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How Photosynthesis in Laminaria digitata and Saccharina latissima is Affected by Water Temperature

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Marine Coastal Development
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Inga Arnesen Aamot

Front page foto: Kelp forest in the Trondheimsfjord; photo by Geir Johnsen

Abstract

The short-term response of photosynthesis to an elevated water temperature was studied in *Laminaria digitata* and *Saccharina latissima* from Svalbard and the Trondheimsfjord. In addition the effect of sampling time(April *vs.* August) on the response of photosynthesis was investigated for Trondheimsfjord kelp. Maximum net oxygen production rate and dark respiration rate was measured and $rETR_{max}$, α and E_k was estimated on the basis of fluorescence measurements for both species. Photosynthesis in kelp form Svalbard responded differently to the elevated water temperature in comparison with kelp from the Trondheimsfjord, with a clear maximum in $rETR_{max}$ at temperatures from 20 to30°C for the Svalbard kelp. In kelp from Trondheimsfjord $rETR_{max}$ increased from 5 to 30°C. Maximum net oxygen production rate and dark respiration rate show that there is a seasonal dependence of the response to an increased water temperature, with a larger increase in the dark respiration rate with temperature in the kelp sampled at TBS in August. At the highest temperatures investigated, respiration rate exceeded the net oxygen production rate resulting in no release of oxygen to the water (negative maximum net oxygen production rate). Results from this MSc thesis indicate that a rise in ocean temperature caused by climate change will have an effect on photosynthesis in the two kelp species *Laminaria digitata* and *Saccharina latissima*. The response in photosynthesis to an elevated water temperature will be different in kelp from different geographical locations and in kelp sampled at different times of the year.

1 Introduction

The kelp forest is an important ecosystem along the Norwegian coast and around Svalbard. It is the home and shelter for the juvenile stages and adults of many important species of fish and crustaceans (Christie et al. 2003, Norderhaug et al. 2005). Kelp provides an important substrate that epiflora and epifauna live on (Lippert et al. 2001, Carlsen et al. 2004, Hartvig et al. 2009). Kelp forest is also important in the costal primary production and in the marine biogeochemical turnover of carbon (Abdullah and Fredriksen 2004, Moy et al. 2009). Today there is a decrease in the kelp biomass/areal coverage and a shift in the species composition of the kelp forest in the southernmost parts of Norway (Dahl et al. 2007, Moy et al. 2009). One of the suggested reasons for this change is an increase in the ocean temperature imposed by a changing global climate (Gerard and Du Bois 1988, Moy et al. 2009). Because of this a better knowledge of how photosynthetic/primary production and oxygen respiration in important kelp species responds to an elevated ocean temperature is needed.

The aim of this thesis is to give insight into how a short term exposure to elevated water temperature affect photosynthesis and dark respiration in two of the most common and important species of the Norwegian kelp forest; *Laminaria digitata* and *Saccharina latissima*. The response of elevated water temperature will be compared between kelp sampled at different geographical locations (a northern-temperate site, Trondheimsfjorden, Norway, and a high-Arctic fjord, Svalbard) and at different seasons of the year.

Below section, [Theory] provides a general introduction to the mechanisms in photosynthesis, light-harvesting processes and electron generation, to fluorescence as phenomenon and how it is utilized for estimating photosynthetic activity, as well as a brief overview of the principals and methods applied. Section [M&M] describes sampling and experimental setup as well as the equations used in calculations of photosynthetic parameters. All findings are presented in the [Results] along with a general explanation of the graphs and tables. Section [Discussion] explains the trends and responses shown in results and provides suggestions to how research on photosynthesis in kelp should proceed to give a more detailed picture of how water temperature affects photosynthesis in kelp. [Conclusion] summarizes the most important findings from the experiments done during this master thesis.

2 Theory

2.1 Climate change

Though there is a debate about the causes of climate change, it is certain that the atmosphere and ocean temperature is rising (Hansen et al. 2005, Levitus et al. 2005, IPCC 2007). In addition to a higher water temperature a warmer climate will contribute to changes in turbidity, stratification and nutrient supply to the coastal zone (IPCC 2007). A rise in ocean temperature is expected to effect the primary production of algae through temperature regulation of photosynthesis, in particular carbon assimilation in the Calvin-Benson cycle and oxygen respiration rate (Davison and Davison 1987, Hancke et al. 2008a).

2. 2 Photosynthesis

Photosynthesis is the process where photosynthetic organisms absorb energy in the form of light (radiation energy) and store it in organic molecules. Carbon dioxide and water is used in the process, and oxygen is a byproduct. The organic molecules are to be used as the energy source for metabolism – the release of energy that is used in different energy dependent processes inside the organism (Taiz and Zeiger 2006).

Light is absorbed by pigment molecules located in the membranes of the thylakoids inside the chloroplasts. The pigments and their associated proteins make up the Light Harvesting Complexes (LHCs). There are several different pigment molecules, the most abundant in brown algae are Chlorophyll *a* and *c* while the accessory pigments are carotenoids and xanthophylls, including fucoxanthin, the pigment that gives kelp its characteristic brown color (Sakshaug et al. 2009). When light is absorbed the energy is transferred from pigment to pigment until it reaches reaction centers of photosystems (PS) I and II. When the energy is transferred to the reaction center molecule it leads to the excitation of an electron that eventually, through the electron transport chain, will lead to the synthesis of high-energy molecules, i.e. ATP and NADPH. The energy stored in these molecules will later be used in the formation of carbohydrates through the Calvin-Benson cycle (Taiz and Zeiger 2006).

2.2.1 Photosystem 1and II

PS I and II and their associated antennae are the light absorbing parts of the photosynthetic machinery. They are located in different parts of the thylakoid membrane and electron transfer between PS I and II is facilitated by electron carriers. Light energy is transferred from the pigment molecules in the antennae to the reaction centers of the photosystems by a process called fluorescence resonance energy transfer (Owens 1991).

The centers of PS I and II are called reaction centers and contain special chlorophyll molecules. In PS II these chlorophyll molecules are called P680 and in PS I they are called P700. 680 and 700 refers to the wavelength of light that gives maximum absorption in these molecules (Taiz and Zeiger 2006). When the energy is absorbed by a reaction center one of its electrons is excited from its ground state to a high-energy state. The excitation energy is unstable and will not last and so the excitation energy has to be dissipated. There are three different pathways for energy dissipation; (i) in photosynthesis (photochemistry) (ii) as heat, (iii) as fluorescence (Maxvell and Johnson 2000). If the energy is to be used in photochemistry, the excited electron is donated to the first molecule in the electron transport chain, pheophytin a.

When an electron is donated from a P680 molecule to the electron transport chain, it will be replaced with an electron from a water molecule that has been broken down into oxygen and hydrogen ions. The splitting of water takes place in the water splitting complex. Electrons donated from P700 are replaced by electrons from a molecule called plastocyanin.

2.2.2. Electron transport chain

The electron transport chain where ATP and NADPH are generated starts with PS II and end with PS I, and can be described in a Z scheme (Figure 1).

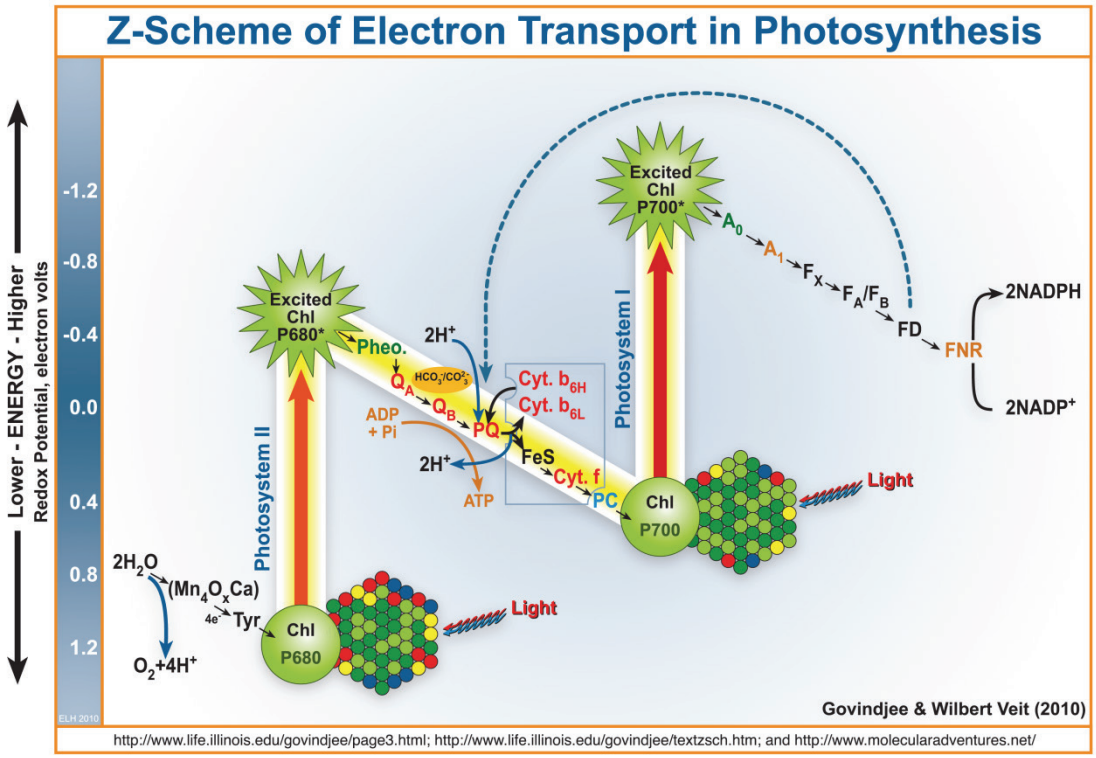

Figure 1. Z-scheme that show the electron pathway through the electron transport chain from P680 to P700, for description see text below. (Figure from www #1).

Light is absorbed by the LHC and transferred to the reaction centers. The excited electrons from P680 and P700 are donated in a redox reaction to an electron carrier. Several more redox reactions transfer the electron through the entire electron transport chain. When the reduction reaction takes place some of the reduced molecules bind hydrogen ions from the stroma side of the thylakoid membrane. The hydrogen ions are released on the inside of the membrane when the electron is given to a new electron carrier. This generates an electrochemical potential gradient over the membrane. Hydrogen ions are allowed to diffuse back over the membrane through the enzyme ATP-synthase that uses the energy from the electrochemical gradient to synthesize ATP from ADP and a phosphate group (Taiz and Zeiger 2006).

NADPH is generated as the last step of the electron transport chain. The ferredoxin-NADP reductase enzyme uses the transferred electron and a hydrogen ion to reduce $NADP^+$ to NADPH (Taiz and Zeiger 2006).

2.2.3 Calvin-Benson cycle

The Calvin-Benson cycle consists of several enzymatic reactions where light energy bound in the form of ATP and NADPH is used in fixation of CO_2. The end product of the Calvin-Benson cycle is carbohydrates that can be used in different metabolic reactions in the organism (Taiz and Zeiger 2006). The first enzyme in these reactions is Rubisco, which catalyses the carboxylation of CO_2. Under light saturation the reaction catalyzed by Rubisco is the rate limiting step in photosynthesis (Feller et al. 1998, Jensen 2000). When Rubisco is operating at highest possible rate, energy from excess light absorbed by the LHC has to be dissipated in other ways than photochemistry as to avoid damage on the photosynthetic machinery.

When the enzymes of the Calvin-Benson cycle are exposed to stress in the form of high light, high temperatures, low CO_2 concentrations etc. the rate of the reaction they facilitate will decrease (Miyake 2010). This leads to a discrepancy between the rate of the light absorption and the rate of products synthesized through the Calvin-Benson cycle. Electrons donated from water to PS680 will then be used in other reactions than NADPH reduction. Such reactions are the Mehler reaction and cyclic electron flow around PS I and PS II (Schreiber and Neubauer 1990, Miyake 2010).

In the Mehler reaction (water-water cycle) electrons on the reduction side of PS I are used in reduction of oxygen and leads to the formation of oxygen radicals (O_2^-). Radicals are very reactive and can do damage to other molecules such as DNA and proteins. This damage is avoided by an enzyme catalyzed reaction where the oxygen radicals are transformed to hydrogen peroxide (H_2O_2) and oxygen. Hydrogen peroxide is then transformed to water, and so the cycle starts again with oxygen as electron acceptor and water as electron donor. The water-water cycle functions as an electron sink that help remove excess energy absorbed at PS II reaction center (Miyake 2010).

In addition to function as an electron sink, the Mehler reaction contributes in the induction of energy dependent quenching of fluorescence, qE (Schreiber and Neubauer 1990, Neubauer and Yamamoto 1992). qE is initiated by the decrease in the cell pH that happens when the photosynthetic rate is high and there is consequently a high transport of H^+-ions from the stroma to the inner side of the thylakoid. The decrease in pH induces protonation of special light harvesting complexes and the xanthophyll cycle. The xanthophylls cycle is the de-epoxidation of diadinoxanthin (in most chromophytes) or violaxanthin (most green algae and also in brown algae) to diatoxanthin and zeaxanthin respectively (Brunet et al. 2011). Protonation and formation of diatoxanthin and zeaxanthin leads to dissipating of excess excitation energy in the LHCs as heat instead of fluorescence (Maxvell and Johnson 2000, Müller et al. 2001, Brunet et al. 2011).

In cyclic electron transport around PS I electrons at PS I are thought to be transferred from NADPH or ferredoxin back to the plastoquinon pool on the reducing side of cyt b6/f complex (Johnson 2011). In the same way as water-water cycle this leads to acidification of thylakoid lumen and initiation of NPQ. In addition this will lead to more production of ATP which can be used in the repair of other molecules damaged under stress (Miyake 2010).

2.2.4 Chl a fluorescence

Fluorescence is light emitted from a molecule when an excited electron goes back from a high-energy state to its ground state. Chlorophyll *a* fluorescence is emitted light at peak wavelength 685 nm (Sakshaug and Johnsen 2006). The fraction of absorbed light emitted as fluorescence from Chlorophyll *a* can be used as an indication of the efficiency of the photosynthesis.

Ground Chl *a* fluorescence, F_0, is fluorescence emitted from a dark-acclimated tissue exposed to non-actinic light, the lowest possible light intensities that induce a measurable fluorescence signal while not inducing electron transport. Maximum fluorescence, F_m, is fluorescence measured on dark acclimated tissue exposed to a pulse of photons high enough to saturate all functional reactions centers with photons (typically with duration of 100 to 800 milliseconds). These fluorescence measurements can then be used in calculation of the maximum quantum yield of Chlorophyll *a* fluorescence, $\Phi_{PSII\ max}$ (see in addition equation 1 below) (Genty et al. 1989). $\Phi_{PSII\ max}$ can be used as an indication of the health of the test organism and measures the fraction of the absorbed light used in photochemistry (Maxvell and Johnson 2000, Hancke et al. 2008b, Zhang and Sharkey 2009).

Ground fluorescence in actinic light conditions, F'_0 ,is the fluorescence the sample is emitting when it is exposed to a particular irradiance. The maximum fluorescence in light, F'_m, is the maximum fluorescence the tissue is emitting after exposure to a saturating light pulse (as for dark-acclimated samples) (Genty et al. 1989, Hancke et al. 2008b). From these two values variable fluorescence, F'_v, can be calculated. The operational quantum yield of Chlorophyll *a* fluorescence, Φ_{PSII}, is calculated from the measured F'_0 and F'_m (see equation 2, materials and methods). Φ_{PSII} is defined as mol electrons generated per mol photons absorbed in PS II (Genty et al. 1989, Maxvell and Johnson 2000, Hancke et al. 2008a).

Measurements of fluorescence can be used to calculate the relative electron transfer rate, rETR, from PS II to PS I. This rate can be used as an indication of the gross photosynthetic rate, e.g. in macroalgal tissue or phytoplankton samples (Hancke et al. 2008a). To calculate rETR Φ_{PSII} is multiplied by the actinic irradiance used when measuring F'_0.

A photosynthesis *versus* irradiance curve, P *vs.* E curve, show change in photosynthetic rate as a function of increasing irradiance (Sakshaug et al. 1997, Sakshaug et al. 2009). As the amount of light increases, photosynthesis will increase linearly up to a given irradiance. From this irradiance the increase in photosynthesis will no longer have a linear response. This

irradiance is called the light saturation parameter, E_k (µmol photons m^{-2} s^{-1}). The initial slope of the P *vs.* E curve is called maximum light utilisation coefficient, α. α is spectrally dependent because efficiency of light absorption in a photosynthetic organism varies with wavelength, e.g. blue light is more efficiently absorbed by chl *a* than green light and so blue light gives a higher α (Sakshaug et al. 2009). If irradiance is increased over E_k photosynthesis will be saturated and reach its maximum rate, P_{max} (Sakshaug et al. 1997).

A rapid light curve is similar to a P *vs*. E curve because it presents photosynthetic rate as a function of irradiance, but it is not a true P *vs*. E curve (Hawes et al. 2003). This is because the exposure time to each irradiance in a RLC is too short for the sample to acclimate fully to the irradiance (Hawes et al. 2003). A RLC-curve is still a useful tool to assess the trend of the photosynthetic response to increasing irradiance, and the same parameters that can be calculated for a P *vs.* E curve can be calculated for a RLC-curve. When measuring rapid light curves, RLCs, with a PhytoPAM, tissue samples or algal cultures are exposed to increasing light intensity. F'_0 and F'_m are measured for each light intensity and rETR calculated for each irradiance (Ralph and Gademann 2005). rETR for each irradiance can be presented in the same way as a P *vs.* E curve, and $rETR_{max}$, E_k and α estimated on the basis of the curve.

The rETR versus irradiance curve will only give relative data on the efficiency of the photosynthesis because it is calculated from relative yield values, ϕ_{PSII}. An absolute measurement of the photosynthesis efficiency can be calculated from the oxygen production rate at the different irradiances. Oxygen production can be measured with a Clark type oxygen microelectrode (at known temperature and known irradiance) and then be compared with rETR values calculated using variable fluorescence.

Studies have shown that oxygen production rate calculated on the basis of rETR measured with PAM will sometimes over-or underestimate the actual oxygen production rate (Flameling and Kromkamp 1998, Morris and Kromkamp 2003). The difference between calculated oxygen production rate and measured oxygen production rate is a result of reactions such as photoprotective cyclic electron flow around PS II and PS I and the Mehler reaction. All these reactions are alternative electron pathways for the electrons generated in PS II and help maintain a high electron transfer rate while not contributing to oxygen production or carbon-assimilation (see 2.2.3 Calvin-Benson cycle).

2. 3 Principles of methods

2.3.1 Pulse amplitude modulated fluorescence

F'_0 and F'_m can be measured in a Phyto-PAM. This PAM- instrument is based on the Pulse Amplitude Modulated measuring protocol, with pulsating light inducing fluorescence (Schreiber et al. 1986). In measurement of F'_0, the sample is exposed to pulses of actinic light with a known irradiance. In addition the organism is exposed to a saturating flash of light with wavelength 655 nm (red light) to measure the maximum fluorescence signal, F'_m. The

fluorescence is detected by a photomultiplier at 90° on the direction of the light inducing the fluorescence. The instrument calculates Φ_{PSII} from the measured F'_m and F'_0 values.

2.3.2 Oxygen microsensor

Oxygen production and respiration rates can be measured using a Clark type O_2-sensor. This is an oxygen microelectrode that consists of a cathode and an anode inside a glass tube with a silicone membrane that separates it from the solution. Oxygen passes from the sample into the head of the microsensor through the membrane, and is reduced at the cathode. The current generated by this reaction is measured by a picoamperemeter and the signal can be used in calculation of oxygen production rate or respiration rate. The signal from an anoxic solution and a 100 % saturated solution must be known for calibration of the sensor in order to obtain absolute measurements of oxygen. The 100 % saturated solution must have the same temperature as the sample, due to the temperature dependency of the partial pressure of oxygen in a solution (Gundersen et al. 1998, Hancke and Glud 2004).

The oxygen microsensor measures the net rate of oxygen released from the water splitting complex in the light. In the dark, when oxygen is only consumed, the measured rate of oxygen removal from the medium is equal to the oxygen respiration rate. If combined the net oxygen production rate and the respiration rate in the dark will give an estimation of the gross oxygen production rate. This estimate will however underestimate the gross oxygen production as the respiration rate in the light is higher than the respiration rate in the dark, due to reactions as Mehler reaction, cyclic electron flow, reparation of parts of the light harvesting machinery etc (Weger et al. 1989, Lewitus and Kana 1995).

3 Material and methods

3. 1 Sampling

The kelp species *Laminaria digitata* (Hudson) J.V.Lamouroux 1813: 42 and *Saccharina latissima (Linnaeus)* C.E.Lane, C.Mayes, Druehl & G.W.Saunders 2006: 509 was sampled by SCUBA divers at Trondheim Biological Station (TBS), Trondheimsfjorden, 63°36'N °10°25'E, Norway (April and August 2010) and Fuglefjellet, Kongsfjorden, 79°N 11°40′E, Svalbard (May, 2010). Sampling was done in the sublittoral zone; depth was approximately 2-4 m (low to high tide). After sampling the kelp was immediately transferred to containers with running seawater. For measurements with Phyto-PAM and oxygen microelectrode subsamples were taken from the growing part of the lamina, the meristem (Figure 2). In between experiments all kelp were kept outside in the running seawater containers to minimize effect of air exposure (Davison and Pearson 1996).

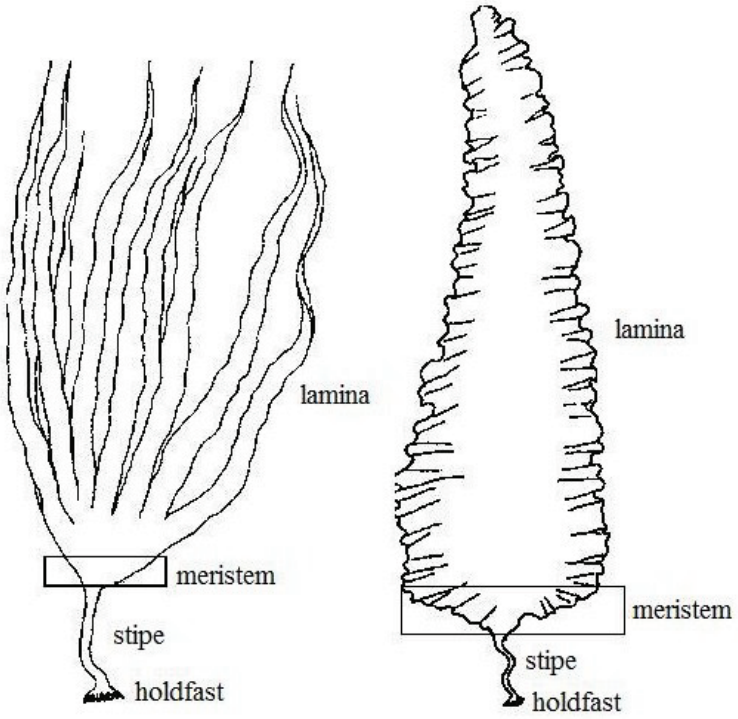

Figure 2. *Laminaria digitata* (left) and *Saccharina latissima* (right). All tissue used in the experiments were taken from the meristem. Figure from www # 2.

Sampling 1 (TBS)

Laminaria digitata and *Saccharina latissima* was sampled at Trondheim Biological Station, Trondheim 06.04.2010. The samples were kept in running seawater for 24 hours and then transported in their containers to Sletvik field station, Agdenes. Upon arrival at Slettvik all kelp was placed in a sheltered area of Hopavågen bay at 40 cm depth (low tides, tidal difference ±50 cm). The samples were kept in the bay for one month, and measurements were done regularly during that time. Tissue pieces from the meristem with a size approximately 5 x 5 cm were collected shortly before measurements and were kept in 10 L containers with seawater after subsamples were taken out. The water in the containers was changed every day to avoid nutrient limitation and CO_2-shortage (Davison and Pearson 1996, Gerard 1997).

Sampling 2 (Svalbard)
L. digitata and *S. latissima* were sampled at Fuglefjellet, Svalbard by Scuba divers 13.05.2010. The samples were kept in a basin with running seawater on the deck of R/V Jan Mayen for 10 days. Measurements were done regularly during the 10 day period.

Sampling 3 (Trondheim Biological Station)
L. digitata and *S. latissima* were sampled at TBS 25.08.2010. The samples were kept in a large outdoor container with running seawater for three weeks, and subsamples were taken from the meristem before every experiment. Experiments were done regularly during the three week period. The container was completely drained and cleaned every third day to avoid contamination of the kelp. During cleaning the kelp was transferred to smaller containers for approximately 20 minutes.

3.2 Phyto-PAM measurements

A Phyto-PAM (Walz, Effeltrich, Germany) equipped with a photomultiplier detector (PM-101P, Walz, Effeltrich, Germany) was used in measurements of changes in fluorescence signal from tissue samples as a function of increasing irradiance and temperature. For fluorescence kinetical measurements, the emitted fluorescence of Chl *a* was probed with a measuring light (Measuring LED-Array-Cone PHYTO-ML Walz, Effeltrich, Germany) and actinic light (Actinic LED-Array-Cone PHYTO-AL, Walz, Effeltrich, Germany) for measurements of F'_0 and F'_m in rapid light curves (RLCs, 1-844 µmol photons m^{-2} s^{-1}) (Ralph and Gademann 2005). Algal tissue of 0.5 x 1 cm were cut out from the meristem and placed in a 4 mL cuvette filled with filtered seawater (0.2 µm sterile filters, Minisart, Santorius, Goettingen, Germany). The cuvette was placed in the cuvette holder so that the Measuring Light cone and the Actinic Light cone of the PhytoPAM faced the surface area of the tissue from each side (Figure 3).

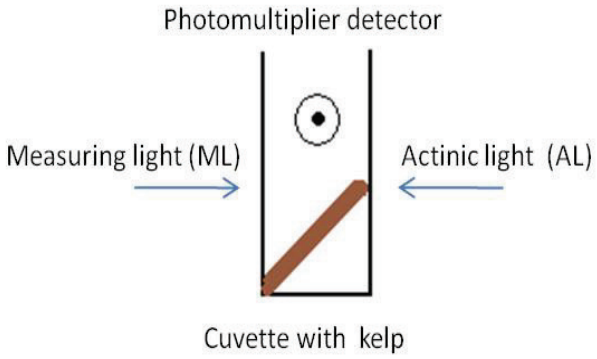

Figure 3. The cuvette was placed in the PhytoPAM so that the AL- and ML-cones faced the surface area of the kelp. The photomultiplier faced the side of the tissue.

The temperature was controlled by a Peltier cell (Temperature Control Unit US-T, Walz, Effeltrich, Germany). Before measuring the RLCs the tissue was kept in darkness for 30 minutes to acclimate to each temperature. During this period F_0 and F_m was measured every five minutes (Hancke et al. 2008b) . The tissue was exposed to temperatures of 5, 10, 15, 20, 25 and 30 ^oC successively, with half hour intervals. Three parallels were done for *L. digitata* and *S. latissima* collected in April (TBS), two parallels were done for each species collected in May (Svalbard) and one measurement was done for each species in August (TBS).

From the measured F_m and F_o signals maximum quantum yield of charge separation in PS II ($\Phi_{PSIImax}$, in the dark) was calculated as follows:

$$\Phi_{PS\ IImax} = \frac{F_m - F_0}{F_m} \quad (1)$$

Operational quantum yield of charge separation (Φ_{PSII}, in the light) was calculated from F'_m and F'_0 measured in the RLCs (Genty et al. 1989):

$$\Phi_{PS\,II} = \frac{F'_m - F'_0}{F'_m} \quad (2)$$

Φ_{PSII} and irradiance emitted from the Actinic Light cone during the RLCs was used in calculation of the relative electron transfer rate (rETR, eq.3):

$$rETR = \Phi_{PS\,II} \times E_{PAR} \quad (3)$$

The calculated rETR values were used to plot P *vs*. E curves). The P *vs*. E curves were then fitted from eq. 4 (Webb et al. 1974).

$$f = a*(1-\exp((-b*x)/a)) \quad (4)$$

From the P *vs* E curves the maximum rETR and α for each curve were calculated using Sigmaplot (SYSTAT Software Inc., San Jose, CA, USA). E_k was calculated from rETRmax and α accordingly.

$$E_k = rETR_{max}/\alpha \quad (5)$$

3.3 Oxygen electrode measurements

Oxygen production and respiration rate measurements were done with a Clark type oxygen microsensor with a guard cathode (Unisense, Aarhus, Denmark) (Revsbech 1989). The sensor current was measured by a picoammeter (Unisense, Aarhus, Denmark) connected to a strip-chart recorder (Kipp & Zonen, Delft, the Netherlands).

Tissue pieces of 0.5 x 1 cm were placed standing up in 4 mL containers filled with filtered seawater (Figure 4). The containers were closed with a lid with a miniature pipe (MicroRespiration System, Unisenses, Denmark) and placed in a rig in a temperature controlled aquarium (Figure 4). Water circulation inside the containers was insured with the use of internal magnetic stirrers. The electrode was inserted into the container through the pipe, which excluded headspace of air and hindered buildup of pressure as a result of photosynthetic oxygen release/gas production (Hancke et al. 2008a).

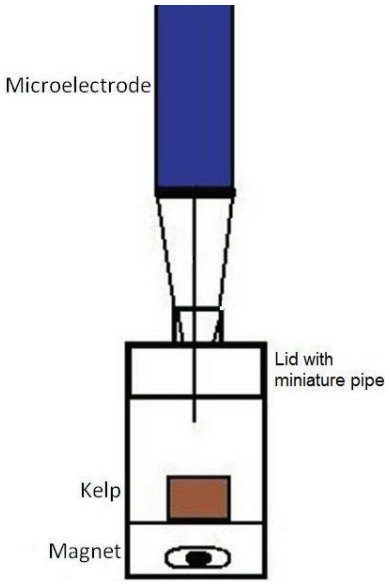

Figure 4. Oxygen microelectrode inserted into a container including a kelp sample through a pipe in the lid. A magnetic stirrer in the bottom of the container ensured water circulation.

During oxygen respiration rate measurements the aquarium was covered with dark plastic. The light source for the maximum net oxygen production rate measurement was a slide projector equipped with a halogen lamp (approx. 960 µmol photons m^{-2} s^{-1}) (Osram, 150 W, Ausburg, Germany). Incubation irradiance (E, PAR) was measured in the aquarium with a RAMSES underwater spectroradiometer (ACC-VIS 320-950 nm, TRIOS, Oldenburg, Germany). The microsensor was calibrated for every temperature in an anoxic and an O_2-saturated solution. Due to the temperature dependent change in solubility of oxygen in water, the saturated solution was made fresh with every temperature change (Gundersen et al. 1998). The temperature in the aquarium was confirmed with a thermometer every time the light was turned on/of.

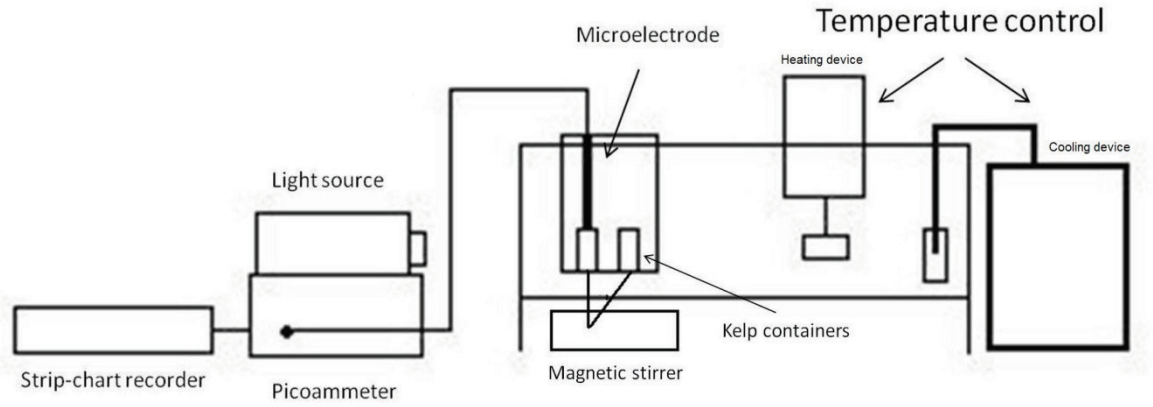

Figure 5. Oxygen measurement setup. When measuring respiration the aquarium was covered in dark plastic. Temperature (±ºC) in the aquarium was obtained by using a cooling device (Hetofrig, Heto, Birkerød, Denmark) and a heating device with a thermostat (Haake D8, Haake, Dieselstrasse, Germany).

In the April-experiments (TBS) the kelp was exposed to temperatures of 5, 10, 15 and 20 °C successively. In the August-experiments the tissue was exposed to temperatures from 5 to 30°C. In each experiment there were meristem tissue from three different individuals. Due to the fact that measurements from 5-20 °C took 12 hours to conduct, measurements from 20-30°C were done in a separate experiment. Adjoining pieces of meristem tissue were used for measuring response from 5 to 20 °C and 20 to 30 °C to minimize differences in physiological state of the samples (Valle 2005).

Before measuring respiration the algal tissue was kept in darkness for 30 minutes to acclimate to the temperature. After the respiration measurements the light was turned on and maximum net production rate was measured after 15 minutes of light exposure. During both O_2 respiration and production measurements a stable linear O_2 exchange rate was ensured for the incubation. Rates of respiration and production were calculated from measuring periods with constant changes in oxygen concentration over a minimum of 10 minutes (Borum et al. 2002). Oxygen concentration was plotted against time of measurement and the production and respiration rate was calculated as the slope of the curve.

Salinity of water in kelp containers was measured to make sure the correct solubility constant for oxygen was used in the calculations of oxygen production and respiration, as oxygen solubility changes with salinity (Garcia and Gordon 1992).

Oxygen concentration in the containers at each measuring point was calculated from the electrode signal according to eq 5. S_s is the electrode signal in the sample, S_0 is the signal in the anoxic solution, Ssat is the signal in a 100 % saturated solution. A is the solubility constant for oxygen.

$$[O_2\ \mu mol\ L^{-1}] = ((S_S-S_0)/(Ssat-S_0))*A \quad (5)$$

The calculated rates of net production and respiration were corrected for Chl *a* content.

No measurements of oxygen production and respiration rate were done at Svalbard due to limited time available for laboratory work aboard the research vessel.

Temperature coefficient (Q_{10}) was calculated for net and gross oxygen production rate, oxygen respiration rate, α and $rETR_{max}$ according to equation 6.

$$Q_{10}= (r_2/r_1)^{(10/t_2-t_1)} \quad (6)$$

In these calculations t_1 was10°C, t_2 was 20°C, and r_1 and r_2 were the corresponding rates at t_1 and t_2.

3. 4 Chl *a* estimation

Chlorophyll *a* was estimated from absorbance measurements (OD, Optical Density, dimensionless) for all pieces of kelp used in oxygen measurements. The absorbance was measured with a Thermo Unicam UV 500 dual beam spectrophotometer. Each piece of kelp was extracted in 4 ml 100 % methanol for 24 hours at – 18 ° C in the dark. Before the absorbance measurements the spectrophotometer was baseline adjusted with a 100 % methanol blank, and all the extracted samples were filtered with 0.2 µm sterile filters to avoid particles. Concentration of Chl *a* was calculated according to equation 7. Extinction coefficient for Chl *a* at red peak OD maximum at 665 using methanol as solvent is 74.5 L g^{-1} cm^{-1} (Mackinney 1941).

$$\frac{(OD665-OD750) * ml\ MeOH * cuvette\ pathlenght * 1000}{extinction\ coefficient\ for\ Chl\ a\ (74.5\ L\ g^{-1}\ cm^{-1}) * piece} = Chl\ a\ \mu g\ piece \quad (7)$$

OD 665 and OD 750 is the OD at 665 and 750 nm respectively and the cuvette pathlength is the distance the light has to travel through the cuvette.

4 Results

4. 1 Oxygen respiration and production

Oxygen respiration and maximum net production rate in *L.digitata* and *S. latissima* was measured with an oxygen microelectrode after short time exposure to water temperatures from 5-20 and 5-30 °C at 5 °C intervals. Respiration rate and production rate was corrected for Chl *a* content in the tissue. All rates were measured in triplicate and standard deviation is shown (Figure 6). Oxygen electrode experiments were conducted in April and August 2010. Kelp was sampled at the pier at TBS for both experiments. Gross oxygen production rate was estimated from the measured dark respiration rate and maximum net production rate (Figure 7).

Temperature effects on dark oxygen respiration rate and net oxygen production rate

Maximum net oxygen production rate increased linearly from 5°C up to 20-25 °C for both species at both sampling times investigated (Figure 6). Q_{10} values showed that the increase with temperature was highest in *L.digitata* from April and in *S. latissima* from August, i.e. no clear seasonal trend in the magnitude of the response to temperature increase (Table 1). At 30 °C there was a decrease in the net oxygen production rate for both species from August. Because 20°C was the highest temperature investigated in April there is no data at 25-30°C in April (Figure 6, C and D). The difference in maximum net production rate between replicates was highest at the highest temperatures, and the difference between replicates was higher in *S. latissima* from August than in any other experiment (Figure 6 D).

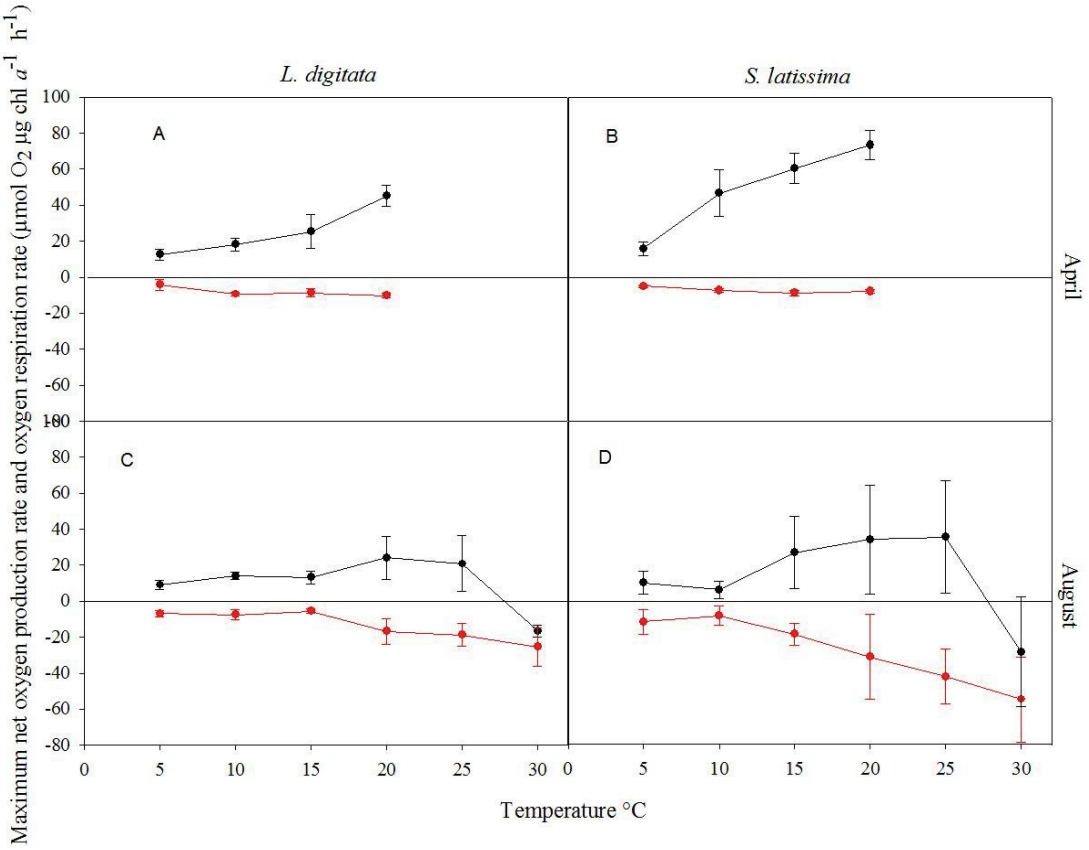

Figure 6. Maximum net oxygen production rate (black) and dark respiration rate(red) in A and C) *L.digitata* sampled in April and August respectively , B and D) *S. latissima* sampled in April and August respectively.

Oxygen respiration rate measured in the dark increased with increasing temperature in both species at both sampling times (Figure 6). From calculated Q_{10} values it was clear that the magnitude of the increase was higher in the August kelp (Table 1). This trend was seen in both species investigated. Overall the increase in respiration rate with temperature was highest in August *S. latissima (*Figure 6 D). As seen in net oxygen production rate the largest difference in response between replicates was found in *S. latissima* sampled in August (Figure 6 D).

The estimate of gross oxygen production rate shows a similar trend to net oxygen production rate. The gross oxygen production rate increased from temperatures 5-20°C in all species at both sampling times (Figure 7). In *L. digiata* from August there was a decrease in gross oxygen production rate at 25-30°C (Figure 7 C). There was a larger drop in gross production rate from 25-30°C compared to the decrease from 20-25°C. In *S. latissmia* from August gross oxygen production rate increases from 5-25°C before there is a large drop at 30°C (Figure 7, D).

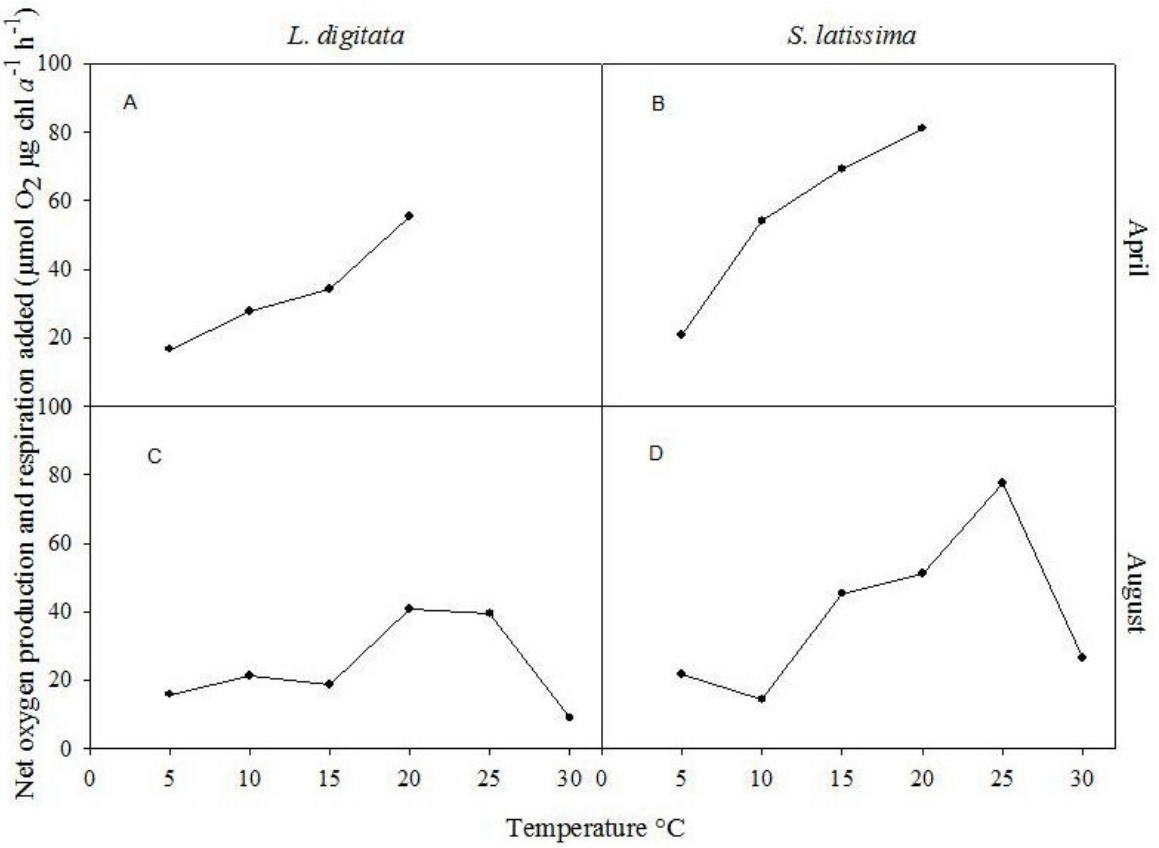

Figure 7. Estimated maximum gross oxygen production calculated from the maximum net oxygen production corrected for the dark respiration rate. Note that the maximum gross production is underestimated as the dark respiration inevitably underestimate the respiration in light (see [Discussion] for details). A and C) *L.digitata* sampled in April and August respectively , B and D) *S. latissima* sampled in April and August respectively.

Table 1. Q_{10} values for oxygen production rate and respiration rate for *L. ditata* and *S. latissima* April and August (TBS). The Q_{10} calculations are based on measured maximum oxygen production and respiration rate for 10 and 20 °C.

Experiment	**Q_{10} respiration rate**	**Q_{10} net production rate**	**Q_{10}gross production rate**
Laminaria digitata April	1,10	2,48	1,50
Saccharina latissima April	1,46	1,57	2,01
Laminaria digitata August	2,25	1,72	1,91
Saccharina latissima August	3,85	5,34	3,53

4.2 Phyto-PAM measurements

RLCs were measured in *L. digitata* and *S. latissima* with a PhytoPAM after short term exposure to water temperatures from 5 to 30°C at 5°C intervals. From the RLCs ϕ_{PSII}, $rETR_{max}$, α and E_k was calculated. Measurements were done on kelp sampled at TBS in April

and in August, and at Svalbard in May. No correction for Chl *a* content was done of the fluorescence signal. All results are presented in Figure 8-11. The error bars shown in all Figures for April (TBS) are standard deviation ± of the mean value, n=3. For May (Svalbard) n=2 and August (TBS) n=1 so no error bars are shown.

4.2.1 rETR$_{max}$

$rETR_{max}$ increased from 5 to 20°C in both species at all times of sampling (Figure 8). Q_{10} values showed that there was no great difference in the magnitude of the response between the sampling times and between the species (Table 2). In *L. digitata* sampled at TBS in April and in both species sampled at TBS in August $rETR_{max}$ was increasing as a function of temperature over the entire temperature regime (Figure 8 A, E, F). In *S. latissima* from TBS in April and both species sampled at Svalbard $rETR_{max}$ reached a maximum at 20 °C, and a subsequent slight decrease at 25 and 30 °C (Figure 8 B, C, D). This indicates a location dependent temperature response in $rETR_{max}$. $rETR_{max}$ values varied between experiments, and the highest $rETR_{max}$ values were found in kelp sampled at TBS in April (Figure 8, A and B). In general the $rETR_{max}$ values for *L.digitata* from TBS were higher than in the *S. latissima* sampled at the same time (Figure 8, A, B, C and D). $rETR_{max}$ values for *L. digitata* sampled at TBS in April were the highest measured (Figure 8 A).

The error bars show that the response of increased temperatures varied most at the two highest temperatures investigated (Figure 8,A). When the different replicates were investigated on their own, the graphs show that the trend in the response is similar between replicates even though the magnitude of the response varied (data not shown).

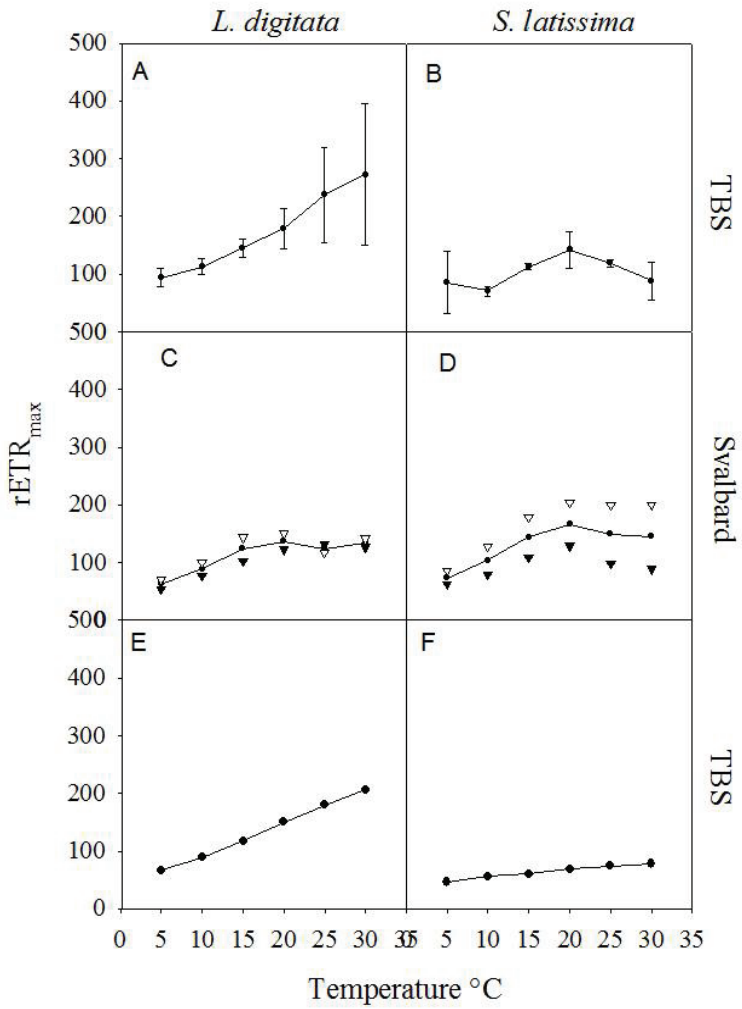

Figure 8. rETRmax as a function of temperature. A) *L. digitata* sampled at TBS in April, B) *S. latissima* sampled at TBS in April, C) *L.digitata* sampled at Svalbard in May D) *S. latissima* sampled at Svalbard in May, E) *L.digitata* sampled at TBS in August and F) *S. latissima* sampled at TBS in August. (Error bars shown for A and B, n=3. No error bars shown for C and D because n=2, and E and F because n=1).

Table 2. Q_{10} values for $rETR_{max}$ for *L.digtata* and *S. latissima* from April, May and August. The Q_{10} calculations are based on measured $rETR_{max}$ for 10 and 20 °C.

Sampling time and place	***Laminaria digitata***	***Saccharina latissima***
April, TBS	1,58	1,88
May, Svalbard	1,52	1,60
August, TBS	1,68	1,23

4.2.2 α

Temperature had a minor effect on effect on α (Figure 9). There was a slight decrease in α with increasing temperature in both species investigated in August (Figure 9 E, F). Q_{10}-values indicated that the decrease with temperature was very small (Table 3). The highest α- values was found in August kelp, and there was no great difference between *L. digitata* and *S. latissima* (Figure 9). In both species sampled at Svalbard there was a small increase with

temperature up to 25 °C and then a drop at 30 °C (Figure 9 C, D). As seen in August kelp the Q_{10}-values were small for these experiments (Table 3). Data in figure 4 B was very scattered and this is believed to be a result of errors in the measurements (Figure 9, B).

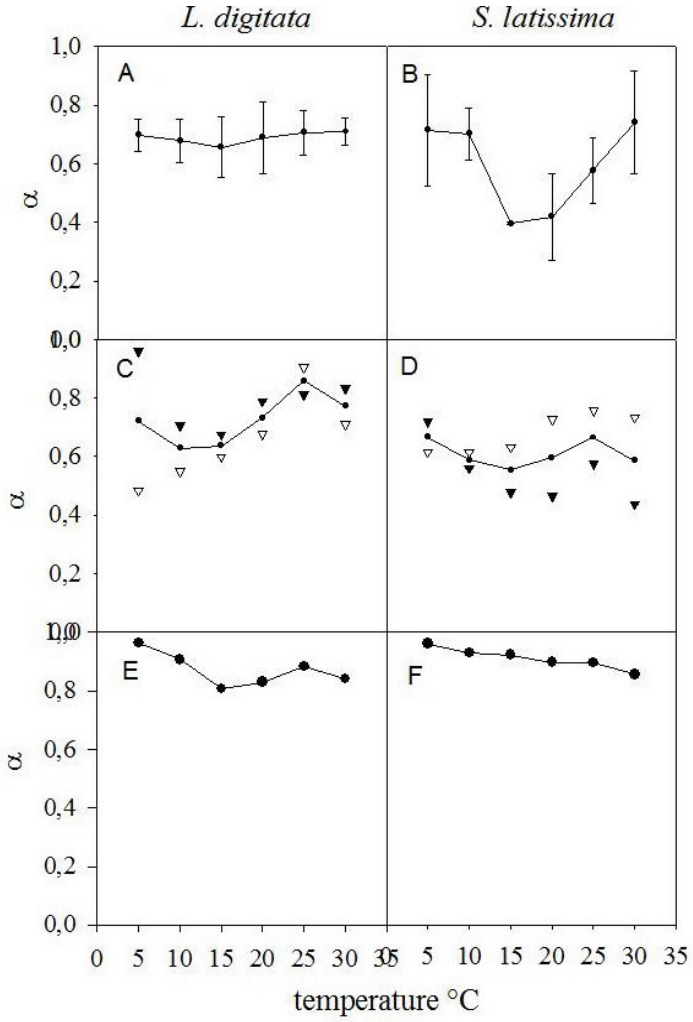

Figure 9. α as a function of temperature for all species. A) *L. digitata* sampled April, B) *S. latissima* sampled April, C) *L. digitata* sampled May D) *S. latissima* sampled May, E*) L. digitata* sampled August and F) *S. latissima* sampled August. (Error bars shown for A and B, n=3. No error bars shown for C and D because n=2, and E and F because n=1).

Table 3. Q_{10} values for α for *L. digitata* and *S. latissima* from April, May and August. The Q_{10} calculations are based on measured P_{max} for 10 and 20 °C.

Sampling time and place	***Laminaria digitata***	***Saccharina latissima***
April, TBS	1,01	0,51
May, Svalbard	1,24	1,07
August, TBS	0,91	0,97

4.2.3 E_k

As the change in α with temperature was very small, the effect of temperature on E_k mirrors the effect on $rETR_{max}$ (Figure 10). The response of E_k as a function of higher temperature is similar in both species investigated when compared for the same sampling time and place. There is a linear increase with temperature in April and August *L. digitata* and *S. latissima* sampled in August (Figure 10, A, E, F). In *S. latissima* from April there is a large increase in E_k from 5°C -20°C. At 25-30°C E_k drops and at 30°C E_k is the same as at 5°C (Figure 10, B). In both speices from Svalbard E_k reaches a plateau at 20-30°C (Figure 10, C, D).

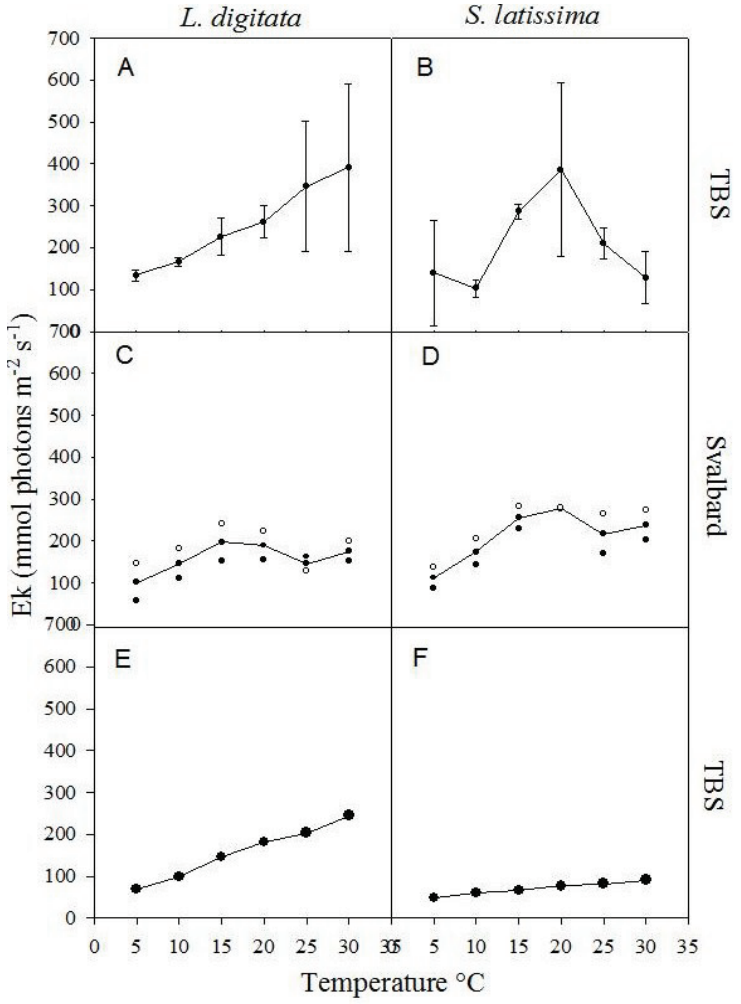

Figure 10. E_k as a function of temperature for all species. A) *L. digitata* sampled April, B) *S. latissima* sampled April, C) *L.digitata* sampled May D) *S. latissima* sampled May, E) *L.digitata* sampled August and F) *S. latissima* sampled August. (Error bars shown for A and B, n=3. No error bars shown for C and D because n=2, and E and F because n=1).

4.2.4 Φ_{PSII}

The response of Φ_{PSII} as a function of increasing irradiance in the RLC changed with increasing temperature. Figure 11 show the response of Φ_{PSII} as a function of increasing irradiance for all temperatures investigated. The response of Φ_{PSII} varied between the two species investigated. In *L. digitata* the yield curve is more linear for the higher temperatures (Figure 11 A,C, E). This is caused by an increased yield value at the higher irradiances at the highest temperatures compared to the lowest temperatures investigated (Figure 11 A, C, E). This trend is also seen in *S. latissima*, but in this species the change in Φ_{PSII} with increasing irradiance is less obvious (Figure 11, B, D, F). The exception from this trend of approaching linearity of the response of Φ_{PSII} as a function of irradiance is *S. latissima* sampled at TBS in April, where the graph for 30 degrees has more similar values as the graphs representing the lowest temperatures (Figure 11, B).

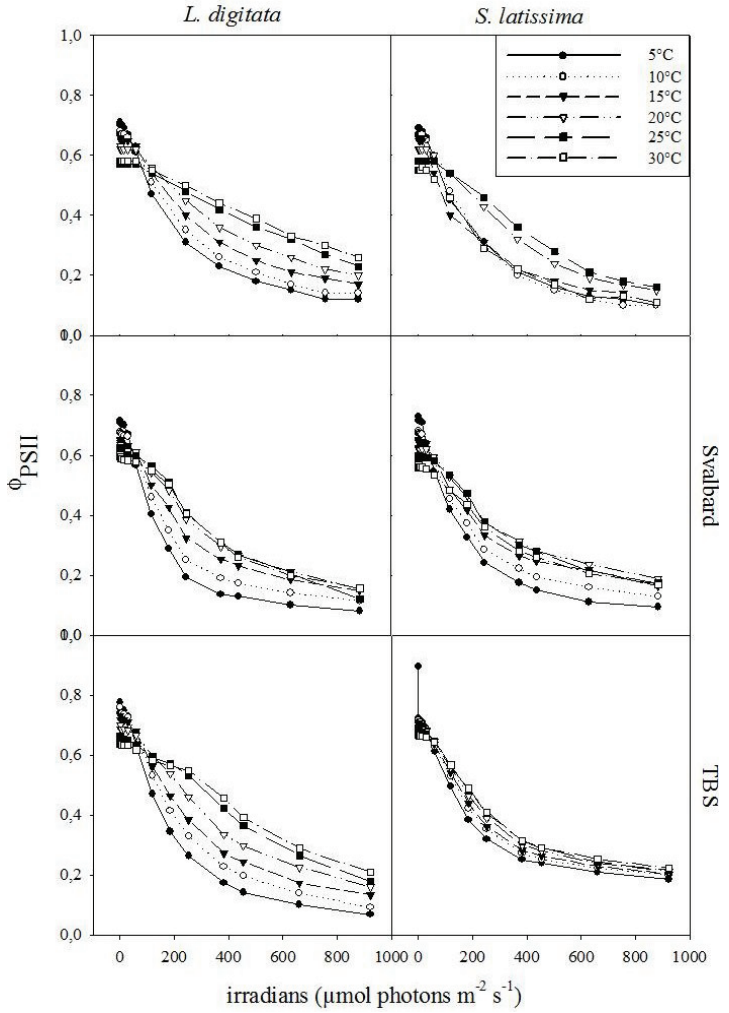

Figure 11. ϕ_{PSII} as a function of increasing irradiace. Graphs for each temperature investigated are shown. A) *L. digitata* sampled April, B) *S. latissima* sampled April, C) *L.digitata* sampled May D) *S. latissima* sampled May, E) *L.digitata* sampled August and F) *S. latissima* sampled August.

5 Discussion

The results show that temperature has a profound effect on photosynthesis in *Laminaria digitata* and *Saccharina latissima.* The effect was similar in the two species, however,

variations were observed between individuals sampled at different geographical locations and during different times of the year.

The results showed differences, most possible due to biological variation between the replicates sampled at the same time and location in the size/severity of the response, although the trend in the temperature response was the same between the replicates. The variation between individuals sampled at the same time and place can be explained by variations in physiological status/in the tissue. Previous studies of kelp have shown that chloroplast density is dependent on the thickness and age of the algae and there can be variations in chloroplast numbers, age and functionality at a given area of the lamina (Forbord 2004, Valle 2005). Kelp has the ability to adapt to the specific environment, and as the kelp forest is dense the light that reaches each individual varies greatly and leads to differences in tissue thickness and composition (Colombo-Pallotta et al. 2006, Gomez et al. 2009). It was observed that the color of *S. latissima* tissue used in the oxygen production measurements went from brow to bright green at 30 °C indicating de-naturation of pigment-proteins and further on degradation of chl *a* (Johnsen and Sakshaug 2000).

5.1 Effect of water temperatures on photosynthesis – seasonal and geographical differences

The results shows a clear temperature optimum at 20-25 °C in maximum net oxygen production in individuals sampled at TBS in August, while no clear optimum in $rETR_{max}$ was observed in the temperature range for the same individuals (Figure 6 and 8). This discrepancy between the two methods used could be accounted to the fact that $rETR_{max}$ is a measure of the gross photosynthetic rate, while the oxygen production rate measured in light represent net photosynthetic rate (Hancke et al. 2008a,b). As the respiration rate increases with temperature (Figure 6, Hancke et al. 2008a), the net oxygen production rate is depressed accordingly compared to the gross production with increasing temperature. Negative net oxygen production rate is seen when respiration exceeds production rate (Figure 6).

The results indicate a geographical dependency in the short term response of photosynthesis to elevated water temperature in *Laminaria digitata* and *Saccharina latissima*. The $rETR_{max}$ data from Svalbard indicates a temperature optimum of 20°C for maximum electron transport rate in these individuals (Figure 8, C and D). In comparison, $rETR_{max}$ data from the Trondheimsfjord gives no clear temperature optimum for 5 to 30° except for *S .latissima* sampled in April where an optimum was observed at 20°C (Figure 8).

Earlier investigation of photosynthesis in algal species from the Antarctica show that these algae are adapted/acclimated to a colder temperature regime, and therefore are more sensitive to higher temperatures (Gomez et al. 2009). This is not necessarily the case for arctic kelp since this is a younger region and the species found there have had shorter time to adapt to the environment (Gomez et al. 2009). Cold adaptation in polar algae species include maintenance of membrane fluidity critical for correct function of the light harvesting apparatus, cold adaption of Calvin-Benson cycle enzymes, antifreeze proteins that prevents freezing of the

cellular fluids and cold adaption of the electron transport chain molecules (Davison and Davison 1987, Morgan-Kiss et al. 2006).

It is not likely that temperature increase in Arctic waters will be so high as to reach the highest temperatures investigated in these experiments, but it is useful to know that kelp growing along the coast of Svalbard will be more sensitive to increase in water temperature than kelp growing along the coast of Mid-Norway. The present data show the effect of short-term exposure to a higher temperature and it is likely that with a longer exposure time the optimum temperature for photosynthesis will be lower than what was seen from these experiments. It has been reported that a short-term exposure to temperature does not give the same response as long term exposure. In an experiment done by Strømgren in 1977 four different intertidal species of seaweed from the Trondheimsfjord was sampled and increase in length was measured with laser diffraction technique at temperatures from 10 to 35 °C after different exposure times (Strømgren 1977). The results showed an increase in growth rate for all temperatures during the first hour. After two days the growth rate stabilised for all temperatures, and after two weeks the growth rate had declined to under the rate of the control samples grown at 6-8 degrees.

Difference in response to short and long time exposure to temperature stress was also seen in an experiment executed by Gerard and Du Bois in 1988. They compared growth in *S. latissima* sampled at its southernmost and central distribution locations at the east coast of USA. Individuals from both sampling sites were incubated at 20 °C for several weeks. All individuals tolerated short time exposure to 20°C, but the individuals from the northernmost sampling died after 3 weeks while the southern individuals died after 6 weeks (Gerard and Du Bois 1988).

A possible explanation for the results seen in the study from Gerard and Du Bois is the high respiration rate seen at 20°C in *S. latissima* from August (Figure 6, D). A high respiration rate will lead to a decrease in assimilated carbon available for growth, and might also lead to an increased bacteria growth than can damage the kelp (Carlsen et al. 2004). In this study the highest respiration rate was found in the August kelp. This kelp had been exposed to the highest *in situ* temperatures in advance of the experiments and was thought to be the most temperature tolerant kelp in comparison to the kelp sampled in April and May (Svalbard). Davison et al. 1991 found that kelp grown at 15°C had a lower respiration rate when exposed to a water temperature of 20°C in comparison with kelp grown at 5°C (Davison et al. 1991). This is the opposite of what was seen in present study. An explanation for the observed differences in response of respiration to temperature can be a difference in epifauna and bacteria on the kelp used in these two studies. In the experiments done by Davison et al. the kelp was grown in a laboratory previous to the experiments, while the kelp in the present study had been growing in the ocean up to the time of sampling. Epigrowth and bacteria growing on the kelp changes from season to season and have been found to be highest in the summer (Carlsen et al. 2004). As the bacteria and epifauna also contribute to respiration this will be in addition to the kelps own respiration rate, and when measuring with oxygen

microelectrode it is impossible to separate respiration by the kelp from respiration in bacteria and epifauna. This additional respiration by bacteria and epifauna can be a reason for the higher respiration rate seen in the August kelp in comparison with the April kelp, and the study done by Davison et al. (Figure 6, Davison et al 1991).

The difference in temperature response of *Saccharina latissima* from April in comparison with the response in August is supported with evidence of the existence of different ecotypes of this particular species (Figure 8, Gerard and Du Bois 1988). Each ecotype is adapted to a certain environment and this will lead to a difference (i.e. biological variation) in the observed effect of changing environmental factors as temperature, salinity and light.

In Breemans review article from 1988 *Saccharina latiissima* is used as an example of a kelp species with no obvious thermal ecotypes in its distribution range (Breeman 1988). I argue that this might not be the case from the observed differences in tolerance of temperatures from 20 – 30 °C observed in Trondheimsjford kelp and Svalbard kelp (Figure 8 and 10, C and D). Sampling sites used in experiments supporting Breemans conclusion does not include latitudes higher than 69° north, while our northernmost sampling site was 79° north. This might be the reason that we observed a latitude difference in temperature tolerance.

The effect of sampling location on response of temperature of photosynthesis show the importance of selecting different sampling sites when investigating the effect of environmental factors on species that has wide distribution. It is also important to consider the sampling time, and preferably sample at several different time of the year. Due to the fact that I only sampled Svalbard kelp in the spring, a valuable addition to the present study would be to sample in the early fall. This would give a more detailed picture of how a rise in water temperature could affect Svalbard kelp.

For *Saccharina latissima* there has been observed a change in distribution along the coasts of Europe during the recent years (SFT 2007, Ottesen 2010). Though it has not been proved that this change is correlated to changes in water temperature, it is a likely contributing reason though the whole explanation most likely will be a complex mix of changes in the environment (Breeman 1988, Muller et al. 2009). Higher temperatures also give an enhanced epigrowth that will highly influence photosynthesis and respiration measurements and can be damaging to the kelp (Forbord 2004,Valle 2005).

The effect of sampling time on response of elevated water temperature is caused by the kelps temperature acclimation over the year. Kelp sampled in April had for several months in advance of the sampling been exposed to winter water temperatures and had optimized its photosynthesis for these cold temperatures (Davison and Davison 1991). Kelp sampled in August had been exposed to much higher in *situ* temperatures in advance of sampling and had acclimated to a higher temperature regime (measured *in situ* temperature at sampling in April 4.6°C versus 14 °C in August). This explains better temperature tolerance found in August individuals in comparison with April individuals seen in $rETR_{max}$ (Figure 8, A, B, E, F). Q_{10} values show that the respiration rate and the gross oxygen production rate of the kelp is

increasing more rapidly in the August kelp in comparison to the April kelp (Table 1). This supports the theory of a seasonal temperature acclimation to water temperature (Table1).

A study of the change in surface water temperature along the Portuguese coast showed that that the increase in water temperature was most pronounced during winter and summer (Lima et al. 2007). Winter is the most critical time for kelp as they reproduce in the winter. The ability to reproduce is also influenced by temperature, and a rise in the surface water temperature from normal *in situ* temperatures might lead to decrease in recruitment (Breerman 1988). This could be a contributing reason for the loss of *Saccharina latissima* along the southern part of Norway (Breeman 1988, Moy et al. 2008, Ottesen 2010).

5.2 Effect of water temperature on photosynthesis – physiological responses to short-term elevated water temperature in *L. digitata* and *S. latissima*

An elevated water temperature had different effects on the $rETR_{max}$ and net oxygen production rate. $rETR_{max}$ values increased over the entire temperature regime investigated (Figure 8, E and F) while net oxygen production rate peaked at 20° C and showed a decrease at temperature above 25 to 30°C in the TBS kelp from August (Figure 6, C and D). $rETR_{max}$ is a measure of the gross photosynthetic rate and the main reason for the different response of $rETR_{max}$ and net oxygen production is the increase in respiration rate with temperature. When the respiration rate increases it uses more of the oxygen produced by the kelp, which results in a decrease in the oxygen released into the water. If the dark respiration rate is added to net oxygen production rate this gives a rough estimate of the gross oxygen production at each temperature. The estimated gross O_2-production show an increased rate up to approximately 20 to 25°C, and a large decrease at 30 °C (Figure 7, C and D). This hampers a direct comparison between the two different estimates of gross photosynthetic rates, $rETR_{max}$ and respiration and maximum net oxygen production rate added.

Adding the dark respiration to the light production rate of oxygen only show a rough estimate of the gross oxygen production rate. This approach is found to underestimate the gross oxygen production rate with as much as 20-30 % (Weger et al. 1989, Lewitus and Kana 1995). The reason for this underestimation of gross photosynthesis is caused by light dependent reactions that consume oxygen, processes as the Mehler reaction, chlororespiration and cyclic electron flow around PS I and PS II in the light (Falkowski et al. 1986, Prasil et al. 1996, Munekage et al. 2004, Miyake 2010). These reactions can all contribute to a high $rETR_{max}$ and increased oxygen consumption in the light compared to the dark. All reactions are correlated with stress, and with temperatures above optimum they are likely to occur in a higher rate than when the kelp is exposed to temperatures close to the normal in *situ* temperature (Kromkamp et al. 2001, Morris and Kromkamp 2003, Miyake 2010).

In the Mehler reaction (water-water cycle) oxygen is used as an electron acceptor at the reducing side of PS I. The product of this reaction is oxygen radical that is transformed to oxygen and hydrogen peroxide by enzymes. The oxygen can be used as a reduction agent, and hydrogen peroxide is reduced to water. The Mehler reaction is dependent upon electrons from

water that can be donated to P680 in PS II reaction center and be transferred through the electron transfer chain to PS I (Miyake 2010). As the Mehler reaction uses electrons from the electron transport chain and oxygen, it will lead to a decrease in the net oxygen release from the tissue while contributing to maintain the electron transport (Heber 2002, Miyake 2010). This will contribute to the observed discrepancy in the measured net and gross photosynthetic rates (Flameling and Kromkamp 1998, Morris and Kromkamp 2003).

The water-water cycle and cyclic electron flow around PS II and PS I contributes to ATP synthesis by acidification of thylakoid lumen. Cyclic electron flow around PS II is donation of electrons from the electron acceptor side of PS II to the donor side of PS II, which leads to a cycling of electrons around P680 (Falkowski et al. 1986, Prasil et al. 1996). In PS I electrons are given from the donor side of PS I back to cyt b6f complex (Johnson 2011). At high temperatures damage is likely to occur in the photosynthetic components and so the increase in ATP production will help in the repair of these molecules (Munekage et al. 2004).

Even if estimated gross O_2 is an underestimation of the real gross oxygen production rate I argue that the trend of the temperature response in this estimate is the most correct measure of temperature effect on gross photosynthetic rate. When measuring with Phyto-PAM the tissue is only exposed to light in the three minutes it takes to measure the RLCs. In the oxygen measurements the tissue is exposed to light for minimum 30 minutes to ensure correct rates of production. As the light exposure in the PAM-measurements is so brief it is possible that the tissue is able to process the light it absorbs as normal even if the photosynthetic machinery is affected by the elevated temperature. If the light exposure had been longer it is possible that the high $rETR_{max}$-values seen at the highest temperatures would show a similar response as estimated gross O_2-production.

Φ_{PSII} is increasing at the highest irradiances for the highest temperatures investigated in these experiments (Figure 11). This has also been seen in model plant Arabidopsis leaves under short term exposure to high temperatures (Zhang and Sharkey 2008). This temperature response can be explained by the increased $rETR_{max}$ observed for all Trondheim fjord kelp. When electron rate increases the need for energy dissipation by fluorescence decreases and so there is a decrease in F'_0 and as a result an increase in Φ_{PSII}. In addition the high rETR will increase acidification of the inside of the thylakoids wich leads to the induction of qE, quenching of fluorescence (Brunet et al. 2011). The reason for the increase in $rETR_{maxI}$ in the Arabidopsis leaves was found to be an increase in cyclic electron flow which resulted in oxidation of quinine A and B, the primary electron donors of the electron transport chain which allowed for rapid transfer of electrons from P680 (Zhang and Sharkey 2008).

From these result there is no clear effect of temperature on α and there is not a great difference between the α values for the different species and the different sampling sites (Figure 9). The highest values of α was found in August kelp sampled in the Trondheimsfjord. A high α indicates that these individuals are adapted to a low light regime and this can be explained by the fact that samples were taken in a very dens kelp forest with high biomass, and so shading from neighboring individuals would affect the amount of available light (

Henely 1993, Gomez et al. 2009). Davison et al. 1991 found high α value in *L. saccharina* to be a function of growth temperature. Kelp grown at 15 °C had higher α-values than kelp grown in 5°C (Davison et al. 1991). This might also be the case in these experiments as the kelp that is assumed to have experienced the highest growth temperature, the August kelp, has the highest α-values but on the basis of the present data it is not possible to disprove or confirm this hypothesis.

Both 25 and 30 ºC are above the observed optimum temperature for net oxygen production (Figure 6), but 30 ºC resulted in a greater decrease in oxygen production than exposure to 25 ºC. Control experiments were executed to make sure that this effect was not caused by the fact that the kelp tissue was exposed to two above optimum temperatures in succession. In the control experiment kelp tissue were first exposed to 10 ºC while measurements of production and respiration were made to make sure that the tissue was healthy. After these measurements the tissue was exposed of 30 ºC directly. This gave the same results as if the kelp had been exposed to 15-25 ºC in advance of 30 ºC. This indicates that the effect of temperature on photosynthesis is higher for temperatures that are significantly above optimum temperature.

5.3 Future perspectives

The result obtained in this thesis indicates that an elevated surface water temperature along the Norwegian coast can lead to a decrease in the total kelp forest photosynthesis and growth rate. If water temperature in the summer is >20°C for short time periods this will have a negative effect on the kelp forest photosynthesis and production which again will affect the kelp forest growth (Valle 2005, Forbord 2004). The duration of exposure to high temperatures will be essential for understanding how production will change, and long-term exposure experiments must be conducted to fully understand how temperature will affect photosynthesis.

My experiments show that there is a need for a more detailed understanding of the effect of increased water temperature on photosynthesis in *Laminaria digitata* and *Saccharina latissima*. This could be achieved with experiments focused on differences in temperature response as a function of geography and season. In addition there is a need for a better understanding of the physiological background for the temperature response of photosynthesis, and a better understanding of the processes that leads to the observed difference in response of photosynthesis measured with different instruments and the difference in the response between individuals sampled at the same time and place. Since the lamina of a given kelp species consist of tissue with different age, from newly formed and up to 3 year old tissue in the Arctic, the corresponding biological variation in chloroplasts may induce large variations in photosynthesis and respiration (Carlsen et al. 2004). A complete understanding of these processes is highly needed for future investigations.

6 Conclusions

- Temperature had a profound effect on the photosynthesis in both *L. digitata* and *S. latissima* species investigated in this study. The most important result is the optimum temperature for net photosynthesis at 20 to 25°C for the kelp sampled in the Trondheimsfjord in early fall.
- Temperature effect in kelp is dependent upon habitat. This is shown clearly in the $rETR_{max}$ results that show gross photosynthesis in the different species at Svalbard and in the Trondheimsfjord. The $rETR_{max}$ in the Svalbard kelp showed a temperature optima at 20°C, while in the Trondheimsfjord kelp the $rETR_{max}$ increased over the entire examined temperature range (5 to 30°C).
- Temperature effect is dependent on time of year of sampling. This is best seen in the results for $rETR_{max}$ and E_k . Both $rETR_{max}$ and E_k are higher in April sampled kelp than in August sampled kelp in both *L. digitata* and *S. latissima.*
- The observed decrease in oxygen production in August may help explain the loss of kelp forest in the southern parts of the Norwegian coast as kelp that grow in this area experience water temperatures as high as 20 °C during summer months
- $rETR_{max}$, net oxygen production and estimated gross oxygen production rate showed the same response to an increased temperatures below 20 °C. At the highest temperatures there was a mismatch between the different methods, net oxygen production and estimated gross oxygen production rate show an optimum temperature between 20 and 25°C while there was no clear optimum in $rETR_{max}$.

7 References

7.1 Litterature

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