy transfer is most efficient when cells are exposed to yellow/red light (Akimoto *et al.,* 2012; Gloag *et al.,* 2007). Therefore, phytoplankton species synthesizing PBS have an advantage in turbid waters with a red shifted light spectrum. Some cyanobacteria such as *Acaryochloris marina* synthesize Chl*d*, a far red light (710nm) absorbing chromophore (Gloag *et al.,* 2007).Hence, the growth rate of cyanobacteria *Acaryochloris marina* is higher when it is exposed to red light compared to green light (Gloag *et al.,* 2007). Yet, not all species are more efficient while exposed to red light. For example, the growth rate of diatoms, when exposed to red light, is lower than when cells are exposed to blue or white light (Costa *et al.,* 2013).

4.4.2 Composition of pigments during blue shifted light

Blue light triggers the synthesizes of photoprotective pigments such as fucoxanthin, MAAs (Cadoret *et al.,* 2004; Costa *et al.,* 2013; Montgomery, 2007; Wilson *et al.,* 2008). Hence, under blue light fucoxanthin amounts are higher compared to other colors, but also Chl*c* increases (Aidar *et al*, 1994; Costa *et al.,* 2013; Mouget *et al.,* 2004; Valle *et al.,* 2014). The increase of Chl*c* and carotenoids results in a strong absorption in the blue part of the spectrum (450-500nm) (Bidigare *et al.,* 1990). The strong absorption of pigments like Chl*c* and carotenoids which occur in diatoms, dinoflagellates and coccolithophores results in a high photosynthetic activity in the blue part of the spectrum by these classes (Jiang *et al.,* 2012; Glover *et al.,* 1997). This coincides with a high growth rate under blue light of in diatoms, dinoflagellates and coccolithophores (Abe & Gianesella-Galvão, 1991; Aidar et al, 1994; Costa *et al.,* 2013; Holdsworth, 1985; Mouget *et al.,* 2004; Oh *et al.,* 2008).

In cyanobacteria the synthesis of the photo protective pigment-complex such as CP43 is triggered by blue light (Cadoret *et al.,* 2004). Furthermore, under blue light PE/Chl ratio increases compared to other colors or white light in red algae (Brody & Emerson, 1959). The cyanobacterium *F. diplosiphon* grown under green light synthesizes ≈4 fold higher amounts of PE compared to PC (Whitaker *et al.,* 2011; Whitaker *et al.,* 2009). However, when light shifts towards UV-B the concentration of PE decreases (Aráoz & Häder, 1999). Furthermore, during blue light exposure the cyanobacteria *Spirulina fussiformis* decreases the number of PBPs and up regulate Chl that can result in Chl*a* numbers that exceed the number of the other pigments (Madhyastha & Vatsala, 2007). Because PBPs are highly efficient in transferring energy, cells lose this efficiency when exposed to blue light (Akimoto *et al.,* 2012) that result in a lower photosynthetic efficiency and therefore a lower growth rate when cyanobacteria and red algae are grown under blue light (Aguilera et al, 2000; Figueroa *et al.,* 1995).

The role of mycosporine like amino acids

Mycosporine like amino acids (MAA) are light absorbing secondary metabolites that are associated with the absorption of UV light and the protection of cells against photo oxidative stress (described in Chapter 3.6.1). The exact role of MAA's in different species of phytoplankton seems to differ. For example, concentrations in symbiotic dinoflagellates did not seem to depend on UV irradiation (Banaszak *et al.,* 2000). However in non-symbiotic oceanic dinoflagellates MAAs do seem to play a role in UV protection (Singh *et al.,* 2008). In the cyanobacteria *Microcystis aeruginosa* MAA's do not seem to play a crucial role in photo protection (Hu *et al.,* 2014). In contrast, MAA concentrations do increase significantly in response to UV-B exposure in *Aulosira fertilissima*, *Scytonema*, *Nostoc*, and *Anabaena* (Mishra & Richa, 2014; Mushir & Fatma, 2011; Sinha *et al.,* 2001). The difference between these cyanobacteria and their reaction to the exposure of UV-B might be due to the differences in habitat. *Aulosira*, *Scytonema*, *Nostoc*, and *Anabaena* occur both in freshwater and terrestrial habitats and must therefore be well adapted to high UV light irradiance. In contrast *Microcystis* only inhabits freshwaters and is therefore less exposed to UV light (Bharadwaja, 1934). However, the adaptation through MAAs to higher UV-B irradiance is a relative slow process and showed no difference in concentration after 24 hours increased UV-B irradiance. After 48 hours of increased UV-B a ≈1.5 fold increase of MAA was observed (Mishra & Richa, 2014). This might indicate that MAA unlike e.g. the xanthophyll cycle is less functional in adapting to high or low light irradiance but might form an important protection in species continuously exposed to

high UV irradiance such as mat forming species, or species habiting alpine lakes (Mishra & Richa, 2014; Mushir & Fatma, 2011; Sinha *et al.,* 2001; Tartarotti & Sommaruga, 2006).

4.4.3 Dynamic pigment composition in changing light quality

Many cyanobacteria are well capable of adjusting to a changes in light quality through chromatic adaption. Chromatic adaption leads to a change in the PUB:PEB ratio. However, strains with a low PUB:PEB ratio tend to adapt more strongly and faster to a changing environment (Palenik, 2001). The *Synechococcus* coastal strains, CC9311 and CC9617 or the motile strain WH8113 double their PUB:PEB ratio when faced with increasing blue light (Palenik, 2001). However, high light adapted strains with high PUB:PEB ratios are not capable of increasing the PUB:PEB ratio even more in response to increased blue light irradiance (Harrison *et al.,* 1990). In contrast, the red or low light adapted species could convert PE rich PUB into PC or into PE rich PEB shifting the light absorption to the blue part of the spectrum.

The high-light adapted open ocean *Synechococcus* strain WH8103 shows only a slight difference in the PUB:PEB ratio when light cells grown in white light are exposed to blue light (from 1.2 to 1.1). In contrast, the coastal strain CC9311 increases the PUB:PEB ratio by two fold when transferred from white to blue light (Palenik, 2001). This adaptation however, is a slow process: only after 200 hours maximum PUB concentrations were reached (Palenik, 2001). This difference in chromatic adaptation between the strains coincides with the necessity to adapt to changes. The coastal strain CC9311 frequently experiences changes in light quality due to the turbidity of its habitat and has overall a greater ability to adapt to a changing environment (Palenik *et al.,* 2006).

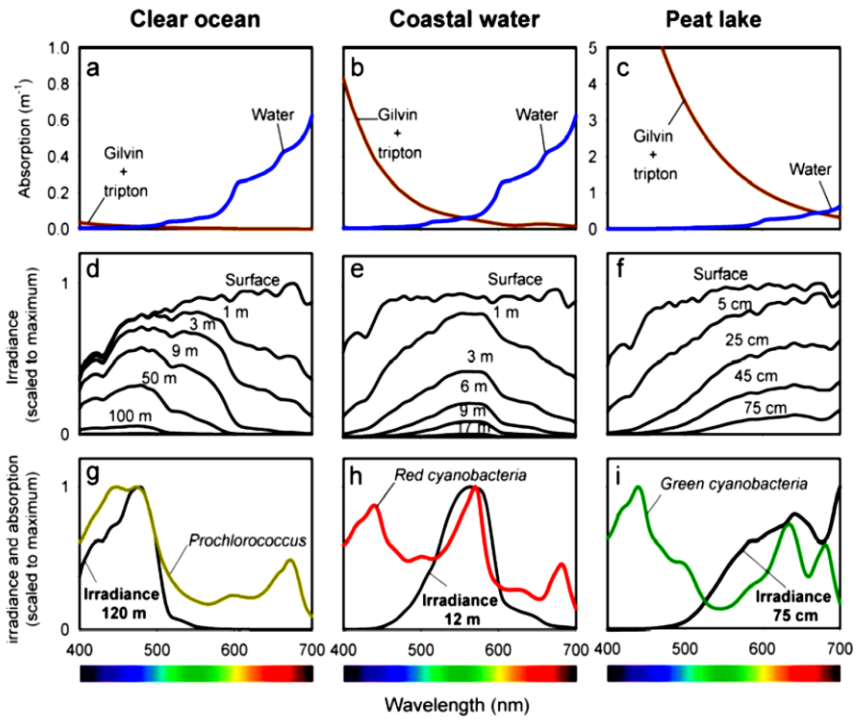


Figure 4-4 The light penetration as a function of available light quantity and quality of clear oceans (subtropical Pacific Ocean), coastal waters (Baltic Sea) and a peat lake (Lake Groote Moost, Netherlands) (d,e,f) as a result of light absorption by dissolved organic matter (a,b,c). Prochlorococcus found in clear oceans has a strong absorption on the blue part of the spectrum (g), while the light absorption of red cyanobacteria is shifted to the green part of the light spectrum (h). Green cyanobacteria found in peat lakes absorb light more strongly in the red part of the light spectrum (I)

Within the marine cyanobacteria *Synechococcus*, light quality did not have an effect on the concentration of APC or the size of the PBS. This implies that the number of PBSs stays the same while exposed to different colors, (De Marsac, 1977; Everroad *et al.,* 2006). But also the far-red light adapted marine species *Acaryochloris marina* adapts less well by chromatic adaptation to light changes compared to freshwater cyanobacteria species (Gloag *et al.,* 2007).

4.6 Phytoplankton distribution in response to light

The distribution of phytoplankton depends on a great number of factors and light quantity and quality is one of them. Pure distilled water absorbs photons with a wavelength higher than 550nm (Morel, 1974), while dissolved substances in water cause more photons to be absorbed in the blue part of the spectrum (≈440nm) (see Figure 4-4 a till c) (Davies-Colley & Vant, 1987; Kirk, 1994). Furthermore, the availability of photons also differ over the depth of the water column. In the deeper layers of the water column less light is available for photosynthesis and in clear water only the blue light is capable of penetrating the column to the deeper layers (Blankenship & Chen, 2013). By synthesizing pigments with an absorption spectrum that fits the light irradiance, phytoplankton can adjust to different light environments (see 4-4 light quantity and 4-5 light quality) (Figure 4-4). However, phytoplankton classes differ in the pigment types they can synthesize. For example, diatoms synthesize protein-pigment complexes that are efficient in light harvesting during exposure to high light or blue light (Glover *et al.*, 1997) but they are not capable of synthesizing PBPs that are efficient in harvesting red light or harvesting light when light is limiting. It is therefore expected that the pigment composition differs per light environment and that some species might not be able to inhabit water with light irradiance that do not suit their absorption spectrum.

4.6.1 Light quantity and phytoplankton distribution

The fast changes induced by the xanthophyll cycle of e.g. diatoms, coccolithophores and dinoflagellates pose an excellent protection during intermittent light exposure, and thus in turbulent waters (Lavaud *et al.,* 2002). Hence, species that possess a xanthophyll cycle and or fucoxanthin are well adapted to environments such as coastal areas, estuaries, or surface mixed layers (Figure 4-5) (Ansotegui *et al.,* 2001; Brunet & Lavaud, 2010; Gévaert *et al.,* 2003; Lavaud *et al.,* 2007; Seoane *et al.,* 2006).

Figure 4-5 Diatoms can thrive well in swallow streams and rivers, such as the film of diatoms formed on the rock (giving the rock its brown color) showed on the right-hand side (Lange-Bertalot & Ulrich, 2014).

Furthermore, species with good NPQ abilities are more resistant to high light. For example, the high amount of LHCsR in diatoms give an advantage in high-light environments (Bailleul *et al.,* 2010; Niyogi & Truong, 2013). Furthermore, the ability to maintain the photosynthetic reaction center (Falkowski, & Owens, 1980) makes diatom cells well-adjusted and well capable of anticipating to fast changes in light

irradiance. In addition, diatoms are capable of coping with 2,000 µmol photons m^2 s^{-1}, corresponding water surface light intensities at noon on a bright day in the Atlantic Ocean, for at least one hour (Lavaud & Kroth, 2006; Lavaud *et al.,* 2002). Dinoflagellates are also well adapted to high (intermittent) light but are also poor competitors for light at low light intensities (Ansotegui *et al.,* 2001; Schwaderer *et al.,* 2011). Phytoplankton that synthesize PBPs like cryptomonads, do not possess a xanthophyll cycle, and are therefore poorly adapted to high light intensities. Hence, the number of cryptomonads cells decreases when light in the environment increases, while the number of diatoms increases (Henriksen *et al.,* 2002).

Distribution of phytoplankton in environments with changing light quantities

Phytoplankton classes that have a xanthophyll cycle such as diatoms, *Xanthophytes*, coccolithophores, and dinoflagellates synthesize photoprotective pigments at a fast rate. Cyanobacteria, cryptomonads and red algae lack such cycles (Goss & Jakob, 2010; Holt *et al.,* 2005; Rascher *et al.,* 2003; Raven, 2011; Schagerl & Müller, 2006). Hence, cyanobacteria exposed to high light, have to include quenching carotenoids in their PBPs or detach their PBPs to protect the reaction center from excess light energy (Niyogi & Truong, 2013). Thus, adjusting to light-changing environments takes longer for cyanobacteria than for species that can activate a xanthophyll cycle such as diatoms or green algae. However, cyanobacteria have better NPQ abilities at the surface, which indicates that they can discard excess energy better than species such as diatoms, green algae and dinoflagellates (Zhang *et al.,* 2008). This suggests that cyanobacteria are favored by stratified water columns that offer a steady light environment (Figure 4-6). Not only are cyanobacteria favored by a non-mixing environment, but also the PBS containing red algae, cryptomonads and even dinoflagellates that have a xanthophyll cycle are dominant in stratified waters or poorly mixed waters (Bleiker & Schanz, 1997). However, compared to other phytoplankton classes, cyanobacteria are relatively bad competitors in waters with a high irradiance and thus a high visibility (Schwaderer *et al.,* 2011).

Figure 4-6 A cyanobacteria bloom (referred to as 'pea soup' because of the thickness and color) in a stratified lake.
Source: http://ks.water.usgs.gov/cyanobacteria

The fast adaptation of the xanthophyll cycle poses a good protection in turbulent waters. However, it might not be the best adaptation to constant high light. Generally, it is efficient for diatoms, to maintain the reaction center during light changes (Anning *et al.,* 2000; Falkowski, & Owens, 1980); for diatoms in a changing light environment, this adaptation might be redundant because it costs too much time. However, many diatoms might therefore not be well adapted to constant high light environments. Since the green algae *D. tertiolecta* mainly lives in habitats that are constantly exposed to high irradiance, it might strategically decrease its number of reaction centers, which might be a slower but a more efficient

adaptation to constant high light exposure. For example, the diatom *Skeletonema costatum* has a low light-saturated rate of photosynthesis and is therefore faster saturated in higher light irradiance than the green algae *Dunaliella tertiolecta* (Falkowski, & Owens, 1980). But, compared to diatoms and coccolithophores, green algae absorb least in the blue part of the spectrum and are therefore less able to absorb light in clear open oceans (Fujiki & Taguchi, 2002).

4.6.2 Light quality and phytoplankton distribution

PC rich cyanobacteria are more likely to be found in the upper layers of the water column, such as the PC rich adapted *Synechococcus* that absorbs strong in the red part of the light spectrum, which matches the light spectrum at the water surface (see blue line Figure 4-7)(Lohscheider *et al.,* 2011). In contrast, PE rich cyanobacteria are found in the deeper layers (Lohscheider *et al.,* 2011; Glover *et al.,* 1985) and absorb more strongly in the green part of the spectrum that matches the light spectrum at this depth (see orange line in Figure 4-7). Because of the high irradiance in the upper ten meters, PC rich strains from the water surface also have significantly higher concentrations of photoprotective β-carotenoid like pigments compared to PE rich strains from several meters depth (Lohscheider *et al.,* 2011).

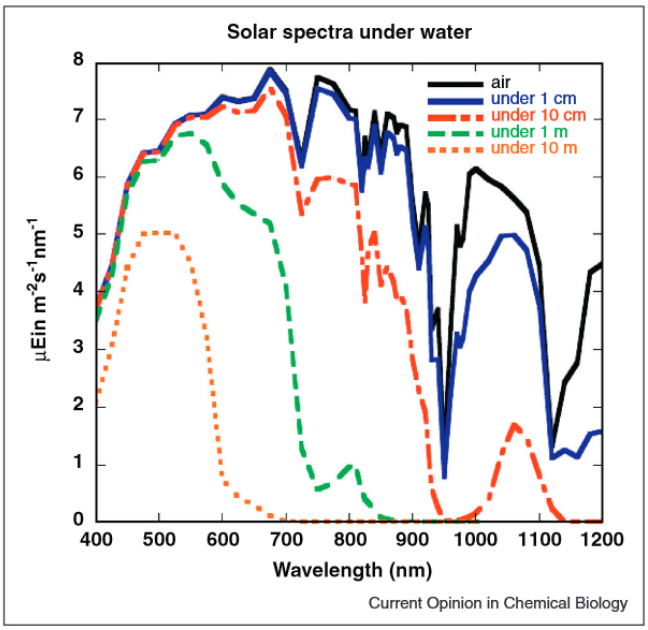


Figure 4-7 Light penetration as a function of μein and wavelength in clear water measured over different depths (Blankenship & Chen, 2013)

4.6.2.1 The distribution of marine cyanobacteria

Open ocean cyanobacterial species often contain high quantities of PE with high levels of PUB or variable PUB:PEB ratios. Whereas freshwater- or coastal marine species contain low concentrations of PE that bind low numbers of PUB or, in some cases species may even lack PE and thus PUB (Everroad, & Wood, 2012; Olson *et al*., 1988). For example, species of *Synechococcus* that inhabit fresh waters or turbid marine waters, are rich in PC compared to PE and often only bind PEB and no PUB to the PE (Haverkamp *et al.,* 2008; Patel *et al.,* 2005; Quinlan & Phlips, 2007). This difference is not only seen between fresh- and marine water species, but also among marine species. The *Synechococcus* strain WH8102 is found in open marine waters and uses PE as the main light harvesting pigment with a high PUB:PEB ratio and thus absorbs light shifted towards the blue part of the spectrum. While the *Synechococcus* strain WH7803, which is mainly found in coastal waters, also uses PE as the main pigment but has a low PUB:PEB ratios and thus absorbs more in the green part of the spectrum (Figure 4-8) (Everroad & Wood, 2012; Scanlan *et al.,* 2003; Six *et al.,* 2007). Furthermore, coastal strains overall have a low PUB:PEB ratio and a greater ability to adapt to changing environments than strains found in open oceans (Palenik *et al.,* 2001, 2006).

Often deeper aquatic environments have low visible light irradiance due to the absorbed photons of organisms in the upper layers of the water column. Therefore, phytoplankton species living in these environments absorb photons in the infrared part of the spectrum up to 750 nm by binding high quantities of Chl *d* and phycobilins (Blankenship & Chen, 2013; Gan *et al.,* 2014). An extreme example of a marine species with a red light shifted absorption spectrum is the cyanobacteria *A. marina* that often lives in these environments with low visible light irradiance (Gan *et al.,* 2014).

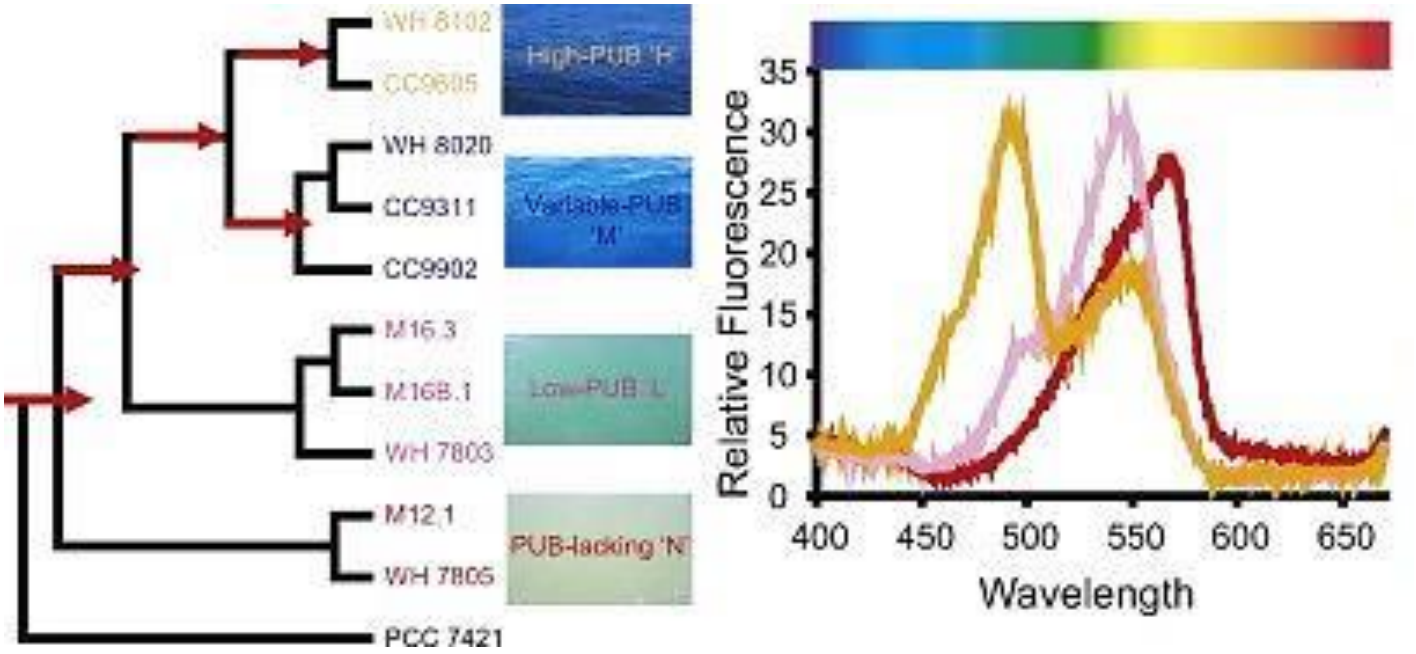


Figure 4-8 The relative PUB:PEB ratio and fluorescence of different *Synechococcus* strains from different habitats. Strains with a high PUB:PEB ratio inhabit clear open oceans and absorb strongly in the blue part of the light spectrum (orange line), while strains with a low PUB:PEB ratio or PUB lacking strain inhabit coastal area and absorb stronger in the red part of the spectrum (pink and red respectively)

As discussed in subsection 4.3, there is a difference in the size and quantity of PBS per cell in different cyanobacterial species and strains. In higher light intensities cells synthesize larger but less PBS. In addition, mesotrophic marine species, such as the strain *Synechococcus* RCC307 have many but smaller PBSs compared to the strains living in oligotrophic waters such as RS9917 (Six *et al.,* 2007b). The difference between the quantities of PBSs is even larger when fresh- and marine species are compared. For example, the freshwater cyanobacterium *Spirulina sp*. has up to 4 fold higher PBS concentrations compared to marine species (Patel *et al.,* 2005). In conclusion, species living in high light or blue light contain larger PBS probably due to the linking of PE to PC. In addition, linking PE to PC gives the advantage of absorbing light shifted to the blue part of the spectrum. However, due to higher irradiance less thylakoid membranes are present in the cells of high-light adapted marine species, so less PBS can be attached to reaction centers (Kana & Glibert, 1987).

In fresh waters, cyanobacteria are likely to be found in waters with a light irradiance between 565 and 620 nm (Ssebiyonga *et al.,* 2013). Considering that turbid waters have green to red light (500-650nm),

this gives cyanobacteria that contain PBSs an advantage over phytoplankton that mainly contain high amounts of chlorophyll and carotenoids. Indeed, most of the cyanobacterial absorption spectrum matches the irradiance (500-650nm) of turbid waters (Bryant *et al.,* 1976; Six *et al.,* 2010; Wang *et al.,* 2014).

4.7 Similar patterns in light availability and light quality

There are several similarities between the adaptation to changes in light quality and light quantity. Phytoplankton cells exposed to high light often adapt by absorbing more light in the blue part of the spectrum (Bidigare *et al.,* 1990; Harrison *et al.,* 1990; Jiang *et al.,* 2012; Tamary *et al., 2012*). An exception to this rule are DV-Chl *a* binding PCB light-harvesting protein complexes and Chl*d* that are prone to damage caused by excess light *(*Andrizhiyevskaya *et al.,* 2005; Domingues *et al.,* 2012; Mella-Flores *et al.,* 2012; Mimuro *et al.,* 2011). Cells exposed to low light absorb more light in the red part of the spectrum (De Marsac, 1977; Kwon *et al.,* 2013; Six *et al.,* 2007a). For example, when cryptomonads cells are exposed to high light (550 µmol quanta m^{-2} s^{-1}) a large part of the light absorption is being absorbed in the blue part of the light spectrum. However, this shifts once cells are exposed to lower light irradiance (18 µmol quanta m^{-2} s^{-1}), when more light is absorbed in the red part of the light spectrum (Sciandra *et al.,* 2000). Cyanobacteria grown in red light or low light availability become smaller in cell size but contain more PBSs, while cyanobacteria grown in blue light or bright light are larger but contain fewer PBSs (Whitaker *et al.,* 2009 & 2011). However, some wavelength of light are also triggers for the synthesis of specific pigments. Such as blue light triggers the synthesis of quenching pigments such as carotenoids, CP43 and OCD (Cadoret *et al.,* 2004; Costa *et al.,* 2013; Wilson *et al.,* 2008) that may cause the concurrence of an adaptation to high light. Therefore, in further research it is advised to take these concurrences between light quantity and quality into account.

5. Nitrogen availability

All proteins and all chromophores contain nitrogen (Figure 5-1). Without nitrogen, photosynthetic activity would not be possible. Consequently, higher nitrogen concentrations result in a higher quantum yield, a higher maximum rate of electron transport and higher oxygen production, which are all signs of improved photosynthetic performance (Ostrowska, 2011; Palmer *et al.*, 2013; Sutherland *et al.*, 2014). In contrast, when phytoplankton species experience a shortage in N availability, the most obvious change is a decrease in nitrogen-rich photosynthetic proteins (reviewed in Turpin, 1991).

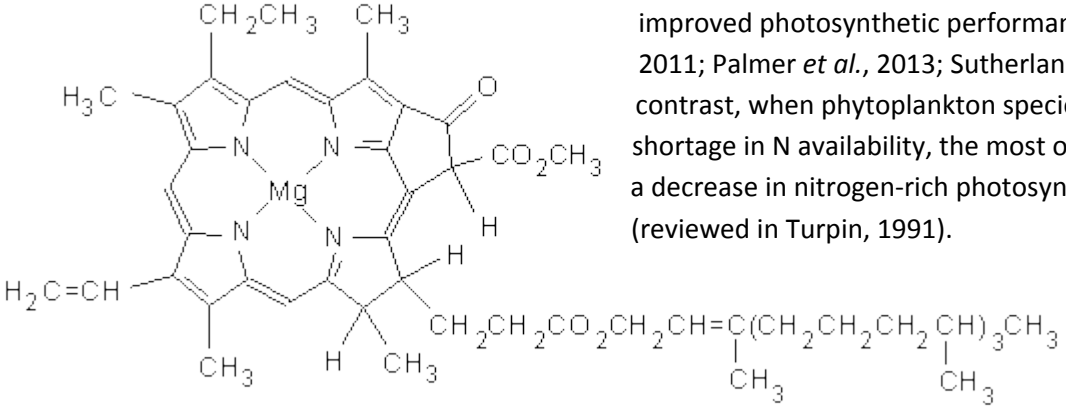
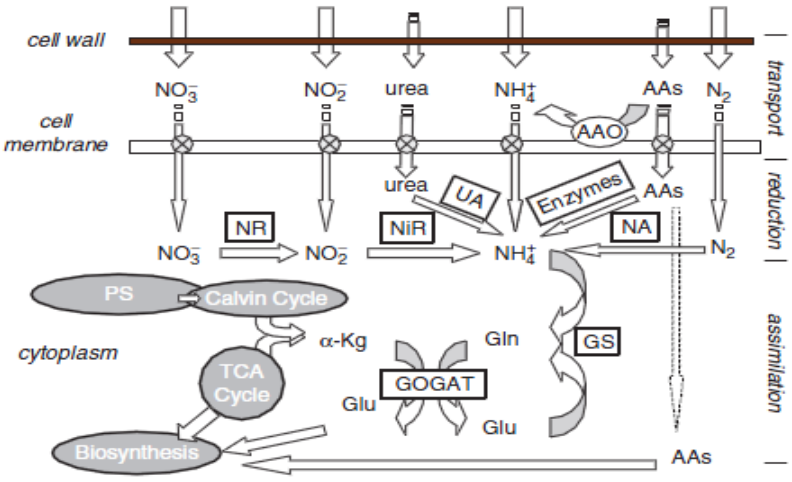


Figure 5-1 The chemical structure of chlorophyll, which contains 4 N atoms per molecule. (Source picture: Chemical of the Week – CHLOROPHYLL scifun.chem.wisc.edu)

Several chemical forms of nitrogen (hereafter referred to as 'N'), such as nitrate (NO_3^-) and ammonium (NH_4) can be used by phytoplankton to build chromophores, proteins and other N-requiring structures. However, the cost in terms of light energy to assimilate the N-atoms depends on the chemical form of nitrogen. If NO_3^- is used as an N-source, 1.5 fold more photons are required for protein synthesis than when NH_4^+ is used (Raven, 1984). Consequently, if nitrogen is available as NO_3^-, cells need to use 1.5 fold more light energy to build the same protein-pigment complex as when NH_4^+ is available (Figure 5-2). Because NH_4^+ is cheaper to assimilate, more photons can be used for growth (Raven & Hurd, 2012). The concentrations of NH_4^+ are usually higher in the top layers of the water column where sufficient light might be available, while NO_3^- is limited in the top layers (Figure 5-3). Furthermore, the uptake of NO_3- can be inhibited by high concentration of NH_4^+, but only when light is not limited (Mulholland & Lomas, 2008). The chemical form of N used to build protein-pigment complexes differs per phytoplankton class, species and even per strain (Lomas, 2004; Maldonado & Price, 1996; Waser *et al.*, 1999). Thus, the variety in qualitative and quantitative N-demands of different phytoplankton species may (partly) explain their ecological distribution.

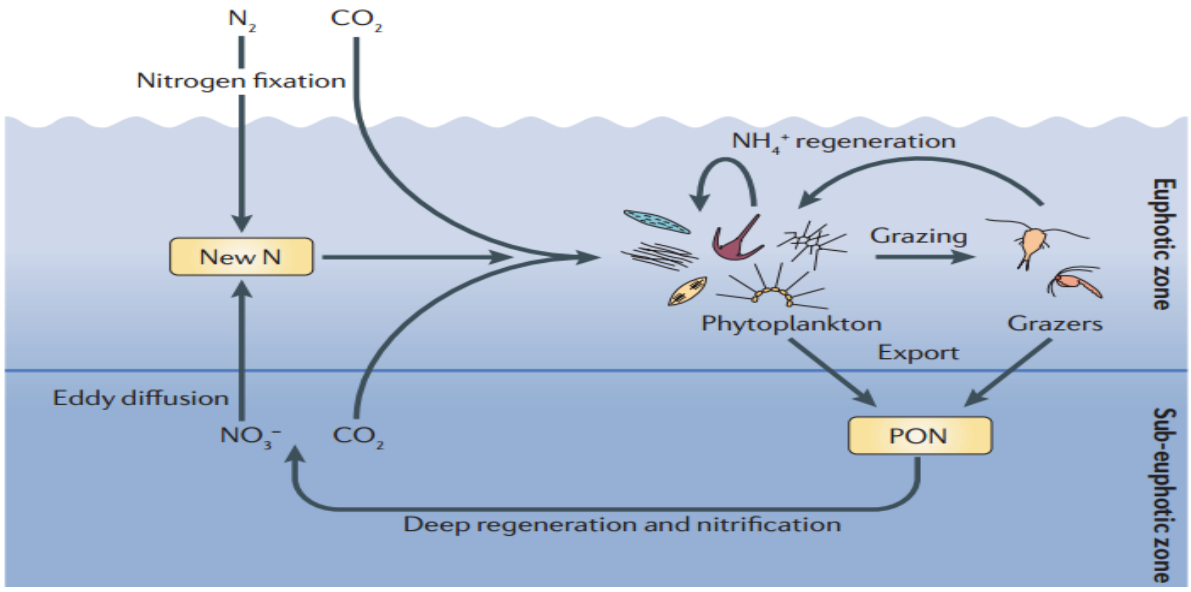


Figuur 5-2 A schematic map of the intracellular pathways if different sources of N are used. If NO_3^- is used, it first has to be reduced to NH_4^+ that in terms requires more light energy (Mulholland & Lomas, 2008)

5.1 Different chemical forms of nitrogen

When discussing N-availability it is of great importance to take the different chemical forms of N into account. Some species prefer NH_4^+ while others prefer NO_3^- (Lomas, 2004; Maldonado & Price, 1996; Waser *et al.*, 1999). In general, cyanobacteria prefer to assimilate the cheaper NH_4^+ as a source for N, even when NO_3- is ≈40 fold more abundant, while diatoms seem to prefer NO_3^- (MacFarlane & Raven, 1990; Kolodny *et al.*, 2006; Raven & Hurd, 2012). For example, even though the cyanobacterium *Synechococcus* can use both NO_3- and NH_4^+, in North Atlantic, *Synechococcus* has a higher growth rate when using NH_4^+ (Maldonado & Price, 1996). In contrast, diatoms in the North Atlantic have a faster growth rate when using NO_3^- (Ludwig & Bryant, 2012; Maldonado & Price, 1996). Furthermore, cyanobacteria grown on NH_4^+ contain a higher PBP concentration per cell than cells grown on NO_3^- (Carmona *et al.*, 2006; Kolodny *et al.*, 2006). Hence, these differences have a great influence on the distribution and competitive qualities of phytoplankton. For example, the *Haptophyte Phaeocystis* also has a higher growth rate when NH_4^+ is used as a source of N, allowing them to outcompete diatoms in NO_3- limited environments (Tungaraza *et al.*, 2003).

These differences in preference for N-source are also seen among different strains of one species, for example among the MED4, MIT9313 and SS120 strains of *Prochlorococcus*. The high-light ocean surface inhabiting strain MED4 has lost its NO_3- and NO_2- transporter and NO_3- reductase genes (Figure 5-2). Consequently MED4 can only use NH_4^+ as a source for N. The mid-light adapted strain MIT9313 has also lost the capacity to use NO_3-, but is able to use NO_2- (Rocap *et al.*, 2003; Scanlan & West, 2002; Ting *et al.*, 2002). The only strain of *Prochlorococcus* capable of using NO_3- and NO_2- is the low-light adapted strain SS120 (Scanlan & West, 2002). Strain SS120 however, originates from the deep waters of the oceans (below 170 meters) where NO_3- becomes more abundant (Malmstrom *et al.*, 2010; World ocean atlas, 2009; Figure 5-5) and where the uptake of NO_3- is not inhibited by NH_4^+ such as in high light environments (Mulholland & Lomas, 2008).



Figuur 5-3 A schematic picture of "the biological pump" where N is being fixated by N-fixating bacteria forming NH_3 and NH_4^+ that is regenerated at the surface. NO_3^- is transported to the top layers by mixing of waterlayers throught waves, currents or changes in watertemperature (Sohm *et al.*, 2011).

5.2 Differences in nitrogen availability and pigment composition

Because all photosynthetic phytoplankton contain chlorophyll *a* and reaction centers, there are some general effects of N-availability that occur among all phytoplankton species. Other effects are more species or pigment specific. In general, when N becomes limiting, the quantity of chlorophylls decrease while the number of carotenoids and MAAs increases (Berges *et al.*, 1996; Frada *et al.*, 2013; Llewellyn *et al.*, 2012; Merzlyak *et al.*, 2007; Peperzak *et al.*, 2014). The increase of carotenoids during N-limitation often coincides with the increase of the lipid/N ratio. Diatoms and coccolithophores from N limited environments store energy absorbed from light in lipids, which function as a high energy source, while less energy is invested in photosystems that are in high demand of N (Frada *et al.*, 2013; Kaffes *et al.*, 2010). In addition, during N-limitation, carotenoids accumulate between lipids in the membrane and diatoms therefore do not have to assemble protective carotenoids in expensive pigment-protein complexes (Deruère *et al.*, 1994; Havaux, 1998; Niyogi, 1999).

Despite the fact that chlorophyll content decreases during N-limitation, the concentrations of different types of chlorophyll such as Chl*a*, Chl*b* and Chl *c* may differ. For example, at decreasing N availability, the Chl*c*:Chl*a* ratio increases, while the ratio Chl*b*:Chl*a* decreases (Herzig & Falkowski, 1989; Silva *et al.*, 2009; Young & Beardall, 2003). Furthermore, the quantity of the D1 reaction center and the protein-pigment complex CP43 (relative expensive pigment-protein complexes (Table 3-4) also decrease as a reaction to N-limitation (Berges *et al.*, 1996; Falkowski *et al.*, 1992; Kolber *et al.*, 1988). As a result of the decrease of reaction centra, the photo efficiency decreases too (Berges *et al.*, 1996; Kolber *et al.*, 1988). PBPs are compared to other pigments expensive in terms of N. Hence, in cyanobacteria, cryptomonads and red algae, PBPs such as PC and PE decrease at a fast rate (≈within 14 to 20 hours) after N becomes depleted (Davies *et al.*, 2014; Ludwig & Bryant, 2012; Sciandra *et al.*, 2000; Silva *et al.*, 2009; Stevens *et al.*, 1981; Wanner *et al.*, 1986).

5.2.1 Cellular adaptation to low nitrogen availability

Exclusive to diatoms is the ornithine-urea cycle with multiple transporters for N. Other than diatoms only animals have a similar urea cycle (Allen *et al.*, 2011). However, diatoms do not use the urea cycle the same way animals do, but use is to redistribute carbon and nitrogen in times of low nitrogen availability. The possibility to redistribute N in an effective manner, gives diatoms the possibility to continue growth when N is limited (Allen *et al.*, 2011). Other N regulating transporters that become active when N is limited have been found in the cell surface of coccolithophores, which also gives coccolithophores an advantage in N-limited environments (Palenik & Koke, 1995).

The high efficiency of diatoms in the uptake and reduction of NO_3^- is shown by the leaking of reduced N, which in turns also has a great impact on the environment. While diatoms take up NO_3-, they release NH_4+ in reaction to increased light irradiance (Lomas *et al.*, 2000). Although leaking of NH_4^+ is common among more species, diatoms reduce NO_3- faster and leak more N from their cells than species less efficient in N uptake and with higher N demand such as dinoflagellates. The fast leaking of N, suggests that diatoms are more efficient in the uptake and reduction of NO_3^- relative to their N demands for growth (Lomas *et al.*, 2000).

Not all phytoplankton are phototrophic, some dinoflagellates are mixotrophic and are therefore capable of ingesting macromolecules (Bockstahler & Coats, 1993; Klut *et al.*, 1987), or can store N in a vacuole (Kromkamp, 1987). Dinoflagellates therefore can maintain their expensive pigment-protein structures during short term N-depletion. Another example of adaptation to low N availability is N_2 fixation by certain cyanobacteria (diazotrophs). When N becomes depleted, diazotrophs have the capability to convert N_2 to NH_3 and maintain or may even increase N expensive structures. When N_2 fixing cyanobacteria are compared to non N_2 fixing cyanobacteria, little difference is found in the amount of Chl and Car. However, where non N_2-fixing cyanobacteria synthesize on average only 1% C-PE of all pigments, N_2 fixing cyanobacteria assemble 8% of C-PE (Rodriguez *et al.*, 1989). However, although N_2-fixation gives an advantage in N-supply (Ferber *et al.*, 2004), it comes with high energetic costs (Jensen *et al.*, 1994; Kirchman, 2012) and is therefore N_2 is not a common source of N.

The function of C-PE as a storage for N

In cyanobacteria, cyanophycin (CP) is a well-known structure that functions as a storage for N (Obst & Steinbüchel, 2006). But in 1985 Wyman *et al.*, suggested that, besides cyanophycin, PE might have an additional function as a storage for N. PE compared to e.g. chlorophyll pigment-complexes are, in terms of N, expensive structures. Hence, in red algae PE is being reduced under N-limitation. In cryptomonads after six days of N depletion, only 10% of the total PE remained (Sciandra *et al.*, 2000; Silva *et al.*, 2009). When the cyanobacteria *Phormidium tenue* is faced with nutritional stress, cells degrade the C-PE content, releasing amino acids for the assembling of essential proteins. However, initially not the complete C-PE is degraded; the alpha subunit of C-PE with a molecular mass of 14kDa (αC-PE) remains (Anwer *et al.*, 2014; Parmar *et al.*, 2011b; Soni *et al.*, 2010). Hence, the total molecular mass of C-PE is strongly reduced because of the reduction in amino acids.

The relative cost of the αC-PE protein-pigment complex is 2 fold lower than its original form (179 mol N per mol chromophore for C-PE and 85 mol N per mol chromophore for the αC-PE) while remaining photosynthetic active (Anwer *et al.*, 2014, 2015; Parmar *et al.*, 2011b; Soni *et al.*, 2010) suggesting that C-PE, in addition to CP, functions as a storage for N. As far as known, within cyanobacteria, only one strain of the halotolerant *Synechococcus* has been found to contain both CPs and PEs (Wingard *et al.*, 2002; Newman *et al.*, 1987). If it is true that cyanobacteria use C-PE to store N (Silva *et al.*, 2009; Wyman *et al.*, 1985), it would answer the question why cyanobacteria synthesize larger C-PE units while one sub-unit is sufficient for sufficient light absorption (Parmar *et al.*, 2011b). Furthermore, the breakdown of C-PE, while remaining photosynthetic activity, results in slow continued growth of Cryptomonad cells for ≈4 days after N got depleted (Silva *et al.*, 2009). While cells lacking C-PE arrest growth immediately after N-depletion (Silva *et al.*, 2009).

Despite the fact that *Prochlorococcus* cells do not synthesize PBS, their cells do contain PE. Even though PE in *Prochlorococcus* might have a photosynthetic function (Lokstein *et al.*, 1999), the quantity is probably too low to have an impact on the absorption spectrum (Steglich *et al.*, 2003). In contrast to the D1 concentration, PE concentrations in *Prochlorococcus* do not change under N-depletion, suggesting that PE in *Prochlorococcus* does not function as N- storage (Steglich *et al.*, 2001), which leaves the function of PE in *Prochlorococcus* unknown.

5.2.2 Photo-oxidative damage from low nitrogen availability

While a great number of phytoplankton species are adapted to cope with a decrease in N-availability, long-term shortage may lead to significant negative effects. Cells in N-limited environments with expensive pigments may not have enough N available to repair photo-oxidative damaged pigment-proteins that has occurred during the day time. Pcb's in *Prochlorococcus* are quickly damaged when exposed to high light irradiance due to the high light sensitivity of DV-Chl (Six *et al.*, 2007).

Under low nitrogen pigment-protein complexes recover slowly or not at all (Six *et al.*, 2007). For example, dinoflagellates with expensive pigment-proteins have a higher maintains respiration than diatoms (Falkowksi and Raven, 2013). As a result, it takes longer for dinoflagellates to recover from photo-oxidative damaged than for diatoms and less energy can be spend in growth. However, with sufficient N available, dinoflagellates are capable of repairing their protein-pigment complexes (Prézelin & Matlick, 1983).

5.3 The dynamics and photochemical efficiency of pigments and the growth rate of cells in response to differences in nitrogen availability

How well different phytoplankton groups thrive in their habitat depends to a large extent on the efficiency of their pigments. This, in turn affects their growth rate. The pigment composition of phytoplankton, and consequently their photosynthetic efficiency depends highly on the availability of nitrogen (Ostrowska, 2011; Sutherland *et al.*, 2014). Furthermore, dynamics in pigment content and composition, and recovery from N depletion may differ greatly among different phytoplankton species.

When red algae receive high loads of N after a period of N-depletion, they will assemble more pigments then during the period of N-depletion. However, the rate at which the pigments are assembled differs. For example, Chl content recovers twice as fast as PE content (40 and 80 hours respectively) (Sciandra *et al.*, 2000). Furthermore, under N-starvation less thylakoid membranes are present in the cell. However, not all species degrade the thylakoid membrane. Species such as *Synechococcus* can decrease their thylakoid membranes by up to 60% under N limitation (Wanner *et al.*, 1986). In contrast, green algae (e.g. *Dunaliella tertiolecta)*, only altered but did not reduce its thylakoid membrane, even under long N starvation of 60 days. Maintaining the thylakoid membrane after N-starvation suggests that after a period of N-deprivation, cells can recover and reassemble their pigment-protein complexes at a fast rate. Therefore, after replenishment of N the maximum quantum yield of photosynthesis recovered within 15 hours (Young & Beardall, 2003). The quick recovery of *D. tertiolecta* after N-starvation suggests that this species is well adapted to N-changing environments.

Different PUB:PEB ratios in cyanobacteria and their response to N-depletion

Often the pigment composition and dynamics of cyanobacteria such as *Synechococcus* are more complex than that of *Prochlorococcus* or other PBP lacking phytoplankton species. Because PBPs can differ in their PUB:PEB ratio (Figure 5-4), each strain of e.g. *Synechococcus* can differ in its response to N-limitation. Hence, to compare different PUB:PEB ratios three strains of *Synechococcus* are compared: the open ocean strain WH8103 adapted to low nitrogen availability, with a high PUB:PEB ratio (>2.0), the oceanic medium N adapted strain WH7803 with a low PUB:PEB ratio (0.39), and the coastal strain WH8018 which does not contain PUB that is adapted to relatively high, but fluctuating N conditions.

Upon N depletion, growth of the *Synechococcus* strain WH7803 (with low PUB:PEB ratio) becomes immediate arrested. In contrast, cells from the WH8018 strain that contains no PUB could maintain slow growth for at least 24 hours (Gilbert *et al.*, 1986; Glibert & Ray, 1990). Furthermore, also the high PUB:PEB ratio strain WH8103 continues growth under N-depletion (Kana *et al.*, 1992). In the cells of the low PUB:PEB WH7803 strain and high PUB:PEB WH8103 strain, 83% and 98% respectively of PE remained after N starvation, whereas only 32% of PE remained in the PUB-lacking WH8018 (Kana *et al.*, 1992). Furthermore, after N depletion uptake of NH_4^+ increased 6-fold in strain WH8018 in comparison NH_4^+ uptake in N depleted cells. In the WH7803 strain however, only a small increase in NH_4^+ uptake was observed after a period of N-depletion (Glibert & Ray, 1990). Strain WH8103 could maintain slow growth even after five days of N starvation, whereas strain WH8018 continued growth but recovered slowly after a long period of N depletion (Table 5-1).

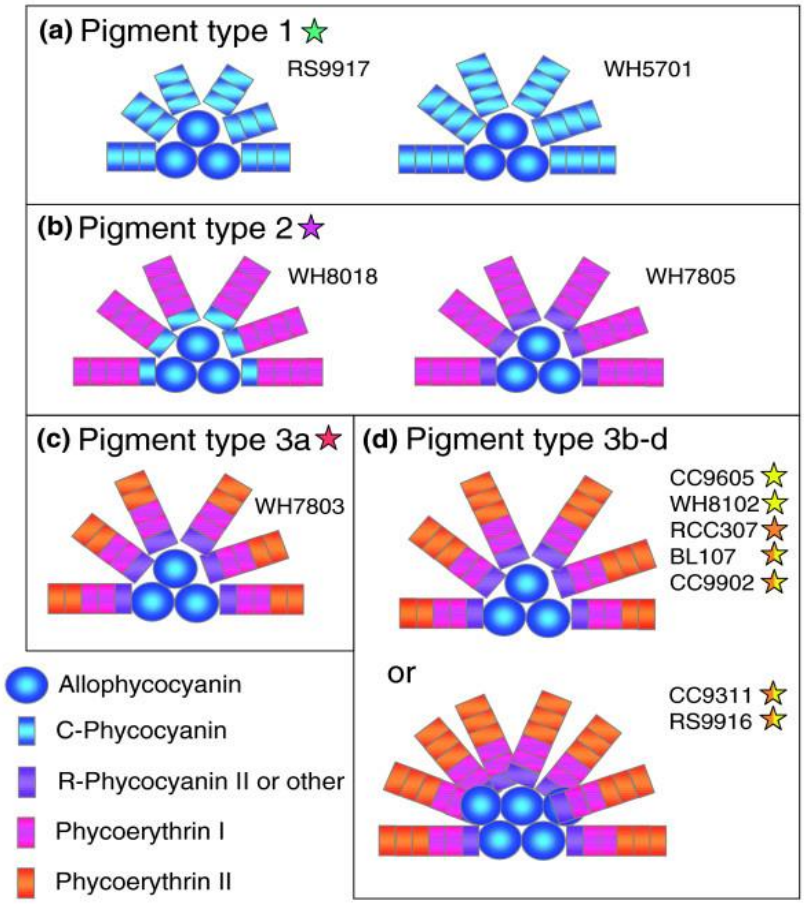


Figure 5-4 A by Six et al, (2007) proposed models of PBS structure of several strains of Synechococcus. The model is divided in 4 group: (a) strains that do not assemble PUB, (b) strains that assemble PEs with a moderate PUB:PEB ratio (c)strains that assemble PE with high PUB:PEB levels (d) strain that can alter their PUB:PEB ratio in response to light.

These results suggest that the PUB-lacking strain WH8018 is better adjusted to short term N depletion while the low PUB:PEB strain WH7803 is better adjusted to long term N depletion. However, this may come with the cost of an overall slower growth of the strain WH7803 (Mackey *et al.* 2013). These results are consistent with the finding that strains from nitrogen rich waters contain low PUB concentrations and therefore possibly more C-PE (see Table 3-2), which might function as nitrogen storage (Silva *et al.*, 2009; Wyman *et al.*, 1985). Therefore, the lower the PUB:PEB ratio the better cells can handle short term N-depletions, but face a longer recovery time after prolonged (a couple of days) N-depletion. The higher the PUB:PEB ratio the faster growth is arrested upon N-depletion, but the faster the recovery upon N-repletion.

In conclusion, the distribution of PUB:PEB coincides with the N-concentrations (Olson *et al.*, 1988; Scanlan *et al.,* 2003) and fluctuations in the environment; coastal species experience high fluctuations in N availability and are often exposed to short term N-depletion, while open oceanic strains are faced with low N-availability. Furthermore, comparing the growth of the strains WH8103, WH7803 and WH8018 of *Synechococcus* shows that the lower the PUB:PEB ratio is, the lower the doubling time of the cell is (Binder & Chisholm, 1995; Kramer & Morris, 1990; Mackey *et al.*, 2013). This would mean that strains adapted to the oligotrophic oceans have fewer and cheaper pigments and are therefore less efficient in increasing their growth rate than the coastal adapted strain (Table 5-1).

Table 5-1 The PUB:PEB ratio of the three Synechococcus strains WH8103, WH7803 and WH8018 compared with the percentage of remaining PE and growth after N-depletion. Growth rate at ≈150 µE m-2 s-1 Reference in texts with the exception of: 1. Binder & Chisholm, (1995)

Strain	WH8103	WH7803	WH8018
Origin sample	Open oceanic waters	Oceanic waters	Coastal water
PUB:PEB N-replete	2.4	0.39	No PUB
% remaining PE after N-depletion	0.98	0.83	0.32
Growth during N-depletion	Continued	Arrested	Continued
Growth after N-recovery	-	Continued	Arrested
N-uptake after starvation	-	Regular	Increased
General cell doubling time (h)	21.6-23.3 [1]	17 [2]	≈14 [3]

5.4 Phytoplankton distribution linked to nitrogen availability

Nitrogen is not uniformly available around the globe. Hence, over the depth of the water column, both the concentrations and the chemical form of nitrogen differs (Figure 2-2). In the open ocean at the surface layer only small amounts of NO_3- (0-0.25 µmol NO_3- /L) are found while at 200 meters depth or more, relatively large amounts of NO_3- (20-25 µmol NO_3-/L) are found (Acker & Leptoukh, 2007; World ocean atlas, 2009; Figure 5-5). Unfortunately, less information is available on the distribution of NH_4^+, or nitrogen sources such as urea and N_2. However, it is known the total dissolve nitrogen (TDN) does not significantly differ over the latitudes (reviewed in: Berman & Bronk 2003). The fact that diatoms preferentially take up nitrate whereas cyanobacteria, cryptomonads and dinoflagellates prefer to take up reduced nitrogen forms such as ammonium, urea, dissolved free amino acids and adenine (Berg *et al.*, 2003), the availability and chemical form of nitrogen are likely to have an impact on phytoplankton distribution.

5.4.1 Marine environments

The most abundant cyanobacteria *Synechococcus* and *Prochlorococcus* occur in the open oceans, where *Prochlorococcus* is responsible for a large part of the total primary production and is the most abounded cyanobacterium in the North Atlantic (McClain, 2009; Lane *et al.*, 1994). Considering the nitrogen costs of their pigment-protein complexes one would expect that these cyanobacteria occur in regions with high amounts of nitrogen. However, when the distribution of cyanobacteria is compared to the global nitrate concentration, such a pattern is not shown.

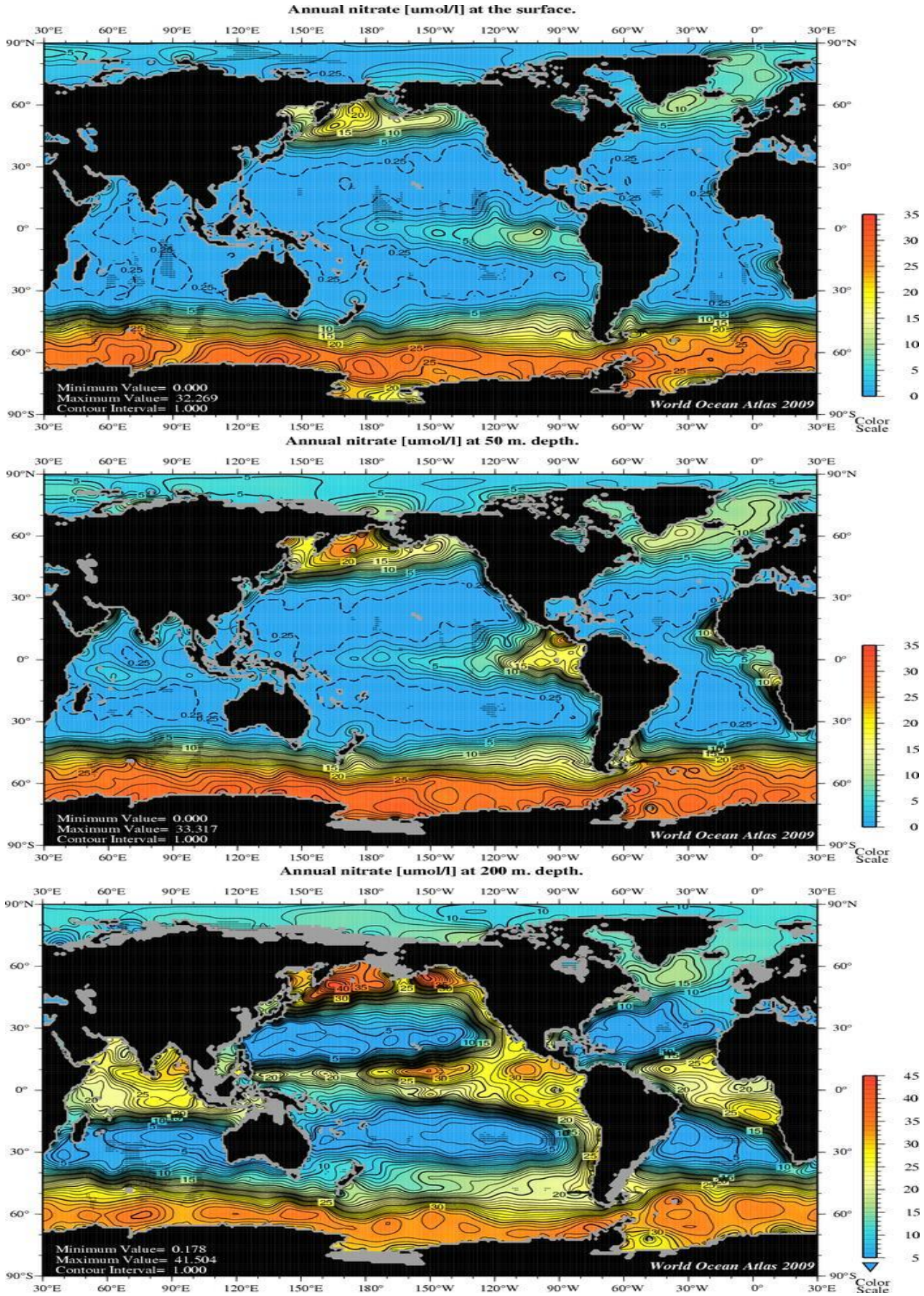


Figure 5-5 Three world maps of the annual nitrate distribution (µmol/l) in the oceanic waters at 50 meters depth, 100m depth and 200 meters depth.

As discussed before in subsection 5.1, there is a great difference in the preference for nitrogen sources between diatoms and cyanobacteria (Lomas, 2004; Maldonado & Price, 1996; Waser *et al.*, 1999). For example, *Synechococcus* can use both NO_3- as NH_4^+ as nitrogen sources, however in Fe limited environments such as the North Atlantic, *Synechococcus* has a higher growth rate when using NH_4^+ than NO_3^-. While the *Prochlorococcus* strains MED4 and MIT931 have lost their NO_3- transporter and NO_3-reductase genes and are not capable of using NH_4^+ (Rocap *et al.*, 2003; Scanlan & West, 2002; Ting *et al.*, 2002). Diatoms, on the other hand have a faster growth rate using NO_3^- as nitrogen sources in Fe limited waters (Maldonado & Price, 1996). Hence, when comparing satellite data from NASA's Ocean Biogeochemical Model no apparent correlation is found between cyanobacteria and NO_3^-. In contrast, diatoms are abundant in NO_3^- rich but Fe limited waters, such as around the equator and in the Antarctic sea (Figure 5-6) where diatoms have the highest growth (Lomas *et al.*, 2000; Maldonado & Price, 1996).

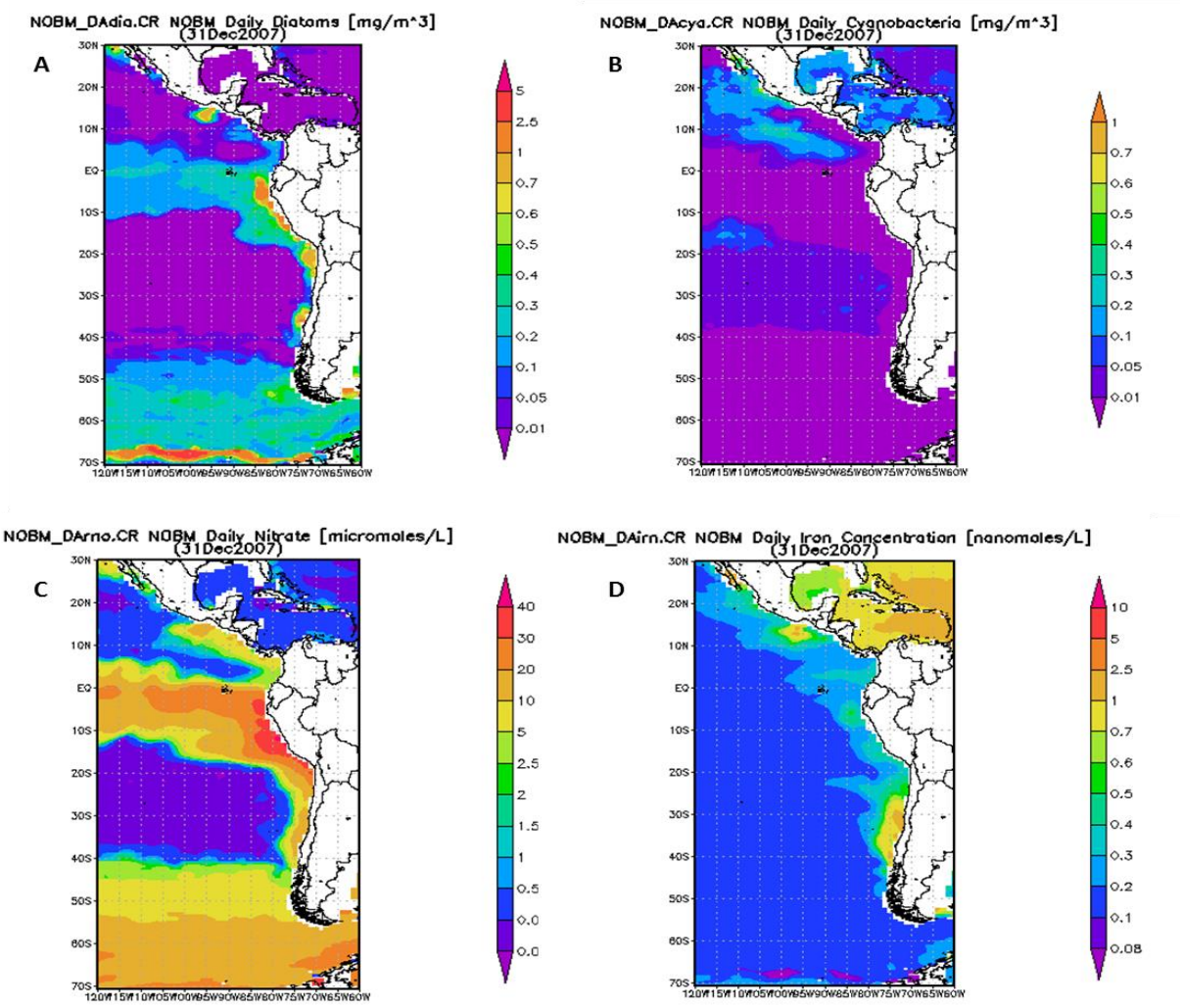


Figure 5-6 Four distribution maps from the date 31-12-2007 of the oceanic water between the latitudes 30N – 70S and 120W-60W. (a) the distribution of diatoms in mg/m^3 (b) the distribution of cyanobacteria in mg/m^3 (c) the concentration of nitrate in the upper 50 meter (micromoles/L) and (d) the iron concentration in the upper 50 meters (nanomoles/L). Graphs created with Giovanni system utilizing data from the MODIS mission (NASA).

Cyanobacteria dominate NO_3^- limited areas such as the Gulf of Mexico and the Caribbean Sea. Areas that are limited in both Fe and NO_3^- may favor small cells that use NH_4^+ as a source of nitrogen (Price *et al.*, 1994). Since the N_2 fixing nitrogenase protein is rich in Fe, waters rich in Fe may stimulate the growth of N_2 fixing cyanobacteria which release vast amounts of dissolved organic nitrogen (DON) such as urea. And indeed, N_2 fixing bacteria are the greatest component of phytoplankton in the Caribbean Sea (that is poor in NO_3^- but rich in Fe) releasing substantial amount of DON (Carpenter & Price, 1977; Glibert & Bronk, 1994 Paerl, *et al.*, 1994) that in turns can be used by other species of cyanobacteria. Furthermore, due to their small size (and possibly due the lack of phycobilisomes) *Prochlorococcus* dominates in oligotrophic tropical oceans (McClain, 2009).

Species distribution does not only differ in space due to differences in N availability but also in due to the availability of other nutrients. In addition to NO_3^-, diatom cells depend on silicate for their shell structures. Therefore, diatoms are also often dominant in nutrient rich waters (DeMaster *et al.*, 1995). Furthermore, because diatoms rely on NO_3^-, while other species are capable of using NH_4^+, changes in NO_3^- concentrations over time can influence diatom abundance. For example, off the coast of Belgium, NO_3^- concentration declined in 1997 and consequently the diatom bloom decreased and got replaced by the *Haptophyte Phaeocystis* that prefers NH_4^+ as an N-source (Tungaraza *et al.*, 2003). Among other factors such as a high sinking rate of the cells, the demand for NO_3^- causes diatoms to bloom when the water column is mixed from late autumn to the beginning of spring (Berg *et al.*, 2003; Ferber *et al.*, 2004; Marty *et al.*, 2002). For example, in the Baltic Sea and the gulf of Riga diatoms, cyanobacteria, cryptomonads and dinoflagellates form the dominate group. However, with increasing temperatures due to the seasons and thus increasing stratification diatoms decreased to the point they were almost not present anymore (Berg *et al.*, 2003).

Dinoflagellates such as *Heterocapsa triquetra* assemble relative expensive pigment complexes in terms of nitrogen: the peridinin-chlorophyll a-protein (Table 3-4) (Waller *et al.*, 2006). Hence, dinoflagellate blooms often occur in coastal regions (Roy *et al.*, 2011). For example, near the coast of Finland cell abundance increases along with nitrogen availability (Lindholm & Nummelin, 1999). Many dinoflagellates however, including *H. triquetra* can become phagotrophic when nutrients are limited (Legrand *et al.*, 1998). In contrast to phytoplankton species with expensive pigments, coccolithophores bloom when nutrients are low and when there is little competition with other phytoplankton (McClain, 2009).

5.4.2 Freshwater habitats

The nitrogen availability in freshwater habitats differs hugely among different water bodies and more extremes are encountered compared to the N-availability in ocean waters (Hecky *et al.*, 1993). Hence, fresh water bodies can be extreme eutrophic and rich in N. Therefore, a major difference between freshwater and marine cyanobacteria is the assembly of PC. With the exception of *Oscillatoria amoera*, freshwater cyanobacteria have higher concentrations of the N-rich PC compared to oceanic cyanobacteria that contain mostly PE (Ong & Glazer, 1991; Patel *et al.*, 2005; Pawar & Puranik, 2014). However, cyanobacteria are not only found in N-rich waters, N-fixing cyanobacteria can also thrive well in waters that are not replete in N (Schindler *et al.*, 2008). It has even been suggested that low N-concentrations favor (N-fixing) cyanobacterial blooms in freshwaters (Havens *et al.*, 2003; Smith, 1983). However, energetically it is more beneficial to use other sources of N instead of fixing N_2. Consequently,

a correlation between N-depleted water and N-fixing cyanobacteria is not always found (Jensen *et al.*, 1994). In freshwater, N_2-fixation by cyanobacteria is only a small percentage (less than 2%) of N uptake, which is minimal compared to the 82–98% uptake of NH_4^+ (Ferber *et al.*, 2004). But N_2-fixing cyanobacteria are capable of assembling more PBS than non N_2-fixing cyanobacteria (Rodriguez *et al.*, 1989). Surprisingly, in NO_3 limited environments the diatom *Nitzschia perminuta* outcompetes the N_2-fixing cyanobacteria, whereas the N_2-fixing cyanobacteria dominate at high NO_3^- concentration (Van der Grinten *et al.*, 2004). This suggests that diatoms with cheaper pigment-protein complexes are better competitors for NO_3^- than cyanobacteria with relative N-costly pigment-protein complexes. It might therefore not be beneficial for cyanobacteria to compete with phytoplankton species with cheap pigments in NO_3^- limited waters.

Low N-concentrations in lakes might be also a result of cyanobacteria blooms rather than the reason why cyanobacteria blooms occur since NH_4^+ and NO_3^- levels drop by up to 18 times (Carmona *et al.*, 2006; Ferber *et al.*, 2004; Xie *et al.*, 2003). For example, *Porphyra* spp. was able to deplete 150 µM of N within four days (Carmona *et al.*, 2006). In fact, most freshwater cyanobacteria are capable of storing a substantial amounts of N in cyanophycin (Whitton & Potts, 2000) and that therefore at that moment not all N has be assembled in protein structures such as pigments.

6. Nitrogen and light

Light absorbed for photosynthesis mainly depends on the pigment composition, while the pigment composition depends largely on the availability of N (Geider *et al.*, 1998). However, light and nitrogen availability across different habitats are negatively correlated. For example, with increasing depth in the ocean, light gradually decreases while nitrogen rapidly increases. How cells deal with (fluctuations in) light and N-limitation depends per phytoplankton class (Anderson *et al.*, 2006; Figueroa *et al.*, 2010; Berges *et al.*, 1996). These differences between phytoplankton classes might be explained by the costs and absorption spectrum of the pigments. If photosynthetic pigment-protein complexes require high amounts of N, they tend to absorb more in the red part of the spectrum (Figure 6-1: r_s 0.851, p=<0.001). This is most likely due to the lattice structure of phycobilins in the phycobiliprotein complexes. In this lattice structure the phycobilins are strongly stretched, making them more unstable reacting to low energy light such as red light (Fetisova *et al.*, 1988; Zehetmayer *et al.*, 2004) and therefore absorb light in the red part of the spectrum. Expensive pigment-protein complexes such as phycobiliprotein are probably more affected by a reduction in N-availability than the cheaper pigment-protein complexes such as LHCsR (light harvesting complex stress response). However, these cheap protein-pigment complexes are less adapted to absorb light in turbid water because of their strong absorption in the blue part of the spectrum. Therefore, cheap pigment-protein complexes which absorb mainly in the blue part of the spectrum might be most limited by light availability in contrast to N. The interplay between light and nitrogen availability is phytoplankton class specific. Therefore, in the following section the interplay between light and nitrogen availability is discussed per phytoplankton class.

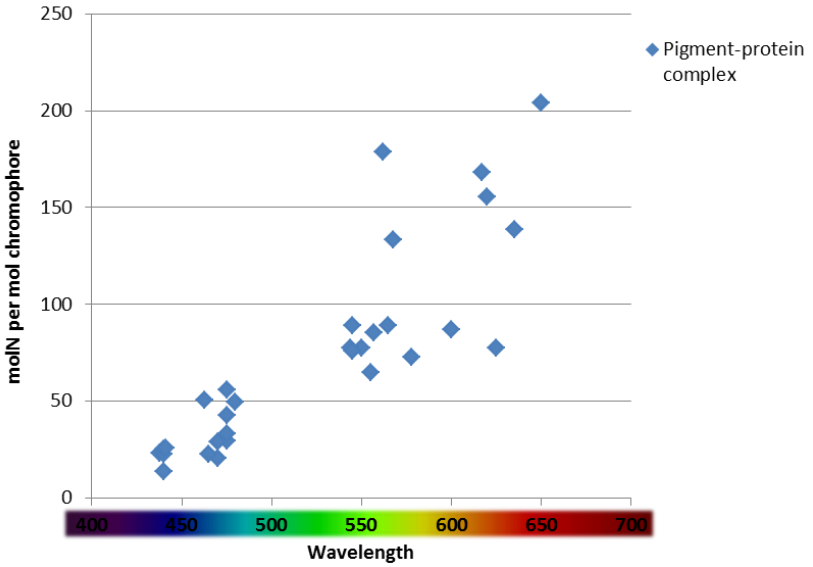


Figure 6-1 The costs of photosynthtic pigment-protein complexes in mol nitrogen per mol chromophore as a function of the maximum peak of light absortption. Pigments with higher N requirements absorb in the red part of the spectrum while pigments withlower N requirements absorb in the blue part of the spectrum. Full data including references is attached in appendix II.

6.1 Cyanobacteria

Cyanobacteria are considered to be superior light competitors and will therefore become abundant in enviroments with high nutrition loads (since there is no competition over nutrients but light will become limited)(Brauer *et al.*, 2012; Yang & Jin, 2008). A low nitrogen availability, some cyanobacterial species are strongly limited by N-depletion, while others dominate in N-limitated waters depending on the PE/PC ratio, PUB:PEB ratios, the concentrations of PBS associated with the thylakoid membrane and therefore the costs of photosyntesis in terms of N (Everroad, & Wood, 2012; Olson et a., 1988). An example of a

strain adapted to low N-availability is *Synechococcus* CCMP 839 that continued to grow under N-depletion and only showed a decreased growth rate at a light irradiance lower than 10 mol photons m^{-2} s^{-1}. However, there is a trade-off in being a good competitor for N and or light; compared to other phytoplankton species, CCMP 839 grows slow at high N, high light conditions (Timmermans *et al.*, 2005).

Under N limited conditions, more light is absorbed in the blue part of the spectrum, compared to N replete conditions. This shift is most notable in the *Synechococcus* strain WH 5701 with high quantities of PC. At high N availability, light is being absorbed in the red part of the spectrum. However, when N becomes depleted the light absorption shifts to the blue part of the spectrum (Scanlan, 2003). This is due to the fact that under nitrogen starvation PBPs which absorb in the red part of the spectrum are being degraded first (Sciandra *et al.*, 2000) while relatively more chlorophyll which absorb light in the blue part of the spectrum, remain.

Cyanobacteria inhabiting the open surface oceans often rely more on NH_4^+ than on NO_3^- as a nitrogen source (Maldonado & Price, 1996; Scanlan & West, 2002; Findlay *et al.*, 2014). *Synechococcus* grown on NH_4^+ contained more Chl*a* and less PC compared to cells grown on NO_3^- (Forchhammer & de Marsac, 1995). One would expect that NH_4^+ would be used to assemble more PC, because PC is more abundant in surface layers and in turbid waters with low light irradiance compared to PE (Lohscheider *et al.*, 2011; Glover *et al.*, 1985). Assimilation of NH_4+ requires less photons to build new pigment-protein complexes (Raven, 1984) and is therefore considered to be a cheaper source of N in turbid waters. However, the surface oceans also contain high-light adapted cyanobacteria. Under high light conditions, cyanobacteria produce more chlorophyll than PBS (Glover *et al.*, 1987). The assembling of different pigments when different N-sources are available could be therefore an additional adaptive advantage for cyanobacteria, thus absorbing more in red part of the spectrum when more NO_3- is available. Another change in use of nitrogen sources is seen in N-fixating cyanobacteria. N fixation inceases with increasing light intensities, and decreases with increasing inorganic nitrogen concentrations (de Tezanos Pinto & Litchman, 2010; Lehtimaki *et al.*, 1997).

Light absorption of the relative 'cheap' Pcb of *Prochlorococcus* shows a strong absorption in the blue part of the spectrum matching the deep layers of oligotrophic waters (Lohscheider *et al.*, 2011). In contrast, expensive pigment-protein complexes tend to have less sharp absorption peaks and absorb less in the blue part of the spectrum (Sciandra *et al.*, 2000) matching the underwater light environment of turbid waters. Therefore, in oligotrophic waters *Prochlorococcus* dominates over *Synechococcus* (Six *et al.*, 2007) and vice a versa.

Pico-cyanobacteria such as *Synechococcus* often assemble high levels of PUB or variable PUB:PEB ratios in their PE, whereas freshwater- or turbulent marine species lack PUB in their PE or even lack PE (Everroad, & Wood, 2012). PUB rich PEs are cheaper in terms of nitrogen and absorb more light in the blue part of the spectrum than low PUB containing PEs. Furthermore, it seems that cyanobacteria with more expensive PBP and thus with lower PUB:PEB ratio tend to adapt better in fluctuating light environments. Furthermore, high PUB containing PEs can stand N-limitation for a short time but suffer from long recovery times (see subunit 5.3). The cheaper PBPs with a high PUB:PEB ratio have an absorption spectrum shifted to the blue part and are often found in N-limited waters (Gilbert *et al.*,

1986; Glibert & Ray, 1990; Kana *et al.*, 1992). When *Synechococcus* sp. WH7803 with low PUB:PEB ratio was cultured under non-limiting light and N supply, cells assembled PE. However, the PE did not transfer the energy of the light to the reaction center suggesting that this excess PE functioned as N storage (Heathcote *et al.*, 1992). Therefore, assembly of PE when N is not yet depleted and light is still available seems to give the cells an advantage and make them better adapted to possible N and light limitation (Barrett *et al.*, 1996).

6.2 Cryptomonads

At high N-availability and low light the Cryptomonad *Rhodomonas marina* has the highest chlorophyll content. Furthermore, the change in Chl content in response to changes in light was greater than the change in Chl content in response to changes in nitrogen availability (Sciandra *et al.*, 2000). Hence, Chl increases strong with decreasing light while Chl increases less strong with increasing N-loads. This suggests that chlorophyll is more sensitive to changes in light than to changes in nitrogen supply. In contrast, levels of the photoprotective pigment alloxanthin remain constant and do not change in response to differences in N- or light availability (Henriksen *et al.*, 2002). The PE content of cryptomonads does not change significantly in response to changes in light irradiance, but the PE content decreases when nitrogen availability becomes limiting. In conclusion, chlorophyll content responds strongly to changes in to light, whereas PE content respond strongly to changes in N-supply (Sciandra *et al.*, 2000). These changes in pigment composition due to N availability impact the light absorption strongly. At high NO_3^- levels, light is more strongly absorbed in the blue part of the spectrum compared to low NO_3^- levels (Sciandra *et al.*, 2000). Furthermore, the density of cryptomonads is influenced more by nitrogen concentration than by light irradiance (Cruz *et al.*, 2006).

6.3 Red algae

Red algae in N-replete media can tolerate sudden changes in the environment, like short term irradiance stress, better than N-depleted cells. When N becomes limiting and irradiance is high, MAAs increase (Figueroa *et al.*, 2010). However, despite the increase in MAA, the red alga *Gracilaria conferta* still suffered from photo inhibition (Figueroa *et al.*, 2010). Because N-repletion stimulates the accumulation of PBPs, red algae might recover faster form light stress than cells exposed to low N-availability (Anderson *et al.*, 2006; Figueroa *et al.*, 2010). Furthermore, when both light and nitrogen is limiting, the growth rate decreases (Lapointe & Duke, 1984). In conclusion, red algae with expensive pigments are able to recover from high-light stress or low light availability as long as sufficient N is available.

6.4 Diatoms

In diatoms, the amount of diadinoxanthin doubled while the amount of chlorophyll and the antenna size remained constant under intermittent light exposure compared to constant light exposure. This suggests that in diatoms, little nitrogen costs are involved in adaptation to changing light conditions (Lavaud *et al.*, 2002). Diatoms mainly dominate in high-latitude eutrophic waters (appendix II). As described in chapter 4 and 5, diatoms are capable of adjusting their pigment composition, and contain high levels of fucoxanthin and diatoxanthin which function in the xanthophyll cycle and protect the cell from photo induced damage (Ikeda *et al.*, 2013). Xanthophyll cycle pigments might have low nitrogen costs because the molecule structure changes but no proteins are synthesized. Therefore, diatoms have an advantage

in high light – low nitrogen areas areas.

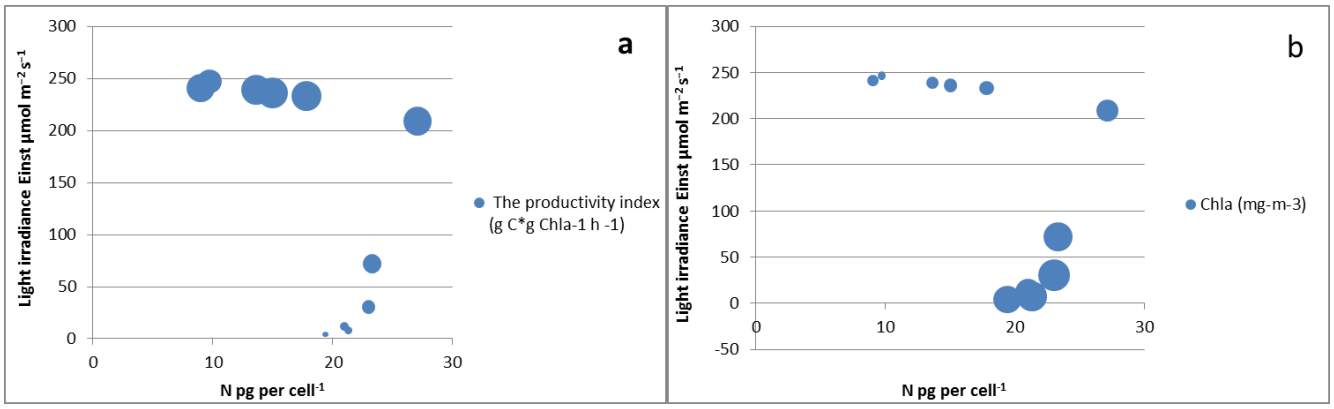


Figure 6-2 (a) The chlorophyll specific productivity (g C*g Chla-1 h -1) and (b) volumetric Chl*a* content of the diatom *Thalassiosira fluviatilis* given as a function of light irradiance and N content, data retrieved from Laws & Bannister (1980).

However, the chlorophyll specific productivity diatom responds more strongly to light limitation than to N-limitation (Figure 6-2; Laws & Bannister, 1980). Hence, cell density increases up to three fold under high-light even when N is limited (Leonardos & Geider, 2004). In addition, the highest growth rate is measured when cells are grown under bright blue light (Abe & Gianesella-Galvão, 1991). When light irradiance is low the chlorophyll specific productivity is low while the chlorophyll content in the diatom cells is high. Furthermore, the metabolic cycling of N increases from 1% in low light to 14% in high light (Li *et al.*, 2014). Therefore, under high light, diatoms might be capable of repairing PSII without additional N costs and can continue photosynthesis in N-limited environments.

6.5 Green algae

Green alga such as *Dunaliella* and *Scenedesmus quadricauda* become dominant under high- to moderate light intensities at a relative low N load (Sciandra *et al.*, 1997; de Tezanos Pinto & Litchman, 2010). Yet, too much of high light can cause photo oxidative damage that brings extra costs in terms of N (Zhang *et al.*, 1997). Also, a strong N depletion results in lower chlorophyll synthesis, C fixation rates and respiration rates and therefore growth decreases (Sciandra *et al.*, 1997). In contrast to photosynthetic pigments the concentration of β-Car is almost three times higher when cells are exposed to both high light and N starvation, than when cells are exposed to only high light or N depletion (Lamers *et al.*, 2012).

6.6 Dinoflagellates

Results discussed in chapter 4 showed that dinoflagellates are capable of adjusting to low light by decreasing blue light absorbing pigments such as Chl*c* and peridinin and that cells usually contain high amounts of chlorophylls (Berdalet *et al.*, 1992; Vaillancourt *et al.*, 2004). However, when cells were exposed to low light irradiance (80 µE m^2 s^{-1}) after being adapted to high-light irradiance (330 µE m^2 s^{-1}), without the addition of N, chlorophyll content temporarily increased and then decreased. In contrast, when cells received additional N, chlorophyll concentration rose during the first 3 days and thereafter the quantity remained steady. Furthermore, under nitrogen depletion dinoflagellates are also more sensitive to UV induces damage, because cells are less efficient in repairing damaged proteins (Litchman *et al.*, 2002). Because the assembly of new pigment-protein complexes requires nitrogen, dinoflagellates are only under high nitrogen availability well capable of adjusting to lower or higher light irradiance

(Prézelin & Matlick, 1983). The high demand for N during photoadaptation might explain why dinoflagellates are considered to be poor competitors for light (Schwaderer *et al.*, 2011) and why red tides are often found in N rich environments near the coastline (Prézelin & Matlick, 1983).

7. Application

Concerns regarding environmental anthropogenic changes are gradually increasing. Such concerns include the decrease in and the pollution by fossil fuels, doubling of atmospheric CO_2 since 1870, the large energy and water consumption of wheat production and water pollution and eutrophication (Höök & Tang, 2013; IPCC, 2007; Khoshnevisan *et al.*, 2013; Obilonu *et al.*, 2013). To predict the impact of global change the Intergovernmental Panel on Climate Change (IPCC) defined four scenarios known as the Special Report on Emissions Scenarios (SRES). These four scenarios are based on economic (A) versus environment (B) development, and global (1) versus regional (2) development. Within the SRES the B1 scenario is predicted to cause the least climate change in the future (see text box). To be able to carry out the B1 scenario, problems such as energy demand, CO_2 emission and water pollution, may be solved by phytoplankton. Phytoplankton can meet the demands for resource efficient and environmental sustainable solutions without the additional negative impact on the environment. Solutions can be found in phytoplankton cultures grown for biofuel and food production, but also for waste water treatment and possibly prevention of harmful algae blooms. This chapter will describe how the information on the impact of nitrogen and light on phytoplankton pigments may be used in sustainable and innovative solutions.

B1 scenario: "The B1 scenario family describes a convergent world with the same global population, but with a rapid change in economic structures toward a service and information economy, with reductions in material intensity and the introduction of clean and resource-efficient technologies. The emphasis is on global solutions to economic, social and environmental sustainability, including improved equity, but without additional climate initiatives."

Text adapted from IPCC, 2007

7.1 Phytoplankton cultures

Culturing phytoplankton is of interest for many products such as phycocyanin used for its blue color, carotenoids used in health supplements and lipids used for biofuels (Table 7-1). Furthermore, harvesting products such as biofuel from phytoplankton is more sustainable than the current harvested fossil fuels. Depending on the product different demands for N and light may be required. One of the key problems in massive dense phytoplankton cultures that use natural light is that they require high irradiances, which may only be available near the equator. Furthermore, to stimulate the synthesis of specific pigments, specific wavelengths are required. Using artificial light in phytoplankton cultures may solve these problems, but artificial light requires additional energy. Nevertheless, using artificial light allows more control over the culture, and may increase productivity and CO_2 uptake while requiring less space for installations (De Buisonjé & Aarnink, 2011).

Table 7-1 An example of phytoplankton (species) products cultivated for commercial product. Table adapted from Pulz & Gross (2004)

Species (group)	Product of interest	Example of application area
***Spirulina platensis* (Cyanobacteria)**	Phycocyanin	Cosmetics and make-up
***Chlorella vulgaris* (*Chlorophyta*)**	Biomass	Food surrogates/color enhancers
***Dunaliella salina* (*Chlorophyta*)**	Carotenoids	Health food supplement
***Porphyridium cruentum* (*Rhodophyta*)**	Polysaccharides	Pharmaceuticals
***Isochrysis galbana* (*Chlorophyta*)**	Fatty acids	Animal nutrition/health products
***Phaedactylum tricornutum* (*Bacillariohyta*)**	Lipids	Fuel production

Difficulties with phytoplankton cultures not only concern their energy use, but also the establishment of mass production. Algal mass culture systems can be infected by algal parasites such as viruses, or by herbivores. The larger the culture, the higher the chance of infections, and the higher the losses (Lane & Carney, 2014 Smith & Crews, 2014). Despite the fact that the chance of infection by unwanted species is high in open ponds, for now open pond cultures are the only economical possible culturing method for biofuels considering that biofuel production costs may not exceed $0.14US per kilogram dry biomass to compete with gasoline. However, future hope lies culturing phytoplankton in photobioreactors because it offers more control over the development of the cultures. Current research, like research conducted by Wetsus (the Netherlands), focuses on an energy efficient way of culturing phytoplankton in photobioreactors. Solutions are seeked in for example intermittent light exposure to limit energy costs. Also, flat-plate photobioreactors (Figure 7-1) may be economically feasible because more energy is gained from this system than invested into it, and flat-plate photobioreactors are therefore cheaper than other photobioreactors (Jonathan & Mordechai, 2013; Sun *et al.*, 2011). Therefore, in the three following sections phytoplankton culture for lipid production, carotenoids and phycobiliproteins are discussed that are grown in photobioreactors with light

Figure 7-1 A small flat photobioreactor used for cultivation of phytoplankton (picture retreived from: http://www.psi.cz/)

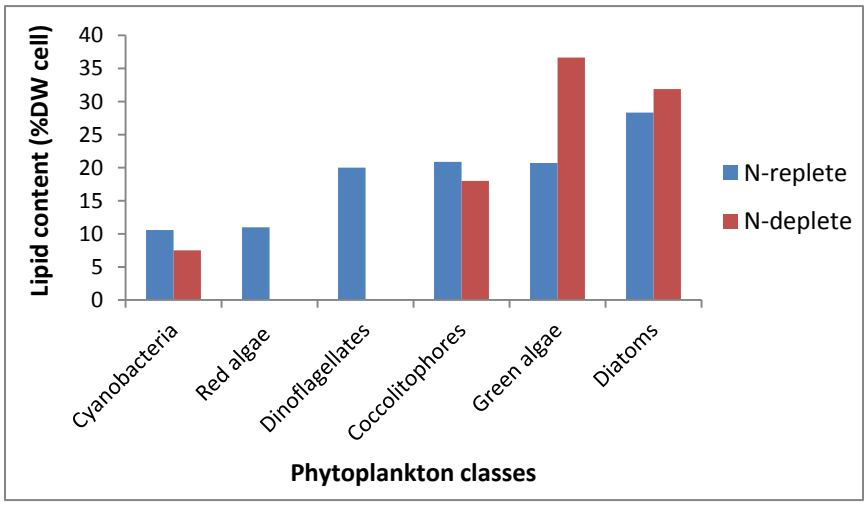


Figure 7-2 The lipid content of phytoplankton classes in N-repelted or N-depleted cultures. Full data and references are attached in appendix IV Griffiths & Harrison (2009), Griffiths *et al.* (2012), Zheng *et al.* (2013)

emitted from light-emitting diodes (LEDs).

7.1.1 Culturing phytoplankton for lipid production

Lipids are of major importance for the production of biofuels and dietary supplements. It is therefore important to know which phytoplankton species are capable of producing high amount of lipids and under which conditions these lipids are formed. Overall there seems to be a trend in high synthesis of lipid when phytoplankton contain cheap pigments (Figure 7-2). Compared to other species (also from other classes) the diatoms *Amphora* sp., *Nitzschia palea* and *Chaetoceros calcitrans* produce the highest percentage of lipids per dry weight. In contrast, the cyanobacterium *Anabaena cylindrica* produces the least percentage (90% less than the diatom *Amphora* sp.) of lipids per dry weight (Griffiths & Harrison, 2009). In all classes except for cyanobacteria (and dinoflagellates) the percentage of lipid increased when cell were grown in N-depleted media (Deruère *et al.*, 1994; Griffiths *et al.*, 2012; McGinnis *et al.*, 1997; Xin *et al.*, 2010). In the green alga *Scenedesmus* sp. lipid percentage could increase up to 4-fold when cells were N-limited (Griffiths & Harrison, 2009; Griffiths *et al.*, 2012). Another important aspect of green algae exposed to N-deprivation, is that cells also contain unsaturated lipids (Merzlyak *et al.*, 2007) that are beneficial for human health and thus a desirable substance in a daily diet (Horrocks & Yeo, 1999; Nagao & Yanagita, 2005).

Under high (blue intermittent) light and low N-concentration stress adapted species with cheap pigments such as diatoms and green algae, increase their growth rate and lipid content (See Figure 7-3, Abe & Gianesella-Galvão, 1991; Anning *et al.*, 2000; Deblois *et al.*, 2013; Glover *et al.*, 1997; Yoshioka *et al.*, 2012). Hence, under these conditions, chlorophyll content in green algae decreases and carotenoids accumulate in oily globules within interthylakoid spaces of the chloroplasts or outside the chloroplast (Bar *et al.*, 1995; Hejazi *et al.*, 2004; Kleinegris *et al.*, 2010; Lamers *et al.*, 2012; Mendoza *et al.*, 1999) and may function as a protection mechanism against high light irradiance (Ben Amotz *et al.*, 1982). Hence, green algae, seem more suitable for biofuel production than e.g. diatoms because of their high lipid production and their high growth rate (Gouveia & Oliveira, 2009; Griffiths *et al.*, 2012). Phytoplankton with expensive pigments seem least suitable for lipid production, especially in N-limited cultures. However, prokaryotes are easier to genetically modify than eukaryotes such as green algae. Therefore, a lot of research is conducted on genetic engineering of cyanobacteria (Hays & Ducat, 2014; Liu *et al.*, 2011; Rosenberg *et al.*, 2008). Considering that most cyanobacteria are superior light competitors, they might need less light to produce a voluminous amount of lipid. Hence, using modified cyanobacterial strains that require less light will eventually

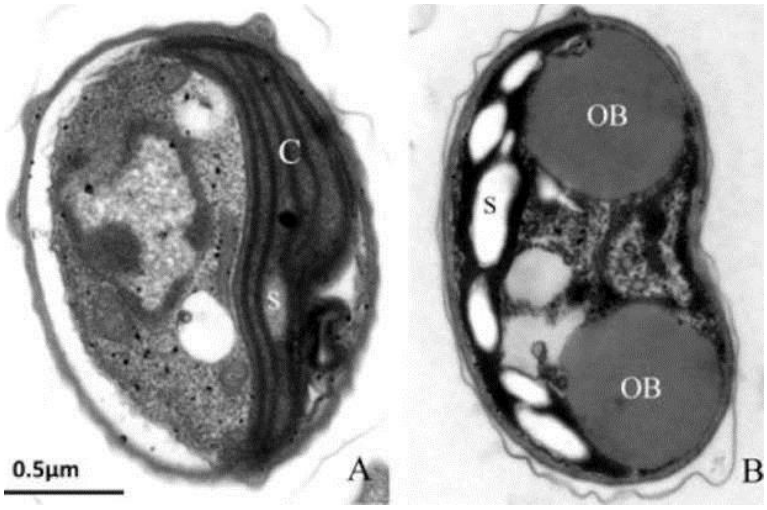


Figure 7-3 A picture of green algae before (A) and after nitrogen starvation (B). Before nitrogen starvation more chloroplast (C) is visible in the cell. After nitrogen starvation the chloroplast decreases in size while the starch granules (S) and oil bodies (OB) increase in size (Wang *et al.*, 2011).

cost less energy and will be more profitable than using natural green algae. Nevertheless, in the hope to produce affordable lipids that can be used for biofuel in the future, research on modification of eukaryotic phytoplankton continues, because in general they produce more lipids (Radakovits *et al.*, 2010).

7.1.2 Culturing phytoplankton for carotenoids

Carotenoids are used for a broad range of applications. Some of these applications include food coloring and enhancing the color of egg yolk, but carotenoids are also used as antioxidant, sun protection and cancer prevention (Raja *et al.*, 2007; Zhang *et al.*, 2014). Carotenoid production is, like the accumulation of lipids, a stress response to environmental factors such as high light and low N-availability (Lavaud & Kroth, 2006; Merzlyak *et al.*, 2007; Partensky *et al.*, 1993; Zhu *et al.*, 2010). Similar to lipid production, carotenoid production is high in stress adapted strains with cheap protein-pigment complexes (Takaichi, 2011). Carotenoids of major importance for commercial use are β-carotenes and the powerful antioxidant astaxanthin, which are both assembled by green algae such as *Chlorella* and *Scenedesmus* (Takaichi, 2011; Spolaore *et al.*, 2006). The highest amounts of astaxanthin per cell are achieved when cells are grown in nutrient limited media under high light irradiance (Bar *et al.*, 1995; Ben Amotz *et al.*, 1982; Phillips *et al.*, 1995). However, low N also inhibits the growth rate of different species of green algae, which results in a lower total carotenoid yield (Borowitzka *et al.*, 1991; Orosa *et al.*, 2000; Xia *et al.*, 2013). Therefore, the highest concentration of carotenoids was achieved under high light (≈300 µmol photons m^{-2} s^{-1}), while the optimal quantity of N used for the total yield of carotenoids is still debatable and differs greatly per species.

7.1.3 Culturing phytoplankton for phycobiliproteins

The most important use of phycobiliproteins is the use of phycocyanin as a food coloring product due to their unique blue color that is for example used for coloring blue Smarties. C-phycocyanin may also be effective in preventing cancer and is thus of interest for medical purposes (Marzieh Hosseini *et al.*, 2013).

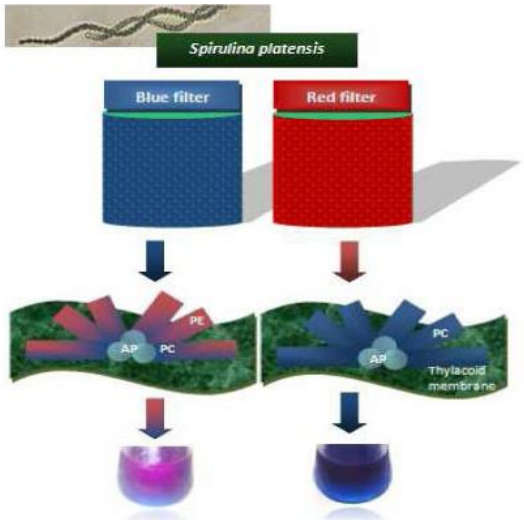


Figure 7-4 Two cultures of S. platensis grown under blue light and red light. Cultures grown under blue light assamble more PE while cultures grown under red light assamble more PC. (Picture adapted from:Walter *et al.*, 2011)

Often, the freshwater cyanobacteria *Spirulina platensis* is used for the production of phycocyanin. To favor the assembly of phycobiliproteins, N-depletion should be avoided since N-depletion decreases the number PBS and size of PBS (Berges *et al.*, 1996; Stevens *et al.*, 1981). Furthermore, more photosynthetic pigments are assembled when cells are grown in lower light irradiance (Geider *et al.*, 1996). When cyanobacteria are exposed to light irradiances ranging from 7 to 42 µmol m^{-2} s^{-1}, the highest PBP production per µg/ml cells is found at 28 µmol m^{-2} s^{-1} (Maurya *et al.*, 2014).

Phytoplankton can adjust the composition of PBPs to a change in light quality. Hence, the production of PBPs can be influenced by the color of irradiance (Figure 7-4). The production of PC is highest under red- and yellow light while these light colors inhibit cell growth (Chainapong *et*

al., 2012; Whitaker *et al.*, 2011; Whitaker *et al.*, 2009). Under white light growth rate is maximum, but PC concentrations are relatively low (Chainapong *et al.*, 2012; Walter *et al.*, 2011; Whitaker *et al.*, 2011; Whitaker *et al.*, 2009). This trade-off between pigment composition and growth rate is also seen in the production of carotenoids. Where high light is limiting the growth rate. Therefore, a trade-off between pigment content and growth rate may be a general phenomenon.

7.2 Water management

A growing concern within water management is the increasing eutrophication of water bodies. The annual atmospheric deposition and groundwater discharge of NO_3- and NH_4- has been increasing over the past years and is expected to continue to increase (Beusen et al, 2013; Paerl, 1997) and increases more rapidly than P concentrations (Anderson *et al.*, 2002). Atmospheric N deposited (AND) is of a major contributor of N enrichment in the oceans and coastal waters, while near estuaries the runoff of N is as important as AND in the eutrophication of coastal waters (Kim *et al.*, 2011). Because N-enrichment increases CO_2 uptake by phytoplankton and the total Chl*a* concentration (Paerl, 1995), it may also increase the occurrence of toxic- and non-toxic blooms of phytoplankton (Zhang, 1994). Increasing N in oligotrophic oceans may shift the composition of phytoplankton blooms from N-limited species to light-limited species, and favor phytoplankton with expensive pigments such as cyanobacteria and dinoflagellates (Domingues *et al.*, 2011a; Domingues *et al.*, 2011b; Kim *et al.*, 2011).

Important tasks of water management are increasing the ecological value of water bodies and guaranteeing the safety of waters for recreational, agricultural and industrial purposes. Consequently, water managers should try to prevent the development of Harmful Algal Blooms (HABs). HABs can be a great risk to human health. HABs can cause direct toxic reactions when people get in contact with phytoplankton through e.g. swimming, but HABs can also cause indirect toxic reactions through ingestion of food which was exposed to HABs. For example, HAB's can be responsible for seafood-borne illnesses such as neurotoxic shellfish poisoning or ciguatera fish poisoning (Schoelinck *et al.*, 2014; Watkins *et al.*, 2008). Cyanobacteria are often responsible for intoxication caused by skin contact while dinoflagellates are often responsible for food-borne illnesses. Both phytoplankton groups are mostly responsible for HABs. In particular, cyanobacteria often cause HABs in freshwaters, while dinoflagellates tend to cause HABs in saline environments (>5% salinity) (Paerl, 1988). Toxic blooms of dinoflagellates may be difficult to battle because blooms in oceans and coastal waters are difficult to manage.

7.2.1 Preventing or predicting cyanobacteria blooms

Cyanobacteria blooms occur mostly in eutrophic freshwaters (Davis *et al.*, 2009; Sellner, 1997). The occurrence of cyanobacterial blooms have increased over the last years and are expected to increase with increasing anthropogenic eutrophication and climate change (Figure 7-5) (Heisler *et al.*, 2008; O'Neil *et al.*, 2012; Paerl & Huisman, 2009; Paerl & Paul, 2012). Toxins produced by cyanobacteria can damage the nerves, liver and skin of mammals and are tumor promoters (Falconer & Humpage, 2005; and reviewed in: O'Neil *et al.*, 2012). Blooms often start in early summer when waters are stratified and nutrient concentrations are adequate (Berg *et al.*, 2003; Deng *et al.*, 2014). Therefore, cyanobacteria are favored by warmer springs and bloom earlier in warm springs (Deng *et al.*, 2014). Many cyanobacterial species contain gas vesicles, which give them buoyancy: the ability to float up in the water column. When lakes are stratified and water mixing is poor, dense blooms of cyanobacteria can form scums in

the top layer of the water column, thereby shading other phytoplankton (Berg *et al.*, 2003; Carr & Whitton 1982). Hence, sinking species like diatoms and green algae are outcompeted by buoyant cyanobacteria when lakes are stratified. The occurrence of cyanobacterial blooms in stratified- N-enriched lakes corresponds with the effect of light and N on cyanobacteria. Because cyanobacteria are better adapted to long term light changes, a stable, stratified environment is favorable where (N-fixating) cyanobacteria are capable of using NH_4+ or in lesser amounts N_2 as their source of nitrogen (Ferber *et al.*, 2004). Therefore, artificial water mixing as applied in eutrophic lakes (such as Nieuwe Meer, Amsterdam) with cyanobacteria blooms is a successful management strategy that has a negative impact on buoyant cyanobacterial blooms but favors diatom and green algae growth (Jungo *et al.*, 2001; Huisman *et al.*, 2004).

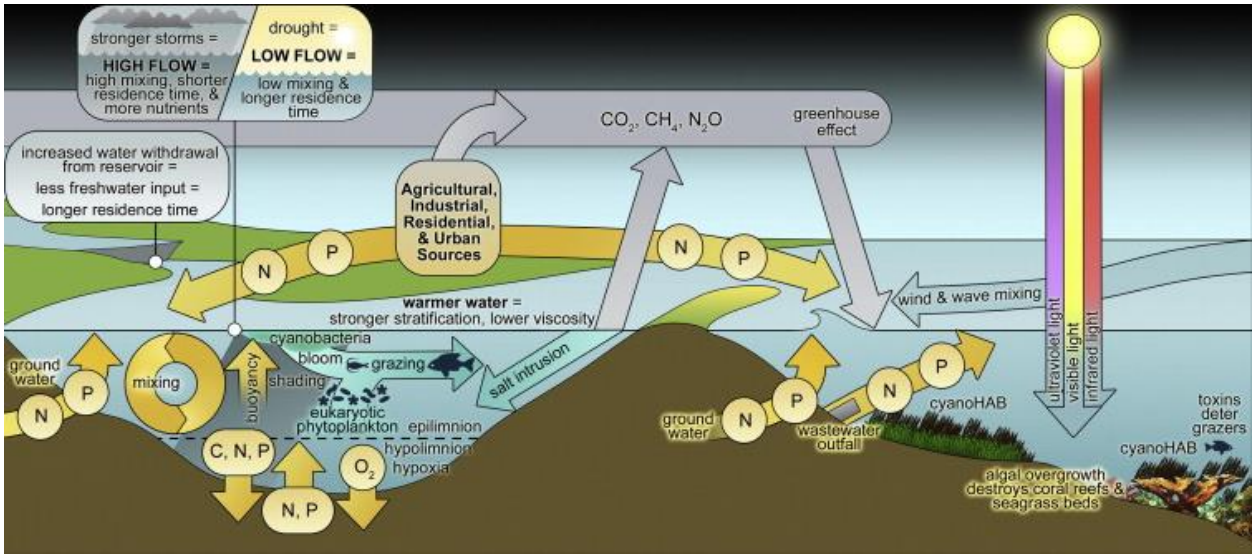


Figure 7-5 A conceptual picture, of interacting environmental factor controlling cyanobacteria blooms, such as: nutrient availability, water column transparency and shading by cyanobateria, water residence times, temperature and mixing conditions (Paerl & Paul, 2012).

Extensive cyanobacterial blooms are often a result of eutrophication (Figure 7-5, Paerl *et al.*, 2011). However, the strategy to limit eutrophication of lakes and coastal waters is still under debate. Although reduction of P is generally accepted as a powerful strategy against phytoplankton blooms, it is not always feasible or sufficient to reduce only P concentrations (Abell *et al.*, 2010; Jeppesen *et al.*, 2005; Raudsepp *et al.*, 2013; Wang & Wang, 2009). For example, N-enrichment has been found to favor cyanobacterial blooms in freshwaters and oceanic waters (Scott & McCarthy, 2011; Stal *et al.*, 1999). Therefore, beside P reduction, a long term solution would be to also reduce N-concentrations in the water. Since phytoplankton growth rates respond more strongly to increases in NH_4^+, not only NO_3- concentrations should be abated (Chaffin & Bridgeman, 2014).

7.2.2 Preventing or predicting dinoflagellates blooms

90% of HABs consist of flagellate phytoplankton, and 75% of the flagellate phytoplankton are dinoflagellates. The toxins dinoflagellates produce can be much more toxic than cyanobacterial toxins. For example, ciguatoxin produced by the dinoflagellate *Gambierdiscus toxicus,* is 22.000 more toxic than cyanide produced by cyanobacteria (Zingone & Oksfeldt Enevoldsen, 2000). Dinoflagellate HABs have

increased over the last years as a result of anthropogenic eutrophication (Brand & Compton, 2007; Zingone & Oksfeldt Enevoldsen, 2000). Toxic dinoflagellate blooms are mainly found when the turbulence of the coastal waters is low and are often triggered by rainfall events (Hallegraeff *et al.*, 1995). Blooming of the toxic dinoflagellate *Karenia selliformis* is strongly promoted by NO_3- (Feki *et al.*, 2013). Rainfall fuels coastal areas with terrestrial runoff rich inNO_3- and NH_4- (Beusen et al, 2013). Since dinoflagellates are only capable of adapting to different light environments when sufficient N is available (Prézelin & Matlick, 1983), stratified N-rich waters are favorable considering the sensitivity of dinoflagellates for turbulence, velocity and low nutrient affinity (Smayda, 1997; Smayda, 2002). Therefore, to prevent dinoflagellate blooms near coastal areas that are used to produce or to collect seafood it is favored to stimulate water mixing, causing dinoflagellates to be exposed to changing light environments and thus creating a disadvantageous environment for dinoflagellates. However, this solution would treat the symptoms but not the cause of the problem. Treating the cause of the blooms, namely eutrophication, would involve limiting run-off of nutrients favoring phytoplankton growth (Figure 7-6). Only limiting PO_4^{3-} run-off into rivers would not be sufficient because of limited growth and limited uptake of N by phytoplankton in the rivers. This will cause a larger N run-off in the coastal areas that are usually N-limited (Conley *et al.*, 2009) favoring dinoflagellate blooms. Regardless of reductions in nutrient loads it remains important to monitor the weakly weather conditions such as wind, rain and temperature and predict the chances for the development of dinoflagellate blooms and thus toxic seafood catches.

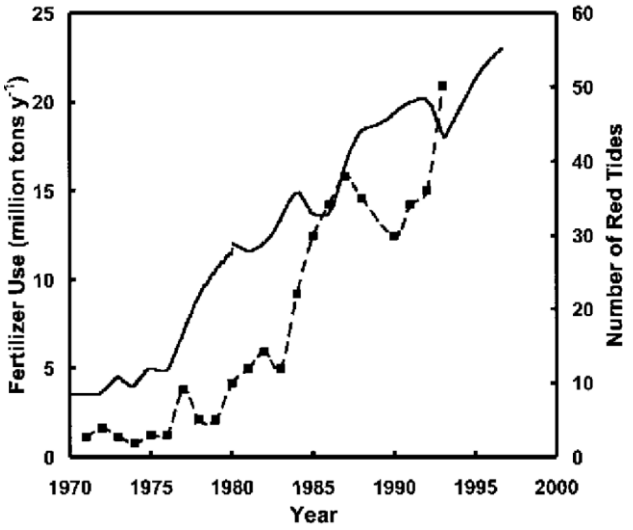


Figure 7-6 The trend of fertilizer use and the occurrence of red tides of the coast of China between the years 1970 to 2000. While there is a trend between fertilizer use and the number of red tides, it is thought that atmospheric deposit also plays a role in the occurrence of these blooms (Anderson *et al.*, 2002)

7.2. Waste water treatment

Cyanobacteria have a high growth rate when urea is used as a source of N compared to NO_3-. Cyanobacteria would be therefore suitable to be cultured on urine. More importantly for the environment, cyanobacteria remove within seven days 97% of NH_4+, 96.5% of total phosphorus (TP) and 85–98% of urea and are more efficient in removing N than the green algae *Chlorella sorokiniana* (Chang *et al.*, 2013; Tuantet *et al.*, 2014a). When glucose is added to the urine compounds in the cell increases to a level that makes the cells suitable for conversion into to bio-crude oil (a substitute for petroleum) (Chang *et al.*, 2013). Even though phytoplankton can efficiently remove nutrients from waste water and produce useful products, conventional waste water treatment plants can treat about 20000 m^3 d^{-1} (Sotirakou *et al.*, 1999), while waste water treatment using phytoplankton needs further adaptations to reach this amount (in a flow through of a ≈0.95 L^{-1} tank with 1:1 urine dilution is about 0.97 L^{-1} d^{-1}) (Tuantet *et al.*, 2014). However, the future perspective of using urine for algae growth and water

purification looks positive and plausible especially because growth, protein concentration and pigments are not affected by the use of urine (Chang *et al.*, 2013; Tuantet *et al.*, 2014a; Tuantet *et al.*, 2014b; Zhang *et al.*, 2014). However, although adding glucose to the waste water makes the cells suitable for bio-crude oil, it does not reach the same quantity as when a cell is stressed by i.e. nutrient limitation. Therefore, secondary effluents that have been already purified of nutrients could be a potential solution for mass-cultivation and biofuel production (Cho *et al.*, 2010).Limiting N (flow) in the environment might not decrease the total chlorophyll amount, but will influences the species composition. When N is reduced the total biomass of cyanobacteria decreases but is replaced by phytoplankton groups with less expensive pigments such as cryptomonads (Scott & McCarthy, 2011). Therefore, it is expected that by decreasing the N load, diatoms and green algae with cheaper pigment might become dominant. Since often cyanobacteria and dinoflagellates are responsible for HABs decreasing N would prevent most of the HABs (Cai *et al.*, 2013).

7.2.4 Global change and future perspective of phytoplankton distribution

Eutrophication might help dinoflagellates, red algae and cyanobacteria with expensive pigments to recover after environmental stress such as sudden high light irradiance (Figueroa *et al.*, 2010). In agreement with other diversity models, the diversity of phytoplankton is the highest when resources are limited (Interlandi & Kilham, 2001). In this case, light could also be considered as a limiting resource, decreasing with depth and might favor niche partitioning. However, the shading of other species as seen during cyanobacteria blooms and thus limiting light irradiance does not favor species diversity. Considering future perspectives, with eutrophication due to anthropogenic activities, phytoplankton with expensive pigments could become dominant, favored by high N-availability and stratified water due to an increased temperature, over shading other phytoplankton species in freshwater bodies. Whereas strong winds will stimulate upwelling and therefore increase the N-availability that might stimulate HABs, such as dinoflagellates, along the coastlines. However, the extra N-load due to runoff and even increased inorganic nitrogen deposit is not climate change depended and mainly depends on anthropogenic activities (Baron *et al.*, 2013).

The distribution of phytoplankton is, however, not only depending on light and N availability or N:P ratios. The distribution of phytoplankton is dynamic and also influenced by e.g. pH, temperature, CO_2, and the occurrence of El Niño and La Niña (Calbet *et al.*, 2014; Dandonneau *et al.*, 2004). Furthermore, often the factors are influenced by one another, such as a low pH favors diatoms by a faster uptake of NO_3- (Calbet *et al.*, 2014). Future prediction of the distribution of phytoplankton solely based on pigment composition would not be suitable because genetic adaptation to increased temperatures and increased CO_2 is an even greater component of prediction of future distribution considering climate chance and in that a large interspecific difference is expected (Costas *et al.*, 2014).

8. Conclusion

The effects of light intensity, light color and nitrogen availability on pigment composition often show a similar response. In particular, high light availability causes an increase in carotenoids, MAAs, Chl*c,* PUB (relative to PEB) while Chl*b*, Chl*d*, PC and PS decrease. This same reaction is seen when cells are exposed to blue light or low nitrogen availability (Figure 8-1). In contrast, when cells are exposed to low light, they synthesize more phycobilins, PBS (especially PC) Chl*b* and Chl*d,* while photoprotective pigments decrease. This response is very similar to the response of cells exposed to red light or high nitrogen loads. Also N-rich protein-pigment complexes tend to absorb strong in the red part of the light spectrum while N-poor protein-pigment complexes absorb more strongly in the blue part of the spectrum.

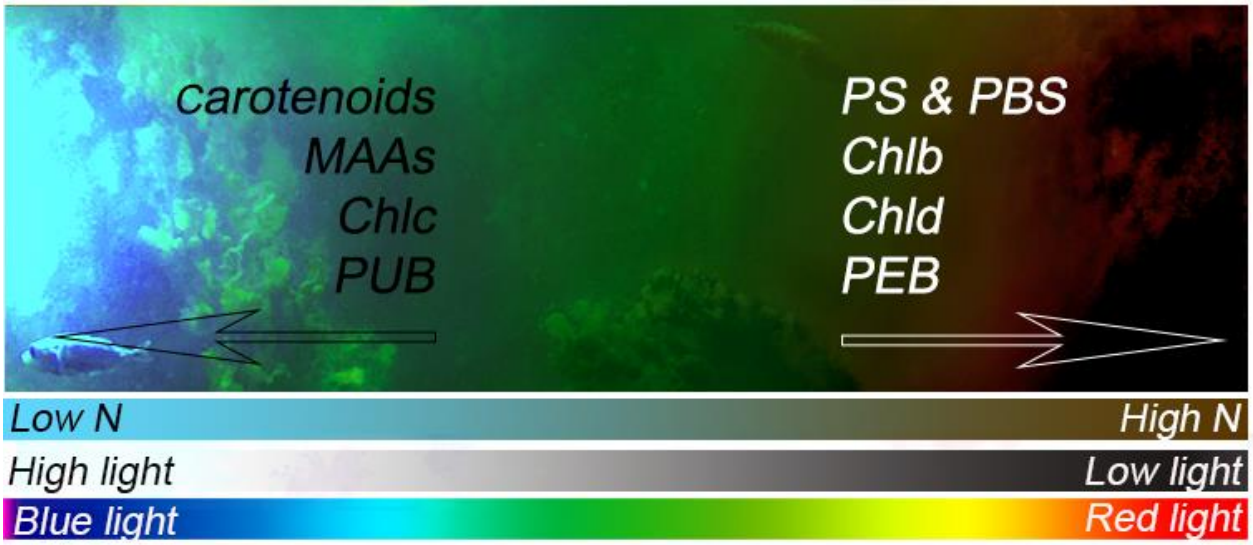


Figure 8-1 The effects of light intensity, light color and nitrogen availability on pigment composition shown in a conceptual picture. If nitrogen availibilty increases, light decreases or red light availability increases then Chl*b*, Chl*d*, PEB and complexes such as PS and PBS relatively increase. While a relative increase of carotenoids, MAAs Chl*c* and PUB is seen when nitrogen is depleted, high light irradiance or blue light is available.

Species such as diatoms and green algae that synthesize cheap pigment-protein structures in terms of nitrogen such LHCaR and LHCsR respond fast to changes in the light intensity. Both of these LCHsR are capable of a xanthophyll cycle due to the binding of diadinoxanthin and violaxanthin that will protect the cells within one hour from excess light. Hence, species with cheaper pigments generally thrive well in high light environments and can deal well with short fluctuations in light and NO_3^- availability. Dinoflagellates also possess a xanthophyll cycle and are therefore well protected against intermittent high light. However, they also synthesize N-rich structure for photosynthesis and, therefore, require high loads of reduced nitrogen. Structures that are expensive in terms of nitrogen are also often large protein structures that take time to assemble. Therefore, expensive structures such as phycobilosomes and reaction centers may be decoupled fast in reaction to high light or nitrogen depletion but take time to synthesize when light becomes limited or nitrogen availability increases. Therefore, phytoplankton classes such as cyanobacteria, red algae and cryptomonads that synthesize N-rich structures and do not possess a xanthophyll cycle thrive better in stable environments with sufficient reduced nitrogen. Species with expensive pigment-protein complexes in these environments are good competitors for

light. In contrast to fast changes in light availably, most cyanobacteria are well capable of alternating the composition of phycobilins to changes in light quality. Coastal or freshwater cyanobacteria with a low PUB:PEB ratio are often better in adapting to changes in light quality than cyanobacteria inhabiting the open ocean with high PUB:PEB ratios (Palenik, 2001). However, this adaptation also takes time because structures have to synthesize and therefore also costs nitrogen.

Application of the results

Phytoplankton species with cheap pigments in terms of nitrogen synthesize more carotenoids which can be used in food processing or health care and also contain lipids that can be used to produce biofuels. By stressing the cells, more of these product are synthesized, however, the total biomass decreases due to lower growth rates. If pigments from species with expensive pigment-protein structures are of interest, cells should be exposed to low or moderate light and should have sufficient nitrogen available. Furthermore, classes with expensive pigment-protein complexes can be used to deplete nutrients from water and can be used in sustainable waste water treatment plants. This will reduce the N-load in the environment and limit HABs caused by classes with expensive pigments such as cyanobacteria and dinoflagellates. Due to increasing temperatures and therefore increased stratification, increased rainfall and therefore increased N-deposit HABs might increase over the years. Therefore, the possibilities of limiting the N-load and limiting HABs caused by cyanobacteria and dinoflagellates are of great importance to investigate.

Further research

This report shows that the effects of light quantity, light quality and nitrogen demand on pigment composition often coincide in phytoplankton. This raises the question if these similar responses is an evolutionary adaptation because low nutrient waters are often clear waters with high blue light availability, or if the absorption of red and low light requires more nitrogen? To this day, little research has been done on the combined effects of light and nitrogen availibility. So far it is unkown if by combining these factors their impact on pigments composition is intensified or not. When conducting future experiments it is recommended to take the availibilty of different chemical forms of nitrogen into acount since different classes have different preferences. For example, future competition experiment can be conducted under different light environments with different chemical sources of nitrogen.

Appendix I The costs of pigment-protein complexes

Appendix IA

	g/mol	mol protein algae	g/mol protein	aandeel	
C	12	4,43	53,16	53,42670612	
H	1	7	7	7,035119316	
O	16	1,44	23,04	23,15559272	
N	14	1,16	16,24	16,32147681	
S	32	0,0019	0,0608	0,061105036	

Appendix IB

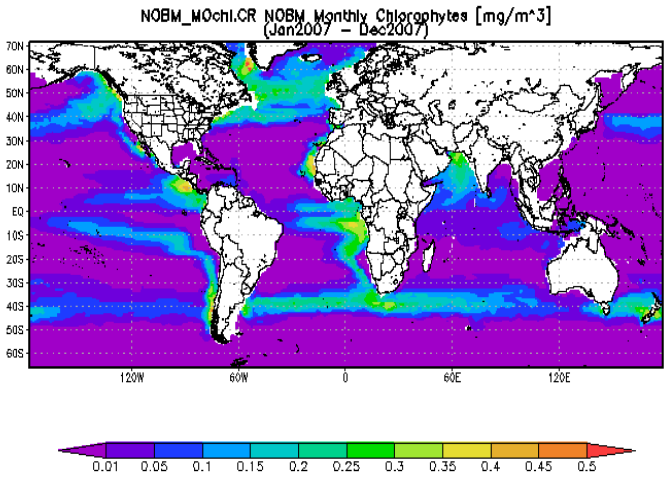
Pigment-protein complex	composition	molecular mass protein complex (g/mol)	kg proteins per mol	chromophores	kg protein per mol chromophore
peridinin-chlorophyll a-protein (PCP)	2 Chl *a* ; 6 peridinin	34688	34.688	8	4.336
fucoxanthin chlorophyll a/c2proteins (FCP	8 Chl *a* ; 8 Fuco; 2 Chl-c2	36000	36	18	2
violaxanthin–chlorophyll a	Chl *a* ; violaxanthin	22000	22	unkown	-
LHCaR1 (Rhodophyceae)	8 Chl *a* ; 4 Zea	21300	21.3	12	1.775
LHCaR1 (Bacillariophyceae)	7 Chl *a* ; 1 Chl *c* ; 1 Fuco; 2 diadin	21300	21.3	11	1.936363636
LHCsR3	6 Chl *a* ; 1 Chl *b* ; 3 β-car; 1 Viox; 2 Lut	25000	25	13	1.923076923
PcbA gene (prochlorococcus)	6 Chl *a* ; 2 pheophytin *a*	38511	38.511	8	4.813875
PcbB gene (prochlorococcus)	16 Chl *a*	40737	40.737	16	2.5460625
PcbC gene (prochlorococcus)	14 Chl *a* ; βCar	43000	43	15	2.866666667
PcbD gene(prochlorococcus)	6 Chl *a* ; 2 pheophytin *a* ; β-car	33000	33	9	3.666666667
R -Phycoerythrin (R-PE)	α 2 PEB; β 2 PEB; β PUB; γ 1 PUB; 3 γ PEB	260000	260	34	7.647058824
B-Phycoerythrin (B-PE)	α 2 PEB; β 3 PEB; γ 2 PEB; γ 2 PUB	260000	260	34	7.647058824
C-Phycoerythrin (C-PE)	α 2 PEB; β 3 PEB	230000	230	15	15.33
Phycoerythrocyanin (PEC)	α PVB; β 2 PCB	103000	103	9	11.44
R-Phycocyanin (RPC)	α PCB; β PCB; β PEB	130000	130	9	14.44444444
C-Phycocyanin (CPC)	α PCB; β 2PCB	120000	120	9	13.33333333
allophycocyanin (APC)	α PCB; β PCB	105000	105	6	17.5
FCP1-PSI	252 Chl *a* ; 23 Chl *c* ; 56 Fux; 34 Diax; 1Vio; 21 β-car; 2 MK4	1050000	1050	389	2.699228792
FCP1-PSI-core (minus)	205 Chl *a* ; 13 Chl *c* ; 30 Fux; 23 Diax; 1Vio; 20 β-car; 2 MK4	725000	725	294	2.465986395
FCP1-1	8 Chl *a* ; 2 Chl *c* ; 5 Fux; 2 Diax; 1 β-car		0	18	0
FCP1-2	8 Chl *a* ; 1 Chl *c* ; 3 Fux; 2 Diax		0	14	0
Cr-PE545	6 PEB; 2DBV	52240	52.24	8	6.53
Cr-PE555	6 PEB; 2DBV	44657	44.657	8	5.582125
Cr-PC612	6 PCB; 2DBV	40950	40.95	8	5.11875
Cr-PC645	4 PCB; 2 DBV; 2 MBV	53056	53.056	8	6.632
Cr-PC577	6 PCB; 2DBV	50000	50	8	6.25
Cr-PC612	6 PCB; 2DBV	59507	59.507	8	7.438375
peridinin-chlorophyll a-protein (PCP) II	2 Chl *a* ; 6 Per	34000	34	8	4.25
PSI + core Cyanobacteria	96 Chl *a* ; 22 β-car	257161	257.161	118	2.179330508
Starved C-PE	2 PEB	14645	14.645	2	7.3225
Diatom LHC	2 Chl *a* ; 1 Chl *c* ; 5 fux	17750	17.75	8	2.21875
C-PC	18 PCB	214128	214.128	18	11.896
Orange carotenoid protein (OCP)	2 Car	34622	34.622	2	17.311
Red carotenoid protein (RCP)	3'-hydroxyechinenone	16000	16	1	16

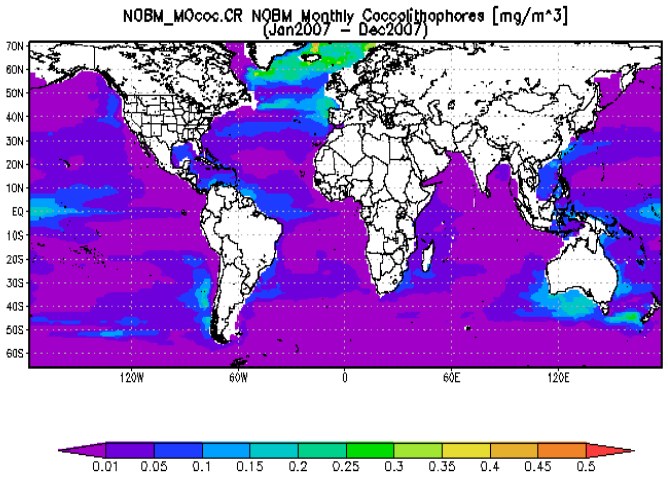
kg N per mol chromophore	g N per mol chromphore	molN per mol chromophore	Source organism	found in classes	ref	pdb reference
0.707699235	707.6992346	50.550	Amphidinium carterae	dinoflagellate	Haxo et al., (1976); Kamiya & Shen (2003)	2C9E
0.326429536	326.4295362	23.316	Cyclotella meneghiniana	diatom and cocco	Premvardhan et al., (2000)	
-	-	-				
0.289706213	289.7062134	20.693	Porphyridium cruentum	red algea	Grabowski et al., (2001)	
0.316043142	316.0431419	22.575	Thalassiosira fluviatilis	diatoms	Grabowski et al., (2001)	
0.313874554	313.8745541	22.420	Chlamydomonas reinhardtii	green and brown	Bonente et al., (2011)	
0.785695492	785.6954919	56.121	MIT 9313	Cyanobacteria	Bibby et al., (2013); Dekker & Boekema, (2005)	
0.415555001	415.5550006	29.683	MIT 9313	Cyanobacteria	Bibby et al., (2013); Dekker & Boekema, (2005);	Kamiya & She
0.467882335	467.8823353	33.420	MIT 9313	Cyanobacteria	Rocap et al., (2003); Kamiya & Shen, (2003)	TIGR01153
0.59845415	598.4541498	42.747	MIT 9313	Cyanobacteria	Bibby et al., (2013); Dekker & Boekema, (2005)	
1.248112933	1248.112933	89.151	Gracilaria chilensis	Cyanobacteria	Gantt (1981)	
1.248112933	1248.112933	89.151		Cyanobacteria	Gantt (1981)	
2.502626445	2502.626445	178.759		Cyanobacteria	Gantt (1981)	
1.867902346	1867.902346	133.422		Cyanobacteria	Gantt (1981)	
2.357546651	2357.546651	168.396		Cyanobacteria	Gantt (1981)	
2.176196908	2176.196908	155.443	Lyngbya Spp. (Marine) and Sp	Cyanobacteria	Satyanarayana et al. (2011)	
2.856258442	2856.258442	204.018		Cyanobacteria	Marx & Adir (2013)	
0.440554001	440.5540014	13.767	Chaetoceros gracilis	Diatoms	Ikeda et al., (2013)	
0.402485398	402.4853976	28.749	Chaetoceros gracilis	Diatoms	Ikeda et al., (2013)	
0	0	0.000	Chaetoceros gracilis	Diatoms	Ikeda et al., (2013)	
0	0	0.000	Chaetoceros gracilis	Diatoms	Ikeda et al., (2013)	
1.065792436	1065.792436	76.128	Chroomonas sp. CS24	cryptophyta	Doust et al., 2004	
0.911085238	911.0852375	65.078	Hemiselmis andersenii CCMP	cryptophyta	Harrop et al., (2014)	
0.835455594	835.4555943	59.675	Hemiselmis virescens M1635	cryptophyta	Harrop et al., (2014)	
1.082440342	1082.440342	77.317	Chroomonas sp. CCMP 270	cryptophyta	Harrop et al., (2014)	4LM6
1.020092301	1020.092301	72.864	Hemiselmis pacifica CCMP 70	cryptophyta	McClure et al., (2014); Overkamp et al,. (2014)	
1.214052651	1214.052651	86.718	Hemiselmis virescens M1635	cryptophyta	Harrop et al., (2014)	
0.693662765	693.6627645	49.547	Amphidinium carterae	dinoflagellate	Schulte et al., (2008); Sharples et al., (1996)	
0.355698924	355.6989236	25.407	Synechococcus elongatus	Cyanobacteria	Jordan et al., (2001)	
1.19514014	1195.14014	85.367	Phormidium Tenue	Cyanobacteria	Soni et al., (2010)	3MWN
0.362132767	362.1327668	25.867	Phaeodactylm ticornntam	Diatoms	Lepetit et al., (2007)	
1.941602882	1941.602882	138.686	Thermosynechococcus vulcan	Cyanobacteria	David et al., (2014)	
2.825410851	2825.410851	201.815	Synechocystis PCC 6803	Cyanobacteria	Wu & Krogmann (1997)	
2.61143629	2611.43629	186.531	Arthrospira maxima	Cyanobacteria	Chábera et al., (2011)	

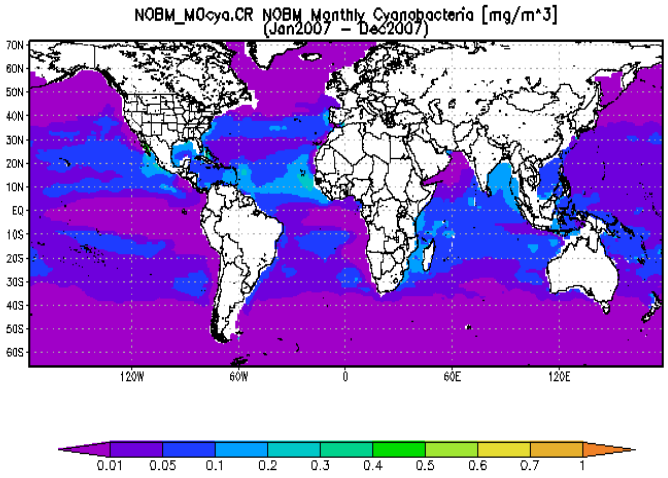
Appendix II The costs of photosynthetic pigment-protein complexes in terms of nitrogen as a function of the maximum absorption peak.

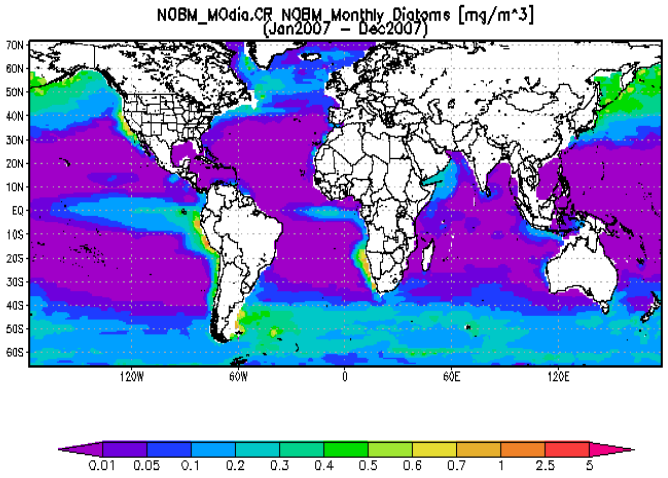
Pigment-protein complex	molN per mol chromophore	λ^{A}_{max}	Reference
peridinin-chlorophyll a-protein	50,55	463	Song et al., (1976)
fucoxanthin chlorophyll a/c2 complex (FCPa+b)	23,32	438	Premvardhan et al., (2010)
LHCaR1 (Rhodophyceae)	20,69	470	Grabowski et al., (2001)
LHCaR1 (Bacillariophyceae)	22,57	465	Grabowski et al., (2001)
LHCsR3	22,42	440	Bonente et al., (2011)
PEI	77,72	550	Six et al., (2010)
PEII	77,72	544	Six et al., (2010)
R -Phycoerythrin (R-PE)	89,15	565	Contreras-Martel et al., (2001)
B-Phycoerythrin (B-PE)	89,15	545	Camara-Artigas et al., (2012)
C-Phycoerythrin (C-PE)	178,76	562	Gantt (1981)
Phycoerythrocyanin (PEC)	133,42	568	Bryant et al., (1976)
R-Phycocyanin (RPC)	168,40	617	Wang et al., (2014)
C-Phycocyanin (CPC)	155,44	620	Six et al., (2010)
allophycocyanin (APC)	204,02	650	Gantt (1981)
FCP1-PSI	13,767	440	Ikeda et al., (2013)
FCP1-PSI-core (minus)	28,749	470	Ikeda et al., (2013)
Cr-PE545	76,128	545	Doust et al., (2004)
Cr-PE555	65,078	555	Harrop et al., (2014)
Cr-PC612	86,718	600	Harrop et al., (2014)
Cr-PC645	77,317	625	Harrop et al., (2014)
Cr-PC577	72,864	578	McClure et al., (2014)
peridinin-chlorophyll a-protein (PCP) II	49,547	480	Schulte et al., (2008)
peridinin-chlorophyll a/c-protein (PCP) II	25,407	440	Kennis et al., (2001)
Starved PE	85,367	557	Soni et al., (2010)
Diatom LHC	25,867	441	Lepetit et al., (2007)
C-PC	138,686	635	David et al., (2014)

Appendix III Distribution maps of the phytoplankton classes *Chlorophytes*, *Coccolithophores*, cyanobacteria and diatoms









Appendix IV The average lipid content in percentage dry weight per cell of cyanobacteria, diatoms, green algae dinoflagellates, coccolithophores, red algae and green algae

Phytoplankton Class	lipid content (%DW per cell)		Phytoplankton Class	lipid content (%DW per cell)	
	N-replete	N-deplete		N-replete	N-deplete
Cyanobacteria	5	13	**Green algae**	19	50
	7	10		24	42
	27	5		18	28
	7			21	26
	13			29	32
	11			31	33
	4	2		13	63
Diatoms	22	26		16	57
	51	25		18	23
	27	26		25	64
	33	24		36	18
	27	16		22	42
	24	28		23	14
	28	27		19	10
	26	34		15	18
	47	27		12	42
	40	35		14	35
	18	45		13	52
	21	51		9	30
	16	46		26	41
		40		21	46
	27	32		18	
	18	28		21	
dinoflagellates	15			17	
	25			16	
	20			22	
coccolithophores	20	14		12	
	25	29		12	30
	7			14	57
	36			13	44
	31			9	43
	30			9	13
	7	15		48,7	
	11	14		50,3	
red algae	11			38,7	

Reference

Abe, D., & Gianesella-Galvão, S. (1991). Pigment chromatic adaptation in Cyclotella caspia Grunow (*Bacillariophyta*). *Boletim do Instituto Oceanográfico*,*39*(2). doi:10.1590/S0373-55241991000200003

Abell, J. M., Özkundakci, D., & Hamilton, D. P. (2010). Nitrogen and phosphorus limitation of phytoplankton growth in New Zealand lakes: implications for eutrophication control.*Ecosystems*, *13*(7), 966-977.

Acker J. G. and Leptoukh, G. (2007) "Online Analysis Enhances Use of NASA Earth Science Data", Eos, Trans. AGU, Vol. 88, No. 2 (9 January 2007), pages 14 and 17.

Adams, D. G. (2000). Heterocyst formation in cyanobacteria.*Current opinion in microbiology*, *3*(6), 618-624.

Adeloye, A., & Ajibade, P. (2011). A high molar extinction coefficient mono-anthracenyl bipyridyl heteroleptic ruthenium(II) complex: synthesis, photophysical and electrochemical properties. *Molecules (Basel, Switzerland)*,*16*(6), 4615–31. doi:10.3390/molecules16064615

Aguilera, J., Francisco, J., Gordillo, L., Karsten, U., Figueroa, F. L., & Niell, F. X. (2000). Light quality effect on photosynthesis and efficiency of carbon assimilation in the red alga *Porphyra leucosticta*. *Journal of plant physiology*, *157*(1), 86-92.

Agustí, S. (2004). Viability and niche segregation of *Prochlorococcus* and *Synechococcus* cells across the Central Atlantic Ocean. *Aquatic microbial ecology*, *36*(1), 53-59.

Aidar, E., Gianesella-Galvão, S. M. F., Sigaud, T. C. S., Asano, C. S., Liang, T. H., Rezende, K. R. V., ... & Sandes, M. A. L. (1994). Effects of light quality on growth, biochemical composition and photo synthetic production in *Cyclotella caspia* Grunow and *Tetraselmis gracilis* (Kylin) Butcher. *Journal of experimental marine biology and ecology*, *180*(2), 175-187.

Akimoto, S., Yokono, M., Hamada, F., Teshigahara, A., Aikawa, S., & Kondo, A. (2012). Adaptation of light-harvesting systems of *Arthrospira platensis* to light conditions, probed by time-resolved fluorescence spectroscopy. *Biochimica et biophysica acta*, *1817*(8), 1483–9. doi:10.1016/j.bbabio.2012.01.006

Allen, A. E., Dupont, C. L., Oborník, M., Horák, A., Nunes-Nesi, A., McCrow, J. P., ... & Bowler, C. (2011). Evolution and metabolic significance of the urea cycle in photosynthetic diatoms. *Nature*, *473*(7346), 203-207

Amengual-Morro, C., Moyà Niell, G., & Martínez-Taberner, A. (2012). Phytoplankton as bioindicator for waste stabilization ponds.*Journal of environmental management*, *95*, S71-S76.

Amengual-Morro, C., Moyà Niell, G., & Martínez-Taberner, A. (2012). Phytoplankton as bioindicator for waste stabilization ponds.*Journal of environmental management*, *95*, S71-S76.

Anderson, D. M., Glibert, P. M., & Burkholder, J. M. (2002). Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries*, *25*(4), 704-726.

Andersson, M., Schubert, H., Pedersén, M., & Snoeijs, P. (2006). Different patterns of carotenoid composition and photosynthesis acclimation in two tropical red algae. *Marine Biology*, *149*(3), 653-665.

Andrizhiyevskaya, E. G., Chojnicka, A., Bautista, J. A., Diner, B. A., van Grondelle, R., & Dekker, J. P. (2005). Origin of the F685 and F695 fluorescence in photosystem II. *Photosynthesis research*,*84*(1-3), 173-180.

Anning, T., MacIntyre, H., Pratt, S., Sammes, P., Gibb, S., & Geider, R. (2000). Photoacclimation in the marine diatom *Skeletonema costatum*. *Limnology and Oceanography*, *45*(8), 18071817. doi:10.4319/lo.2000.45.8.1807

Ansotegui, A., Trigueros, J. M., & Orive, E. (2001). The use of pigment signatures to assess phytoplankton assemblage structure in estuarine waters. *Estuarine, Coastal and Shelf Science*, *52*(6), 689-703.

Anwer, K., Parmar, A., Rahman, S., Kaushal, A., Madamwar, D., Islam, A., ... & Ahmad, F. (2014). Folding and stability studies on C-PE and its natural N-terminal truncant. *Archives of biochemistry and biophysics*, *545*, 9-21.

Anwer, K., Sonani, R., Madamwar, D., Singh, P., Khan, F., Bisetty, K., ... & Hassan, M. I. (2015). Role of N-terminal residues on folding and stability of C-phycoerythrin: simulation and urea-induced denaturation studies. *Journal of Biomolecular Structure and Dynamics*, *33*(1), 121-133.

Aourahoun, K. A. K., Fazouane, F., Benayad, T., Bettache, Z., & Denni, N. (2014). The synthetic antioxidant Butylated Hydroxytoluene, a naturally occurring constituent of the broom *Cytisus triflorus* L'Hérit. *Journal of Natural Products*, *7*.

Aráoz, R., & Häder, D.-P. (1999). Phycoerythrin synthesis is induced by solar UV-B in the cyanobacterium Nostoc. *Plant Physiology and Biochemistry*, *37*(3), 223229. doi:10.1016/S0981-9428(99)80037-0

Armbrecht, L. H., Smetacek, V., Assmy, P., & Klaas, C. (2014). Cell death and aggregate formation in the giant diatom *Coscinodiscus wailesii* (Gran & Angst, 1931). *Journal of Experimental Marine Biology and Ecology*, *452*, 31-39.

Aro, E. M., Kettunen, R., & Tyystjärvi, E. (1992). ATP and light regulate D1 protein modification and degradation Role of D1* in photoinhibition. *FEBS letters*, *297*(1), 29-33.

Baden, D. G., & Mende, T. J. (1982). Toxicity of two toxins from the Florida red tide marine dinoflagellate, *Ptychodiscus brevis*. *Toxicon*, *20*(2), 457-461.

Banaszak, T., LaJeunesse, T. C., & Trench, R. K. (2000). The synthesis of mycosporine-like amino acids (MAAs) by cultured, symbiotic dinoflagellates. *Journal of experimental marine biology and ecology*, *249*(2), 219-233.

Bar, E., Rise, M., Vishkautsan, M., & Arad, S. M. (1995). Pigment and Structural Changes in *Chlorella zofingiensis* upon Light and Nitrogen Stress. *Journal of plant physiology*, *146*(4), 527-534.

Baron, J. S., Hall, E. K., Nolan, B. T., Finlay, J. C., Bernhardt, E. S., Harrison, J. A., ... & Boyer, E. W. (2013). The interactive effects of excess reactive nitrogen and climate change on aquatic ecosystems and water resources of the United States. *Biogeochemistry*, *114*(1-3), 71-92.

Barrett, P. R. F., Curnow, J. C., & Littlejohn, J. W. (1996). The control of diatom and cyanobacterial blooms in reservoirs using barley straw. In *Management and Ecology of Freshwater Plants*(pp. 307-311). Springer Netherlands.

Bautista, A., & Necchi-Júnior, O. (2008). Photoacclimation in a tropical population of *Cladophora glomerata* (L.) Kützing 1843 (*Chlorophyta*) from southeastern Brazil. *Brazilian Journal of Biology*, *68*(1), 129136. doi:10.1590/S1519-69842008000100018

Beale, S. I. (1994). Biosynthesis of Open-Chain Tetrapyrroles in Plants, Algae, and Cyanobacteria. In *Ciba Foundation Symposium 180-The Biosynthesis of the Tetrapyrrole Pigments* (pp. 156-176). John Wiley & Sons, Ltd.

Beaujuge, P. M., Amb, C. M., & Reynolds, J. R. (2010). Spectral engineering in π-conjugated polymers with intramolecular donor− acceptor interactions. *Accounts of chemical research*, *43*(11), 1396-1407.

Ben Amotz, A., Katz, A., & Avron, M. (1982). Accumulation of beta-carotene in halotolerant algae: purification and characterization of beta-carotene-rich globules from *Dunaliella bardawil* (*Chlorophyceae*)[Algae]. *Journal of Phycology*.

Berdalet, E., Latasa, M., & Estrada, M. (1992). Variations in biochemical parameters of *Heterocapsa* sp. and *Olisthodiscus luteus* grown in 12: 12 light: dark cycles. In *The Daily Growth Cycle of Phytoplankton* (pp. 139-147). Springer Netherlands.

Berg, G., Balode, M., Purina, I., Bekere, S., Béchemin, C., & Maestrini, S. (2003). Plankton community composition in relation to availability and uptake of oxidized and reduced nitrogen. *Aquatic Microbial Ecology*, *30*, 263–274. doi:10.3354/ame030263

Berges, J. A., Charlebois, D. O., Mauzerall, D. C., & Falkowski, P. G. (1996). Differential effects of nitrogen limitation on photosynthetic efficiency of photosystems I and II in microalgae. *Plant Physiology*, *110*(2), 689-696.

Berman, T., & Bronk, D. A. (2003). Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. *Aquatic microbial ecology*, *31*(3), 279-305.

Beusen, A. H. W., Slomp, C. P., & Bouwman, A. F. (2013). Global land–ocean linkage: direct inputs of nitrogen to coastal waters via submarine groundwater discharge. *Environmental Research Letters*, *8*(3), 034035.

Bharadwaja, Y. (1934). The taxonomy of *Scytonema* and *Tolypothrix*, including some new records and some new species from India and Ceylon. *Revue Algologique* 7: 149-178.

Bibby, T. S., Mary, I., Nield, J., Partensky, F., & Barber, J. (2003). Low-light-adapted *Prochlorococcus* species possess specific antennae for each photosystem. Nature, 424(6952), 1051-1054.

Binder, B. J., & Chisholm, S. W. (1995). Cell cycle regulation in marine *Synechococcus* sp. strains. *Applied and environmental microbiology*, *61*(2), 708-717.

Blankenship, R., & Chen, M. (2013). Spectral expansion and antenna reduction can enhance photosynthesis for energy production. *Current opinion in chemical biology*, *17*(3), 457–61. doi:10.1016/j.cbpa.2013.03.031

Bleiker, W., & Schanz, F. (1997). Light climate as the key factor controlling the spring dynamics of phytoplankton in Lake Zürich. *Aquatic sciences*, *59*(2), 135-157.

Bockstahler, K. R., & Coats, D. W. (1993). Grazing of the mixotrophic dinoflagellate *Gymnodinium sanguineum* on ciliate populations of Chesapeake Bay. *Marine Biology*, *116*(3), 477-487.

Bogorad, L. (1975). Phycobiliproteins and complementary chromatic adaptation. *Annual review of plant physiology*, 26(1), 369-401.

Bonente, G., Ballottari, M., Truong, T. B., Morosinotto, T., Ahn, T. K., Fleming, G. R., ... & Bassi, R. (2011). Analysis of LhcSR3, a protein essential for feedback de-excitation in the green alga *Chlamydomonas reinhardtii*. *PLoS biology*, 9(1), e1000577.

Borowitzka, M. A., Huisman, J. M., & Osborn, A. (1991). Culture of the astaxanthin-producing green alga *Haematococcus pluvialis*. Effects of nutrients on growth and cell type. *Journal of Applied Phycology*, *3*(4), 295-304.

Bown, P. R., Lees, J. A., & Young, J. R. (2004). Calcareous nannoplankton evolution and diversity through time. In*Coccolithophores* (pp. 481-508). Springer Berlin Heidelberg.

Bracher, A., Vountas, M., Dinter, T., Burrows, J. P., Röttgers, R., & Peeken, I. (2009). Quantitative observation of cyanobacteria and diatoms from space using PhytoDOAS on SCIAMACHY data.*Biogeosciences*, *6*, 751-764.

Brand, L. E., & Compton, A. (2007). Long-term increase in Karenia brevis abundance along the Southwest Florida Coast. *Harmful Algae*, *6*, 232-252.

Brauer, V. S., Stomp, M., & Huisman, J. (2012). The nutrient-load hypothesis: patterns of resource limitation and community structure driven by competition for nutrients and light. *The American Naturalist*, *179*(6), 721-740.

Britton, G., Liaaen-Jensen, S., & Pfander, H. (Eds.). (2004). *Carotenoids: handbook*. Springer.

Broadwater, S. T., & Scott, J. L. (1994). Ultrastructure of unicellular red algae. In Evolutionary pathways and enigmatic algae: *Cyanidium caldarium (Rhodophyta)* and related cells (pp. 215-230). Springer Netherlands.

Brody, M., & Emerson, R. (1959). The Effect of Wavelength Intensity of Light on the Proportion of Pigments in *Porphyridium cruentum*. *American Journal of Botany*, 433-440.

Brotas, V., Brewin, R. J., Sá, C., Brito, A. C., Silva, A., Mendes, C. R., ... & Sathyendranath, S. (2013). Deriving phytoplankton size classes from satellite data: Validation along a trophic gradient in the eastern Atlantic Ocean. *Remote Sensing of Environment*,*134*, 66-77.

Brunet, C., & Lavaud, J. (2010). Can the xanthophyll cycle help extract the essence of the microalgal functional response to a variable light environment?. *Journal of plankton research*,*32*(12), 1609-1617.

Bryant, D. A., Glazer, A. N., & Eiserling, F. A. (1976). Characterization and structural properties of the major biliproteins of *Anabaena* sp. Archives of microbiology, 110(1), 61-75.

Burton, G. W., Daroszewski, J., Nickerson, J. G., Johnston, J. B., Mogg, T. J., & Nikiforov, G. B. (2014). β-Carotene autoxidation: oxygen copolymerization, non-vitamin A products, and immunological activity. *Canadian Journal of Chemistry*, *92*(4), 305-316.

Cadoret, J. C., Demoulière, R., Lavaud, J., van Gorkom, H. J., Houmard, J., & Etienne, A. L. (2004). Dissipation of excess energy triggered by blue light in cyanobacteria with CP43'(*isiA*). *Biochimica et Biophysica Acta (BBA)-Bioenergetics*,*1659*(1), 100-104.

Cai, T., Park, S. Y., & Li, Y. (2013). Nutrient recovery from wastewater streams by microalgae: status and prospects.*Renewable and Sustainable Energy Reviews*, *19*, 360-369.

Calbet, A., Sazhin, A. F., Nejstgaard, J. C., Berger, S. A., Tait, Z. S., Olmos, L., ... & Jakobsen, H. H. (2014). Future Climate Scenarios for a Coastal Productive Planktonic Food Web Resulting in Microplankton Phenology Changes and Decreased Trophic Transfer Efficiency. *PloS one*, *9*(4), e94388.

Caljon, A. G. (1987). A recently landlocked brackish-water lagoon of Lake Tanganyika: physical and chemical characteristics, and spatio-temporal distribution of phytoplankton. *Hydrobiologia*,*153*(1), 55-70.

Camacho, A. (2006). On the occurrence and ecological features of deep chlorophyll maxima (DCM) in Spanish stratified lakes.*Limnetica*, *25*(1-2), 453-478.

Camara-Artigas A, Bacarizo J, Andujar-Sanchez M, Ortiz-Salmeron E,... Allen JP. (2012) Ph-dependent structural, conformations of b-phycoerythrin from porphyridium cruentum. Febs J. 279 p.3680

Campbell, D., Eriksson, M. J., Öquist, G., Gustafsson, P., & Clarke, A. K. (1998). The cyanobacterium *Synechococcus* resists UV-B by exchanging photosystem II reaction-center D1 proteins. *Proceedings of the National Academy of Sciences*, *95*(1), 364-369.

Campbell, E. L., & Meeks, J. C. (1989). Characteristics of hormogonia formation by symbiotic *Nostoc* spp. in response to the presence of *Anthoceros punctatus* or its extracellular products.*Applied and environmental microbiology*, *55*(1), 125-131.

Campbell, L., & Vaulot, D. (1993). Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (station ALOHA). *Deep Sea Research Part I: Oceanographic Research Papers*, *40*(10), 2043-2060.

Carbonera, D., Agostini, A., Di Valentin, M., Gerotto, C., Basso, S., Giacometti, G. M., & Morosinotto, T. (2014). Photoprotective sites in the violaxanthin–chlorophyllabinding Protein (VCP) from *Nannochloropsis gaditana*. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1837(8), 1235-1246.

Carmona, R., Kraemer, G., & Yarish, C. (2006). Exploring Northeast American and Asian species of *Porphyra* for use in an integrated finfish–algal aquaculture system. *Aquaculture*, *252*(1), 5465. doi:10.1016/j.aquaculture.2005.11.049

Carpenter, E. J., & Foster, R. A. (2002). Marine cyanobacterial symbioses. In *Cyanobacteria in symbiosis* (pp. 11-17). Springer Netherlands.

Carpenter, E. J., & Price, C. C. (1977). Nitrogen fixation, distribution, and production of *Oscillatoria* (*Trichodesmium*) spp. in the western Sargasso and Caribbean Seas1. *Limnology and Oceanography*, *22*(1), 60-72.

Carr, N. G., & Whitton, B. A. (Eds.). (1982). *The biology of cyanobacteria* (Vol. 19). Univ of California Press.

Carreto, J. I., & Carignan, M. O. (2011). Mycosporine-like amino acids: relevant secondary metabolites. Chemical and ecological aspects. *Marine drugs*, *9*(3), 387-446.

Catalan, J., Camarero, L., Felip, M., Pla, S., Ventura, M., Buchaca, T., ... & de Quijano, D. D. (2006). High mountain lakes: extreme habitats and witnesses of environmental changes. *Limnetica*,*25*(1-2), 551-584.

Chábera, P., Durchan, M., Shih, P. M., Kerfeld, C. A., & Polívka, T. (2011). Excited-state properties of the 16kDa red carotenoid protein from *Arthrospira maxima*. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1807*(1), 30-35.

Chaffin, J. D., & Bridgeman, T. B. (2014). Organic and inorganic nitrogen utilization by nitrogen-stressed cyanobacteria during bloom conditions. *Journal of Applied Phycology*, *26*(1), 299-309.

Chainapong, T., Traichaiyaporn, S., & Deming, R. L. (2012). Effect of light quality on biomass and pigment production in photoautotrophic and mixotrophic cultures of *Spirulina platensis*. *Journal of Agricultural Technology*, *8*(5), 1593-1604.

Chang, W. R., Jiang, T., Wan, Z. L., Zhang, J. P., Yang, Z. X., & Liang, D. C. (1996). Crystal Structure of R-phycoerythrin from *Polysiphonia urceolata* at 2.8 Å Resolution. *Journal of molecular biology*, *262*(5), 721-722.

Chang, Y., Wu, Z., Bian, L., Feng, D., & Leung, D. Y. (2013). Cultivation of *Spirulina platensis* for biomass production and nutrient removal from synthetic human urine. *Applied Energy*, *102*, 427-431.

Charlson, R. J., Lovelock, J. E., Andreae, M. O., & Warren, S. G. (1987). Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. *Nature*, 326(6114), 655-661.

Chen, H.-B., Wu, J.-Y., Wang, C.-F., Fu, C.-C., Shieh, C.-J., Chen, C.-I., … Liu, Y.-C. (2010). Modeling on chlorophyll a and phycocyanin production by Spirulina platensis under various light-emitting diodes. *Biochemical Engineering Journal*,*53*(1), 5256. doi:10.1016/j.bej.2010.09.004

Cho, S., Luong, T. T., Lee, D., Oh, Y. K., & Lee, T. (2011). Reuse of effluent water from a municipal wastewater treatment plant in microalgae cultivation for biofuel production. *Bioresource technology*, *102*(18), 8639-8645.

Clarke, A. K., Soitamo, A., Gustafsson, P., & Oquist, G. (1993). Rapid interchange between two distinct forms of cyanobacterial photosystem II reaction-center protein D1 in response to photoinhibition. *Proceedings of the National Academy of Sciences*, *90*(21), 9973-9977.

Cohen-Bazire, G., Beguin, S., Rimon, S., Glazer, A. N., & Brown, D. M. (1977). Physico-chemical and immunological properties of allophycocyanins. *Archives of microbiology*, 111(3), 225-238.

Colijn, F., & Cadée, G. C. (2003). Is phytoplankton growth in the Wadden Sea light or nitrogen limited?. *Journal of Sea Research*,*49*(2), 83-93.

Colyer, C. L., Kinkade, C. S., Viskari, P. J., & Landers, J. P. (2005). Analysis of cyanobacterial pigments and proteins by electrophoretic and chromatographic methods. *Analytical and bioanalytical chemistry*, *382*(3), 559-569.

Conley, D. J., Paerl, H. W., Howarth, R. W., Boesch, D. F., Seitzinger, S. P., Havens, K. E., ... & Likens, G. E. (2009). Controlling eutrophication: nitrogen and phosphorus. *Science*,*323*(5917), 1014-1015.

Contreras-Martel, C., Martinez-Oyanedel, J., Bunster, M., Legrand, P., Piras, C., Vernede, X., & Fontecilla-Camps, J. C. (2001). Crystallization and 2.2 A resolution structure of R-phycoerythrin from Gracilaria chilensis: a case of perfect hemihedral twinning. *Acta Crystallographica Section D: Biological Crystallography*,57(1), 52-60.

Cordeiro, T. A., Brandini, F. P., Rosa, R. S., & Sassi, R. (2013). Deep Chlorophyll Maximum in Western Equatorial Atlantic-How does it Interact with Islands Slopes and Seamounts?. *Marine Science*, *3*(1), 30-37.

Costa, B. S., Jungandreas, A., Jakob, T., Weisheit, W., Mittag, M., & Wilhelm, C. (2013). Blue light is essential for high light acclimation and photoprotection in the diatom *Phaeodactylum tricornutum*. *Journal of experimental botany*, *64*(2), 483-493.

Costas, E., Baselga-Cervera, B., García-Balboa, C., & López-Rodas, V. (2014). Estimating the genetic capability of different phytoplankton organisms to adapt to climate warning. *Environmental Science group, Oceanography Open Access*.

Croce, R., & van Amerongen, H. (2014). Natural strategies for photosynthetic light harvesting. *Nature chemical biology*, *10*(7), 492-501.

Croome, R. L., & Tyler, P. A. (1985). Distribution of silica-scaled *Chrysophyceae* (*Paraphysomonadaceae* and *Mallomonadaceae*) in Australian inland waters. *Marine and Freshwater Research*, *36*(6), 839-853.

Cruz, F., Valenzuela-Espinoza, E., Millán-Núñez, R., Trees, C., Santamaría-del-Ángel, E., & Núñez-Cebrero, F. (2006). Nutrient uptake, chlorophyll a and carbon fixation by *Rhodomonas* sp. (Cryptophyceae) cultured at different irradiance and nutrient concentrations. *Aquacultural Engineering*, *35*(1), 5160. doi:10.1016/j.aquaeng.2005.08.004

Cruz, S., & Serôdio, J. (2008). Relationship of rapid light curves of variable fluorescence to photoacclimation and non-photochemical quenching in a benthic diatom. *Aquatic Botany*,*88*(3), 256264. doi:10.1016/j.aquabot.2007.11.00

Cullen, J. J., & Horrigan, S. G. (1981). Effects of nitrate on the diurnal vertical migration, carbon to nitrogen ratio, and the photosynthetic capacity of the dinoflagellate *Gymnodinium splendens*. *Marine biology*, *62*(2-3), 81-89.

D'Agnolo, E., Rizzo, R., Paoletti, S., & Murano, E. (1994). R-phycoerythrin from the red alga *Gracilaria longa*. *Phytochemistry*, 35(3), 693-696.

Dandonneau, Y., Deschamps, P.-Y., Nicolas, J.-M., Loisel, H., Blanchot, J., Montel, Y., … Bécu, G. (2004). Seasonal and interannual variability of ocean color and composition of phytoplankton communities in the North Atlantic, equatorial Pacific and South Pacific. *Deep Sea Research Part II: Topical Studies in Oceanography*, *51*(1-3), 303318. doi:10.1016/j.dsr2.2003.07.018

David, L., Prado, M., Arteni, A. A., Elmlund, D. A., Blankenship, R. E., & Adir, N. (2014). Structural studies show energy transfer within stabilized phycobilisomes independent of the mode of rod–core assembly. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1837(3), 385-395.

Davies, F. K., Work, V. H., Beliaev, A. S., & Posewitz, M. C. (2014). Engineering limonene and bisabolene production in wild type and a glycogen-deficient mutant of *Synechococcus* sp. PCC 7002.*Frontiers in bioengineering and biotechnology*, *2*.

Davies-Colley, R. J., & Vant, W. N. (1987). Absorption of light by yellow substance in freshwater lakes. *Limnol. Oceanogr*, *32*(2), 416-425.

Davis, T. W., Berry, D. L., Boyer, G. L., & Gobler, C. J. (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*(5), 715-725.

De Buisonjé, F. E., & Aarnink, A. J. A. (2011). Algenkweek op stallucht. *Journal of Microbiology and Biotechnology*, *20*(3), 609-614.

de Domitrovic, Y. Z., Devercelli, M., & Forastier, M. E. (2014). Phytoplankton of the Paraguay and Bermejo rivers. *Advances in Limnology*, 67-80.

De Marsac, N. T. (1977). Occurrence and nature of chromatic adaptation in cyanobacteria. *Journal of Bacteriology*, *130*(1), 82-91.

De Marsac, N. T. (1994). Differentiation of hormogonia and relationships with other biological processes. In *The molecular biology of cyanobacteria* (pp. 825-842). Springer Netherlands.

De Marsac, N. T. (2003). Phycobiliproteins and phycobilisomes: the early observations. *Photosynthesis research*, *76*(1-3), 193-205.

De Marsac, N. T., & Houmard, J. (1988). [34] Complementary chromatic adaptation: Physiological conditions and action spectra. *Methods in enzymology*, 167, 318-328.

de Paula, J. C., Robblee, J. H., & Pasternack, R. F. (1995). Aggregation of chlorophyll a probed by resonance light scattering spectroscopy. *Biophysical journal*, 68(1), 335.

De Tezanos Pinto, P., & Litchman, E. (2010). Interactive effects of N: P ratios and light on nitrogen-fixer abundance. *Oikos*,*119*(3), 567-575.

Dekker, J. P., & Boekema, E. J. (2005). Supramolecular organization of thylakoid membrane proteins in green plants. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1706(1), 12-39.

DeMaster, D. J., Leynaert, A., & Queguiner, B. (1995). The silica balance in the world ocean: a reestimate. *Science*, *268*(5209), 375-379.

Deng, J., Qin, B., Paerl, H. W., Zhang, Y., Ma, J., & Chen, Y. (2014). Earlier and warmer springs increase cyanobacterial (Microcystis spp.) blooms in subtropical Lake Taihu, China. *Freshwater Biology*, *59*(5), 1076-1085.

Deruère, J., Römer, S., d'Harlingue, A., Backhaus, R. A., Kuntz, M., & Camara, B. (1994). Fibril assembly and carotenoid overaccumulation in chromoplasts: a model for supramolecular lipoprotein structures. *The Plant Cell Online*, *6*(1), 119-133.

Devred, E., Turpie, K. R., Moses, W., Klemas, V. V., Moisan, T., Babin, M., ... & Jo, Y. H. (2013). Future retrievals of water column bio-optical properties using the Hyperspectral Infrared Imager (HyspIRI). *Remote Sensing*, *5*(12), 6812-6837.

DeYoe, H. R., Lowe, R. L., & Marks, J. C. (1992). Effects of nitrogen and phosphorus on the endosymbiont load of *Rhopalodia gibba* and *Epithemia turgida* (Bacillariophyceae) 1.*Journal of Phycology*, *28*(6), 773-777.

Di Mascio, P., Devasagayam, T. P., Kaiser, S., & Sies, H. (1990). Carotenoids, tocopherols and thiols as biological singlet molecular oxygen quenchers. *Biochemical Society Transactions*, *18*(6), 1054-1056.

Dierssen, H. M., Kudela, R. M., Ryan, J. P., & Zimmerman, R. C. (2006). Red and black tides: Quantitative analysis of water-leaving radiance and perceived color for phytoplankton, colored dissolved organic matter, and suspended sediments. *Limnology and Oceanography*, 51(6), 2646-2659.

Dignum M., Hoogveld H. L., Gons H.J., Laanbroek H. J. (2004). *Flowcytometrische bepaling van de fytoplankton voedingsstatus in Nederlandse meren.* (ISBN. 90.5773.237.8). (2004). Utrecht: Kruyt Grafisch Advies Bureau.

Diversé-Pierluissi, M., & Krogmann, D. W. (1988). A zeaxanthin protein from< i> Anacystis nidulans</i>. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 933(2), 372-377.

Dodge, J. D. (1972). The ultrastructure of the dinoflagellate pusule: a unique osmo-regulatory organelle. *Protoplasma*, *75*(3), 285-302.

Domingues, N., Matos, A., Silva, J., & Cartaxana, P. (2012). Response of the diatom *Phaeodactylum tricornutum* to photooxidative stress resulting from high light exposure. *PloS one*, *7*(6), e38162. doi:10.1371/journal.pone.0038162

Domingues, R. B., Anselmo, T. P., Barbosa, A. B., Sommer, U., & Galvão, H. M. (2011a). Nutrient limitation of phytoplankton growth in the freshwater tidal zone of a turbid, Mediterranean estuary.*Estuarine, Coastal and Shelf Science*, *91*(2), 282-297.

Domingues, R. B., Anselmo, T. P., Barbosa, A. B., Sommer, U., & Galvão, H. M. (2011b). Light as a driver of phytoplankton growth and production in the freshwater tidal zone of a turbid estuary.*Estuarine, Coastal and Shelf Science*, *91*(4), 526-535.

Doust, A. B., Marai, C. N., Harrop, S. J., Wilk, K. E., Curmi, P. M., & Scholes, G. D. (2004). Developing a structure–function model for the cryptophyte phycoerythrin 545 using ultrahigh resolution crystallography and ultrafast laser spectroscopy. Journal of molecular biology, 344(1), 135-153.

Downing, J., Watson, S., & McCauley, E. (2001). Predicting Cyanobacteria dominance in lakes. *Canadian Journal of Fisheries and Aquatic Sciences*,*58*(10), 19051908. doi:10.1139/cjfas-58-10-1905

Dubinsky, Z., & Stambler, N. (2009). Photoacclimation processes in phytoplankton: mechanisms, consequences, and applications.Aquat*. Microb. Ecol*, 56, 163-176.

Ducklow, H. W., Hansell, D. A., & Morgan, J. A. (2007). Dissolved organic carbon and nitrogen in the Western Black Sea. *Marine chemistry*, *105*(1), 140-150.

Dufresne, A., Salanoubat, M., Partensky, F., Artiguenave, F., Axmann, I. M., Barbe, V., ... & Hess, W. R. (2003). Genome sequence of the cyanobacterium *Prochlorococcus* marinus SS120, a nearly minimal oxyphototrophic genome. *Proceedings of the National Academy of Sciences*, *100*(17), 10020-10025.

Elser, J. J., Marzolf, E. R., & Goldman, C. R. (1990). Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. *Canadian Journal of fisheries and aquatic sciences*, *47*(7), 1468-1477.

Everroad, C., Six, C., Partensky, F., Thomas, J. C., Holtzendorff, J., & Wood, A. M. (2006). Biochemical bases of type IV chromatic adaptation in marine *Synechococcus* spp. *Journal of bacteriology*, *188*(9), 3345-3356.

Everroad, R. C., & Wood, A. M. (2012). Phycoerythrin evolution and diversification of spectral phenotype in marine *Synechococcus* and related picocyanobacteria. *Molecular phylogenetics and evolution*, *64*(3), 381-392.

Falconer, I. R., & Humpage, A. R. (2005). Health risk assessment of cyanobacterial (blue-green algal) toxins in drinking water. *International Journal of Environmental Research and Public Health*, *2*(1), 43-50.

Falkowski, P. (2012). Ocean science: the power of plankton. *Nature*, 483(7387), S17-S20.

Falkowski, P. G. (1984). Physiological responses of phytoplankton to natural light regimes. *Journal of Plankton Research*, *6*(2), 295-307.

Falkowski, P. G., & Raven, J. A. (2013). *Aquatic photosynthesis*. (page 16) Princeton University Press.

Falkowski, P. G., Greene, R. M., & Geider, R. J. (1992). Physiological limitations on phytoplankton productivity in the ocean. *Oceanography*, *5*(2), 84-91.

Falkowski, P. G., Katz, M. E., Knoll, A. H., Quigg, A., Raven, J. A., Schofield, O., & Taylor, F. J. R. (2004). The evolution of modern eukaryotic phytoplankton. *science*, *305*(5682), 354-360.

Falkowski, P., & Owens, T. (1980). Light-Shade Adaptation : Two strategies in marine phytoplankton. *Plant physiology, 66*(4), 592595. doi:10.1104/pp.66.4.592

Fay, P. (1992). Oxygen relations of nitrogen fixation in cyanobacteria. *Microbiological Reviews*, *56*(2), 340.

Feki, W., Hamza, A., Frossard, V., Abdennadher, M., Hannachi, I., Jacquot, M., ... & Aleya, L. (2013). What are the potential drivers of blooms of the toxic dinoflagellate *Karenia selliformis*? A 10-year study in the Gulf of Gabes, Tunisia, southwestern Mediterranean Sea. *Harmful Algae*, *23*, 8-18.

Felip, M., Sattler, B., Psenner, R., & Catalan, J. (1995). Highly active microbial communities in the ice and snow cover of high mountain lakes. *Applied and environmental microbiology*, *61*(6), 2394-2401.

Ferber, L., Levine, S., Lini, A., & Livingston, G. (2004). Do cyanobacteria dominate in eutrophic lakes because they fix atmospheric nitrogen?*Freshwater Biology*, *49*(6), 690708. doi:10.1111/j.1365-2427.2004.01218.x

Fetisova, Z. G., Freiberg, A. M., & Timpmann, K. E. (1988). Long-range molecular order as an efficient strategy for light harvesting in photosynthesis. *Nature*, *334*(6183), 633-634.

Ficek, D., Kaczmarek, S., Ston-Egiert, J., Wozniak, B., Majchrowski, R., & Dera, J. (2004). Spectra of light absorption by phytoplankton pigments in the Baltic; conclusions to be drawn from a Gaussian analysis of empirical data. *Oceanologia*, 46(4).

Figueroa, F. L., Aguilera, J., & Niell, F. X. (1995). Red and blue light regulation of growth and photosynthetic metabolism in *Porphyra umbilicalis* (Bangiales, *Rhodophyta*). *European Journal of Phycology*, *30*(1), 11-18.

Figueroa, F., Israel, A., Neori, A., Martínez, B., Malta, E., Put, A., … Korbee, N. (2010). Effect of nutrient supply on photosynthesis and pigmentation to short-term stress (UV radiation) in *Gracilaria conferta* (Rhodophyta). *Marine pollution bulletin*, *60*(10), 1768–78. doi:10.1016/j.marpolbul.2010.06.009

Findlay, H. S., Hennige, S. J., Wicks, L. C., Navas, J. M., Woodward, E. M. S., & Roberts, J. M. (2014). Fine-scale nutrient and carbonate system dynamics around cold-water coral reefs in the northeast Atlantic. *Scientific reports*, 4.

Fiore, C. L., Jarett, J. K., Olson, N. D., & Lesser, M. P. (2010). Nitrogen fixation and nitrogen transformations in marine symbioses. *Trends in microbiology*, *18*(10), 455-463.

Flores, E., & Herrero, A. (2009). Compartmentalized function through cell differentiation in filamentous cyanobacteria. *Nature Reviews Microbiology*, *8*(1), 39-50.

Forchhammer, K., & de Marsac, N. T. (1995). Functional analysis of the phosphoprotein PII (glnB gene product) in the cyanobacterium *Synechococcus* sp. strain PCC 7942. *Journal of bacteriology*, *177*(8), 2033-2040.

Frada, M., Burrows, E., Wyman, K., & Falkowski, P. (2013). Quantum requirements for growth and fatty acid biosynthesis in the marine diatom *Phaeodactylum tricornutum* (*Bacillariophyceae*) in nitrogen replete and limited conditions. *Journal of Phycology*, *49*(2), 381388. doi:10.1111/jpy.12046

Fujiki, T., & Taguchi, S. (2002). Variability in chlorophyll a specific absorption coefficient in marine phytoplankton as a function of cell size and irradiance. *Journal of Plankton Research*, *24*(9), 859-874.

Fujita, R. M., Wheeler, P. A., & Edwards, R. L. (1989). Assessment of macroalgal nitrogen limitation in a seasonal upwelling region.

Gan, F., Zhang, S., Rockwell, N. C., Martin, S. S., Lagarias, J. C., & Bryant, D. A. (2014). Extensive remodeling of a cyanobacterial photosynthetic apparatus in far-red light. *Science*, *345*(6202), 1312-1317.
Gantt, E. (1981). Phycobilisomes. *Annual Review of Plant Physiology*, *32*(1), 327-347.

Gantt, E. (1981). Phycobilisomes. *Annual Review of Plant Physiology*, 32(1), 327-347.

Garczarek, L., Dufresne, A., Blot, N., Cockshutt, A. M., Peyrat, A., Campbell, D. A., ... & Six, C. (2008). Function and evolution of the psbA gene family in marine *Synechococcus*: *Synechococcus* sp. WH7803 as a case study. *The ISME journal*, *2*(9), 937-953.

Garczarek, L., van der Staay, G. W., Hess, W. R., Le Gall, F., & Partensky, F. (2001). Expression and phylogeny of the multiple antenna genes of the low-light-adapted strain *Prochlorococcus* marinus SS120 (Oxyphotobacteria). *Plant molecular biology*,*46*(6), 683-693.

Geider, R. J., MacIntyre, H. L., & Kana, T. M. (1996). A dynamic model of photoadaptation in phytoplankton. *Limnology and Oceanography*, *41*(1), 1-15.

Geider, R. J., MacIntyre, H. L., & Kana, T. M. (1998). A dynamic regulatory model of phytoplanktonic acclimation to light, nutrients, and temperature. *Limnology and Oceanography*, 43(4), 679-694.

Gerber, S., & Häder, D. P. (1993). Effects of solar irradiation on motility and pigmentation of three species of phytoplankton. *Environmental and experimental botany*, 33(4), 515-521.

Gévaert, F., Créach, A., Davoult, D., Migné, A., Levavasseur, G., Arzel, P., ... & Lemoine, Y. (2003). Laminaria saccharina photosynthesis measured in situ: photoinhibition and xanthophyll cycle during a tidal cycle. *Marine Ecology Progress Series*, *247*, 43-50.

Glazer, A. N. (1985). Light harvesting by phycobilisomes. *Annual review of biophysics and biophysical chemistry*, *14*(1), 47-77.

Glazer, A. N. (1990). [14] Phycoerythrin fluorescence-based assay for reactive oxygen species. *Methods in enzymology*, 186, 161-168.

Glazer, A. N., & Hixson, C. S. (1975). Characterization of R-phycocyanin. Chromophore content of R-phycocyanin and C-phycoerythrin. *Journal of Biological Chemistry*, 250(14), 5487-5495.

Glazer, A. N., & Hixson, C. S. (1977). Subunit structure and chromophore composition of rhodophytan phycoerythrins. Porphyridium cruentum B-phycoerythrin and b-phycoerythrin. *Journal of Biological Chemistry*, 252(1), 32-42.

Glibert, P. M., & Bronk, D. A. (1994). Release of dissolved organic nitrogen by marine diazotrophic cyanobacteria, Trichodesmium spp. *Applied and Environmental microbiology*, *60*(11), 3996-4000.

Glibert, P. M., & Ray, R. T. (1990). Different patterns of growth and nitrogen uptake in two clones of marine *Synechococcus* spp. *Marine Biology*, *107*(2), 273-280.

Glibert, P. M., Kana, T. M., Olson, R. J., Kirchman, D. L., & Alberte, R. S. (1986). Clonal comparisons of growth and photosynthetic responses to nitrogen availability in marine *Synechococcus* spp. *Journal of experimental marine biology and ecology*, *101*(1), 199-208.

Gloag, R., Ritchie, R., Chen, M., Larkum, A., & Quinnell, R. (2007). Chromatic photoacclimation, photosynthetic electron transport and oxygen evolution in the Chlorophyll d-containing oxyphotobacterium *Acaryochloris marina*. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*,*1767*(2), 127135. doi:10.1016/j.bbabio.2006.11.014

Glover, H. E., Phinney, D. A., & Yentsch, C. S. (1985). Photosynthetic characteristics of picoplankton compared with those of larger phytoplankton populations, in various water masses in the Gulf of Maine. *Biological oceanography*, *3*(3), 223-248.

Glover, H., Keller, M., & Spinrad, R. (1987). The effects of light quality and intensity on photosynthesis and growth of marine eukaryotic and prokaryotic phytoplankton clones. *Journal of Experimental Marine Biology and Ecology*, *105*(2-3), 137159. doi:10.1016/0022-0981(87)90168-7

Gobler, C. J., Koch, F., Kang, Y., Berry, D. L., Tang, Y. Z., Lasi, M., ... & Miller, J. D. (2013). Expansion of harmful brown tides caused by the pelagophyte, *Aureoumbra lagunensis* DeYoe et Stockwell, to the US east coast. *Harmful Algae*, *27*, 29-41.

Gobler, C. J., Lonsdale, D. J., & Boyer, G. L. (2005). A review of the causes, effects, and potential management of harmful brown tide blooms caused by *Aureococcus anophagefferens* (Hargraves et sieburth). *Estuaries*, *28*(5), 726-749.

Gómez Garreta, A., Gallardo, T., Ribera, M. A., Cormaci, M., Furnari, G., Giaccone, G., & Boudouresque, C. F. (2001). Checklist of Mediterranean Seaweeds. III. *Rhodophyceae* Rabenh. 1. Ceramiales Oltm. *Botanica marina*, *44*(5), 425-460.

Goss, R., & Jakob, T. (2010). Regulation and function of xanthophyll cycle-dependent photoprotection in algae. *Photosynthesis Research*, *106*(1-2), 103-122.

Gouveia, L., & Oliveira, A. C. (2009). Microalgae as a raw material for biofuels production. *Journal of industrial microbiology & biotechnology*, *36*(2), 269-274.

Govindjee, D. S. (2011). Adventures with cyanobacteria: a personal perspective. *Frontiers in plant science*, *2*.

Grabowski, B., Cunningham, F. X., & Gantt, E. (2001). Chlorophyll and carotenoid binding in a simple red algal light-harvesting complex crosses phylogenetic lines. *Proceedings of the National Academy of Sciences*, 98(5), 2911-2916.

Green, B. R., & Durnford, D. G. (1996). The chlorophyll-carotenoid proteins of oxygenic photosynthesis. *Annual review of plant biology*, *47*(1), 685-714.

Green, B., & Parson, W. W. (Eds.). (2003). Light-harvesting antennas in photosynthesis (Vol. 13). Springer.

Griffiths, M. J., & Harrison, S. T. (2009). Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *Journal of Applied Phycology*, *21*(5), 493-507.

Griffiths, M. J., van Hille, R. P., & Harrison, S. T. (2012). Lipid productivity, settling potential and fatty acid profile of 11 microalgal species grown under nitrogen replete and limited conditions. *Journal of Applied Phycology*, *24*(5), 989-1001.

Gröniger, A., & Häder, D. P. (2002). Induction of the synthesis of an UV-absorbing substance in the green alga *Prasiola stipitata*. *Journal of Photochemistry and Photobiology B: Biology*, *66*(1), 54-59.

Grossman, A. R., Schaefer, M. R., Chiang, G. G., & Collier, J. L. (1993). The phycobilisome, a light-harvesting complex responsive to environmental conditions. *Microbiological reviews*, 57(3), 725-749.

Gruszecki, W. I., & Strzałka, K. (2005). Carotenoids as modulators of lipid membrane physical properties. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1740*(2), 108-115.

Hallegraeff, G. M., McCausland, M. A., & Brown, R. K. (1995). Early warning of toxic dinoflagellate blooms of *Gymnodinium catenatum* in southern Tasmanian waters. *Journal of plankton research*, *17*(6), 1163-1176.

Hamm, C. E., Merkel, R., Springer, O., Jurkojc, P., Maier, C., Prechtel, K., & Smetacek, V. (2003). Architecture and material properties of diatom shells provide effective mechanical protection. *Nature*, *421*(6925), 841-843.

Hansen, P. (1996). Silica-scaled *Chrysophyceae* and *Synurophyceae* from Madagascar. *Archiv für Protistenkunde*,*147*(2), 145-172.

Harrison, P. J., Thompson, P. A., & Calderwood, G. S. (1990). Effects of nutrient and light limitation on the biochemical composition of phytoplankton. *Journal of Applied Phycology*,*2*(1), 45-56.

Harrop, S. J., Wilk, K. E., Dinshaw, R., Collini, E., Mirkovic, T., Teng, C. Y., ... & Curmi, P. M. (2014). Single-residue insertion switches the quaternary structure and exciton states of cryptophyte light-harvesting proteins. Proceedings of the National Academy of Sciences, 111(26), E2666-E2675.

Häubner, N., Sylvander, P., Vuori, K., & Snoeijs, P. (2014). Abiotic stress modifies the synthesis of alpha-tocopherol and beta-carotene in phytoplankton species. *Journal of Phycology*.

Haugan, J. A., & Liaaen-Jensen, S. (1994a). Blue Carotenoids. Part 2. The Chemistry of the Classical Colour Reaction of Common Carotenoid 5, 6-Epoxides with Acid. Acta Chemica Scandinavica,48, 152-152.

Haugan, J. A., & Liaaen-Jensen, S. (1994b). Total Synthesis of Acetylenic Carotenoids. 2. Synthesis of Diatoxanthin and 7, 8-Didehydrocryptoxanthin. *Acta Chemìca Scand*inavica, 48(19941), 899-904.

Haugan, J., Glinz, E., & Liaen-Jensen, S. (1992). Algal carotenoids. XXXVIII: Structural assignments of geometrical isomers of fucoxanthin. *Acta chemica scandi*navica, 46(4), 389-395.

Hausmann, K. (1978). Extrusive organelles in protists. *International review of cytology*, *52*, 197-276.

Havaux, M. (1998). Carotenoids as membrane stabilizers in chloroplasts. *Trends in Plant Science*, *3*(4), 147-151.

Havaux, M., Guedeney, G., Hagemann, M., Yeremenko, N., Matthijs, H. C., & Jeanjean, R. (2005). The chlorophyll-binding protein *IsiA* is inducible by high light and protects the cyanobacterium Synechocystis PCC6803 from photooxidative stress. *FEBS letters*, *579*(11), 2289-2293.

Havens, K. E., James, R. T., East, T. L., & Smith, V. H. (2003). N: P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by non-point source nutrient pollution. *Environmental Pollution*, *122*(3), 379-390.

Haverkamp T, SG Acinas, M Doeleman, M Stomp, J Huisman, LJ Stal (2008) Diversity and phylogeny of Baltic Sea picocyanobacteria inferred from their ITS and phycobiliprotein operons. *Environmental Microbiology* 10: 174-188.

Haxo, F. T., Kycia, J. H., Somers, G. F., Bennett, A., & Siegelman, H. W. (1976). Peridinin-chlorophyll a proteins of the dinoflagellate *Amphidinium carterae* (Plymouth 450). *Plant physiology*, 57(2), 297-303.

Hays, S. G., & Ducat, D. C. (2014). Engineering cyanobacteria as photosynthetic feedstock factories. *Photosynthesis research*, 1-11.

Heathcote, P., Wyman, M. Carr, N. G., & Beddard, G. S. (1992). Partial uncoupling of energy transfer from phycoerythrin in the marine cyanobacterium *Synechococcus* sp. WH7803.*Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1099*(3), 267-270.

Hecky, R. E., Campbell, P., & Hendzel, L. L. (1993). The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans. *Limnology and Oceanography*, *38*(4), 709-724.

Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., ... & Suddleson, M. (2008). Eutrophication and harmful algal blooms: a scientific consensus.*Harmful algae*, *8*(1), 3-13.

Hejazi, M. A., Kleinegris, D., & Wijffels, R. H., (2004). Mechanism of extraction of beta-carotene from microalga *Dunaliellea salina* in two-phase bioreactors. *Biotechnol. Bioeng*. 88:593-600

Henriksen, P., Riemann, B., Kaas, H., Sørensen, H. M., & Sørensen, H. L. (2002). Effects of nutrient-limitation and irradiance on marine phytoplankton pigments. *Journal of Plankton Research*,*24*(9), 835-858.

Herzig, R., & Falkowski, P. (1989). Nitrogen limitation in *Isochrysis galbana* (*Haptophyceae*). I: Photosynthetic energy conversion and growth efficiencies. *Journal of phycology*, *25*(3), 462-471.

Hiyama, T., Nishimura, M., and Chance, B., (1969). Determination of carotenoids by a thin-layer chromatography. *Anal. Biochem*. 29, 339-50

Hoffmann, L., Billard, C., Janssens, M., Leruth, M., & Demoulin, V. (2000). Mass development of marine benthic *Sarcinochrysidales* (*Chrysophyceae* sl) in Corsica. *Botanica Marina*, *43*(3), 223-231.

Holdsworth, E. S. (1985). Effect of growth factors and light quality on the growth, pigmentation and photosynthesis of two diatoms, *Thalassiosira gravida* and *Phaeodactylum tricornutum*. *Marine Biology*, *86*(3), 253-262.

Holt, N., Zigmantas, D., Valkunas, L., Li, X.-P., Niyogi, K., & Fleming, G. (2005). Carotenoid Cation Formation and the Regulation of Photosynthetic Light Harvesting. *Science*,*307*(5708), 433–436. doi:10.1126/science.1105833

Honjo, S. (1976). Coccoliths: production, transportation and sedimentation. *Marine Micropaleontology*, *1*, 65-79.

Höök, M., & Tang, X. (2013). Depletion of fossil fuels and anthropogenic climate change—A review. *Energy Policy*, *52*, 797-809.

Horner, R., Ackley, S. F., Dieckmann, G. S., Gulliksen, B., Hoshiai, T., Legendre, L., ... & Sullivan, C. W. (1992). Ecology of sea ice biota. *Polar Biology*, *12*(3-4), 417-427.

Horrocks, L. A., & Yeo, Y. K. (1999). Health benefits of docosahexaenoic acid (DHA). *Pharmacological Research*, *40*(3), 211-225.

Hu, C., Völler, G., Süßmuth, R., Dittmann, E., & Kehr, J. C. (2014). Functional assessment of mycosporine-like amino acids in *Microcystis aeruginosa* strain PCC 7806. *Environmental microbiology*.

Huisman, J., Jonker, R., Zonneveld, C., & Weissing, F. (1999). Competition for Light between Phytoplankton Species: Experimental Tests of Mechanistic Theory. *Ecology*, *80*(1), 211. doi:10.2307/176991

Huisman, J., Sharples, J., Stroom, J. M., Visser, P. M., Kardinaal, W. E. A., Verspagen, J. M., & Sommeijer, B. (2004). Changes in turbulent mixing shift competition for light between phytoplankton species. *Ecology*, *85*(11), 2960-2970.

Hunter, P. D., Tyler, A. N., Présing, M., Kovács, A. W., & Preston, T. (2008). Spectral discrimination of phytoplankton colour groups: The effect of suspended particulate matter and sensor spectral resolution. *Remote Sensing of Environment*, 112(4), 1527-1544.

Ikeda, Y., Yamagishi, A., Komura, M., Suzuki, T., Dohmae, N., Shibata, Y., ... & Satoh, K. (2013). Two types of fucoxanthin-chlorophyll-binding proteins I tightly bound to the photosystem I core complex in marine centric diatoms. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1827(4), 529-539.

Imai, I., Sunahara, T., Nishikawa, T., Hori, Y., Kondo, R., & Hiroishi, S. (2001). Fluctuations of the red tide flagellates *Chattonella* spp.(*Raphidophyceae*) and the algicidal bacterium *Cytophaga* sp. in the Seto Inland Sea, Japan. *Marine Biology*, *138*(5), 1043-1049.

Interlandi, S. J., & Kilham, S. S. (2001). Limiting resources and the regulation of diversity in phytoplankton communities. *Ecology*,*82*(5), 1270-1282.

IPCC, 2007: Summary for Policymakers. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change

Ivanov, A. G., Krol, M., Selstam, E., Sane, P. V., Sveshnikov, D., Park, Y. I., ... & Huner, N. P. (2007). The induction of CP43' by iron-stress in *Synechococcus* sp. PCC 7942 is associated with carotenoid accumulation and enhanced fatty acid unsaturation. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1767(6), 807-813.

Jahns, P., Latowski, D., & Strzalka, K. (2009). Mechanism and regulation of the violaxanthin cycle: the role of antenna proteins and membrane lipids. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1787*(1), 3-14.

Jeffrey, S. W. (1972). Preparation and some properties of crystalline chlorophyll c1 and c 2 from marine algae. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 279(1), 15-33.

Jeffrey, S. W., Mantoura, R. F. C., & Bjørnland, T. (1997). Data for the identification of 47 key phytoplankton pigments. *Phytoplankton pigments in oceanography: guidelines to modern methods.* UNESCO, Paris, 449-559.

Jeffrey, S. W., Wright, S. W., & Zapata, M. (2011). Microalgal classes and their signature pigments.

Jensen, J. P., Jeppesen, E., Olrik, K., & Kristensen, P. (1994). Impact of nutrients and physical factors on the shift from cyanobacterial to chlorophyte dominance in shallow Danish lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, *51*(8), 1692-1699.

Jeong, H. J., Yoo, Y. D., Lim, A. S., Kim, T. W., Lee, K., & Kang, C. K. (2013). *Raphidophyte* red tides in Korean waters. *Harmful Algae*, *30*, S41-S52.

Jeppesen, E., Søndergaard, M., Jensen, J. P., Havens, K. E., Anneville, O., Carvalho, L., ... & Winder, M. (2005). Lake responses to reduced nutrient loading–an analysis of contemporary long-term data from 35 case studies. *Freshwater Biology*, *50*(10), 1747-1771.

Jiang, Z., Liu, J., Chen, J., Chen, Q., Yan, X., Xuan, J., & Zeng, J. (2014). Responses of summer phytoplankton community to drastic environmental changes in the Changjiang (Yangtze River) estuary during the past 50 years. *Water research*, *54*, 1-11.

Jonathan, G., & Mordechai. G., (2013). Novel Photobioreactor for Enclosed Horizontal Cultivation of Microalgae. assignee. Patent WO 2014064602 A2. 22 Oct. 2013. Print.

Jones, R. I. (2000). Mixotrophy in planktonic protists: an overview.*Freshwater Biology*, *45*(2), 219-226.

Jordan, P., Fromme, P., Witt, H. T., Klukas, O., Saenger, W., & Krauß, N. (2001). Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution. Nature, 411(6840), 909-917.

Jordan, R. W., & Iwataki, M. (2012). Chrysophyceae and Synurophyceae. *eLS*.

Jørgensen, M., Norrman, K., & Krebs, F. C. (2008). Stability/degradation of polymer solar cells. *Solar Energy Materials and Solar Cells*, *92*(7), 686-714.

Jungo, E., Visser, P. M., Stroom, J., & Mur, L. R. (2001). Artificial mixing to reduce growth of the blue-green alga *Microcystis* in Lake Nieuwe Meer, Amsterdam: an evaluation of 7 years of experience. *Water Science & Technology: Water Supply*, *1*(1), 17-23.

Kaffes, A., Thoms, S., Trimborn, S., Rost, B., Langer, G., Richter, K.-U., … Giordano, M. (2010). Carbon and nitrogen fluxes in the marine coccolithophore Emiliania huxleyi grown under different nitrate concentrations. *Journal of Experimental Marine Biology and Ecology*, *393*(1-2), 18. doi:10.1016/j.jembe.2010.06.004

Kamiya, N., & Shen, J. R. (2003). Crystal structure of oxygen-evolving photosystem II from *ThermoSynechococcus vulcanus* at 3.7-Å resolution. *Proceedings of the National Academy of Sciences*, 100(1), 98-103.

Kamjunke, N., Gaedke, U., Weithoff, G., & Bell, E. M. (2004). Strong vertical differences in the plankton composition of an extremely acidic lake. *Archiv für Hydrobiologie*, *161*(3), 289-306.

Kana, T. M., & Glibert, P. M. (1987). Effect of irradiances up to 2000 μE on marine *Synechococcus* WH7803—I. Growth, pigmentation, and cell composition. *Deep Sea Research Part A. Oceanographic Research Papers*, *34*(4), 479-495.

Kana, T. M., Feiwel, N. L., & Flynn, L. C. (1992). Nitrogen starvation in marine *Synechococcus* strains: clonal differences in phycobiliprotein breakdown and energy coupling. *MARINE ECOLOGY-PROGRESS SERIES*, *88*, 75-75.

Ke, B. (2003). Phycobiliproteins and Phycobilisomes.*Photosynthesis: Photobiochemistry and Photobiophysics*, 251-269.

Kennis, J. T., Gobets, B., van Stokkum, I. H., Dekker, J. P., van Grondelle, R., & Fleming, G. R. (2001). Light harvesting by chlorophylls and carotenoids in the photosystem I core complex of *Synechococcus* elongatus: a fluorescence upconversion study*. The Journal of Physical Chemistry* B, 105(19), 4485-4494.

Kerfeld, C. A. (2004a). Water-soluble carotenoid proteins of cyanobacteria. *Archives of biochemistry and biophysics*, *430*(1), 2-9.

Kerfeld, C. A. (2004b). Structure and function of the water-soluble carotenoid-binding proteins of cyanobacteria. *Photosynthesis research*, *81*(3), 215-225.

Kerimoglu, O., Straile, D., & Peeters, F. (2012). Role of phytoplankton cell size on the competition for nutrients and light in incompletely mixed systems. *Journal of theoretical biology*,*300*, 330-343.

Kettler, G. C., Martiny, A. C., Huang, K., Zucker, J., Coleman, M. L., Rodrigue, S., ... & Chisholm, S. W. (2007). Patterns and implications of gene gain and loss in the evolution of *Prochlorococcus*. *PLoS genetics*, *3*(12), e231.

Khoshnevisan, B., Rafiee, S., Omid, M., Yousefi, M., & Movahedi, M. (2013). Modeling of energy consumption and GHG (greenhouse gas) emissions in wheat production in Esfahan province of Iran using artificial neural networks. *Energy*, *52*, 333-338.

Kilham, P., & Hecky, R. E. (1988). Comparative ecology of marine and freshwater phytoplankton. *Limnology and Oceanography*,*33*(4), 776-795.

Kim, H., Spivack, A. J., & Menden-Deuer, S. (2013). pH alters the swimming behaviors of the raphidophyte *Heterosigma akashiwo*: Implications for bloom formation in an acidified ocean. *Harmful Algae*, *26*, 1-11.

Kim, T. W., Lee, K., Najjar, R. G., Jeong, H. D., & Jeong, H. J. (2011). Increasing N abundance in the northwestern Pacific Ocean due to atmospheric nitrogen deposition. *Science*, *334*(6055), 505-509.

Kirchman, D. L. (2012). *Processes in microbial ecology*. Oxford University Press.

Kirk, J. T. O. (1994). *Light and photosynthesis in aquatic ecosystems*. (Page 83-84) Cambridge university press.

Kivic, P. A., & Walne, P. L. (1984). An evaluation of a possible phylogenetic relationship between the *Euglenophyta* and *Kinetoplastida*. In *Evolutionary Protistology* (pp. 269-288). Springer Netherlands.

Kjøsen, H., Norgard, S., Liaaen-Jensen, S., Svec, W.A., Strain, H.H., Wegfahrt, P., Rapoport, H. and Haxo, F.T. (1976) Algal carotenoids XV structural studies on peridinin. Part 2. *Supporting evidence. Acta Chem. Scand*.30, 157-64

Kleinegris, D. M., van Es, M. A., Janssen, M., Brandenburg, W. A., & Wijffels, R. H. (2010). Carotenoid fluorescence in *Dunaliella salina*. *Journal of applied phycology*, *22*(5), 645-649.

Klisch, M., & Häder, D. P. (2002). Wavelength dependence of mycosporine-like amino acid synthesis in *Gyrodinium dorsum*. *Journal of Photochemistry and Photobiology B: Biology*, *66*(1), 60-66.

Klut, M. E., Bisalputra, T., & Antia, N. J. (1987). Some observations on the structure and function of the dinoflagellate pusule. *Canadian journal of botany*, *65*(4), 736-744.

Kolber, Z., Zehr, J., & Falkowski, P. (1988). Effects of growth irradiance and nitrogen limitation on photosynthetic energy conversion in photosystem II. *Plant Physiology*, *88*(3), 923-929.

Kolodny, N. H., Bauer, D., Bryce, K., Klucevsek, K., Lane, A., Medeiros, L., ... & Allen, M. M. (2006). Effect of nitrogen source on cyanophycin synthesis in *Synechocystis* sp. strain PCC 6308.*Journal of bacteriology*, *188*(3), 934-940.

Kramer, J. G., & Morris, I. (1990). Growth regulation in irradiance limited marine *Synechococcus* sp. WH 7803. *Archives of microbiology*, *154*(3), 286-293.

Krinsky, N. I. (1989). Antioxidant functions of carotenoids. *Free Radical Biology and Medicine*, *7*(6), 617-635.

Kromkamp, J. (1987). Formation and functional significance of storage products in cyanobacteria. *New Zealand Journal of Marine and Freshwater Research*, 21(3), 457-465.

Kunath, C., Jakob, T., & Wilhelm, C. (2012). Different phycobilin antenna organisations affect the balance between light use and growth rate in the cyanobacterium *Microcystis aeruginosa* and in the cryptophyte *Cryptomonas ovata*. *Photosynthesis research*,*111*(1-2), 173-183.

Küpper, H., Andresen, E., Wiegert, S., Šimek, M., Leitenmaier, B., & Šetlík, I. (2009). Reversible coupling of individual phycobiliprotein isoforms during state transitions in the cyanobacterium *Trichodesmium* analysed by single-cell fluorescence kinetic measurements. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1787*(3), 155-167.

Kutser, T. (2004). Quantitative detection of chlorophyll in cyanobacterial blooms by satellite remote sensing. *Limnology and Oceanography*, *49*(6), 2179-2189.

Kwon, J.-H., Bernát, G., Wagner, H., Rögner, M., & Rexroth, S. (2013). Reduced light-harvesting antenna: Consequences on cyanobacterial metabolism and photosynthetic productivity. *Algal Research*, *2*(3), 188195. doi:10.1016/j.algal.2013.04.008

Lafabrie, C., Garrido, M., Leboulanger, C., Cecchi, P., Grégori, G., Pasqualini, V., & Pringault, O. (2013). Impact of contaminated-sediment resuspension on phytoplankton in the Biguglia lagoon (Corsica, Mediterranean Sea). *Estuarine, Coastal and Shelf Science*, *130*, 70-80.

Lamers, P. P., Janssen, M., De Vos, R. C., Bino, R. J., & Wijffels, R. H. (2012). Carotenoid and fatty acid metabolism in nitrogen-starved *Dunaliella salina*, a unicellular green microalga.*Journal of biotechnology*, *162*(1), 21-27.

Lamers, P. P., Laak, C. C., Kaasenbrood, P. S., Lorier, J., Janssen, M., Vos, R. C., … Wijffels, R. H. (2010). Carotenoid and fatty acid metabolism in light stressed *Dunaliella salina*. *Biotechnology and bioengineering*, *106*(4), 638–648. doi:10.1002/bit.22725

Lane, D., Beaumont, A., & Hunter, J. (1994). Primary production of prochlorophytes, cyanobacteria, and eucaryotic ultraphytoplankton: measurements from flow cytometric sorting. *Mar. Biol*, *114*, 85-95.
Lane, T. W., & Carney, L. T. (2014). Parasites in algae mass culture. *Aquatic Microbiology*, *5*, 278.

Lange-Bertalot, H. & Ulrich, S. (2014). Contributions to the taxonomy of needle shaped *Fragilaria* and *Ulnaria* species 1. *Lauterbornia* 78:

Lapointe, B., & Duke, C. (1984). Biochemical strategies for growth of *Gracilaria tikvahiae* (*Rhodophyta*) in relation to light intensity and nitrogen availability. *Journal of phycology*, *20*(4), 488-495.

Lavaud, J., & Kroth, P. G. (2006). In diatoms, the transthylakoid proton gradient regulates the photoprotective non-photochemical fluorescence quenching beyond its control on the xanthophyll cycle. *Plant and cell physiology*, *47*(7), 1010-1016.

Lavaud, J., Rousseau, B., van Gorkom, H. J., & Etienne, A. L. (2002). Influence of the diadinoxanthin pool size on photoprotection in the marine planktonic diatom *Phaeodactylum tricornutum*. *Plant Physiology*, *129*(3), 1398-1406.

Lavaud, J., Strzepek, R. F., & Kroth, P. G. (2007). Photoprotection capacity differs among diatoms: possible consequences on the spatial distribution of diatoms related to fluctuations in the underwater light climate. *Limnology and Oceanography*, *52*(3), 1188-1194.

Legrand, C., Graneli, E., & Carlsson, P. (1998). Induced phagotrophy in the photosynthetic dinoflagellate *Heterocapsa triquetra*. *Aquatic Microbial Ecology*, *15*(1), 65-75.

Lehtimaki, J., Moisander, P., Sivonen, K., & Kononen, K. (1997). Growth, nitrogen fixation, and nodularin production by two balticsea cyanobacteria. *Applied and environmental microbiology*,*63*(5), 1647-1656.

Leonardos, N., & Geider, R. (2004). Responses of elemental and biochemical composition of *Chaetoceros muelleri* to growth under varying light and nitrate:phosphate supply ratios and their influence on critical N : P. *Limnology and Oceanography*, *49*(6), 21052114. doi:10.4319/lo.2004.49.6.2105

Lepetit, B., Volke, D., Szabó, M., Hoffmann, R., Garab, G., Wilhelm, C., & Goss, R. (2007). Spectroscopic and molecular characterization of the oligomeric antenna of the diatom *Phaeodactylum tricornutum*. Biochemistry, 46(34), 9813-9822.

Li, G., Brown, C. M., Jeans, J. A., Donaher, N. A., McCarthy, A., & Campbell, D. A. (2014). The nitrogen costs of photosynthesis in a diatom under current and future pCO2. *New Phytologist*.

Li, Y., Lin, Y., Loughlin, P. C., & Chen, M. (2014). Optimization and effects of different culture conditions on growth of Halomicronema hongdechloris–a filamentous cyanobacterium containing chlorophyll f. *Frontiers in plant science*, *5*.

Lindholm, T., & Nummelin, C. (1999). Red tide of the dinoflagellate *Heterocapsa triquetra* (*Dinophyta*) in a ferry-mixed coastal inlet. In *Biological, Physical and Geochemical Features of Enclosed and Semi-enclosed Marine Systems* (pp. 245-251). Springer Netherlands.

Litchman, E., Neale, P. J., & Banaszak, A. T. (2002). Increased sensitivity to ultraviolet radiation in nitrogen-limited dinoflagellates: Photoprotection and repair. *Limnology and Oceanography*, 47(1), 86-94.

Liu, X., Sheng, J., & Curtiss III, R. (2011). Fatty acid production in genetically modified cyanobacteria. *Proceedings of the National Academy of Sciences*, *108*(17), 6899-6904.

Llewellyn, C. A., White, D. A., Martinez-Vincente, V., Tarran, G., & Smyth, T. J. (2012). Distribution of mycosporine-like amino acids along a surface water meridional transect of the Atlantic. *Microbial ecology*, *64*(2), 320-333.

Lohscheider, J. N., Strittmatter, M., Küpper, H., & Adamska, I. (2011). Vertical distribution of epibenthic freshwater cyanobacterial *Synechococcus* spp. strains depends on their ability for photoprotection. *PloS one*, *6*(5), e20134.

Lokstein, H., Steglich, C., & Hess, W. R. (1999). Light-harvesting antenna function of phycoerythrin in *Prochlorococcus marinus*. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1410*(1), 97-98.

Lomas, M. W. (2004). Nitrate reductase and urease enzyme activity in the marine diatom *Thalassiosira weissflogii* (*Bacillariophyceae*): interactions among nitrogen substrates.*Marine Biology*, *144*(1), 37-44.

Lomas, M. W., Glibert, P. M., Clougherty, D. A., Huber, D. R., Jones, J., Alexander, J., & Haramoto, E. (2001). Elevated organic nutrient ratios associated with brown tide algal blooms of *Aureococcus anophagefferens* (*Pelagophyceae*). *Journal of Plankton Research*,*23*(12), 1339-1344.

Lomas, M. W., Rumbley, C. J., & Glibert, P. M. (2000). Ammonium release by nitrogen sufficient diatoms in response to rapid increases in irradiance. *Journal of Plankton Research*, *22*(12), 2351-2366.
López-García, P., Rodríguez-Valera, F., Pedrós-Alió, C., & Moreira, D. (2001). Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. *Nature*, *409*(6820), 603-607.

Loquay, N., Wylezich, C., & Arndt, H. (2009). Composition of groundwater nanoprotist communities in different aquifers based on aliquot cultivation and genotype assessment of cercomonads. *Fundamental and Applied Limnology/Archiv für Hydrobiologie*,*174*(3), 261-269.

Ludwig, M., & Bryant, D. A. (2012). Acclimation of the global transcriptome of the cyanobacterium *Synechococcus* sp. strain PCC 7002 to nutrient limitations and different nitrogen sources. *Frontiers in microbiology*, *3*.

Lv, J., Li, N., & Niu, D. K. (2008) . Association between the availability of environmental resources and the atomic composition of organismal proteomes: evidence from *Prochlorococcus* strains living at different depths. *Biochemical and biophysical research communications*, *375*(2), 241.

MacFarlane, J. J., & Raven, J. A. (1990). C, N and P nutrition of *Lemanea mamillosa* Kütz. (Batrachospermales, *Rhodophyta*) in the Dighty Burn, Angus, UK. *Plant, Cell & Environment*, *13*(1), 1-13.

Macintyre, H., Kana, T. M., Anning, T., & Geider, R. J. (2002). Photoacclimation of photosynthesis irradiance response curves and photosynthetic pigments in microalgae and cyanobacteria. J. *Phycol.*, 38, 17-38.

Mackey, K. R., Paytan, A., Caldeira, K., Grossman, A. R., Moran, D., McIlvin, M., & Saito, M. A. (2013). Effect of temperature on photosynthesis and growth in marine *Synechococcus* spp. *Plant physiology*, *163*(2), 815-829.

Madhyastha, H. K., & Vatsala, T. M. (2007). Pigment production in Spirulina fussiformis in different photophysical conditions. *Biomolecular engineering*, *24*(3), 301-305.

Maldonado, M. T., & Price, N. M. (1996). Influence of N substrate on Fe requirements of marine centric diatoms. *Marine ecology progress series. Oldendorf*, *141*(1), 161-172.

Malmstrom, R. R., Coe, A., Kettler, G. C., Martiny, A. C., Frias-Lopez, J., Zinser, E. R., & Chisholm, S. W. (2010). Temporal dynamics of *Prochlorococcus* ecotypes in the Atlantic and Pacific oceans. *The ISME journal*, *4*(10), 1252-1264.

Manceau, M., Bundgaard, E., Carlé, J. E., Hagemann, O., Helgesen, M., Søndergaard, R., ... & Krebs, F. C. (2011). Photochemical stability of π-conjugated polymers for polymer solar cells: a rule of thumb. *Journal of Materials Chemistry*, *21*(12), 4132-4141.

Marosvölgyi, M., & Gorkom, H. (2010). Cost and color of photosynthesis. *Photosynthesis research*,*103*(2), 105–9. doi:10.1007/s11120-009-9522-3

Marshall, W., & Laybourn-Parry, J. (2002). The balance between photosynthesis and grazing in Antarctic mixotrophic cryptophytes during summer. *Freshwater biology*, *47*(11), 2060-2070.

Marty, J. C., Chiavérini, J., Pizay, M. D., & Avril, B. (2002). Seasonal and interannual dynamics of nutrients and phytoplankton pigments in the western Mediterranean Sea at the DYFAMED time-series station (1991–1999). *Deep Sea Research Part II: Topical Studies in Oceanography*, *49*(11), 1965-1985.

Marx, A., & Adir, N. (2013). Allophycocyanin and phycocyanin crystal structures reveal facets of phycobilisome assembly.*Biochimica et Biophysica Acta* (BBA)-Bioenergetics, 1827(3), 311-318.

Marzieh Hosseini, S., Shahbazizadeh, S., Khosravi-Darani, K., & Reza Mozafari, M. (2013). *Spirulina paltensis*: Food and function. *Current Nutrition & Food Science*, *9*(3), 189-193.

Matthijs, H. C., Balke, H., Van Hes, U. M., Kroon, B., Mur, L. R., & Binot, R. A. (1996). Application of light-emitting diodes in bioreactors: Flashing light effects and energy economy in algal culture (*Chlorella pyrenoidosa*). *Biotechnology and bioengineering*, *50*(1), 98-107.

Matthijs, H. C., Visser, P. M., Reeze, B., Meeuse, J., Slot, P. C., Wijn, G., ... & Huisman, J. (2012). Selective suppression of harmful cyanobacteria in an entire lake with hydrogen peroxide. *Water research*, *46*(5), 1460-1472.

Maurya, S. S., Maurya, J. N., & Pandey, V. D. (2014). Factors regulating phycobiliprotein production in cyanobacteria. *Int. J. Curr. Microbiol. App. Sci*, *3*(5), 764-771.

McClain, C. R. (2009). A decade of satellite ocean color observations*. *Annual Review of Marine Science*, *1*, 19-42.

McClure, S. D., Turner, D. B., Arpin, P. C., Mirkovic, T., & Scholes, G. D. (2014). Coherent Oscillations in the PC577 *Cryptophyte* Antenna Occur in the Excited Electronic State. *The Journal of Physical Chemistry* B, 118(5), 1296-1308.

McGinnis, K. M., Dempster, T. A., & Sommerfeld, M. R. (1997). Characterization of the growth and lipid content of the diatom *Chaetoceros muelleri*. *Journal of Applied Phycology*, *9*(1), 19-24.

McGrory, C. B., & Leadbeater, B. S. C. (1981). Ultrastructure and deposition of silica in the *Chrysophyceae*. In *Silicon and siliceous structures in biological systems* (pp. 201-230). Springer New York.

Melis, A., Neidhardt, J., & Benemann, J. R. (1998). *Dunaliella salina* (*Chlorophyta*) with small chlorophyll antenna sizes exhibit higher photosynthetic productivities and photon use efficiencies than normally pigmented cells. *Journal of Applied Phycology*, *10*(6), 515-525.

Mella-Flores, D., Six, C., Ratin, M., Partensky, F., Boutte, C., Le Corguillé, G., ... & Garczarek, L. (2012). *Prochlorococcus* and *Synechococcus* have evolved different adaptive mechanisms to cope with light and UV stress. *Frontiers in microbiology*, *3*.

Mendoza, H., Martel, A., Del Río, M. J., & Reina, G. G. (1999). Oleic acid is the main fatty acid related with carotenogenesis in *Dunaliella salina*. *Journal of Applied Phycology*, *11*(1), 15-19.

Menezes, M., & Bicudo, C. E. D. M. (2010). Freshwater *Raphidophyceae* from the State of Rio de Janeiro, Southeast Brazil. *Biota Neotropica*, *10*(3), 323-331.

Merzlyak, M. N., Chivkunova, O. B., Gorelova, O. A., Reshetnikova, I. V., Solovchenko, A. E., Khozin-Goldberg, I., & Cohen, Z. (2007). Effect of nitrogen starvation on optical properties, pigments, and arachidonic acid content of the unicellular green alga parietochloris incisa (*Trebouxiophyceae, Chlorophyta*). *Journal of phycology*.

Meyer, R. L. (1969). The freshwater algae of Arkansas. In *Arkansas Academy of Science Proceedings* (Vol. 23, pp. 145-156).

Mimuro, M., Murakami, A., Tomo, T., Tsuchiya, T., Watabe, K., Yokono, M., & Akimoto, S. (2011). Molecular environments of divinyl chlorophylls in *Prochlorococcus* and Synechocystis: Differences in fluorescence properties with chlorophyll replacement. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1807*(5), 471481. doi:10.1016/j.bbabio.2011.02.011

Mishra, S., & Richa, R. P. S. (2014). Irradiation dependent mycosporine-like amino acids synthesis in the cyanobacterium *Scytonema geitleri*.

Moisan TA, MH Ellisman, CW Buitenhuys and GA Sosinsky (2006). Differences in chloroplast ultrastructure of *Phaeocystis antarctica* in low and high light. *Marine Biology* 149(6):1281-1290

Montgomery, B. L. (2007). Sensing the light: photoreceptive systems and signal transduction in cyanobacteria. *Molecular microbiology*, 64(1), 16-27.

Montoya, J. P., Holl, C. M., Zehr, J. P., Hansen, A., Villareal, T. A., & Capone, D. G. (2004). High rates of N2 fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean. *Nature*, *430*(7003), 1027-1032.

Morar, A. S., Olteanu, A., Young, G. B., & Pielak, G. J. (2008). Production of natural butylated hydroxytoluene as an antioxidant by freshwater phytoplankton. *Journal of Phycology*.

Morel, A. (1974). Optical properties of pure water and pure sea water. *Optical aspects of oceanography*, *1*.

Moser, M., Callieri, C., & Weisse, T. (2009). Photosynthetic and growth response of freshwater picocyanobacteria are strain-specific and sensitive to photoacclimation. *Journal of plankton research*, *31*(4), 349-357.

Motten, A. F. 2004. Diversity of photosynthetic pigments. *Tested studies for laboratory teaching,* Volume 25 (M. A. O'Donnell, Editor), 159-177.

Mouget, J. L., Rosa, P., & Tremblin, G. (2004). Acclimation of *Haslea ostrearia* to light of different spectral qualities–confirmation ofchromatic adaptation'in diatoms. *Journal of Photochemistry and Photobiology B: Biology*, *75*(1), 1-11.

Mozzo, M., Mantelli, M., Passarini, F., Caffarri, S., Croce, R., & Bassi, R. (2010). Functional analysis of Photosystem I light-harvesting complexes (Lhca) gene products of *Chlamydomonas reinhardtii*. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1797(2), 212-221.

Mulholland, M. R., & Lomas, M. W. (2008). Nitrogen uptake and assimilation. *Nitrogen in the marine environment*, 303-384.

Müller, P., Li, X. P., & Niyogi, K. K. (2001). Non-photochemical quenching. A response to excess light energy. *Plant Physiology*,*125*(4), 1558-1566.

Mushir, S., & Fatma, T. (2011). Ultraviolet radiation-absorbing mycosporine-like amino acids in cyanobacterium *Aulosira fertilissma*: Environmental perspective and characterization, current research. *J Biol Sci*, *3*, 165-171.

Nagao, K., & Yanagita, T. (2005). Conjugated fatty acids in food and their health benefits. *Journal of bioscience and bioengineering*, *100*(2), 152-157.

Nakajima, Y., & Ueda, R. (2000). The effect of reducing light-harvesting pigment on marine microalgal productivity. *Journal of applied phycology*, *12*(3-5), 285-290.

Nakajima, Y., Tsuzuki, M., & Ueda, R. (1998). Reduced photoinhibition of a phycocyanin-deficient mutant of Synechocystis PCC 6714. *Journal of applied phycology*, *10*(5), 447-452.

Newman, J., Wyman, M., & Carr, N. G. (1987). Absence of the nitrogen reserve polymer cyanophycin from marine *Synechococcus* species. *FEMS microbiology letters*, *44*(2), 221-224.

Niyogi, K. K. (1999). Photoprotection revisited: genetic and molecular approaches. *Annual review of plant biology*, *50*(1), 333-359.

Niyogi, K. K., & Truong, T. B. (2013). Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis. *Current opinion in plant biology*, *16*(3), 307-314.

Noy, D., Moser, C. C., & Dutton, P. L. (2006). Design and engineering of photosynthetic light-harvesting and electron transfer using length, time, and energy scales. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1757*(2), 90-105.

O'Neil, J. M., Davis, T. W., Burford, M. A., & Gobler, C. J. (2012). The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae*, *14*, 313-334.

Obilonu, A. N., Chijioke, C., Igwegbe, W. E., Ibearugbulem, O. I., & Abubakar, Y. F. (2013). Water quality challenges and impact.*Academic Journal of Interdisciplinary Studies*, *2*(7), 69.

Obst, M., & Steinbüchel, A. (2006). Cyanophycin—an ideal bacterial nitrogen storage material with unique chemical properties. In Inclusions in prokaryotes (pp. 167-193). *Springer Berlin Heidelberg*.

Oh, S. J., KIM, D. I., Sajima, T., Shimasaki, Y., Matsuyama, Y., Oshima, Y., ... & YANG, H. S. (2008). Effects of irradiance of various wavelengths from light-emitting diodes on the growth of the harmful dinoflagellate *Heterocapsa circularisquama* and the diatom *Skeletonema costatum*. *Fisheries science*, *74*(1), 137-145.

Oi, V. T., Glazer, A. N., & Stryer, L. (1982). Fluorescent phycobiliprotein conjugates for analyses of cells and molecules.*The Journal of cell biology*, 93(3), 981-986.

Ojaveer, H., Jaanus, A., MacKenzie, B. R., Martin, G., Olenin, S., Radziejewska, T., ... & Zaiko, A. (2010). Status of biodiversity in the Baltic Sea. *PLoS One*, 5(9), e12467.

Olson, R. J., Chisholm, S. W., Zettler, E. R., & Armbrust, E. V. (1988). Analysis of *Synechococcus* pigment types in the sea using single and dual beam flow cytometry. *Deep Sea Research Part A. Oceanographic Research Papers*, *35*(3), 425-440.

Ong, L. J., & Glazer, A. N. (1991). Phycoerythrins of marine unicellular cyanobacteria. I. Bilin types and locations and energy transfer pathways in *Synechococcus* spp. phycoerythrins.*Journal of Biological Chemistry*, *266*(15), 9515-9527.

Orosa, M., Torres, E., Fidalgo, P., & Abalde, J. (2000). Production and analysis of secondary carotenoids in green algae. *Journal of Applied Phycology*, *12*(3-5), 553-556.

Ostrowska, M. (2011). Dependence between the quantum yield of chlorophyll a fluorescence in marine phytoplankton and trophicity in low irradiance level. *Optica Applicata*, *41*(3), 567-577.

Overkamp, K. E., Langklotz, S., Aras, M., Helling, S., Marcus, K., Bandow, J. E., ... & Frankenberg-Dinkel, N. (2014). Chromophore composition of the phycobiliprotein Cr-PC577 from the cryptophyte *Hemiselmis pacifica*. Photosynthesis research, 1-12.

Paasche, E. (1968). Biology and physiology of coccolithophorids.*Annual Reviews in Microbiology*, *22*(1), 71-86.

Paasche, E. (2001). A review of the coccolithophorid *Emiliania huxleyi* (*Prymnesiophyceae*), with particular reference to growth, coccolith formation, and calcification-photosynthesis interactions. *Phycologia*, *40*(6), 503-529.

Paerl, H. W. (1984). Cyanobacterial carotenoids: their roles in maintaining optimal photosynthetic production among aquatic bloom forming genera. *Oecologia*, *61*(2), 143-149.

Paerl, H. W. (1988). Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. *Limnology and Oceanography*,*33*(4), 823-847.

Paerl, H. W. (1990). Physiological ecology and regulation of N2 fixation in natural waters. In *Advances in microbial ecology* (pp. 305-344). Springer US.

Paerl, H. W. (1995). Coastal eutrophication in relation to atmospheric nitrogen deposition: current perspectives. *Ophelia*,*41*(1), 237-259.

Paerl, H. W. (1997). Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as" new" nitrogen and other nutrient sources. *Limnology and oceanography*, *42*(5), 1154-1165.

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

Paerl, H. W., & Paul, V. J. (2012). Climate change: links to global expansion of harmful cyanobacteria. *Water research*, *46*(5), 1349-1363.

Paerl, H. W., Fulton, R. S., Moisander, P. H., & Dyble, J. (2001). Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *The Scientific World Journal*, *1*, 76-113.

Paerl, H. W., Prufert-Bebout, L. E., & Guo, C. (1994). Iron-stimulated N2 fixation and growth in natural and cultured populations of the planktonic marine cyanobacteria *Trichodesmium* spp. *Applied and Environmental Microbiology*,*60*(3), 1044-1047

Paerl, H. W., Xu, H., McCarthy, M. J., Zhu, G., Qin, B., Li, Y., & Gardner, W. S. (2011). Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): The need for a dual nutrient (N & P) management strategy. *Water Research*,*45*(5), 1973-1983.

Palenik, B. (2001). Chromatic Adaptation in Marine *Synechococcus* Strains. *Applied and environmental microbiology*, *67*(2), 991-994.

Palenik, B., & Koke, J. A. (1995). Characterization of a nitrogen-regulated protein identified by cell surface biotinylation of a marine phytoplankton. *Applied and environmental microbiology*,*61*(9), 3311-3315.

Palenik, B., Ren, Q., Dupont, C. L., Myers, G. S., Heidelberg, J. F., Badger, J. H., ... & Paulsen, I. T. (2006). Genome sequence of *Synechococcus* CC9311: insights into adaptation to a coastal environment. *Proceedings of the National Academy of Sciences*,*103*(36), 13555-13559.

Pallela, R. (2014). Antioxidants from Marine Organisms and Skin Care. *Systems Biology of Free Radicals and Antioxidants*, 3771-3783.

Palmer, M. A., van Dijken, G. L., Mitchell, B. G., Seegers, B. J., Lowry, K. E., Mills, M. M., & Arrigo, K. R. (2013). Light and nutrient control of photosynthesis in natural phytoplankton populations from the Chukchi and Beaufort seas, Arctic Ocean. *Limnol. Oceanogr*, *58*(6), 2185-2205.

Park, Y. I., Sandström, S., Gustafsson, P., & Öquist, G. (1999). Expression of the *isiA* gene is essential for the survival of the cyanobacterium *Synechococcus* sp. PCC 7942 by protecting photosystem II from excess light under iron limitation. *Molecular microbiology*, *32*(1), 123-129.

Parmar, A., Singh, N. K., Kaushal, A., & Madamwar, D. (2011). Characterization of an intact phycoerythrin and its cleaved 14kDa functional subunit from marine cyanobacterium *Phormidium* sp. A27DM. *Process Biochemistry*, *46*(9), 1793-1799.

Partensky, F., Hess, W. R., & Vaulot, D. (1999). *Prochlorococcus*, a marine photosynthetic prokaryote of global significance.*Microbiology and molecular biology reviews*, *63*(1), 106-127.

Partensky, F., Hoepffner, N., Li, W. K., Ulloa, O., & Vaulot, D. (1993). Photoacclimation of *Prochlorococcus* sp.(*Prochlorophyta*) strains isolated from the North Atlantic and the Mediterranean Sea. *Plant Physiology*, *101*(1), 285-296.

Partensky, F., La Roche, J., Wyman, K., & Falkowski, P. G. (1997). The divinyl-chlorophyll a/b-protein complexes of two strains of the oxyphototrophic marine prokaryote *Prochlorococcus*–characterization and response to changes in growth irradiance.*Photosynthesis research*, *51*(3), 209-222.

Partensky, F., Roche, J., Wyman, K., & Falkowski, P. (1997). The divinyl-chlorophyll a/b-protein complexes of two strains of the oxyphototrophic marine prokaryote *Prochlorococcus* – characterization and response to changes in growth irradiance. *Photosynthesis Research*, *51*(3), 209222. doi:10.1023/A:1005807408161

Patel, A., Mishra, S., Pawar, R., & Ghosh, P. (2005). Purification and characterization of C-Phycocyanin from cyanobacterial species of marine and freshwater habitat. *Protein Expression and Purification*, *40*(2), 248255. doi:10.1016/j.pep.2004.10.028

Pattanaik, B., Whitaker, M. J., & Montgomery, B. L. (2012). Light quantity affects the regulation of cell shape in *Fremyella diplosiphon*. *Frontiers in microbiology*, *3*.

Pawar, S. T., & Puranik, (2014) P. R. C-phycocyanin production by halotolerant cyanobacteria., *Phykos* 44 (1): 25-32

Peperzak, L., Woerd, H., & Timmermans, K. (2014). Disparities between *Phaeocystis* in situ and optically-derived carbon biomass and growth rates: potential effect on remote-sensing primary production estimates. *Biogeosciences Discussions*,*11*(4), 61196149.

Perrine, Z., Negi, S., & Sayre, R. (2012). Optimization of photosynthetic light energy utilization by microalgae. *Algal Research*, *1*(2). doi:10.1016/j.algal.2012.07.002

Phillips, L. G., Cowan, A. K., Rose, P. D., & Logie, M. R. R. (1995). Operation of the Xanthophyll Cycle in Non-Stressed and Stressed Cells of *Dunaliella salina* Teod. in Response to Diurnal Changes in Incident Irradiation: A Correlation with Intracellular β-Carotene Content. *Journal of Plant Physiology*, *146*(4), 547-553.

Pondaven, P., Gallinari, M., Chollet, S., Bucciarelli, E., Sarthou, G., Schultes, S., & Jean, F. (2007). Grazing-induced changes in cell wall silicification in a marine diatom. *Protist*, *158*(1), 21-28.

Post, A. F., Dubinsky, Z., Wyman, K., & Falkowski, P. G. (1984). Kinetics of light-intensity adaptation in a marine planktonic diatom. *Marine Biology*, *83*(3), 231-238.

Postius, C., Kenter, U., Wacker, A., Ernst, A., & Boger, P. (1998). Light causes selection among two phycoerythrin-rich *Synechococcus* isolates from Lake Constance. *FEMS Microbiology Ecology*,*25*(2), 171178. doi:10.1111/j.1574-6941.1998.tb00470.x

Prathapan, S., Johnson, T. E., & Lindsey, J. S. (1993). Building-block synthesis of porphyrin light-harvesting arrays. *Journal of the American Chemical Society*, *115*(16), 7519-7520.

Premvardhan, L., Robert, B., Beer, A., & Büchel, C. (2010). Pigment organization in fucoxanthin chlorophyll a/c 2 proteins (FCP) based on resonance Raman spectroscopy and sequence analysis. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1797(9), 1647-1656.

Prézelin, B. B., & Matlick, H. A. (1983). Nutrient-dependent low-light adaptation in the dinoflagellate *Gonyaulax polyedra*. *Marine Biology*, 74(2), 141-150.

Price, N. M., Ahner, B. A., & Morel, F. M. M. (1994). The equatorial Pacific Ocean: Grazer-controlled phytoplankton populations in an iron-limited ecosystem1. *Limnology and Oceanography*, *39*(3), 520-534.

Punginelli, C., Wilson, A., Routaboul, J. M., & Kirilovsky, D. (2009). Influence of zeaxanthin and echinenone binding on the activity of the orange carotenoid protein. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1787*(4), 280-288.

Qin, S., Liu, G. X., & Hu, Z. Y. (2008). The accumulation and metabolism of astaxanthin in *Scenedesmus obliquus* (*Chlorophyceae*). *Process Biochemistry*, *43*(8), 795-802.

Quinlan, E. L., & Phlips, E. J. (2007). Phytoplankton assemblages across the marine to low-salinity transition zone in a blackwater dominated estuary. *Journal of Plankton Research*, *29*(5), 401-416.

Quiroga, M. V., Unrein, F., Garraza, G. G., Küppers, G., Lombardo, R., Marinone, M. C., ... & Mataloni, G. (2013). The plankton communities from peat bog pools: structure, temporal variation and environmental factors. *Journal of plankton research*, *35*(6), 1234-1253.

Rabinowitch, E., and Govindjee, (1969). *Photosynthesis*, John Wiley & Sons Inc, New York (1969) Chap. 9

Radakovits, R., Jinkerson, R. E., Darzins, A., & Posewitz, M. C. (2010). Genetic engineering of algae for enhanced biofuel production. *Eukaryotic cell*, *9*(4), 486-501.

Rai, A. N., Bergman, B., & Rasmussen, U. (Eds.). (2002).*Cyanobacteria in symbiosis*. Kluwer Academic Pub..

Raja, R., Hemaiswarya, S., & Rengasamy, R. (2007). Exploitation of *Dunaliella* for β-carotene production. *Applied microbiology and biotechnology*, *74*(3), 517-523.

Rascher, U., Lakatos, M., Büdel, B., & Lüttge, U. (2003). Photosynthetic field capacity of cyanobacteria of a tropical inselberg of the Guiana Highlands. *European Journal of Phycology*, *38*(3), 247-256.

Raudsepp, U., Laanemets, J., Maljutenko, I., Hongisto, M., & Jalkanen, J. P. (2013). Impact of ship-borne nitrogen deposition on the Gulf of Finland ecosystem: an evaluation. *Oceanologia*,*55*(4).

Raven, J. A. (1984). A cost-benefit analysis of photon absorption by photosynthetic unicells. (page 621-622) *New Phytologist*,*98*(4), 593-625.

Raven, J. A. (2002). Evolution of cyanobacterial symbioses. In *Cyanobacteria in symbiosis* (pp. 329-346). Springer Netherlands.

Raven, J. A., & Waite, A. M. (2004). The evolution of silicification in diatoms: inescapable sinking and sinking as escape?. *New phytologist*, *162*(1), 45-61.

Raven, J., & Hurd, C. (2012). Ecophysiology of photosynthesis in macroalgae. *Photosynthesis research*,*113*(1-3), 105–25. doi:10.1007/s11120-012-9768-z

Riegman, R., & Kraay, G. W. (2001). Phytoplankton community structure derived from HPLC analysis of pigments in the Faroe-Shetland Channel during summer 1999: the distribution of taxonomic groups in relation to physical/chemical conditions in the photic zone. *Journal of Plankton Research*, *23*(2), 191-205.

Roberts, E. C., & Laybourn-Parry, J. (1999). Mixotrophic cryptophytes and their predators in the Dry Valley lakes of Antarctica. *Freshwater Biology*, *41*(4), 737-746.

Rocap, G., Larimer, F. W., Lamerdin, J., Malfatti, S., Chain, P., Ahlgren, N. A., ... & Chisholm, S. W. (2003). Genome divergence in two *Prochlorococcus* ecotypes reflects oceanic niche differentiation. *Nature*, *424*(6952), 1042-1047.

Rodriguez, H., Rivas, J., Guerrero, M. G., & Losada, M. (1989). Nitrogen-fixing cyanobacterium with a high phycoerythrin content. *Applied and environmental microbiology*, *55*(3), 758-760.

Rojo, C., Ortega-Mayagoitia, E., Rodrigo, M. A., & Alvarez-Cobelas, M. (2000). Phytoplankton structure and dynamics in a semiarid wetland, the National Park Las Tablas de Daimiel (Spain). *Archiv für Hydrobiologie*, *148*(3), 397-419.

ROSATI, G., & MODEO, L. (2003). Extrusomes in ciliates: diversification, distribution, and phylogenetic implications. *Journal of Eukaryotic Microbiology*, *50*(6), 383-402.

Rosenberg, J. N., Oyler, G. A., Wilkinson, L., & Betenbaugh, M. J. (2008). A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. *Current opinion in Biotechnology*, *19*(5), 430-436.

Round, F. E., Crawford, R. M., & Mann, D. G. (1990). *The diatoms: biology & morphology of the genera*. Cambridge University Press.

Rowan, K. S. (1989). Photosynthetic pigments of algae. *CUP Archive*.

Roy, S., Llewellyn, C. A., Egeland, E. S., & Johnsen, G. (Eds.). (2011). *Phytoplankton pigments: characterization, chemotaxonomy and applications in oceanography*. (page 3-77) Cambridge University Press.

Ruban, A., Lavaud, J., Rousseau, B., Guglielmi, G., Horton, P., & Etienne, A.-L. (2004). The super-excess energy dissipation in diatom algae: comparative analysis with higher plants.*Photosynthesis Research*. doi:10.1007/s11120-004-1456-1

Ryther, J. H., & Dunstan, W. M. (1971). Nitrogen, phosphorus, and eutrophication in the coastal marine environment. *Science*,*171*(3975), 1008-1013.

Sandgren, C. D., Hall, S. A., & Barlow, S. B. (1996). Siliceous scale production in *Chrysophyte* and *Synurophyte* algae I. Effects of silica-limited growth on cell silica content, scale morphology and the construction of the scale layer of *Synura petersenii* Korsh. I1. *Journal of Phycology*, *32*(4), 675-692.

Sandmann, G., Kuhn, M., & Böger, P. (1993). Carotenoids in photosynthesis: Protection of D1 degradation in the light. *Photosynthesis research*, *35*(2), 185-190.

Sathyendranath, S., Lazzara, L., & Prieur, L. (1987). Variations in the spectral values of specific absorption of phytoplankton. *Limnology and Oceanography*, 32(2), 403-415.

Satyanarayana L, Patel A, Mishra S, Ghosh PK, Suresh CG. (2011) Crystal structure of c-phycocyanin from phormidium, lyngbya spp. (Marine) and spirulina sp. (Fresh water) shows two different ways of energy. transfer between two hexamers. Nucleic Acids Res. 2012 Jan; 40(Database issue):D461-4

Scanlan, D. J. (2003). Physiological diversity and niche adaptation in marine *Synechococcus*. *Advances in microbial physiology*, *47*, 1-64.

Scanlan, D., & West, N. (2002). Molecular ecology of the marine cyanobacterial genera *Prochlorococcus* and *Synechococcus*. *FEMS Microbiology Ecology*, *40*(1), 1–12. doi:10.1111/j.1574-6941.2002.tb00930.x

Schagerl, M., & Müller, B. (2006). Acclimation of chlorophyll a and carotenoid levels to different irradiances in four freshwater cyanobacteria. *Journal of Plant Physiology*, *163*(7), 709716. doi:10.1016/j.jplph.2005.09.015

Schindler, D. W., Hecky, R. E., Findlay, D. L., Stainton, M. P., Parker, B. R., Paterson, M. J., ... & Kasian, S. E. M. (2008). Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. *Proceedings of the National Academy of Sciences*,*105*(32), 11254-11258.

Schnepf, E., & Elbrächter, M. (1992). Nutritional strategies in dinoflagellates: a review with emphasis on cell biological aspects.*European journal of protistology*, *28*(1), 3-24.

Schoelinck, C., Hinsinger, D. D., Dettaï, A., Cruaud, C., & Justine, J. L. (2014). A Phylogenetic Re-Analysis of Groupers with Applications for Ciguatera Fish Poisoning. *PloS one*, *9*(8), e98198.

Scholes, G. D., Fleming, G. R., Olaya-Castro, A., & van Grondelle, R. (2011). Lessons from nature about solar light harvesting.*Nature chemistry*, *3*(10), 763-774.

Schulte, T., Sharples, F. P., Hiller, R. G., & Hofmann, E. (2009). X-ray structure of the high-salt form of the peridinin-chlorophyll a-protein from the dinoflagellate *Amphidinium carterae*: Modulation of the spectral properties of pigments by the protein environment. Biochemistry, 48(21), 4466-4475.

Sciandra, A., Gostan, J., Collos, Y., Descolas-Gros, C., Leboulanger, C., Martin-Jézéquel, V., ... & Avril, B. (1997). Growth-compensating phenomena in continuous cultures of *Dunaliella tertiolecta* limited simultaneously by light and nitrate.*Limnology and oceanography*, *42*(6), 1325-1339.

Sciandra, A., Lazzara, L., Claustre, H., & Babin, M. (2000). Responses of growth rate, pigment composition and optical properties of *Cryptomonas* sp. to light and nitrogen stresses. *Marine Ecology Progress Series*, *201*, 107–120. doi:10.3354/meps201107

Scott, J. T., & McCarthy, M. J. (2011). Response to Comment: Nitrogen fixation has not offset declines in the Lake 227 nitrogen pool and shows that nitrogen control deserves consideration in aquatic ecosystems. *Limnol. Oceanogr*, *56*(4), 1548-1550.

Seager, S., Turner, E. L., Schafer, J., & Ford, E. B. (2005). Vegetation's red edge: a possible spectroscopic biosignature of extraterrestrial plants. *Astrobiology*, 5(3), 372-390.

Sellner, K. G. (1997). Physiology, ecology, and toxic properties of marine cyanobacteria blooms. *Limnology and Oceanography*,*42*(5), 1089-1104.

Seoane, S., Laza, A., & Orive, E. (2006). Monitoring phytoplankton assemblages in estuarine waters: The application of pigment analysis and microscopy to size-fractionated samples. *Estuarine, Coastal and Shelf Science*, *67*(3), 343-354.

Sharples, F. P., Wrench, P. M., Ou, K., & Hiller, R. G. (1996). Two distinct forms of the peridinin-chlorophyll *a* protein from *Amphidinium carterae*. *Biochimica et Biophysica Acta* (BBA)-Bioenergetics, 1276(2), 117-123.

Shibl, A. A., Thompson, L. R., Ngugi, D. K., & Stingl, U. (2014). Distribution and diversity of *Prochlorococcus* ecotypes in the Red Sea. *FEMS Microbiology Letters*.

Sidler, W. A. (2004). Phycobilisome and phycobiliprotein structures. In *The molecular biology of cyanobacteria* (pp. 139-216). Springer Netherlands.

Silva, A., Lourenço, S., & Chaloub, R. (2009). Effects of nitrogen starvation on the photosynthetic physiology of a tropical marine microalga *Rhodomonas* sp. (*Cryptophyceae*).*Aquatic Botany*, *91*(4), 291297. doi:10.1016/j.aquabot.2009.08.001

Singh, S. P., Kumari, S., Rastogi, R. P., Singh, K. L., & Sinha, R. P. (2008). Mycosporine-like amino acids (MAAs): chemical structure, biosynthesis and significance as UV-absorbing/screening compounds. *Indian journal of experimental biology*, *46*(1), 7.

Sinha, R. P., Klisch, M., Walter Helbling, E., & Häder, D. P. (2001). Induction of mycosporine-like amino acids (MAAs) in cyanobacteria by solar ultraviolet-B radiation. *Journal of Photochemistry and Photobiology B: Biology*, *60*(2), 129-135.

Six, C., Thomas, J. C., Garczarek, L., Ostrowski, M., Dufresne, A., Blot, N., ... & Partensky, F. (2007a). Diversity and evolution of phycobilisomes in marine *Synechococcus* spp.: a comparative genomics study. *Genome Biol*, *8*(12), R259.

Six, C., Finkel, Z., Irwin, A., & Campbell, D. (2007b). Light Variability Illuminates Niche-Partitioning among Marine Picocyanobacteria. *PLoS ONE*, *2*(12). doi:10.1371/journal.pone.0001341

Smayda, T. J. (1997). Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnology and oceanography*, *42*(5), 1137-1153.

Smayda, T. J. (2002). Turbulence, watermass stratification and harmful algal blooms: an alternative view and frontal zones as “pelagic seed banks”. *Harmful Algae*, *1*(1), 95-112.

Smith, V. H. (1983). Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. *Science*,*221*(4611), 669-671.

Smith, V. H., & Crews, T. (2014). Applying ecological principles of crop cultivation in large-scale algal biomass production. *Algal Research*, *4*, 23-34.

Snoeijs, P., & Häubner, N. (2014). Astaxanthin dynamics in Baltic Sea mesozooplankton communities. *Journal of Sea Research*, *85*, 131-143.

Sohm, J. A., Webb, E. A., & Capone, D. G. (2011). Emerging patterns of marine nitrogen fixation. *Nature Reviews Microbiology*, *9*(7), 499-508.

Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Miller (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Soni, B. R., Hasan, M. I., Parmar, A., Ethayathulla, A. S., Kumar, R. P., Singh, N. K., ... & Singh, T. P. (2010). Structure of the novel 14kDa fragment of α-subunit of phycoerythrin from the starving cyanobacterium *Phormidium tenue*. *Journal of structural biology*, *171*(3), 247-255.

Sotirakou, E., Kladitis, G., Diamantis, N., & Grigoropoulou, H. (1999). Ammonia and phosphorus removal in municipal wastewater treatment plant with extended aeration. *The Int. J*,*1*(1), 47-53.

Spolaore, P., Joannis-Cassan, C., Duran, E., & Isambert, A. (2006). Commercial applications of microalgae. *Journal of bioscience and bioengineering*, *101*(2), 87-96.

Ssebiyonga, N., Erga, S. R., Hamre, B., Stamnes, J. J., & Frette, Y. (2013). Light conditions and photosynthetic efficiency of phytoplankton in Murchison Bay, Lake Victoria, Uganda.*Limnologica-Ecology and Management of Inland Waters*, *43*(3), 185-193.

Stadnichuk, I., Bulychev, A., Lukashev, E., Sinetova, M., Khristin, M., Johnson, M., & Ruban, A. (2011). Far-red light-regulated efficient energy transfer from phycobilisomes to photosystem I in the red microalga Galdieria sulphuraria and photosystems-related heterogeneity of phycobilisome population. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1807*(2), 227235. doi:10.1016/j.bbabio.2010.10.018

Stal, L. J., Staal, M. J., & Villbrandt, M. (1999). Nutrient control of cyanobacterial blooms in the Baltic Sea. *Aquatic Microbial Ecology*, *18*.

Steffen, M. M., Belisle, B. S., Watson, S. B., Boyer, G. L., & Wilhelm, S. W. (2014). Status, causes and controls of cyanobacterial blooms in Lake Erie. *Journal of Great Lakes Research*, *40*(2), 215-225.

Steglich, C., Behrenfeld, M., Koblizek, M., Claustre, H., Penno, S., Prasil, O., ... & Hess, W. R. (2001). Nitrogen deprivation strongly affects Photosystem II but not phycoerythrin level in the divinyl-chlorophyll b-containing cyanobacterium *Prochlorococcus* marinus . *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1503*(3), 341-349.

Steglich, C., Mullineaux, C. W., Teuchner, K., Hess, W. R., & Lokstein, H. (2003). Photophysical properties of *Prochlorococcus marinus* SS120 divinyl chlorophylls and phycoerythrin in vitro and in vivo. *FEBS letters*, *553*(1), 79-84.

Steinhoff, F. S., Karlberg, M., Graeve, M., & Wulff, A. (2014). Cyanobacteria in Scandinavian coastal waters—A potential source for biofuels and fatty acids?. *Algal Research*, *5*, 42-51.

Stevens Jr, S. E., Balkwill, D. L., & Paone, D. A. M. (1981). The effects of nitrogen limitation on the ultrastructure of the cyanobacterium *Agmenellum quadruplicatum*. *Archives of microbiology*, *130*(3), 204-212.

Stomp, M. (2008). *Colourful coexistence: a new solution to the plankton paradox*.

Stomp, M., Huisman, J., de Jongh, F., Veraart, A. J., Gerla, D., Rijkeboer, M., ... & Stal, L. J. (2004). Adaptive divergence in pigment composition promotes phytoplankton biodiversity. *Nature*, 432(7013), 104-107.

Stomp, M., Huisman, J., Stal, L. J., & Matthijs, H. C. (2007a). Colorful niches of phototrophic microorganisms shaped by vibrations of the water molecule. *The ISME journal*, 1(4), 271-282.

Stomp, M., Huisman, J., Vörös, L., Pick, F. R., Laamanen, M., Haverkamp, T., & Stal, L. J. (2007b). Colourful coexistence of red and green picocyanobacteria in lakes and seas. *Ecology Letters*,10(4), 290-298.

Stramski, D., & Morel, A. (1990). Optical properties of photosynthetic picoplankton in different physiological states as affected by growth irradiance. *Deep Sea Research Part A. Oceanographic Research Papers*, *37*(2), 245-266.

Sun, A., Davis, R., Starbuck, M., Ben-Amotz, A., Pate, R., & Pienkos, P. T. (2011). Comparative cost analysis of algal oil production for biofuels. *Energy*, *36*(8), 5169-5179.

Sutherland, D., Turnbull, M., Broady, P., & Craggs, R. (2014). Effects of two different nutrient loads on microalgal production, nutrient removal and photosynthetic efficiency in pilot-scale wastewater high rate algal ponds. *Water Research*, *66*, 5362. doi:10.1016/j.watres.2014.08.010

Takaichi, S. (2011). Carotenoids in algae: distributions, biosyntheses and functions. *Marine drugs*, *9*(6), 1101-1118.

Tamary, E., Kiss, V., Nevo, R., Adam, Z., Bernát, G., Rexroth, S., ... & Reich, Z. (2012). Structural and functional alterations of cyanobacterial phycobilisomes induced by high-light stress.*Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1817*(2), 319-327.

Tartarotti, B., & Sommaruga, R. (2006). Seasonal and ontogenetic changes of mycosporine-like amino acids in planktonic organisms from an alpine lake. *Limnology and oceanography*, *51*(3), 1530.

Taylor, D. L., & Lee, C. C. (1971). A new cryptomonad from antarctica: *Cryptomonas cryophila* sp. nov. *Archiv für Mikrobiologie*, *75*(4), 269-280.

Thiele, A., Schirwitz, K., Winter, K., & Krause, G. H. (1996). Increased xanthophyll cycle activity and reduced D1 protein inactivation related to photoinhibition in two plant systems acclimated to excess light. *Plant science*, *115*(2), 237-250.

Thomas, D. N., & Dieckmann, G. S. (2002). Antarctic sea ice--a habitat for extremophiles. *Science*, *295*(5555), 641-644.

Tilman, D. (1985). The resource-ratio hypothesis of plant succession. *American Naturalist*, 827-852.

Timmermans, K., Wagt, B., Veldhuis, M., Maatman, A., & Baar, H. (2005). Physiological responses of three species of marine pico-phytoplankton to ammonium, phosphate, iron and light limitation. *Journal of Sea Research*, *53*(1-2), 109120. doi:10.1016/j.seares.2004.05.003

Ting, C., Rocap, G., King, J., & Chisholm, S. (2002). Cyanobacterial photosynthesis in the oceans: the origins and significance of divergent light-harvesting strategies. *Trends in Microbiology*,*10*(3), 134142. doi:10.1016/S0966-842X(02)02319-3

Tranvik, L. J., Porter, K. G., & Sieburth, J. M. (1989). Occurrence of bacterivory in *Cryptomonas*, a common freshwater phytoplankter. *Oecologia*, *78*(4), 473-476.

Tuantet, K., Janssen, M., Temmink, H., Zeeman, G., Wijffels, R. H., & Buisman, C. J. (2014). Microalgae growth on concentrated human urine. *Journal of Applied Phycology*, *26*(1), 287-297.

Tungaraza, C., Rousseau, V., Brion, N., Lancelot, C., Gichuki, J., Baeyens, W., & Goeyens, L. (2003). Contrasting nitrogen uptake by diatom and Phaeocystis -dominated phytoplankton assemblages in the North Sea. *Journal of experimental marine biology and ecology*, *292*(1), 19-41.

Turpin, D. H. (1991). Effects of inorganic N availability on algal photosynthesis and carbon metabolism. *Journal of Phycology*,*27*(1), 14-20.

Tyler, M. A., Coats, D. W., & Anderson, D. M. (1982). Encystment in a dynamic environment: Deposition of dinoflagellate cysts by a frontal convergence. *Marine ecology progress series. Oldendorf*,*7*(2), 163-178.

Usher, K. M., Bergman, B., & Raven, J. A. (2007). Exploring cyanobacterial mutualisms. *Annual review of ecology, evolution, and systematics*, 255-273.

Vaillancourt, R. D., Brown, C. W., Guillard, R. R., & Balch, W. M. (2004). Light backscattering properties of marine phytoplankton: relationships to cell size, chemical composition and taxonomy.*Journal of plankton research*, *26*(2), 191-212.

Valeur, B., & Berberan-Santos, M. N. (2012). *Molecular fluorescence: principles and applications*. (Page 38) John Wiley & Sons.

Valle, K. C., Nymark, M., Aamot, I., Hancke, K., Winge, P., Andresen, K., ... & Bones, A. M. (2014). System Responses to Equal Doses of Photosynthetically Usable Radiation of Blue, Green, and Red Light in the Marine Diatom *Phaeodactylum tricornutum*. *PloS one*, *9*(12), e114211.

Van der Grinten, E., Simis, S. G. H., Barranguet, C., & Admiraal, W. (2004). Dominance of diatoms over cyanobacterial species in nitrogen-limited biofilms. *Archiv für Hydrobiologie*, *161*(1), 98-111.

Vecktel, B., Kallmann, U., & Ruppel, H. G. (1992). Secondary Carotenoids of *Eremosphaera viridis* De Bary (*Chlorophyceae*) Under Nitrogen Deficiency*. *Botanica acta*, *105*(3), 219-222.

Vershinin, A. (1999). Biological functions of carotenoids–diversity and evolution. *Biofactors*, *10*(2), 99-104.

Visser, P. M., Ibelings, B. W., van der Veer, B., Koedood, J., & Mur, L. R. (1996). Artificial mixing prevents nuisance blooms of the cyanobacterium Microcystis in Lake Nieuwe Meer, the Netherlands. *Freshwater Biology*, *36*, 435-450.

Waller, R. F., Patron, N. J., & Keeling, P. J. (2006). Phylogenetic history of plastid-targeted proteins in the peridinin-containing dinoflagellate *Heterocapsa triquetra*. *International journal of systematic and evolutionary microbiology*, *56*(6), 1439-1447.

Walter, A., Carvalho, J. C. D., Soccol, V. T., Faria, A. B. B. D., Ghiggi, V., & Soccol, C. R. (2011). Study of phycocyanin production from Spirulina platensis under different light spectra. *Brazilian Archives of Biology and Technology*, *54*(4), 675-682.

Wang, H., & Wang, H. (2009). Mitigation of lake eutrophication: Loosen nitrogen control and focus on phosphorus abatement. *Progress in Natural Science*, *19*(10), 1445-1451.

Wang, L., Qu, Y., Fu, X., Zhao, M., Wang, S., & Sun, L. (2014). Isolation, Purification and Properties of an R-Phycocyanin from the Phycobilisomes of a Marine Red *Macroalga Polysiphonia* urceolata. PloS one, 9(2), e87833.

Wang, S. T., Pan, Y. Y., Liu, C. C., Chuang, L. T., & Chen, C. N. (2011). Characterization of a green microalga UTEX 2219-4: effects of photosynthesis and osmotic stress on oil body formation. *Bot Stud*, 52(3), 305-312.

Wanner, G., Henkelmann, G., Schmidt, A., & Kost, H. P. (1986). Nitrogen and sulfur starvation of the cyanobacterium *Synechococcus* 6301. An ultrastructural, morphometrical, and biochemical comparison. *Z Naturforsch*, *41*, 741-750.

Waser, N. A., Yu, Z., Yin, K., Nielsen, B., Harrison, P. J., Turpin, D. H., & Calvert, S. E. (1999). Nitrogen isotopic fractionation during a simulated diatom spring bloom: importance of N-starvation in controlling fractionation. *Marine Ecology Progress Series*, *179*, 291-296.

Waterbury, J. B., Willey, J. M., Franks, D. G., Valois, F. W., & Watson, S. W. (1985). A cyanobacterium capable of swimming motility. *Science*, *230*(4721), 74-76.

Watkins, S. M., Reich, A., Fleming, L. E., & Hammond, R. (2008). Neurotoxic shellfish poisoning. *Marine Drugs*, *6*(3), 431-455.

Werner, D. (Ed.). (1977). *The biology of diatoms* (Vol. 13). (page 117-142) Univ of California Press.

Whitaker, M. J., Bordowitz, J. R., & Montgomery, B. L. (2009). CpcF-dependent regulation of pigmentation and development in Fremyella diplosiphon. *Biochemical and biophysical research communications*, *389*(4), 602-606.

Whitaker, M., Pattanaik, B., & Montgomery, B. (2011). Characterization of green mutants in Fremyella diplosiphon provides insight into the impact of phycoerythrin deficiency and linker function on complementary chromatic adaptation. *Biochemical and Biophysical Research Communications*,*404*(1), 5256. doi:10.1016/j.bbrc.2010.11.056

Whitton, B. A., & Potts, M. (Eds.). (2000). *The ecology of cyanobacteria: their diversity in time and space*. Springer.

Wilson, A., Punginelli, C., Gall, A., Bonetti, C., Alexandre, M., Routaboul, J. M., ... & Kirilovsky, D. (2008). A photoactive carotenoid protein acting as light intensity sensor. *Proceedings of the National Academy of Sciences*, *105*(33), 12075-12080.

Wingard, L. L., Miller, S. R., Sellker, J. M., Stenn, E., Allen, M. M., & Wood, A. M. (2002). Cyanophycin production in a phycoerythrin-containing marine *Synechococcus* strain of unusual phylogenetic affinity. *Applied and environmental microbiology*, *68*(4), 1772-1777.

Wolk, C. P., Ernst, A., & Elhai, J. (2004). Heterocyst metabolism and development. In *The molecular biology of cyanobacteria* (pp. 769-823). Springer Netherlands.

Wright, S. W. (2005, June). Analysis of phytoplankton populations using pigment markers. In Workshop on pigment analysis of Antarctic microorganisms.

Wu, H., Abasova, L., Cheregi, O., Deák, Z., Gao, K., & Vass, I. (2011). D1 protein turnover is involved in protection of Photosystem II against UV-B induced damage in the cyanobacterium *Arthrospira platensis*. *Journal of Photochemistry and Photobiology B: Biology*, *104*(1), 320-325.

Wu, Y. P., & Krogmann, D. W. (1997). The orange carotenoid protein of *Synechocystis* PCC 6803. *Biochimica et Biophysica Acta* (BBA)-Bioenergetics, 1322(1), 1-7.

Wyman, M., Gregory, R. P. F., & Carr, N. G. (1985). Novel role for phycoerythrin in a marine cyanobacterium, *Synechococcus* strain DC2. *Science*, *230*(4727), 818-820.

Xia, S., Wang, K., Wan, L., Li, A., Hu, Q., & Zhang, C. (2013). Production, Characterization, and antioxidant activity of fucoxanthin from the marine diatom *Odontella aurita*. *Marine drugs*, *11*(7), 2667-2681.

Xie, L., Xie, P., Li, S., Tang, H., & Liu, H. (2003). The low TN: TP ratio, a cause or a result of Microcystis blooms?. *Water Research*, *37*(9), 2073-2080.

Xin, L., Hu, H., Ke, G., & Sun, Y. (2010). Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus* sp. *Bioresource technology*, *101*(14), 5494–500. doi:10.1016/j.biortech.2010.02.016

Yang, S., & Jin, X. (2008). Critical light intensities for Microcystis aeruginosa, *Scenedesmus quadricauda* and *Cyclotella* sp. and competitive growth patterns under different light: N: P ratios.*Journal of Freshwater Ecology*, *23*(3), 387-396.

Yokono, M., Tomo, T., Nagao, R., Ito, H., Tanaka, A., & Akimoto, S. (2012). Alterations in photosynthetic pigments and amino acid composition of D1 protein change energy distribution in photosystem II. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1817*(5), 754-759.

Yoshioka, M., Yago, T., Yoshie-Stark, Y., Arakawa, H., & Morinaga, T. (2012). Effect of high frequency of intermittent light on the growth and fatty acid profile of Isochrysis galbana. *Aquaculture*, *338*, 111-117.

Young, E. B., & Beardall, J. (2003). Photosynthetic function in *Dunaliella tertiolecta* (*Chlorophyta*) during a nitrogen starvation and recovery cycle. *Journal of Phycology*, 39(5), 897-905.

Zehetmayer, P., Kupka, M., Scheer, H., & Zumbusch, A. (2004). Energy transfer in monomeric phycoerythrocyanin. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1608*(1), 35-44.

Zhang L, Niyogi KK, Baroli I, Nemson JA, Grossman AR, Melis A. 1997. DNA insertional mutagenesis for the elucidation of a Photosystem II repair process in the green alga *Chlamydomonas reinhardtii*. *Photosynth. Res.*53:173–84

Zhang, J. (1994). Atmospheric wet deposition of nutrient elements: correlation with harmful biological blooms in northwest Pacific coastal zones. *Ambio*, 464-468.

Zhang, J., Sun, Z., Sun, P., Chen, T., & Chen, F. (2014). Microalgal carotenoids: beneficial effects and potential in human health.*Food & function*, *5*(3), 413-425.

Zhang, S., Lim, C. Y., Chen, C. L., Liu, H., & Wang, J. Y. (2014). Urban nutrient recovery from fresh human urine through cultivation of *Chlorella sorokiniana*. *Journal of environmental management*, *145*, 129-136.

Zheng, Y., Li, T., Yu, X., Bates, P. D., Dong, T., & Chen, S. (2013). High-density fed-batch culture of a thermotolerant microalga *Chlorella sorokiniana* for biofuel production. *Applied Energy*, 108, 281-287.

Zhu, Y., Graham, J. E., Ludwig, M., Xiong, W., Alvey, R. M., Shen, G., & Bryant, D. A. (2010). Roles of xanthophyll carotenoids in protection against photoinhibition and oxidative stress in the cyanobacterium *Synechococcus* sp. strain PCC 7002.*Archives of biochemistry and biophysics*, *504*(1), 86-99.

Zingone, A., & Oksfeldt Enevoldsen, H. (2000). The diversity of harmful algal blooms: a challenge for science and management.*Ocean & Coastal Management*, *43*(8), 725-748.

Zondervan, I. (2007). The effects of light, macronutrients, trace metals and CO2 on the production of calcium carbonate and organic carbon in coccolithophores—A review.*Deep Sea Research Part II: Topical Studies in Oceanography*,*54*(5), 521-537.

Zou, D., & Gao, K. (2009). Photosynthetic acclimation to different light levels in the brown marine macroalga, *Hizikia fusiformis* (Sargassaceae, Phaeophyta). *Journal of Applied Phycology*, *22*(4), 395404. doi:10.1007/s10811-009-9471-4