Photosynthetic responses as a function of light and temperature: Field and laboratory studies on marine microalgae

Thesis for the degree philosophiae doctor

Trondheim, May 2007

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Preface and acknowledgements

My thesis is focused on light attenuation in the water column, light absorption by phytoplankton and photosynthesis in microalgae, as a function of temperature. It has been a great challenge and a valuable experience trying to grasp such a wide subject and put it into text with a clear structure. I have learnt a lot along the way and owe a thank you to a lot of skilled colleagues and kind friends.

I will like to sincerely thank my two supervisors Prof. Geir Johnsen and Prof. Egil Sakshaug for the opportunity to fulfil my PhD work at Trondhjem Biological Station (TBS) and for skilful guiding through the stormy waters of science. Geir deserves a dedicated thank you for his endless enthusiasm, countless ideas and significant inputs to my work and wonders. Egil, is especially thanked for his scientific questioning, and lectures on miscellaneous topics. It has been a pleasure (most of the time).

My PhD has been a part of the project ‘Carbon flux and ecosystem feedback in the northern Barents Sea in an era of climate change’ (CABANERA), headed by Prof. Paul Wassmann at the Norwegian College of Fishery Science, University of Tromsø. I will like to address a sincere gratitude to Paul and everybody involved in CABANERA for three educational cruises to the Barents Sea and for fruitful collaborations. My fellow PhD candidates involved in CABANERA deserves a special appreciation for the many discussions, workshop sessions and social activities that have served as an important source of inspiration and motivation during the project period.

Thanks are due to my co-authors for their interest in the work and for the rewarding collaboration. Especially, I will like to express my gratitude to Prof. Ronnie Glud at the Marine Biological Laboratory (University of Copenhagen, Denmark) for his long-distance supervision and considerable contribution to my achievements. At TBS especially Nils Tokle, Johanna Järnegren, Lasse Olsen, Jussi Evertsen, Mathilde Chauton and Sten Karlsson are thanked for numerous educational discussions on a range of subjects including scientific matters and the challenge of working within a
scientific environment. Nils Tokle, additionally, deserves dedicated thanks for introducing me to the secrets of after-dark cross-country skiing and for being a devoted skiing companion through countless trips, on all kinds of skis. Kjersti Andresen is thanked for HPLC analyses. Colleagues, staff and students at TBS are acknowledged for creating a pleasurable working environment at TBS.

At last I want to thank my family and friends for understanding and support, in particular my father for many fruitful discussions of the scientific process and comprehension.

Outstanding all others I want to thank my dear and wonderful wife, colleague and co-author Torunn B. Hancke. It has been a fantastic inspiring and great experience to work with you on both experimental work and through the writing of our two joint papers. More importantly, I want to thank you deeply for the patience and support you have offered during the entire, and especially final stages, of my PhD writing. Thea Emilie, my daughter of 19 month, you are simply wonderful and inspire me daily, never missing a change to amuse and cheer me up in a moody moment.

Funding for this study was provided by the Norwegian Research Council through CABANERA to the Norwegian University of Science and Technology (NTNU) and The University Centre in Svalbard (UNIS). The support is greatly acknowledged.

It is my hope that I through this thesis can contribute to our understanding of aquatic photosynthesis and to the comprehension of the important processes of primary production and its relevance in the Barents Sea. In light of the increased human activity in the Arctic region, an understanding of the ecosystem is becoming increasingly important.

Trondheim, March 2007 Kasper Hancke
List of papers

This thesis is based on the following papers, referred to by their respective numbers:


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**Papers 1 - 4**
1. Introduction

Photosynthesis has been of scientific interest since the mid eighteenth century (J. Priestly). Since then several Nobel Prizes have been given in photosynthesis-related research, from H. Fischer in 1930 (porphyrins and leaf pigments), M. Calvin (and his student A. Benson, CO2-assimilation in photosynthesis) in 1961, and R. Marcus for his contribution to the theory of electron transfer reactions in photosynthesis in 1992.

Photosynthesis supports the bulk of life on Earth and thereby underpins the biomass and biodiversity of the planet. Approximately 45 % of the photosynthesis each year occurs in aquatic environments (Falkowski 1994, Field et al. 1998). The Arctic region contributes considerably to the global primary production. The annual production of the Barents Sea is estimated to ~90 g C m\(^{-2}\) (Sakshaug 2004). In comparison the average for the world oceans is ~140 g C m\(^{-2}\) y\(^{-1}\) (Field et al. 1998). Irradiance and temperature are important variables controlling the rates of photosynthesis. This also pertains for respiration, which can be considered the opposite process. In temperate and arctic seas (including coastal shallow waters) both variables show marked seasonal and diurnal variation (Papers 1 & 4, Cahoon 1999, Glud et al. 2002, Sakshaug 2004).

Primary production is typically measured as O\(_2\)-evolution or \(^{14}\)C-assimilation, but can also be estimated using variable fluorescence as a proxy (Marra 2002). The techniques, however, measure different physiological processes with potentially different response to environmental variables such as light and temperature (Paper 3, Geider & Osborne 1992, Morris & Kromkamp 2003). Accurate estimation of the marine primary production is important on both local and global scale because primary production is a ‘cornerstone’ in marine food webs and in the ecosystem carbon budget. Primary production will inevitably be affected by climate change which is likely to alter sea temperature and irradiance (cloudiness and ice cover). Possible changes are suspected to be amplified in the Arctic (Sakshaug 2004, Holland et al. 2006).
My thesis focuses on the flux of photons, i.e. irradiance\(^1\) originating from the Sun, as it ‘travels’ down the water column, being absorbed by microalgae fuelling photosynthesis (Fig. 1.1). Each of the sections in this thesis presents an introduction to the subject in question, followed by a brief presentation of the relevant underlying theory, concluding with a review of my most important findings. The theory part is meant to review the underlying theories on which the papers are based, and to provide assistance in interpreting the results.

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\(^1\) Irradiance (denoted $E$, μmol photons m\(^{-2}\) s\(^{-1}\)) is the flux of radiant energy on a (small) surface, divided by the area of the surface, per time unit.
Fig. 1.1. A schematic illustration of the most important processes, ‘products’ and ‘costs’, which are yielded from solar energy: as the light penetrates into the water column, subsequently is absorb by microalgae, until the energy is bound as organic molecules, i.e. microalgae biomass, through the pathways of photosynthesis. The figure outlines the focus of the present thesis, as Paper 1 deals with water column attenuation and algae absorption in relation to primary production. Paper 2 deals with Photosystem II (PSII) and I (PSI) absorption, the corresponding electron transfer rate (ETR) and O$_2$ production in microalgae. Paper 3 then deals with O$_2$ production, ETR and carbon fixation as a function of irradiance and temperature in microalgae. Finally Paper 4 is a comparison study of the temperature response on photosynthesis and respiration between intact benthic microalgae-dominated communities from one arctic and two temperate sites.
2. Scope of my thesis

The aim of my thesis is to elucidate the different pathways of light in the marine environment, from underwater irradiance to the absorption of photons in microalgae. The pathway is followed through light harvesting and the subsequent electron transfer, to the fuelling of the photosynthetic process (Fig. 1.1, Papers 1, 2 & 3). In addition, the effect of temperature on photosynthesis and respiration in pelagic and benthic microalgae has been investigated (Papers 3 & 4). A novel approach to estimate the light absorption in Photosystem II (PSII) is evaluated in combination with Pulse Amplitude Modulated (PAM) fluorescence measurements, to calculate the rate of photosynthetic oxygen production (Paper 2). The approach was evaluated against measured rates of oxygen production and $^{14}$C-assimilation, as a function of temperature (Papers 2 & 3).

Paper 1 is an in situ study of water column processes in the Marginal Ice zone (MIZ) of the Barents Sea, Paper 2 & 3 are laboratory studies on culture-grown phytoplankton species, and Paper 4 is a comparison study of intact temperate and arctic diatom-dominated benthic communities from shallow-water sites.

The aims of the papers were:

1) to analyse the significance of spectral composition of irradiance in relation to the concentration and vertical distribution of chl $a$, dissolved oxygen and phytoplankton productivity in the water column. *Spectral attenuation is related to optical depth and discussed in a photo-physiological context, including the concentration and composition of phytoplankton pigments and productivity*

2) to determine the absolute rates of photosynthetic O$_2$ production from variable fluorescence (PAM) measurements by testing three bio-optical approaches to

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Throughout the thesis, the term ‘microalgae’ is used referring to both pelagic and benthic microalgae. ‘Phytoplankton’ or ‘microphytobenthos’ are used referring to pelagic or benthic microalgae, specifically.
estimate the light absorption in PSII, against measured O₂ production rates. A spectral-related approach using PSII-specific light absorption is recommended.

3) to investigate the relationship between temperature and photosynthetic parameters derived from measurements of 1) O₂-production by O₂-microsensors, 2) calculated rates of O₂-production based on variable fluorescence combined with bio-optical determined PSII absorption, and 3) measured rates of ¹⁴C-assimilation. The temperature influence on photosynthetic parameters is discussed in a physiological context.

4) to evaluate possible differences in the temperature adaptation strategy between arctic and temperate benthic microalgal-dominated communities, during short-term temperature incubation studies. The study includes rate measurements of the sediment community respiration, gross photosynthesis and net photosynthesis as determined from O₂ microsensor measurements in intact sediments.
3. Light regime in water columns and sediments

Sunlight is essential to primary producers being the energy source driving photosynthesis (Falkowski & Raven 1997). Light available for photosynthesis is referred to as photosynthetically active radiation (PAR) and includes radiation at wavelengths from 400 to 700 nm (Kirk 1994). The underwater light regime ultimately determines the vertical distribution, abundance and photosynthetic activity of phototrophic microalgae in the water column (phytoplankton) and in the benthic sediments (microphytobenthos) beneath. The Arctic light regime offers extreme seasonal variation, from midnight sun to winter darkness. Moreover, phytoplankton in the water column are subject to a strong vertical light gradient, which is amplified in the MIZ by the sea ice cover. The focus on light regime in the present thesis begins immediately beneath the sea surface. The variables that affect the light regime above the sea surface will, thus, not be treated further than mentioning that day length, zenith sun angle, cloud cover, albedo (i.e. the reflection of light) and ice cover in the Arctic and Antarctic, are major key variables (Sakshaug et al. 1989, Sakshaug & Slagstad 1992, Kirk 1994).

3.1. Downwelling irradiance and attenuation

Downwelling irradiance, \( E_d \) (in this work termed \( E \), since only downwelling irradiance is considered), in a water column diminishes in an approximately exponential manner with depth (Kirk 1994). This can be described as

\[
E_z = E_0 e^{-K_d z} \quad (3.1)
\]

where \( E_z \) and \( E_0 \) are the values of downwelling irradiance at depth \( z \) m and just below the surface, respectively, and \( K_d \, (m^{-1}) \) is the vertical diffuse attenuation coefficient for downwelling irradiance.

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3 Downwelling irradiance \( (E_d) \) is defined as the flux of photons received by a flat collector with a cosine response, facing upwards (Kirk 1994).
The attenuation of light in water is wavelength specific, having the highest attenuation in the long-waved red spectrum, subsequently decreasing with wavelength. Pure seawater is transparent mainly to blue light (clearest at 475 nm), followed by green light, and is nearly opaque to red light and UVB (Paper 1, Kirk 1994). With focus on $K_d$, the spectral attenuation for downwelling irradiance can be rewritten from equation 3.1 as

$$K_d(\lambda) = \frac{-\ln\left(\frac{E_0(\lambda)}{E_z(\lambda)}\right)}{z}$$

(3.2)

where $E_0(\lambda)$, $E_z(\lambda)$ and $K_d(\lambda)$ have a spectral distribution. Light is attenuated in the water column as a consequence of both absorption and scattering. The attenuation coefficient $K_d(\lambda)$ is thus related to the absorption and scattering by water molecules, chromophoric dissolved organic matter (cDOM), particulate organic and inorganic material, and the living plankton themselves (Sathyendranath et al. 2000). In clear oceanic water masses, $K_d(\lambda)$ is mainly influenced by the absorption and scattering of phytoplankton, by the sea water itself, and in some cases by marine cDOM (Case I waters), while terrigenous cDOM and suspended matter additionally influence the optical properties in coastal water masses and fjords (Case II waters, Jerlov 1976, Sathyendranath et al. 2000).

In the strictest sense, $K_d(\lambda)$ (as an apparent\(^4\) optical property) is dependent on the angular distribution of the light field and lacks the additive quality of inherent\(^3\) optical properties. Nonetheless, $K_d(\lambda)$ is often considered to be a ‘quasi-inherent’ optical property and treated as such, and is therefore commonly considered independent of the solar zenith angle (Smith & Baker 1978, Kirk 1994, Sosik in press), which is the case in this work (Paper 1).

In oceanic waters, typical $K_d$ values for PAR, $K_d(\text{PAR})$, are in the range of 0.03 to 0.10 m\(^{-1}\) measured during low chl $a$ concentrations ($<0.1$ mg m\(^{-3}\)), e.g. in the Sargasso Sea

\(^4\) The optical properties that govern the underwater irradiance regime are divided into so-called ‘inherent’ and ‘apparent’ properties. The former is independent of the solar zenith angle and includes properties of absorption and attenuation. The latter is dependent on solar angle, and includes backscatter and reflectance (Mobley 1994, Light and Water).
(Smith et al. 1989) and in the Pacific Ocean (Siegel & Dickey 1987). $K_{d(PAR)}$ of 0.07 m$^{-1}$ have been reported for ‘clearest’ Arctic waters during pre-bloom (Dalløkken et al. 1995). This implies that 1 % of the surface light reaches a depth of 66 m, assuming that $K_{d(PAR)}$ is constant down through the water column. In the Barents Sea, I obtained $K_{d(PAR)}$ values of 0.06 to 0.2 m$^{-1}$ in early bloom waters with [chl $a$] $<$1 mg m$^{-3}$, and of 0.3 to 0.5 m$^{-1}$ during peak bloom conditions with [chl $a$] $\sim$12 mg m$^{-3}$. This resulted in 1 % irradiance depths of $>90$ m during the earliest bloom conditions to $<12$ m during peak bloom (Paper 1). In practise, $K_{d(PAR)}$ is not constant with depth because of the spectral discrimination of attenuation and the distribution of e.g. phytoplankton.

Microalgae and photosynthetic algae in general, absorb light mainly in the blue to blue-green and red wavelength bands, while they are virtually transparent in the green-orange waveband. Thus, when a phytoplankton bloom develops, the available irradiance and hence the 1 % light penetration depth decreases more rapidly for blue light (400 – 500 nm) than for green-orange light (500 – 600 nm), turning the water greenish. This can clearly be observed from the spectral irradiance distribution, and the corresponding $K_{d(\lambda)}$, in water columns with low and high phytoplankton biomass, respectively, as illustrated in Fig. 3.1 (Paper 1). It follows that shading of the water column by phytoplankton is considerably more pronounced at 400 – 500 nm than is apparent from PAR data (Bricaud & Morel 1986, Nelson et al. 1993, Sakshaug 2004).

The attenuation of light in sediments is different than in the water column, as attenuation is strongest at 450 to 500 nm and decreases towards the longer wavelengths (Kühl et al. 1994). This results in a favoured red light penetration into sediments, caused primarily by scattering and reflection. The combination of scattering by sediment grains and high density of light-absorbing microalgae pigments leads to a strong light attenuation within sediments. Conclusively, the light availability within benthic microphyte communities is restricted and significantly smaller than the incident irradiance on the sea surface, even at shallow water habitats. As a consequence, photosynthetic activity in benthic sediments is limited to the upper sediment zone, usually a couple of millimetres at the most (Paper 4).
3.2. Optical depth

Phytoplankton biomass (e.g. chl \(a\)) and productivity are related to optical depth (Paper 1). The optical depth, \(\xi(\lambda)\), in a water column is defined from the vertical diffuse attenuation coefficient, and can be calculated for PAR and any wavelength band as (Morel 1988):

\[
\xi(\lambda) = K_d(\lambda)z
\]

Note that \(\xi(\lambda)\) is dimensionless as \(K_d(\lambda)\) is in m\(^{-1}\) and \(z\) is in m. Thus, the optical depth differs from the physical depth and is independent of this. A given optical depth will correspond to different physical depths in waters of different optical properties, yet to the same overall attenuation of irradiance (Kirk 1994). Thus, in a chl \(a\)-rich water
column, a given optical depth will correspond to a much shallower physical depth than in a chl $a$-poor water column.

Profiles of chl $a$, dissolved oxygen and primary production showed large natural variations in the Barents Sea. Yet the variables showed a trend of aligning by forming profiles with comparable curvatures when plotted as a function of optical depth for PAR, $\xi(PAR)$, instead of physical depth. If plotted as a function of the attenuation at 490 nm, $\xi(490)$ (blue light), uniformity in the curvature became increasingly clear (Paper 1, Behrenfeld & Falkowski 1997). **Conclusion, optical depth proved to be valuable in the attempt to describe the dynamics of chl $a$, dissolved oxygen and primary production in the water column of the MIZ in the Barents Sea (Paper 1).**

### 3.3. Spectral irradiance versus PAR: the relationship to primary production

Paper 1 shows that the accumulated\(^5\) chl $a$ concentration in the water column during bloom conditions in the Barents Sea is correlated with the optical depth, $\xi(PAR)$. Furthermore, I found that when the optical depth was calculated from a single wavelength at 490 nm, the coefficient of determination ($r^2$) increased from 0.41 to 0.50 (entire data set, Fig 3.2). Focusing on only the chl $a$-rich peak-bloom stations with [chl $a]$ $>$9 mg m\(^{-3}\), the correlation between accumulated chl $a$ and optical depth was close to 100 % ($r^2 = 0.99$, insert in Fig 3.2). *This shows that chl $a$, representing the phytoplankton biomass, correlates to the total light absorption down to an optical depth of $\sim$9, corresponding to $\sim$0.01 % of the surface irradiance at 490 nm (Paper 1).* These results are consistent with findings in the North Water Polynya, where chl $a$ and particulate organic carbon (POC) were the components that most influenced K$_{a(\lambda)}$, accounting for 36 to 83 % of the variance in light attenuation (Vasseur et al. 2003).

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\(^5\) The accumulated chl $a$ concentration (mg m\(^{-2}\)) was calculated from accumulating (summarising) trapezoidal integrated volumetric values for each measuring interval from the surface and down through the water column.
It is important to note that chl \( a \) is a biomass estimate and is therefore not directly correlated to rates of production. Hence, I analysed the relationship between downwelling irradiance and the chl \( a \)-normalised primary production rates. The results showed that the primary production was strongly related to optical depth, and hence the water column light regime. I concluded that the chl \( a \)-normalised primary production was closer related to the irradiance at 490 nm (blue light) than to PAR (Paper 1). The conclusion was supported when all data of chl \( a \)-normalised production rates were plotted as a function of downwelling irradiance for PAR, \( E_z(\text{PAR}) \), and at 490 nm, \( E_z(490) \), respectively (Fig. 3.3). The compiled data showed that 66% \( (r^2 = 0.66) \) of the variance...
in the normalised production could be explained by PAR (Fig 3.3a), while 81 % ($r^2 = 0.81$) could be explained from the downwelling irradiance at 490 nm (Fig 3.3b). A strong correlation between the irradiance at 490 nm and primary production is consistent with the average absorption spectrum for the identified dominating phytoplankton groups (Paper 1, Johnsen & Sakshaug in press) and illustrate that the phytoplankton community of the MIZ respond spectrally equivalent to temperate and tropical phytoplankton ecosystems (Bouman et al. 2000, Bricaud et al. 2004). In conclusion, by fitting chl a-normalised production rates to downwelling irradiance at 490 nm, instead of PAR, improved the correlation ~15 % (Paper 1). It follows, as mentioned in 3.1, that shading of the water column by phytoplankton is considerably more pronounced in blue light than for PAR. This is of relevance for modelling the 1 % irradiance depth and critical depth (see Paper 1 for details).

Fig. 3.3. Chl a-normalised primary production rates plotted as a function of available irradiance as a) PAR and b) at 490 nm in per cent of the immediate sub-surface irradiance. Data are compiled from 12 stations visited during summer months 2003-5 in the Barents Sea. Lines are linear regressions and the coefficient of determination ($r^2$) is given. Regression lines are forced through origo.
4. Light absorption in microalgae and Photosystem II (PSII)

This section includes a presentation of the absorption properties of microalgae and their light-harvesting and photo-protective pigments. The presentation includes the absorption properties of PSII and an evaluation of three bio-optical approaches to quantify the PSII-specific light absorption in microalgae.

4.1 Light absorption in microalgae

The rate of light absorption sets an upper limit for algal productivity, i.e. photosynthetic activity. The photosynthetic unit is composed of PSII, PSI and their respective light-harvesting complexes (LHC II and I, Green et al. 2003). The different pigments in LHC II and I, both chlorophylls and carotenoids (see section 4.2), have different absorption properties, and the bulk properties reflects a composite spectrum of the summed contributions from all absorbing molecules presented, i.e. $\phi_a(\lambda)$. The absorption properties of single-isolated pigments is generally well described and understood and can be used to identify and model microalgae absorption under both laboratory and field conditions (Johnsen et al. 1994a, Jeffrey et al. 1997a, Jeffrey et al. 1997b).

As mentioned earlier, light absorption in a water column is characterised as an inherent optical property, and as such holds properties of being additive. This means that, for a water sample containing a mixture of constituents, the absorption and scattering coefficients of the various constituents are independent. Thus, the total coefficient can be determined by summation. The total absorption, $a(\lambda)$ can then be calculated from the summarised absorption by sea water, $a_w(\lambda)$, phytoplankton $a_p(\lambda)$, cDOM, $a_{cDOM}(\lambda)$, and non-algal particles, $a_{nap}(\lambda)$ (Prieur & Sathyendranath 1981). The non-algal particles essentially include virus, heterotrophic bacteria and other heterotrophs, as well as debris from these organisms. In the open ocean, far from terrestrial influence, phytoplankton are generally the principle agents responsible for the optical properties of a water column (Morel & Prieur 1977, Morel 2006).
In the present study I measured absorption in laboratory-grown monocultures of phytoplankton to obtain the \textit{in vivo} chl $a$-specific absorption coefficient, $a_a^v(\lambda)$ (m$^2$ (mg chl $a$)$^{-1}$). The \textit{in vivo} absorption coefficient yields information about total absorption of photosynthetic and photo-protective pigments and reflects the photo-acclimation status of the algae (Paper 2 & 3, Johnsen & Sakshaug 1993).

4.2. Light harvesting and photo-protective pigments

The three main pigment classes that determine the bio-optical properties of algae are the chlorophylls (chl’s), the carotenoids and the phycobiliproteins (Johnsen et al. 1994b, Jeffrey et al. 1997b). The two major functions of microalgae pigments are light harvesting and photo-protection (Scheer 2003).

The chl’s and phycobiliproteins are involved mainly in light harvesting. The carotenoids play an import role both in light harvesting and in photo protection for degrading potentially damaging excess excitation energy to (mostly) harmless heat (Scheer 2003). The major light-harvesting carotenoids are fucoxanthin and the 19’-acyloxy-fucoxanthins, along with peridinin (specific for some dinophytes) and prasinoxanthin (specific for some Prasinophytes) (Sathyendranath et al. 1987, e.g. Johnsen et al. 1994b, Jeffrey et al. 1997b).

The major \textit{in vivo} absorption signature caused by the chlorophylls (chl $a$, $b$ and $c$) is in the blue (400 – 500 nm) and in the red (580 – 700 nm) regions of the PAR spectra. The major light-harvesting carotenoids absorb \textit{in vivo} mainly at 450 – 550 nm (Johnsen & Sakshaug in press, and references herein). Figure 4.1 illustrates the absorption of individual pigments and the effect of the photoprotective carotenoid diadinoxanthin in high and low light adapted cells of \textit{Prorocentrum minimum}. The general absorption maxima for light-harvesting and photo-protective carotenoids at 490 nm motivated the choice of 490 nm when relating primary production to a single wavelength (section 3.3, Paper 1, see also Fig. 4.2 and Paper 2) (Johnsen et al. 1994a, Johnsen et al. 1994b).
The composition and ratio of pigments and carotenoids can be used as chemotaxonomic markers for microalgae identification, and to elucidate the photo-acclimation status of algal cells (Johnsen et al. 1994b, Jeffrey et al. 1997b). This can be studied with HPLC (High Performance Liquid Chromatography) techniques, and important pigment-group markers can be used to differentiate between major phytoplankton groups since chlorophyll $c_3$ and 19'-acyl-oxy-fucoxanthins are major pigment markers for Haptophytes, chl $b$ and prasinoxanthin for prasinoxanthin-containing Prasinophytes, while a high fucoxanthin to chl $a$ ratio (w:w) indicates the presence of diatoms (Paper 1, Jeffrey et al. 1997a). As mentioned above, the different chl’s and carotenoids have absorption maxima at different wavelengths and thus $K_d$ (in Case I waters with low cDOM) will reflect the concentration and composition of phytoplankton pigment groups (Bricaud et al. 1988, Bricaud et al. 1998).

Fig. 4.1. Fractional unpacked absorption (obtained from a pigment model) of individual pigments and the effect of the photoprotective carotenoid diadinoxanthin in (A) high light- and (B) low light-adapted cells of *Prorocentrum minimum*. 1: total pigments; 2: photosynthetic pigments (total pigments minus diadinoxanthin); 3: chl $a$; 4: chl $c_2$; 5: peridinin; 6: diadinoxanthin (From Johnsen et al 1994a, MEPS 114:245-258, with permission).

4.3. Absorption in Photosystem II

During photosynthesis ~20% of the absorbed light is utilised in the photochemical process, while ~75 - 77% is lost as heat (thermal decay), and 3 - 5% is emitted as chl $a$ fluorescence of which about 95% arises from PSII (Owens 1991, Kirk 1994).
Fluorescence emission intensity (at a fixed wavelength) is dependent on the wavelength of the excitation light. By measuring fluorescence emission at 730 nm against a wavelength-specific excitation light, a fluorescence excitation spectrum\(^6\) can be obtained (Blankenship 2002). The shape of the fluorescence excitation spectrum resembles that of the corresponding action spectrum for oxygen, as well as arises from PSII, and thus represents the fraction of light received by PSII (Haxo 1985, Neori et al. 1988). The distribution of light absorption between PSII and PSI is pigment-group specific; this is also the case for the fluorescence excitation spectrum caused by the cell composition of chl’s and carotenoids (Johnsen & Sakshaug in press).

From a theoretical viewpoint, Johnsen et al. (1997) suggested that the PSII-specific light absorption for photosynthesis can be calculated by scaling the \textit{in vivo} fluorescence excitation spectrum to the \textit{in vivo} absorption spectrum, \(a^*_p(\lambda)\), by the ‘no-overshoot’ procedure (Fig 4.2, Paper 2). By matching the fluorescence spectra to \(a^*_p(\lambda)\) between 540 and 650 nm, assuming a 100 % energy conversion efficiency, the obtained spectrum equals the PSII absorption spectrum, \(F^\text{PSII}_p(\lambda)\) (Johnsen et al. 1997). In contrast to \(a^*_p(\lambda)\), the \(F^\text{PSII}_p(\lambda)\) does not include the signatures from photo-protective carotenoids and PSI (Johnsen & Sakshaug 1993, in press).

\(^6\) A plot of the intensity of fluorescence emission at a fixed wavelength versus the wavelength of excitation is called a fluorescence excitation spectrum (Haxo 1985).
Fig. 4.2. *In vivo* absorption (thick line) and PSII-scaled fluorescence excitation (thin line) spectra for the dinoflagellate *P. minimum* (upper panel), the haptophyte *P. parvum* (middle panel) and the diatom *P. tricornutum* (lower panel). The fluorescence excitation spectrum was scaled to the absorption spectrum by the ‘no-overshoot’ procedure, to estimate the light absorption by PSII. The difference spectra (dotted line) were obtained by subtracting the excitation from the absorption spectra and hence denote the light absorption by PSI and photoprotective pigments.

The amount of photons absorbed by PSII, $\bar{a}_{\text{PSII}}$, was computed by spectrally weighting $F_{\text{PSII}}(\lambda)$ against the incubator light source according to eq. 4.1, as illustrated in Fig. 4.3.
\[
\bar{a}_{\text{PSII}} = \frac{\sum_{400}^{700} F_{\text{PSII}}(\lambda) \cdot E(\lambda) \, d\lambda}{E(\text{PAR})}
\]  

(4.1)

where \(E(\lambda)\) is the spectral irradiance of the incubator light source and \(E(\text{PAR})\) is the integrated irradiance from 400 to 700 nm (Paper 2 & 3).

Fig. 4.3. An illustration of the calculation of the light absorption by PSII, \(\bar{a}_{\text{PSII}}\). The \textit{in vivo} fluorescence excitation spectrum was scaled to match the \textit{in vivo} absorption spectrum by the ‘no-overshoot’ procedure (as in Fig. 4.2). The light absorption by PSII equals the shaded area, which is obtained by spectrally weighting (eq. 4.1) the scaled excitation spectrum against the spectral irradiance of the incubator light source \((E(\text{PAR}) = 2 \, \mu\text{mol photons m}^{-2} \text{s}^{-1})\). Data are from Papers 2 & 3.

Most studies dealing with PSII absorption for measurements of photosynthesis assume that the light absorption by PSII and PSI, respectively, is divided equally giving a ratio of 0.5 (e.g. Schreiber et al. 1986, Kolber & Falkowski 1993, Gilbert et al. 2000). However, this imposes an error as the distribution of chl \(a\) between PSII and PSI has a
The distribution of chl $a$ between PSII and PSI is pigment-group specific and related to the light-harvesting complex and the distribution of chl $a$ and pigments within the cell (Johnsen & Sakshaug in press). Chromophytes, the algae class I worked with, has an average PSII to PSI ratio of 0.72, as recently found by Johnsen & Sakshaug (in press). This is in agreement with the PSII to PSI ratio of 0.75 to 0.82 reported in Paper 2.

In Paper 2, we tested the ‘no-overshoot’ approach to calculate the fraction of light received by PSII in absolute units. To evaluate the practical implications of this theoretical approach, the outcome was tested along with two other commonly applied bio-optical approaches for estimating light absorption in PSII (Paper 2, Kromkamp & Forster 2003, Johnsen & Sakshaug in press). The results were then applied in combination with measurements of the quantum yield for PSII to obtain rates of photosynthetic O$_2$ production from PAM measurements.

**4.4. Evaluating three bio-optical approaches to estimate the light absorption in PSII**

In Paper 2, we tested three bio-optical approaches to estimate the fraction of light absorbed by PSII. These estimates were to be used in combination with the operational quantum yield for PSII, derived from PAM measurements, to calculate rates of O$_2$ production. The three approaches were: 1) the factor 0.5 which implies that absorbed light is equally distributed among PSI and PSII, 2) the fraction of chl $a$ in PSII, determined as the ratio between the red-peak ratios between PSII-scaled fluorescence excitation and the corresponding absorption spectrum (Fig. 4.3) and 3) the measure of light absorbed by PSII, determined from the scaling of fluorescence excitation spectra to absorption spectra by the ‘no-overshoot’ procedure (Fig. 4.2). By calculating photosynthesis vs. irradiance (P vs. E, see box 5.1) parameters using the three approaches, we compared the results against simultaneously measured rates of oxygen production.

---

Cyanobacteria, however not microalgae, represent an important group of phototrophs with the major part of chl $a$ associated with PSI, giving a ratio between PSII and PSI of ~0.12 (Johnsen & Sakshaug 1996).
production. Generally, approach 1) underestimated while approach 2) overestimated the gross O₂ production rate. **In conclusion, approach 3 gave the best approximation to estimate quanta absorbed by PSII.** Hence, we recommend approach 3) for estimation of gross O₂ production rates based on PAM fluorescence measurements (Paper 2).
5. Photosynthesis and respiration

This section includes a brief presentation of the fundamental theories in photosynthesis and respiration underlying my initial interest for studying these processes by applying different methodological approaches. The introduction is meant to provide essential information on the subject and to assist the understanding of the papers included in this thesis. The most important findings from the comparison of variable fluorescence measurements and O₂ production measurements for studying photosynthesis are presented towards the end of the section. Section 6 reviews the achieved results concerning temperature effects on photosynthesis and respiration.

5.1. Photosynthesis

Photosynthesis is the process of capturing radiation energy from the sun and transforming it into chemically bound energy (Fig. 1.1). The processes of photosynthesis are responsible for the energy supply in the formation of organic carbon compounds and for the metabolism in primary producers. The overall oxygenic\textsuperscript{8} photosynthetic process can be represented as (Falkowski & Raven 1997):

\[
2H_2O + CO_2 \xrightleftharpoons{\text{sunlight}} (CH_2O) + H_2O + O_2 \quad (5.1)
\]

The photosynthesis process within the chloroplasts can be divided into two parts: the ‘light reactions’ and the ‘dark reactions’.

The light reactions can be described by the formula:

\[
2H_2O + \text{Light} \xrightarrow{\text{LHP} + \text{Chla}} 4H^+ + 4e^- + O_2 \quad (5.2)
\]

and is the process in which light energy, via chl a, is used to withdraw hydrogen from water to generate electrons, and liberate oxygen. In this process, chl a fluorescence is emitted when the excited electrons decay to the ground state. The reactions drive the ATPase and take place in the thylakoid membranes.

\textsuperscript{8} The photosynthetic process can, additionally to oxygenic, be carried out during anoxic condition by exchanging the oxygen in eq. 5.1 by e.g. an atom of sulphur to run anoxic photosynthesis. Most photosynthetic bacteria, with exception of cyanobacteria and prochlorophytes, are obligate anaerobes. In the present thesis, the term photosynthesis will address only the oxygenic process.
The dark reaction responsible for the reduction of CO₂ can be described as:

\[
CO_2 + 4H^+ + 4e^- \xrightarrow{\text{enzymes}} CH_2O + H_2O
\]  

(5.3)

and is the process in which the ATP (and NADPH₂), produced from the light reactions, are used for reduction of CO₂ to form carbohydrates in the Calvin cycle. This reaction takes place in the stroma of the chloroplasts.

In general, the light reactions have a low or zero temperature coefficient, limited almost solely by the available irradiance, as being truly photochemical reactions (Emerson & Arnold 1932, Hall & Rao 1999, Paper 3). The dark reactions are highly temperature sensitive as characteristic for enzymatic reactions. It is therefore the dark reactions that set the limitations in the case of low temperatures (Davison 1991, Falkowski & Raven 1997, Paper 3).

The essential processes of photosynthesis are summarised in Fig. 5.1, including the important products and pathways of the light and dark reactions. Even though the
theoretical bases of the respective techniques are reasonably well understood, the relationship between the variable fluorescence kinetics, the O₂ production and the ¹⁴C-assimilation is not completely understood. Nor are the exact effects of temperature on the different process steps understood, which I will come back to in section 6.

**Box 5.1: Photosynthesis versus irradiance curves (P vs. E curves)**

Photosynthetic rates are related to irradiance in a non-linear fashion. To parameterise this relationship, the photosynthesis versus irradiance curve (P vs. E curve) is defined, and is typically divided into three distinct regions: 1) a light-limited region, 2) a light-saturated region, and 3) a photo-inhibited region (Falkowski & Raven 1997, Sakshaug et al. 1997).

1) In the light-limited region, irradiance levels are low and the photosynthetic rate is linearly proportional to the irradiance, as the rate is limited by the rate of photon absorption. The light-limited, initial slope of the P vs. E curve is termed the ‘maximum light utilisation coefficient’, α.

2) As irradiance increases, photosynthetic rates increase towards a saturation level, at which the rate of photon absorption exceeds the rate of electron transport in the Photosystems. The light-saturated region of the P vs. E curve is termed the ‘maximum photosynthetic rate’, P_{max}.

3) The photo-inhibited region described by the parameter β is not included in the figure.

The light saturation parameter, E_k, describes the relationship between P_{max} and α and is calculated as P_{max}/α.

Several equations have been proposed to fit the P vs. E relationship (e.g. Webb et al. 1974, Jassby & Platt 1976, Platt et al. 1980). The figure illustrates a fit by the Webb et al. (1974) equation, including α and P_{max}.

The Web equation was chosen for studies in this thesis because photosynthesis was studied at irradiance below the photo-inhibited region only (Papers 2 + 3).
5.2. Respiration

The reverse reaction of photosynthesis is oxidative respiration\(^9\). This process is the breaking of the high-energy bonds of carbohydrates in an oxidative reaction, supplying energy for metabolism. Both phototrophic and heterotrophic organisms carry out respiration. Whereas photosynthesis proceeds only during periods with sufficient irradiation, respiration is carried out during both light and dark conditions (Raven & Beardall 1981, Hall & Rao 1999).

The respiration rate in phototrophs can be divided into two parts: 1) ‘dark respiration’ which is the metabolic respiration of a cell, phototroph or heterotroph, independent of the electron transport of photosynthesis. Dark respiration is thus, in principle, independent of photosynthetic activity. However, even uncoupled from photosynthetic activity it might be enhanced by the rate of photosynthesis, as a response to a generally enhanced metabolism (Markager et al. 1992, Epping & Jørgensen 1996). Experimentally, dark respiration is very difficult to isolate from the photorespiration under illumination (Raven & Beardall 1981). 2) ‘Photorespiration’ is the 'extra' oxidative respiration, in addition to dark respiration, and is closely linked to photosynthetic activity. Photorespiration is divided into two reactions, the Mehler\(^{10}\) reaction and the oxygenase reaction of RuBPc (ribulose 1,5-bisphosphate carboxylase) (Raven & Beardall 1981, Falkowski & Raven 1997).

5.3. Measuring photosynthesis: three methodological approaches

As seen from the theory above, photosynthesis can be estimated from the variable fluorescence kinetics of PSII, from the rate of O\(_2\) production and from the rate of \(^{14}\)C-assimilation (Paper 3). Each of these methods has its advantages and disadvantages and

---

\(^9\) As with photosynthesis, respiration can also be anoxic. In anoxic respiration, organic molecules are oxidised by an electron accepter other than O\(_2\), e.g. nitrate or sulphate. In this thesis, the term respiration refers to the oxygenic process only.

\(^{10}\) The Mehler reaction, also called pseudocyclic electron transport, involves an electron transport sequence where the O\(_2\) produced at PSII is reduced again at PSI. Consequently, there is no net production of O\(_2\). The process leads to formation of ATP, but not NADPH\(_2\).
have all been applied to access the ecosystem primary production in various environments. The techniques, however, measure different products of the photosynthetic pathway and reflect different physiological processes with potentially different responses to environmental variables, such as temperature (Paper 2 & 3, Geider & Osborne 1992, Geel et al. 1997, Morris & Kromkamp 2003).

Below is a brief presentation of the three measuring techniques, ordered downstream according to the electron flux of the photosynthetic pathway. In the following section, the outcome of the three techniques will be compared.

**Variable fluorescence measurements**

Variable fluorescence from PSII can be measured by e.g. Pulse Amplitude Modulated (PAM) fluorometry and can be used to estimate the operational quantum yield\(^{11}\) of PSII, \(\Phi_{\text{PSII}}\) (Schreiber et al. 1986). The electron transfer rate (ETR, from PS II to PS I) can be quantified from \(\Phi_{\text{PSII}}\) times the absorbed quanta in PSII, as a proxy for the gross photosynthetic rate (Paper 2 & 3, Genty et al. 1989, Kroon et al. 1993). The electrons generated in PSII are closely coupled to the O\(_2\)-evolution, and subsequently follow several pathways, among those the reduction of CO\(_2\) via NADP(H) production (Falkowski & Raven 1997). The PAM technique is fast and non-invasive and can thus yield measurements of photosynthesis with a high temporal and spatial resolution.

In this study, the operational quantum yield of PSII, \(\Phi_{\text{PSII}}\), was calculated from steady-state fluorescence before \((F_s)\) and after exposing the sample to a saturating light pulse \((F_m')\), during actinic illumination by the PAM technique (Eq. 5.4, Genty et al. 1989).

\(^{11}\) The quantum yield is defined as the ratio of moles of product to the moles of photons absorbed in a photochemical reaction (Falkowski & Raven 1997). Thus, the operational quantum yield of PSII, \(\Phi_{\text{PSII}}\), is mol electrons generated in PSII to mol photons absorbed. Likewise, is the quantum yield for O\(_2\), \(\Phi_{\text{O}_2}\), mol O\(_2\) produced to mol photons absorbed. The inverse of the quantum yield \((1/\Phi)\) is called the ‘quantum requirement’, i.e. mol photons absorbed per mol product formed. Because of an inevitable energy loss in the photochemical reactions, the quantum yield is always <1, while the quantum requirement is >1.
The maximum quantum yield, $\Phi_{\text{PSII}}\text{max}$, was calculated in a similar way on dark acclimated (~15 min) cells. See Papers 2 & 3 for a detailed methodological description.

$$
\Phi_{\text{PSII}} = \frac{\Delta F/F_m}{F_m} = \frac{F_m' - F_s}{F_m}
$$

In combination with knowledge of the chl $a$-specific light absorption in PSII (section 4.3), measurements of $\Phi_{\text{PSII}}$ can be used to estimate the photosynthetic rate of gross O$_2$ production, $P_{\text{PSII}}$, as from eq. 5.5 (Kroon et al. 1993);

$$
P_{\text{PSII}} = \Phi_{\text{PSII}} \cdot E \cdot \Gamma \cdot a_{\text{PSII}}
$$

where $\Gamma$ is the stoichiometric ratio of oxygen evolved per electron generated at PSII.

Usually, according to theory of the standard Z-scheme of photosynthesis, $\Gamma$ is assumed to equal 0.25 O$_2$ electrons$^{-1}$ (for PSII, Kroon et al. 1993, Gilbert et al. 2000). However, a lower ratio is usually found when studied empirically (Paper 2 & 3, Kromkamp et al. 2001, Longstaff et al. 2002). For simplicity, I initially assumed $\Gamma$ to be 0.25 in the present study (see section 6.1 and Paper 3 for a discussion on the divergence between the theoretical and empirical ratio).

**Dissolved oxygen measurements**

Measuring the rate of photosynthesis in phytoplankton using concentration changes of dissolved O$_2$ was first proposed by Gaarder & Gran (1927), who invented the light-dark bottle technique. They calculated the concentration of dissolved O$_2$ using the Winkler titration technique (Strickland & Parsons 1968). With the development of the O$_2$-electrode, measurements of dissolved O$_2$ have become faster and possible to apply during incubation experiments. The fast responding and signal-stable Clark type O$_2$-microelectrode (Revsbech 1989) has been widely applied in aquatic science, and allows for continuous measurements of net O$_2$-production in the light, and O$_2$-respiration in the dark (for a review see Glud et al. 2000).

In oxygenic photosynthesis, the term ‘gross photosynthesis’ refers to the rate of oxygen evolution equivalent to the photochemically generated electron flux produced from the oxidation of water, excluding any respiratory losses (Sakshaug et al. 1997). ‘Net photosynthesis’ in the present work is defined as the net evolution of oxygen following
all respiratory losses within the investigated system (i.e. both autotrophic and heterotrophic respiratory oxygen consumption).

All measurements of O₂ production and consumption rates in this study were performed using Clark-type O₂ microelectrodes (Revsbech 1989) with a fast response (90 % response in <10 s for net production/consumption and <0.5 s for gross production measurements), small tip size (external diameter <1 mm) and low stirring sensitivity (<3 %).

Photosynthetic gross O₂ production can be measured in benthic sediments by the light/dark shift method (Paper 4, Revsbech & Jørgensen 1983, Glud et al. 1992). However, because of a much lower biomass per volume, this method has not yet been successfully applied on water samples. In sea water, gross O₂ production can be measured by spiking the water samples with ¹⁸O-labelled water and measuring the amount of ¹⁸O-labelled O₂ produced photosynthetically (Bender et al. 1987). Alternatively, gross production can be estimated from correcting the net O₂ production rates for respiration. The ¹⁸O-labelling method unequivocally measures gross primary production (i.e. there are no respiratory losses of the labelled O₂), while the latter method will lead to gross production being underestimated if respiration in the light is significantly different from respiration in the dark. In the present study, gross O₂ production was measured by the light/dark shift technique in sediments (P_{gross}, Paper 4) and estimated from the net production and dark respiration rates in the studied phytoplankton cultures (P_{O₂}, Paper 2 & 3). Net O₂ production was measured from concentration profiles (P_{n}, Paper 4) and from net changes of the O₂ concentration over time in phytoplankton samples (Paper 2 & 3).

1⁴C-assimilation measurements
The ¹⁴C technique was developed by Steemann-Nielsen (1952) and has probably been the most widely used method in aquatic science for estimating primary production, because of its high sensitivity allowing measurements on low biomass. The method quantifies the rate of ¹⁴C-assimilation and hence the conversion of inorganic C into cell biomass. It reflects an activity intermediate to net and gross photosynthesis, dependent
on the incubation time (Lewis & Smith 1983, Falkowski & Raven 1997, MacIntyre et al. 2002). For 1 hour incubations, the technique is, for convenience, commonly assumed to indicate gross rates ($P_{14C}$). This method, however, is labour-intensive and the quantum yield of carbon fixation varies according to the intermediate steps in photosynthesis, environmental variables and growth phase of the cells (Paper 3, Kroon et al. 1993). As a consequence, models of primary production based on the $^{14}$C method can be inaccurate (Prézelin et al. 1991, Schofield et al. 1993, Kroon et al. 1993).

5.4. Comparing PSII fluorescence and oxygen production

Photosynthetic O$_2$-production, $\Phi_{\text{PSII}}$ and/or $^{14}$C-assimilation have been compared in a number of studies of macroalgae, microphytobenthos, and marine phytoplankton (e.g. Geel et al. 1997, Barranguet & Kromkamp 2000, Longstaff et al. 2002). Although the investigations have been conducted under a variety of experimental conditions, a preponderance of the studies on microalgae find a linear relationship between O$_2$-evolution and $\Phi_{\text{PSII}}$ under moderate irradiance, sometimes with deviation at very low or very high irradiance conditions (e.g. Schreiber et al. 1995, Flameling & Kromkamp 1998). Different explanations for the deviation have been proposed: spectral difference in PAR sources, changes in O$_2$-consumption in the light, cyclic electron transport around PSII and Mehler-type reactions, see Flameling and Kromkamp (1998) for an overview.

In my studies at moderate irradiances below the photoinhibited levels, the relationship between rates of measured ($P_{O2}$) and calculated O$_2$ production ($P_{PSII}$, from PAM and PSII absorption, eq. 5.5) showed approximately linear responses ($r^2 = 0.7–0.97$, Fig. 5.2, Paper 2). As seen from Fig. 5.2, the linear response of $P_{PSII}$ versus $P_{O2}$ showed species-specific slope coefficients for the three microalgae species investigated. Where the diatom ($P. tricornutum$) tended to show a slope coefficient close to unity, $P_{PSII}$ tended to underestimate the O$_2$ production, compared to $P_{O2}$, for the dinoflagellate ($P. minimum$) and overestimate $P_{PSII}$ for the haptophyte ($P. parvum$). The divergence in the slope coefficient was presumably caused by a lower quantum yield for O$_2$, $\Phi_{O2}$, and hence a stoichiometric ratio lower than the 0.25 theoretically assumed for the calculation of $P_{PSII}$.
(eq. 5.4) of oxygen evolved per electron generated at PSII. A careful discussion of this subject is found in Paper 2 & 3.

![Graph showing the relationship between PPSII and PO2 for different species of algae.](image)

Fig. 5.2. Rates of O2 production calculated from $\Phi_{\text{PSII}}$ in combination with $\tilde{a}_{\text{PSII}}$, PPSII, as a function of measured O2 production, P02, for P. minimum, P. parvum and P. tricornutum. The dashed line represents $x = y$ (Paper 2).

The PAM and the O2-microelectrode techniques have their limitations and strengths in terms of sensitivity and noise. In low lights $\left( E < E_k \right)$, the electron transfer rate (ETR) is relatively robust and thus the estimation of $\alpha$ from the PAM technique. Conversely, the microelectrode technique is working near the detection limit, thus yielding a low
accuracy for $\alpha$. In high light ($E > E_k$), the accuracy of the results from the PAM and the O$_2$-microsensor technique, respectively, are the opposite of that for low light ($E < E_k$). As the ratio of $\Phi_{\text{PSII}}$ to $E$ decreases with increasing irradiance the accuracy becomes weak. In contrast, the signal-to-noise ratio of the O$_2$-microsensor increases with increasing irradiance, yielding more reliable results under high light conditions.
6. Temperature effects on photosynthesis and respiration

The present thesis contains two distinctly different approaches to studying temperature effects on processes of photosynthesis and respiration. Paper 3 is a study on laboratory-grown monocultures of pelagic phytoplankton, and Paper 4 is a study on intact diatom-dominated benthic communities, sampled at shallow water. However, both papers are based on physiological response studies in microalgae, and the associated heterotrophic community, imposed by short-term (minutes to hours) temperature experiments. Laboratory-grown cultures of phytoplankton allow for detailed investigations of temperature-imposed responses on light-saturated and light-limited rates of photosynthesis (P vs. E relationship), where intact sediment samples with microphytobenthos allow for ecological relevant, intact-community temperature-response studies. This section contains a review of the achieved results on temperature effects on light-saturated and light-limited photosynthesis, followed by an introduction to the results of the benthic community study. Section 6.4 summarises the ecosystem implications of the obtained results.

6.1. Temperature effects on light-saturated photosynthesis

Calculated and measured O₂-production rates along with ¹⁴C-assimilation rates showed overall the same relative response to a short-term temperature change for all the three phytoplankton species studied (Paper 3). The maximum photosynthetic rate, \( P_{\text{max}}^{C} \), increased with temperature, resulting in an average Q_{10} of 2.1 ± 0.2 (mean ± S.E.). The Q_{10} values showed only small variance between methods and species. This demonstrated that \( \Phi_{\text{PSII}} \) from intact algae cells responded similarly to the rate of O₂-evolution and ¹⁴C-assimilation, to a short-term temperature change. This is consistent with the hypothesis that the overall rate-limiting reaction for light-saturated photosynthesis is carbon fixation rather than electron transport, as suggested by Sukenik et al. (1987). For the present data, this implies that \( \Phi_{\text{PSII}} \) as well as the O₂-production

\[ P_{\text{max}}^{C} \]

The \( 'C' \) on \( P_{\text{max}}^{C} \) denotes that the parameter was normalised to the particulate organic carbon (POC) content of the sample investigated. Likewise, '***' denotes normalisation to the chl \( a \) content.
may be limited by carbon-fixing enzyme activity, i.e. the Rubisco-complex. In addition, the data suggest that rates of $\Phi_{\text{PSII}}$ and $O_2$-production driven by the light reactions were not different from rates of $^{14}C$-fixation, driven by the dark reaction, as a function of short-term temperature changes (Paper 3). Conclusively, the PAM technique, analogous

---

**Box 6.1: Temperature and Q_{10} (temperature coefficient)**

Temperature in an important environment variable for understanding the physiological ecology of microalgae in nature, as it affect key biological processes, including photosynthesis, enzymatic activity and respiration (Davison 1991).

**Calculation of Q_{10}**

Temperature-imposed activity changes are often quantified by the so-called ‘Q_{10} factor’, describing the relative rate of increase for a temperature increase of 10 °C. The temperature response of a given process can be calculated from the apparent activation energy ($E_a$, kJ·mol$^{-1}$) and Q_{10} then from $E_a$. $E_a$ can be calculated from the initial linear slope of an Arrhenius plot where $\ln(k)$ is plotted as a function of temperature $(R\cdot T)^{-1}$, according to Raven and Geider (1988) as:

$$
\ln(k) = \ln(A) + \left[ -E_a \left( \frac{RT}{K} \right)^{-1} \right]
$$

where $k$ is the rate of the reaction, $A$ is the Arrhenius constant, $R$ is the gas constant (8.3144 J·K$^{-1}$·mol$^{-1}$) and $T$ is the absolute temperature (K).

$Q_{10}$ is then calculated for a given temperature interval of interest as (Berry & Bjorkman 1980):

$$
Q_{10} = \exp \left( E_a \cdot 10 \left( \frac{RT}{T + 10} \right) \right)
$$

All $Q_{10}$ values in the present study are calculated from Arrhenius plots, according to the above equation. In the literature, $Q_{10}$ is sometimes alternatively calculated from a more simple equation, which is strictly exponential, as:

$$
Q_{10} = \left( \frac{r_2}{r_1} \right)^{10/(t_2-t_1)}
$$

where $t_1$ and $t_2$ are the lower and upper temperatures of the range of consideration, and $r_1$ and $r_2$ are the metabolic rates corresponding to $t_1$ and $t_2$, respectively (Davis & McIntire 1983).

**Acclimation versus adaptation**

Temperature *acclimation* usually describes phenotypic changes in a community as a response to short-term temperature change, whereas temperature *adaptation* involves genetic differences in metabolism between communities from different thermal environments (Berry & Bjorkman 1980, Davison 1991).
to O$_2$-production and $^{14}$C-assimilation measurements, can be applied to study relative temperature responses of light-saturated photosynthesis.

Overall, the absolute rates of calculated O$_2$-production, P$_{\text{PSII}}$ (based on $\Phi_{\text{PSII}}$, Eq. 5.5), showed a species-specific correlation to and overestimated the measured O$_2$-production rates of ~1 to 3 times, for light-saturated photosynthesis (Fig. 6.1). As I have shown, $a_{\text{PSII}}$ is a good measure for the light absorption in PSII (section 4.3 & 4.4, Papers 2 & 3). Hence, I suggest that the off-set of the $\Phi_{\text{PSII}}$ based measurements (P$_{\text{PSII}}$) is caused by a lower quantum yield for O$_2$-production than the theoretical maximum, and thus the amount of O$_2$ evolved per electron generated in PSII ($I$) is lower than the commonly assumed 0.25 (Paper 3, e.g. Kroon et al. 1993, Suggett et al. 2004). The lower quantum yield for O$_2$-production can possibly be ascribed to irradiance induced cyclic electron transport around PSII, Mehler reactions (Flameling & Kromkamp 1998, Longstaff et al. 2002), and to the difference between the rates of metabolic respiration (‘dark’ respiration) during light and dark conditions, respectively (section 5.2). The off-set of the $\Phi_{\text{PSII}}$ based measurements seemed to be insensitive to temperature (Paper 3).

![Fig. 6.1. Effect of temperature on the absolute values for the maximum photosynthetic rate ($P_{\text{max}}$). The photosynthetic parameters were calculated from rates of measured O$_2$-production ($P_{O2}$, filled circles), $\Phi_{\text{PSII}}$ ($P_{\text{PSII}}$, eq. 5.5, open diamonds), and $^{14}$C-assimilation ($P_{14C}$, grey triangles). The three pelagic algae species were grown at 15 °C and 80 $\mu$mol photons m$^{-2}$ s$^{-1}$ (Paper 3).](image-url)
6.2 Temperature effects on light-limited photosynthesis

The *relative* and *absolute* values of $\alpha^C$ showed an analogous response to a short-term temperature change and showed itself to be insensitive to (*P. minimum*), or possibly slightly decreasing (*P. parvum* and *P. tricornutum*), with increasing temperature resulting in average $Q_{10}$ of $1.0 \pm 0.2$ (mean $\pm$ S.E.). Based on a statistical test of covariance (ANCOVA) I concluded that the temperature response for the three methods was the same for all three species (Fig. 6.2, Paper 3). *The absolute values of $\alpha^C$ demonstrated an off-set of $\alpha^{\text{PSII}}_C$ compared to $\alpha^{O_2}_C$ and $\alpha^{14C}_C$ which was constant for the entire temperature range, arguing for a linear temperature-insensitive relationship between rates obtained from the three methods, in the light limited part of the $P$ vs. $E$ curve. The off-set in the light-limited region was similar to the off-set of $P^{\text{PSII}}_C$ in the light-saturated region (Fig. 6.1) and hence I concluded that the off-set was general for the $\Phi^{\text{PSII}}_C$ based $O_2$-production rates ($P^{C}_{\text{PSII}}$), for the entire irradiance range (Paper 3).*

The possible decrease of $\alpha^C$ with temperature for *P. tricornutum* is explained by an apparent decrease of the chl $a$ to C ratio, as $\alpha^C$ (carbon-specific) is often correlated to this ratio because light absorption is correlated with chl $a$ (MacIntyre et al. 2002).

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**Fig 6.2.** Effect of temperature on the absolute values for the maximum light utilization coefficient ($\alpha^C$). Symbols and calculations as in Fig 6.1 (Paper 3).
6.3 Temperature effects on intact benthic microphyte communities

Studies at subtidal and intertidal sites have shown that temperature can exert tight control on benthic photosynthetic rates, and can lead to seasonal temperature acclimation and/or change in the microphyte community composition (e.g. Grant 1986, Barranguet et al. 1998). In Paper 4, I studied the short-term temperature effects on respiration and photosynthesis in intact diatom-dominated benthic communities collected at two temperate and one high-arctic subtidal sites, to resolve a potential adaptation strategy (Fig. 6.3). Areal rates of both total (TOE) and diffusive (DOE) O$_2$ exchange were determined from O$_2$-microsensor measurements in darkness and at 140 $\mu$mol photons m$^{-2}$ s$^{-1}$. In darkness, the O$_2$ consumption increased exponentially with increasing temperature for both TOE and DOE with $Q_{10}$ ranging between 1.7 and 3.3. Overall, $Q_{10}$ was not correlated to the \textit{in situ} water temperature or geographical position. Accordingly, no difference in the temperature acclimation or adaptation strategy of the microbial community was observed (see Paper 4 for details). Gross photosynthetic rates increased with temperature yielding $Q_{10}$ in the range of 2.2 to 2.6. However, no temperature adaptation was observed between the sites. The present study shows that increasing temperature stimulates the heterotrophic activity more than gross photosynthesis does. \textit{Consequently, the typically mixed benthic community of heterotrophic and phototrophic microbes gradually turns heterotrophic with increasing temperature. In conclusion, no difference in the temperature acclimation response between the sites was observed, suggesting that the temperature adaptation strategy for the benthic microbial communities was similar for the arctic and the temperate communities.}
6.4. Phototrophic versus heterotrophic temperature responses (ecosystem implications)

The results of Paper 3 demonstrate that $P_{\text{max}}^C$ increased and $\alpha_C$ was more or less insensitive to increasing temperature for all the three investigated species, consistent with most eukaryote algae (Davison 1991). Generally, the light-limited photosynthesis, represented by $\alpha_C$, is a function of photochemical light reactions (not enzyme-dependent), whereas the light-saturated part, represented by $P_{\text{max}}^C$, is limited by enzyme activity associated with the carbon metabolism of the dark reactions (Paper 3, Davison 1991, Sakshaug et al. 1997). Intact community responses to temperature, as in benthic microphyte communities, are confounded by both light-saturated and light-limited processes, as the irradiance regime within the sediment is distributed gradiently, and is further complicated by the simultaneous impact on physical, chemical and biological controls (Paper 4, Epping & Jørgensen 1996, Fenchel & Glud 2000). Based on the laboratory results showing that the light-limited part of photosynthesis is temperature insensitive (Paper 3), it is presumed that the temperature response of the intact benthic microphyte communities (quantified from Q_{10}) is controlled by the light-saturated temperature response. Thus the light-saturated temperature response on photosynthesis is responsible for the temperature response of the net community in the sediments.
A dataset extracted from Paper 3, allowed isolation of the effect of temperature on rates of net O₂ production, measured on the laboratory-grown culture of *Prorocentrum minimum*, at five irradiances and in darkness (Fig. 6.4). The data shows that the net O₂ production rate as a function of temperature decreases with decreasing irradiance in a manner so that the temperature of maximum production (not to be confused with the temperature optimum) decreases. This phenomenon illustrates the balance between the phototrophic *versus* the heterotrophic temperature response, and leads me to conclude that the heterotrophic activity increased more than the gross O₂ production, with increasing temperature, in laboratory-grown culture. *In conclusion, the phytoplankton and the intact microphytobenthic community responded similarly, demonstrating a gradual transition from a phototrophic to a heterotrophic dominated community with increasing temperature. This has implications for the carbon cycling in both pelagic and benthic microalgae-dominated communities that experience seasonal and diel temperature fluctuations.*

Similar observations have previously been reported for intertidal sediments (Davis & McIntire 1983) and in temperate planktonic communities (Lefevre et al. 1994, Robinson 2000). The observations have generally been explained by a stronger and more rapid physiological acclimation of heterotrophic compared to phototrophic activity, to temperature changes. *I therefore suggest that the stronger heterotrophic temperature response as observed in my studies is a general rather than exceptional phenomenon.*
Fig. 6.4. Effects of temperature on rates of the net O₂ production (which is the sum of the gross O₂ production and the respiration) in a laboratory-grown culture of *P. minimum*. Each of the six panels represents different irradiances, from 430 μmol m⁻² s⁻¹ (upper left) to darkness (lower right) (based on the dataset of Paper 3).
7. Conclusions

- Profiles of chl \( a \), dissolved oxygen and primary production showed large natural variations in the Barents Sea. Yet the profiles correlated closely to optical depth, i.e. a certain irradiance regime defined from the attenuation coefficient. The chl \( a \)-normalised primary production correlate stronger to the light regime in the blue-green region, at 490 nm, than to PAR, improving the correlation between irradiance and primary production by \( \sim 15 \% \). Hence, I suggest using 490 nm instead of PAR when relating phytoplankton production to a single wavelength band.

- An accurate estimation of light absorption in PSII is essential for calculating rates of photosynthetic O\(_2\) production from the operational quantum yield in PSII, \( \Phi_{\text{PSII}} \), derived from PAM measurements. Three bio-optical approaches to estimate the fraction of light absorbed by PSII were tested. The best estimate was obtained from a spectrally weighted approach based on the PSII-scaled fluorescence excitation spectrum, by the so-called ‘no-overshoot’ procedure. The approach was evaluated by comparing calculated rates of photosynthetic O\(_2\) production based on \( \Phi_{\text{PSII}} \) and the PSII absorption, against simultaneously measured rates of O\(_2\) production. This approach is hence recommended for estimation of gross O\(_2\) production rates from PAM fluorescence recordings.

- Both calculated and measured O\(_2\)-production rates along with \(^{14}\text{C}\)-assimilation rates showed the same relative response to a short-term temperature change, (for the three studied microalgae species.) This implies that the PAM technique analogous to O\(_2\)-production and \(^{14}\text{C}\)-assimilation measurements can be applied to study relative temperature responses of photosynthesis versus irradiance relationships. Absolute rates of calculated O\(_2\)-production based on \( \Phi_{\text{PSII}} \) showed a species-specific correlation and overestimated the measured O\(_2\)-production rates of \( \sim 1 \) to 3 times during both light-limited (\( \alpha^C \)) and light-saturated (\( P^C_{\text{max}} \)) photosynthesis. The off-set of the \( \Phi_{\text{PSII}} \) based measurements was due to a lower
quantum yield for O$_2$-production than the theoretical maximum and seemed to be insensitive to temperature. In conclusion, the PAM technique can be used to study temperature responses of photosynthesis in microalgae when attention is paid to the absorption properties in PSII.

- No difference in the temperature acclimation response was observed between the investigated arctic and temperate diatom-dominated benthic communities. This was observed from similar Q$_{10}$ values, arguing for a similar temperature adaptation strategy between the sites. Overall, temperature stimulated the heterotrophic activity more than the gross photosynthesis, gradually turning the benthic communities heterotrophic with increasing temperature.
8. Some thoughts on photosynthesis and algorithms of primary production

Bio-optical models developed for the estimation of marine primary production \( P \), and often used with remotely sensed ocean colour data (often determined as \( \text{g C m}^{-3} \text{s}^{-1} \)), can be written on the general form (e.g. Platt & Sathyendranath 1988, Claustre et al. 2005)

\[
P = PAR [\text{chl} a] a^*_\phi \Phi_C
\]

(8.1)

where \( a^*_\phi \) is the chl \( a \)-specific absorption coefficient and \( \Phi_C \) is the quantum yield for carbon fixation. Equation 8.1 is in units of moles, which by multiplying with a factor of 12 can be converted into grams of carbon. The estimation of PAR and [chl \( a \)] is generally not an issue, and these variables can be estimated with a good accuracy, even at a global scale from remotely sensed data or models. Estimation of the product of \( a^*_\phi \) and \( \Phi_C \) is in general considered more challenging (e.g. Claustre et al. 2005). Statistical relationships that relate \( a^*_\phi \Phi_C \) to [chl \( a \)] have been described, however, these only reproduce average trends and remain limited in accounting for natural variability (e.g. Bricaud et al. 1995).

Three major findings in the thesis have inspired me to propose some improvements to eq. 8.1, with the aim of estimating \( P \) from natural ecosystems. First, Paper 1 demonstrates that from a simple approach exchanging PAR with the irradiance at 490 nm the correlation of irradiance to measured primary production can be improved by \( \sim 15 \% \) (Fig. 3.3 and section 3.3). Secondly, Paper 2 shows that replacing \( a^*_\phi \) with the PSII-specific absorption coefficient, \( F^*_{\text{PSII}} \), gives a more accurate estimate of the light absorption for oxygenic photosynthesis (origin in PSII), as it corrects for absorption by photo-protective carotenoids (and PSI). Thirdly, Paper 3 quantifies the (maximum) quantum yield of O\(_2\) production based on the light absorption in PSII, \( \Phi_{O2} \). Using \( \Phi_{O2} \) instead of \( \Phi_C \) is in principle not better, however, by applying the bio-optical approach for quantification of the PSII absorption (Paper 2) it is possible to quantify
PSII $\Phi_{O2}$ with a better accuracy than usually applied for $\Phi_C$. Consequently, incorporating these improvements into equation 8.1, it can be rewritten as

$$P_{O2} = kE_{490}[chla]F_{PSII}^{*} PSII \Phi_{O2}$$

(8.2)

where $k$ is a factor correcting the irradiance at 490 nm to the 400 - 700 nm waveband. The outcome of eq. 8.2 is an estimate of the photosynthetic (gross) O$_2$ production in units of mole, and summaries the finding in this thesis synthesised in a single equation. This approached offers improvement to the mentioned uncertainties included in eq. 8.1.

As PSII $\Phi_{O2}$ in this study is based on culture-grown phytoplankton it is not necessarily representative under in situ conditions. Obviously, there exists a challenge in obtaining accurate and reliable measurements of PSII $\Phi_{O2}$ (and similar bio-optical parameters) under natural conditions in phytoplankton and microphytobenthic communities. This task is still recognised as a major challenge (Behrenfeld & Falkowski 1997, Claustre et al. 2005).

Conclusively, to improve models for marine primary production, not least for the Arctic region, further field investigations are required. From simultaneously measurements of photophysiological parameters (P vs. E curves), phytoplankton absorption and taxonomic composition (e.g. HPLC) knowledge of the in situ variability of the bio-optical parameters (e.g. the quantum yield for photosynthesis) can be obtained (Claustre et al. 2005, Johnsen & Sakshaug in press). Such studies would be extremely valuable for future improvement of primary production estimates: perhaps especially in the Arctic with present climate change scenarios predicting a decreasing ice cover and thus increased light availability in the water column (Holland et al. 2006).
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