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How Lamina Age Influence Photosynthetic Response to Light Variations in *Saccharina latissima*

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Marine Coastal Development
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Longyearbyen, May 2014

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Abstract

Photosynthetic rates, measured on the basis of *in vivo* chlorophyll *a* fluorescence, as a function of increasing lamina age was studied in *Saccharina latissima* from Trondheimfjorden, Norway in winter, summer and autumn 2013. Season influence on the relationship between tissue age and photosynthetic acclimation was investigated. Increasing age of tissue was only found to have significant influence on maximum photosynthetic rate (P_{max}), the maximum light utilization coefficient (α) and light saturation parameter (E_k) in summer (May) relative to the other seasons. Changes in photosynthetic parameters throughout the length of the lamina varied between individuals, both between and within seasons. Results from this study indicate that photosynthetic performance, both across the width and the length of lamina, in a single individual of *S. latissima* is dependent on how the lamina is positioned in the water column, and how the available light is illuminating the different parts of the lamina. The difference between tissues of different age was more profound in large individuals, sampled in a dense kelp forest. In this scenario the lamina of one specific individual could experience a variety of irradiances, due to self-shading and shading by the canopy layer. There was a seasonal dependence of photosynthetic rates depending on temperature, nutrient availability and light climate. Significantly lower $P_{max,}$ E_k and α were found in autumn sampled individuals of *S. latissima* compared to individuals sampled in winter and summer, indicating nutrient depletion and LL acclimation. Low light acclimated algae is thought to be a result of a high concentration of optical active components in the water (Inherent Optical Properties comprising absorption and scattering of water, phytoplankton, coloured Dissolved Organic Matter and Total Suspended Matter) in autumn, thus providing a significant attenuation of light in the water column giving lower irradiances than in winter and summer. This study shows that measurements of photosynthesis from meristem tissue may be different from other parts of the lamina due to different age and physiology. Consequently, it is not enough to measure photosynthetic rate and production on one part of *S. latissima* lamina and assume that the rates of these processes are representative for the whole length of lamina.

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Abbreviations

Symbol	Explanation	Unit
PSU	Photosynthetic unit	
RC	Reaction center	
PS	Photosystem	
F_0	Minimum Chl *a* fluorescence of PSII in dark-acclimated cells	
F_0'	Minimum Chl *a* fluorescence of PSII in cells acclimated to actinic light	
F_m	Maximum Chl *a* fluorescence of PSII in dark-acclimated cells	
F_m'	Maximum Chl *a* fluorescence of PSII in cells acclimated to actinic light	
Φ_{PSII_max}	Maximum quantum yield of charge separation in PSII in dark acclimated chloroplasts	
Φ_{PSII}	Operational quantum yield of charge separation in PSII in actinic light	
rETR	Relative electron transfer rate	
P_{max}	Maximum photosynthetic rate	
α	Light utilization coefficient	
E_k	Light saturation index	µmol photons $m^{-2}s^{-1}$
NPQ	Non-photochemical quenching	
E_{PAR}	Irradiance (400-700 nm)	µmol photons $m^{-2}s^{-1}$
HL	High-light	
LL	Low-light	

Introduction

The sugar kelp, *Saccharina latissima* (L.) C.E. Lane, C. Mayes, Druehl and G.W. Saunders, is a brown alga (Phaeophyta) in the order Laminariales. It is a common species along the Norwegian coast and around Spitsbergen, and one of the dominating species in the kelp forest found on rocky shores of Norway (Lüning 1990).

The kelp forest is an important ecosystem along the Norwegian coast as it is the home, shelter and breeding ground for many sessile and motile organisms. In addition, macroalgae affects water motions and light penetration into the water column (Hayward 1980; Kuklinski and Barnes 2005; Carlsen et al. 2007; Graham et al. 2007; Christie et al. 2009; Kowalczuk et al. 2009). Sub-polar and temperate macroalgae such as *Saccharina latissima* are highly productive algae and contribute a major proportion of the primary production (g C m^{-2} $year^{-1}$) of inshore waters (Mann 1972; Johnston et al. 1977). Recently, there has been a decrease in the kelp biomass/areal coverage and a shift in species composition from Laminariales to thin, small ephemeral algae in the southern parts of Norway (Andersen et al. 2011; Moy and Christie 2012). This may have ecological consequences on higher trophic levels due to loss of primary production and the three dimensional kelp habitats (Carlsen et al. 2007; Christie et al. 2009; Andersen et al. 2011). *Saccharina latissima* is also commercially attractive for production of biofuel and a potential source of alginate which has a wide range of applications (e.g. textile printing, food thickening and pharmaceutical products, McHugh 1987).

Saccharina latissima consist of a holdfast (haptera), stipe and lamina. A growth zone called the meristem is placed above the stipe (lowest part of the lamina) and thus the older parts of the lamina are at the distal end (Parke 1948; Sjøtun 1993). The oldest part of the kelp is usually lost through abrasion and necrosis, and the breakdown of the tissue leads to formation of coloured dissolved organic matter (optically active molecules, cDOM) providing valuable carbon sources for associated microbial populations (Drew 1910; Laycock 1974; Carlsen et al. 2007). There is variation in chloroplast content throughout the lamina of Laminariales, causing variations in photosynthesis and respiration rates (Colombo-Pallotta et al. 2006). This can have a significant influence on the net primary production NPP (total amount of chemical energy fixed by the processes of photosynthesis minus the chemical energy lost through light and dark respiration, Vitousek et al. 1986) made available for other trophic levels. Consequently, it is not enough to measure photosynthetic rate (g C pr biomass per time) and

production on one part of *S. latissima* lamina (one age cross-section) and assume that the rates of these processes are representative for the whole length of lamina, when estimating total primary production (g C per area per time) of one individual or the whole kelp forest. In addition, a given algae will change its physiological characteristics throughout the year (seasonal acclimation) as environmental parameters such as light regime (e.g. irradiance, spectral irradiance and day length), temperature and nutrient input changes (Gévaert et al. 2002).

Photosynthesis

Photosynthesis is a photochemical process where Chl *a* containing organisms absorb energy in the form of light (ħv), converting and store it as organic C (Sakshaug et al. 1997; Sakshaug et al. 2009). The overall equation for photosynthesis describes how carbon dioxide and water is used to synthesize carbohydrates such as glucose ($C_6H_{12}O_6$), releasing oxygen as a byproduct (Sakshaug et al. 2009).

$$6CO_2 + 6H_2O + \hbar v = C_6H_{12}O_6 + O_2 + \text{heat} \quad (1)$$

Photosynthesis can be divided into two sets of reactions: light-reactions and dark-reactions. The light-reactions use solar energy to generate ATP and $NADPH_2$, and the dark reactions use the ATP (and $NADPH_2$), produced in the light-reactions, in reduction of CO_2 to form carbohydrates in the Calvin-Benson cycle (Benson and Calvin 1950).

Light absorption leads to changes in the energy state of pigment molecules located in the thylakoid membrane inside the chloroplasts (Falkowski and Raven 2007). The pigment pool consist of two functional categories; pigments used for light harvesting and for photoprotection. The pigments in the light-harvesting complexes in brown algae are chlorophyll (Chl) *a* and *c*, and the carotenoid fucoxanthin and violaxanthin. The photoprotective carotenoids are basically a de-epoxidation of violaxanthin to zeaxanthin through the xanthophyll cycle (Grzymski et al. 1997; Brunet et al. 2011). The excitation energy absorbed by the light harvesting antennae pigments is transferred from pigment to pigment till it reaches the reaction centers of the two photosystems (PS) II and I. This special transfer mechanism is called fluorescence resonance energy transfer (Owens 1991).

Photosystem I and II

The reaction centers in PS I and II consist of special Chl *a* called P680 (PSII) and P700 (PSI). The numbers (680 and 700) refers to the wavelength of light that gives maximum absorption (Falkowski and Raven 2007).

Once a PSII reaction centre captures a photon an excited state of Chl *a* molecule is formed (^{1}Chla*) (Müller et al. 2001). There are three possible de-excitation pathways from an exited state; (i) re-emission of light as fluorescence, (ii) kinetic transfer and dissipation of energy to the environment in the form of heat and (iii) photochemistry which will eventually lead to the synthesis of high-energy molecules i.e. ATP and NADPH (Krause and Weis 1991; Müller et al. 2001; Falkowski and Raven 2007). Excitation energy can also decay through the triplet state (^{3}Chla*) which can transfer the energy to O_2 and create the damaging and reactive oxygen species $^1O_2$* (Müller et al. 2001).

If the energy is to be used in photochemistry the electron must be passed from P680 to the primary electron acceptor of the electron transport chain (Falkowski and Raven 2007).

Electron transport chain

The electron transport chain mediates the transfer of electrons from PSII to PSI in a series of redox reactions and through the process generates ATP and NADPH. A Z-scheme is a schematic representation of the connection between the two photosystems (Fig. 1).

The exited electron in P680 is donated to the first molecule in the electron transport chain (pheophytin a) and the replacement of the electron eventually leads to the oxidation of water which splits into oxygen and hydrogen ions. The splitting of water takes place in the water splitting complex (Metz et al. 1989). Several redox reactions transfer the electron through the electron transport chain between PSII and PSI. Transfer of H^+ from stroma to the inside of the membrane generates a proton gradient across the thylakoid membrane that is used in synthesis of ATP by the ATP synthase complex. Electrons delivered to PSI reduces the Chl *a* (P700) and absorption of a photon leads to the oxidation of P700 to P700*. The electron is rapidly passed through a series of electron carriers and in the last step the membrane-based enzyme ferredoxin-NAPD reductase uses the transferred electron and a hydrogen ion to reduce $NADP^+$ to $NADPH_2$ (Falkowski and Raven 2007).

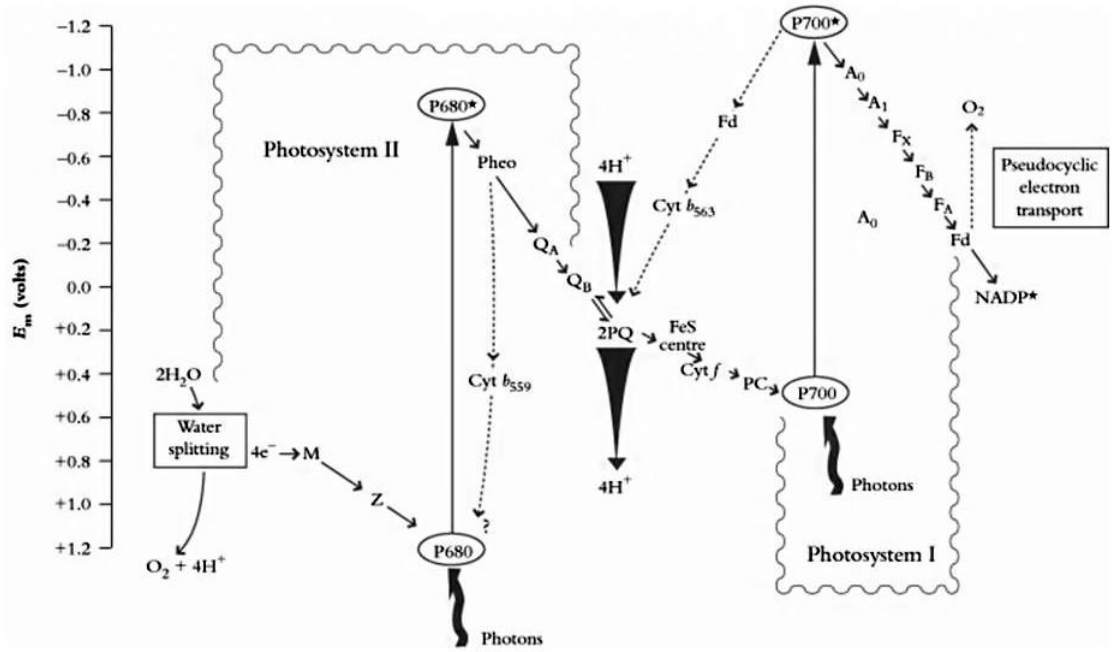

Fig. 1*. The "Z-scheme" for photosynthetic electron transport. The Z-scheme describes the connection between the two photosystems based on the redox potential (vertical axis) of the two reaction centers and the electron carriers. The higher the position of a molecular species, the more negative reduction potential (donates electrons more easily). For more description see text above. Figure modified after Critchley (1997).*

ATP (and $NADPH_2$), produced in the light-reactions, are used for reduction of CO_2 to form carbohydrates in the Calvin cycle (Benson and Calvin 1950). Due to the methodical approach in this thesis (measurements of fluorescence) a detailed description of the dark-reactions will not be given.

In vivo Chl a fluorescence

In vivo Chl *a* fluorescence describes the phenomenon where light absorbed by Chl *a* molecules at one wavelength is re-emitted at another (longer) wavelength with a peak at 685 nm (Johnsen and Sakshaug 2007). By measuring the fraction of absorbed light reemitted as fluorescence the efficiency of photosynthesis can be investigated.

A large number of different techniques have been designed to measure variable Chl *a* fluorescence such as the Pulse Amplitude Modulation technique (PAM) developed by Ulrich Schreiber with Walz industries (Schreiber 1998). The PAM technique uses LED probing light pulsating at different frequencies to detect the minimum and maximum Chl *a* fluorescence from dark and light- acclimated cells. The probe light does not supply sufficient light energy to induce photosynthesis, only enough to induce a background minimum fluorescence (F_0) signal from a dark-acclimated cell. Dark-acclimated cells possess the maximum fraction of

open reaction centers (RCs) and the fluorescence emitted is derived only from light harvesting antenna. A saturation pulse of white light is then applied to the dark acclimated cells to close all RCs in PSII to obtain the maximum fluorescence (F_m) signal (Ralph and Gademann 2005). From F_m and F_0, the maximum quantum yield of PSII charge separation (Φ_{PSII_max}) can be calculated and is a measure of the fraction of healthy RCs (Genty et al. 1989; Hancke et al 2008b).

Ground fluorescence in actinic light (light that induce photosynthesis) conditions, F'_0, is fluorescence emitted from the chloroplasts (about 95% from PSII, Johnsen and Sakshaug 2007) when they are exposed to a given irradiance. The corresponding maximum fluorescence, F'_m, is fluorescence emitted after the tissue is exposed to a saturating light (Genty et al. 1989; Sakshaug et al. 1997; Hancke et al. 2008b). From these two values the operational quantum yield of chlorophyll *a* fluorescence, Φ_{PSII}, can be calculated and it is defined as the proportion of the light absorbed by chlorophyll associated with PSII that is used in photochemistry (Genty et al. 1989; Maxwell and Johnson 2000; Hancke et al. 2008a).

Fluorescence quenching

When light energy absorption exceeds the capacity of light utilization is it necessary to minimize the potential of photooxidative damage (production of $^1O_2$*). Once PSII absorbs light and Q_A has accepted an electron it is not able to accept another until it has passed the electron to the next electron carrier (Q_B) (Fig. 1, Maxwell and Johnson 2000). During this period, the reaction centre is said to be "closed". The light energy transferred to a closed reaction center cannot be used for photosynthesis and will be dissipated as heat and fluorescence (Maxwell and Johnson 2000; Falkowski and Raven 2007).

High quantum efficiency of photochemistry results in decrease, or quenching, of fluorescence and is termed photochemical quenching (qP) (Schreiber et al. 1986). The kinetic transfer and dissipation of energy to the environment in the form of heat, termed Non-photochemical quenching (NPQ), can be seen as the difference between F_m and the measured F_m' (Müller et al. 2001). The major and most rapid NPQ mechanism is related to the buildup of a proton gradient causing lowering of pH in thylakoid lumen. This induces xanthophyll synthesis via the xanthophyll cycle (Johnsen et al. 2011).

Photosynthesis versus irradiance curves (P *vs.* E curves)

Photosynthesis versus irradiance curves (P *vs*. E curves, Fig. 2) show change in photosynthetic rate as a function of increasing irradiance and is typically divided in three distinct regions (i) light-limited region (ii) light saturated region and (iii) photoinhibited region (Sakshaug et al. 1997).

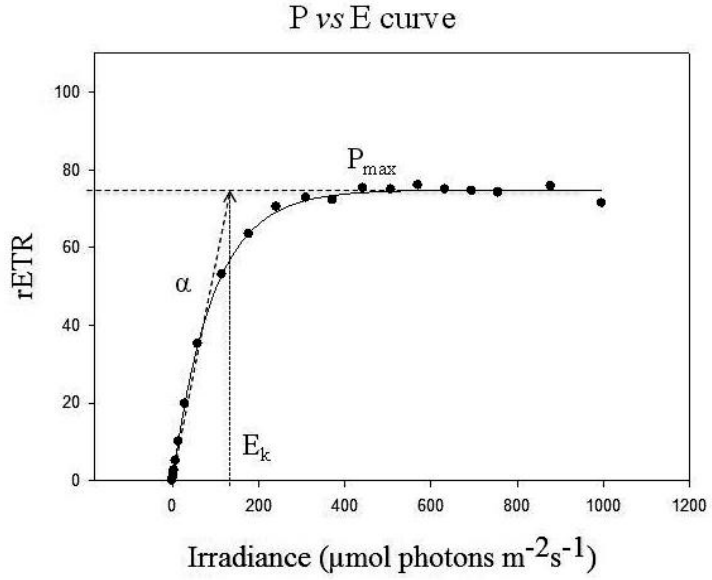

Fig. 2. *P vs. E Curve derived from Saccharina latissima #3. The photosynthetic parameters* P_{max}, α, *and* E_k *is shown.*

Relative electron transfer rate, rETR, is an approximation of the rate of electrons pumped through the photosynthetic chain and can be a proxy for the gross photosynthetic rate (Genty et al. 1989; Kroon et al. 1993). At the lowest irradiances there are still open reaction centers and thus photosynthesis increases linearly with increasing irradiance. This initial slope of the P *vs*. E curve is termed the maximum light utilization coefficient (α) (Sakshaug et al. 1997). As irradiance increases the rate of electron transport levels of and reaches its maximum rate denoted maximum photosynthetic rate (P_{max}) (Ralph and Gademann 2005). The light saturation index, $E_{k,}$ indicates the saturation irradiance, and the photoacclimational state of the algae. E_k is relatively higher in high-light (HL) acclimated cells than in low-light (LL) acclimated cells (Sakshaug et al. 2009). With further increase in irradiance a reduction in photosynthetic rate relative to the saturation level may take place and is referred to as "photoinhibition" comprising several different mechanisms (Kirk 1994).

Several equations have been proposed to fit the P *vs*. E relationship (Webb et al. 1974; Jassby and Platt 1976; Platt et al. 1980). The Webb equation was chosen in this thesis as none of the PAM-derived P *vs*. E curves showed photoinhibition at high irradiances.

Parameters derived from a P *vs.* E curve (P_{max}, α and E_k) can be used to describe the photosynthetic capacity of a photosynthetic organism and the variability in the P *vs*. E curve is commonly used to assess differences in photoacclimation (MacIntyre et al. 2002).

Seasonal changes in light climate

Seasonal variations in abiotic conditions affect the growth and photosynthetic performance in *Saccharina latissima*. Strong season variation in environmental factors such as light regime (irradiance, spectral composition and daylength) is evident at high latitudes and it has been shown that different processes such as growth in *S. latissima* is season dependent; with more rapid growth in winter and spring and little growth in summer and fall (Parke 1948; Kain 1979; Lüning 1979; Sjøtun 1993; Brunet et al. 2011).

Variation in light available for photosynthesis is also associated with concentrations of optical active components, such as coloured dissolved organic material (cDOM), phytoplankton and particulate matter (Kirk 1994). There is high season variation in phytoplankton in Trondheimsfjorden. Generally, during the winter season (November-February) the phytoplankton population is poor with low biomass (Sakshaug 1972). A spring bloom normally reaches a maximum in April and in autumn maximum population densities is typically observed in August (Sakshaug and Myklestad 1973). Drain-off from rivers is also season dependent, and is associated with variation in particulate matter and terrestrial cDOM in the water. Characteristic for Trondheimfjorden is that the drain-off is small in winter, but increases during spring to a maximum in May-June when thawing starts in the mountains (Sakshaug 1972).

Even though *in situ* irradiance in summer may be up to 1600 µmol photons m^{-2} s^{-1} in air and up to 600 µmol photons m^{-2} s^{-1} at 3 m depth the growth rate is low in kelp from June to November (Andersen et al. 2011; Valle 2014). This can be related to the rise in temperature with season, as reduced growth is expected in *Saccharina latissima* exposed to temperatures above 15°C (Bolton and Lüning 1982). Epiphytic growth can also have adverse effects as it may damage tissue and deprive their host of light through shading. Andersen et al. (2011) showed that shading from epiphytes was low in summer and spring but changed dramatically in autumn where fouling was high (Fig. 3). The shading caused by epiphytic cover was substantial and light was reduced with as much as 11% (Bryozoans) to 91% (Tunicates) depending on which species dominating the epiphytic coverage. In addition, reduced growth in macroalgae due to NO_3 deficiency is evident in temperate waters (Lapointe et al. 1992).

NO_3^- and NO_2^- (mg L^{-1}) measurements from "Trollet" sampling location in Trondheimfjorden (January 2002-January 2003) showed that nitrogen in the water typically is highest in winter (from December to February) and declining during summer with minimum values in September. N:C ratio in *Saccharina latissima* corresponds with the ambient nitrogen in the water with highest ratio in winter season and lowest in summer i.e. June, July and August (Hilstad 2005).

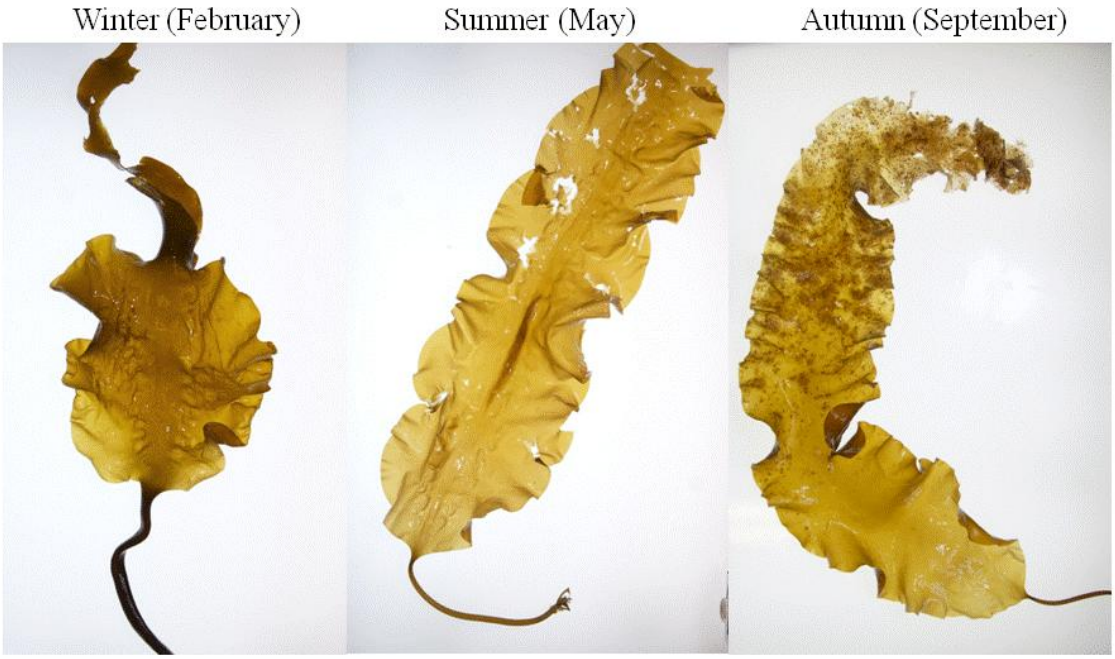

Fig. 3. *Seasonal patterns of epiphytic growth. The Figure shows sampled Saccharina latissima from Brennebukta in February, May and September. Fouling was highest in autumn (Photo: Lene Lund).*

Photo-acclimation

Photo-acclimation describes the phenotypic response that rise following changes in irradiance and represents many processes which serve to optimize cell activities such as photosynthesis, respiration, growth and division (Falkowski and LaRoche 1991; Brunet et al. 2011). Short term (minutes-hours) photo-acclimation mainly concerns the xanthophyll cycle and associated NPQ. Long-term (hours-days) photoacclimation concerns changes of structure and composition of the photosystem and is characterized by changes in pigment composition, photosynthetic parameters, enzymatic activities involved in photosynthesis and respiration, and cell volume and chemical composition. Photo-adaptation indicates a more long-term change to maximize the evolutionary fitness based on genes present in a given species (Brunet et al. 2011).

Lamina acclimated to HL have specific physiological characteristics, such as lower chlorophyll *a* content per unit tissue, higher maximum photosynthetic rate (P_{max}), higher photoprotective capacity through NPQ and higher E_k relative to LL-acclimated cells (Ralph and Gademann 2005; Colombo-Pallotta et al. 2006). Consequently, P_{max} and E_k are frequently used as an indicator of the photoacclimation state, in addition to Chl *a* C^{-1} ratio (Sakshaug et al. 1997; Hennige et al. 2008).

A better overall understanding of the variations in photosynthetic and respiration rates in macroalgae tissue with different age, and how this relationship changes with season is necessary to obtain a complete understanding of how different environmental conditions influence the eco-physiology of *Saccharina latissima.*

The aim of this study was to investigate photosynthetic parameters, derived from measurements of *in vivo* chlorophyll *a* fluorescence, as a function of lamina age using the kelp *Saccharina latissima*. In addition, a season study was carried out to investigate how photosynthetic rates changed throughout the year.

Materials and methods

Sampling

Three individuals of *Saccharina latissima* were sampled at 2-6 meters depth in February, May and September, 2013, in Brennebukta, Trondheimfjorden (63°26'52.44'N; 10°19'45.12'E), Norway. Details of the samplings are given in Table 1, and weather data in addition to average daylength, solar angle and sea temperature is shown in Table 2. The samples were kept dark and cool during transport to the laboratory, and transferred to a large outdoor container (3 m^3) with running seawater with *in situ* temperature (Table 1), immediately after sampling. In winter, kelp was kept in the containers for averagely 16 days, in summer 5 days and in autumn for 2 days. Measurements were done on 4 different parts of the lamina representing different age of the tissue. Subsamples (4 replicates) were measured for every age across the width of the lamina as shown in Figure 4.

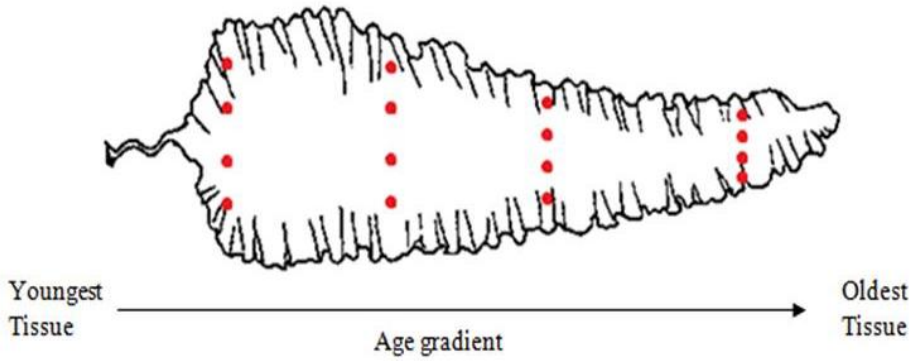

Fig. 4. *Saccharina latissima. Lamina was divided into four age zones from the youngest part (meristem) to the oldest part. Four subsamples (replicates) were measured on lamina tissue with different age. Figure modified from www #1.*

Table 1. Date and depth of sampling of *Saccharina latissima* in Brennebukta, Trondheimfjorden 2013 and description of sampling site, number of specimen and lamina size of the different individuals is listed. Phytoplankton concentration (cells/liter) in Trondheimfjorden and drain-off from Gaula, containing particulate matter and terrestrial cDOM, is listed to make an analysis of light climate with respect to the optical active components.

Date	Depth	Number of individuals	Lamina size distribution	Description of site	Optical active components
28.02. Winter	2-4m (low tide)	*n*=3	#1: 45 cm #2: 41 cm #3: 31 cm	Attached to a rope	Phytoplankton concentration: low* Drain-off: 9.34-9.37m³/s**
06.05. Summer	2-4m (low tide)	*n*=3	#4: 69 cm #5: 66 cm #6: 85 cm	Dense kelp forest	Phytoplankton concentration: moderate-high* Drain-off : March: 39.43-45.9m³/s**
02.09. Autumn	2-4m (low tide)	*n*=3	#7: 66 cm #8: 57 cm #9: 49 cm	Open area	Phytoplankton concentration: moderate* Drain-off: 23.6-120.9m³/s**

*Algeinfo (www #2) **Norges vassdrags- og energidirektorat (www #3)

Table 2. # of days with clear sky, overcast and rain. In addition, average daylength and average solar latitude 1 month before sampling dates and water temperature at sampling day is listed.

Season	# Days fair weather*	# Days overcast*	# Rainy days*	Average Daylength**	Average solar angle**	Sea temperature
February	7	12	7	08h 50m	14.6°	4 °C
April	6	12	8	14h 08m	36.6°	12 °C
August	8	8	12	16h 30m	40.0°	14 °C

*Meterologiske institutts klimadatabase (www #4) **Time and date AS (www #5)

Photosynthetic measurements

In vivo Chl *a* fluorescence kinetics was measured as a function of increasing irradiance using the Pulse Amplitude Modulated (PAM, Walz, Effeltrich, Germany) technique. A Diving-PAM equipped with a 2π cosine corrected Diving_LS PAM irradiance (E_{PAR}) sensor, measuring Photosynthetic Active Radiation (PAR, 400-700 nm, µmol photons m^{-2} s^{-1}) (Walz, Effeltrich, Germany), was used in the experiments.

Due to requirements of a new light source, Chl *a* fluorescence kinetics in the last *Saccharina latissima* sampled in February was measured with Phyto-PAM (Walz, Effeltrich, Germany) equipped with a photomultiplier detector (PM-101P, Walz, Effeltrich, Germany), a measuring and actinic LED (Measuring LED-Array-Cone PHYTO-ML and Actinic LED-Array-Cone PHYTO-AL, Walz, Effeltrich, Germany). Tissue samples of 0.5 x 1 cm were cut out and placed in 4 mL cuvettes with filtered seawater (0.2-µm sterile filters, Minisart, Santorius, Goettingen, Germany). Temperature in the cuvettes was held at a stable level at 4°C using a Peltier cell (Temperature Control Unit US-T, Walz, Effeltrich, Germany).

The Diving-PAM was equipped with a red light emitting diode (giving as little as < 0.15 µmol photons $m^{-2}s^{-1}$, at 0.6 kHz), for measurements of ground fluorescence (F_0) after 5 minutes of dark acclimation. An internal halogen lamp (custom made) provided a saturating flash (>5000 µmol photons $m^{-2}s^{-1}$, 0.8 s duration) ensuring that all reaction centers of PSII (RC_{PSII}) were closed, inducing maximum fluorescence (F_m). From the measured F_m and F_0, the maximum quantum yield of PSII charge separation (Φ_{PSII_max}) in the dark-acclimated cells was calculated as follows (Genty et al. 1989, Eq. 2).

$$\Phi_{PSII\_max} = \frac{F_m - F_0}{F_m} \quad (2)$$

Increasing irradiance (E), as indicated in Appendix B, of three minutes duration per E were used in measurements of P *vs*. E curves; recording changes in minimum (F_0') and maximum (F_m') Chl *a* fluorescence in actinic light. The operational quantum yield of PSII charge separation (Φ_{PSII}) was calculated for each irradiance in the P *vs.* E curves (Genty et al. 1989, Eq. 3).

$$\Phi_{PSII} = \frac{F'_m - F'_0}{F'_m} \quad (3)$$

Φ_{PSII} and irradiance (µmol photons m^{-2} s^{-1}) was used to calculate the relative electron transfer rate (rETR) from PSII to PSI (Genty et al. 1989; Beer et al. 1998, Eq. 4).

$$rETR = \Phi_{PSII} \cdot E_{PAR} \quad (4)$$

The calculated rETR values and corresponding irradiance were used to plot P *vs.* E curves in Sigma plot 12.0 (SYSTAT Software Inc., San Jose, California, USA). The P *vs.* E curve was fitted according to Webb et al. (1974), (Eq. 5):

$$rETR = P_{max}\left(1\text{-}\exp\left(\frac{\alpha E}{P_{max}}\right)\right) \quad (5)$$

Photosynthetic parameters such as the light utilization coefficient (α) and the maximum photosynthetic rate (P_{max}) were estimated from the P *vs.* E curves using Sigmaplot. The light saturation index (E_k) was calculated as follows (Eq. 6):

$$E_K = \frac{P_{max}}{\alpha} \quad (6)$$

Non-photochemical quenching (NPQ) was calculated according to the Stern-Volmer equation as the difference between F_m and F_m' (Maxwell and Johnson 2000; Müller et al. 2001, Eq. 7):

$$NPQ = \frac{F_m - F'_m}{F'_m} \quad (7)$$

A multifactor ANOVA test was conducted in Statgraphics (STATPOINT Technologies Inc., Warrenton, Virginia, USA) to elucidate age variations (intraseasonal variations) of the photosynthetic parameters (α, P_{max}, E_k) and between seasons for interseasonal variations. Coefficient of variation (±CV% of mean value) was calculated for each photosynthetic parameter for all individuals and for winter, summer and autumn to show the extent of variation.

Experimental setup

All measurements were performed in a 60 liter aquarium filled with sand-filtered (600-µm sand filters, Aster 99, Fluidra, Sabadell, Barcelona, Spain) seawater at *in situ* temperature (Fig. 5a). The water was running during all photosynthetic measurements to avoid depletion of nutrients and CO_2/HCO_3^- (Davison and Pearson 1996). The lamina was divided in four zones from the youngest part (meristemic tissue) to the oldest part at the distal end (Fig. 4).

Measured algal tissue was exposed to increasing irradiance while the rest of the kelp was kept in dark by black polyethylene plates (Fig. 5b).

Fig. 5. *Overview over (A) experimental setup with 60 liter aquarium with running sea-water, fluorescent light tubes and mesh, and (B) Saccharina latissima; meristem tissue was exposed for different irradiances while the rest of the specimen was kept in dark by black polyethylene plates.*

Six fluorescent light tubes in winter and eight in summer (Philips Master TL-D Super 80 30W/865, Guilford, Surrey, UK), equal amount on each side of the aquarium, were used as light source. Five alternating layers of black (mesh width: 1.5 mm) and grey (mesh width: 1mm) mesh covering the fluorescent tubes were used to adjust irradiance in the aquarium, in addition to turning one or several of the tubes on each side on and off (Fig. 5a). Average irradiance at each step in the P *vs*. E curve is given in Appendix B.

Due to light acclimation throughout the year, and consequently need for higher saturating irradiance, twelve fluorescent tubes (Philips Master TL-D Super 90 36W/965, Guilford, Surrey, UK), four on each side and four above the aquarium, were used in photosynthesis measurements in September to achieve photosynthetic saturation of the lamina tissue. Average irradiance is given in Appendix B.

Results

Photosynthetic performance was measured as changes in *in vivo* Chl *a* fluorescence as the tissue was exposed to different actinic irradiances. P *vs.* E curves was measured on four tissues of different age, from 9 different individuals of *Saccharina latissima.* For each P_{max}, α, E_k and NPQ were estimated. Photosynthetic parameters, sorted by season, are presented in Figure 6-8 with standard deviation ± of the mean value (n=3) for winter (February), summer (May), and autumn (September). Coefficient of variation (±CV% of mean value) was calculated for all individuals and each season and is presented in Table 3-5. A multifactor ANOVA test was carried out and data is presented in Appendix A.

Intraseasonal variations

P vs. E parameters, winter (February)

The individuals sampled in winter were smaller compared to the individuals collected in other seasons (lamina length: 30-45cm, Table 1). Tissue age was not found to have significant influence on either P_{max}, E_k or α in winter season. The initial slope of the P *vs.* E curve (α) was relatively constant throughout the lamina (Table 3). Thus, the light saturation parameter (E_k) mainly followed the changes of P_{max} (Fig. 6). No clear pattern of the photosynthetic parameters as a function of age was evident between individuals measured in winter. The highest variation in all parameters was evident in the individual with largest lamina size (*Saccharina latissima* #1) and lowest variation was found in the smallest individual (*S. latissima* #3).

Table 3. Coefficient of variation (±CV % of mean value) of P_{max}, α, E_k and NPQ within each individual (#1-3) and combined CV % for the winter season is listed.

Specimen	CV (%) in P_{max}	CV (%) in α	CV (%) in E_k	CV (%) in NPQ
#1	15.9	4.6	19.5	70.1
#2	6.6	2.9	12.7	28.5
#3	5.1	2.7	8.9	21.9
Combined winter	12.1	6.3	14.6	47.0

P_{max} (Fig. 6a-c) and E_k (Fig. 6g-i) showed no significant variation (P=0.088 and P=0.16 respectively) with tissue age and followed a similar pattern within individuals; P_{max} and E_k increased from meristem tissue (tissue 1) to tissue 2 in all individuals. A peak was evident in tissue 4 in *Saccharina latissima* 1, while the second and third individual showed a fairly stable P_{max} (CV of 6.6% and 5.1%) and E_k (CV of 12.7% and 8.9%, respectively) throughout lamina. Tissue age had no significant effect on α (P=0.53) in all three individuals and variations with lamina age was low ranging from 2.7-4.6% variation. α showed an inverse trend compared to P_{max} and E_k (Fig. 6d-f). High variation in NPQ with age was found in winter, with CV up to 70.1% in the first individual. Highest NPQ values were found in the youngest tissue (tissue 1 and 2; NPQ ranging from 0.6-1.5) and decreased as a function of tissue age (Fig. 6j-l).

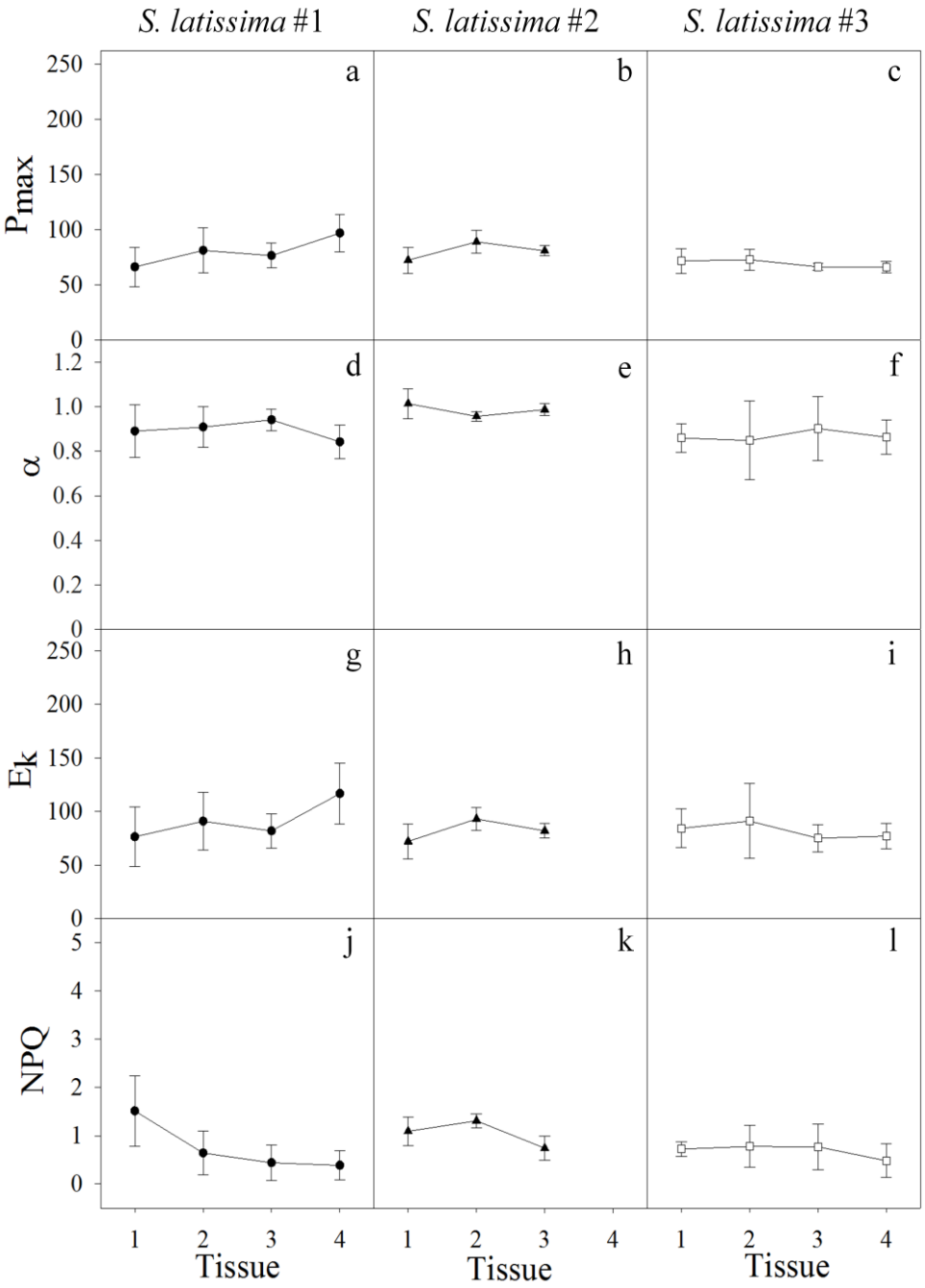

Fig. 6. *Photosynthetic parameters in S. latissima in February, P_{max} (a-c), α (d-f), E_k (g-i) and NPQ (j-l) as a function of lamina age from meristem tissue (tissue 1) to the oldest tissue (tissue 4.) Brennebukta, Trondheim.*

P vs. E parameters, summer (May)

The individuals from May were collected from a dense part of the kelp forest. Lamina length ranged from 69-85cm in *Saccharina latissima* #4-6, and was up to two times longer compared to other seasons (Table 1). Individuals displayed high variation in P_{max}, α and E_k as a function of tissue age from meristem tissue (tissue 1) to the oldest tissue (tissue 4) (Table 4). Thus, age had a significant effect on all the photosynthetic parameters. The same changes in all the photosynthetic parameters were evident throughout the lamina in *S. latissima* 4 and 5. α was more constant throughout the lamina compared to P_{max}, and therefore E_k followed the same trend as P_{max} in all individuals (Fig. 7).

Table 4. Coefficient of variation (±CV% of mean value) of P_{max}, α, E_k and NPQ within each individual (#4-6) and combined CV% for the summer season is listed.

Specimen	CV (%) in P_{max}	CV (%) in α	CV (%) in E_k	CV (%) in NPQ
#4	45.9	23.1	32.0	61.7
#5	43.0	11.5	34.9	24.2
#6	28.8	10.7	27.7	56.2
Combined summer	46.7	17.3	40.0	55.0

Summer was the only season were P_{max} and E_k variation with age was highly significant (P=0.001 and P=0.007), and both parameters showed high variation throughout lamina. A common trait for the three investigated individuals was that E_k (Fig. 7g-i) possessed the same trend as P_{max} (Fig. 7a-c), where *Saccharina latissima* #4 and 5 reached their optimum P_{max} and E_k in tissue 2 and thereafter decreased as a function of age. In *S. latissima* #6, P_{max} and E_k increased from meristem to tissue 3, and then dropped in tissue 4. α displayed the smallest variation throughout lamina (CV of 11% – 23%) compared to the other photosynthetic parameters. All individuals had a significantly decreasing α (P=0.00) with increasing tissue age (Fig. 7d-f). NPQ varied greatly between individuals; it decreased with age in *Saccharina latissima* 4 (from 3.6 to 0.6), and reached a peak in tissue 3 and 2 in *S. latissima* 5 and 6 respectively.

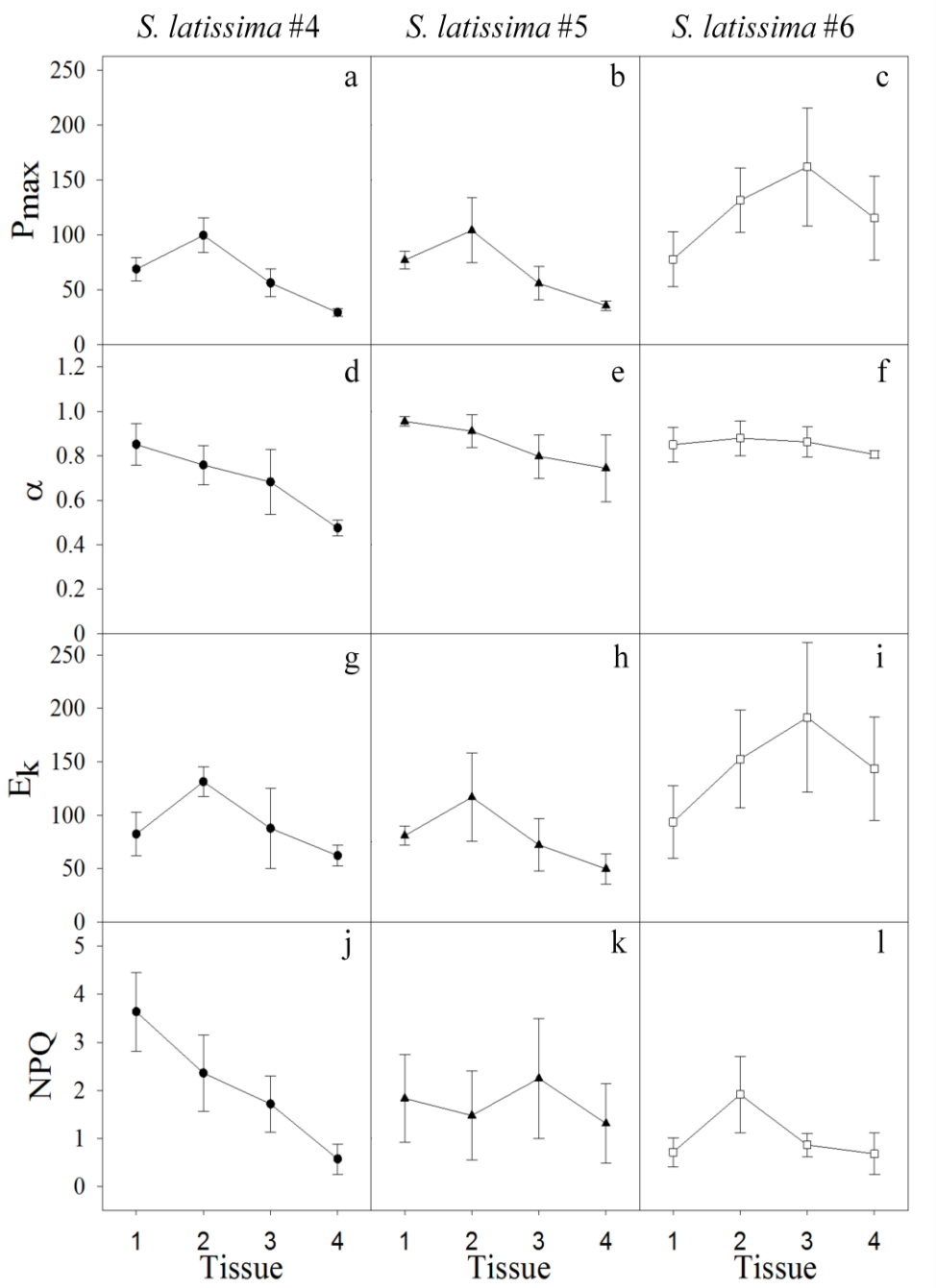

Fig. 7. *Photosynthetic parameters in S. latissima in May, P_{max} (a-c), α (d-f), E_k (g-i) and NPQ (j-l) as a function of lamina age from meristem tissue (tissue 1) to the oldest tissue (tissue 4. Brennebukta, Trondheim.*

P vs. E parameters, autumn (September)

Individuals sampled in autumn (#7-9) were collected from a part of the kelp forest with low density and had intermediate lamina length compared to the other seasons (49-66cm). The sampled *Saccharina latissima* had the highest cover of epifauna compared to individuals collected in winter and summer season (personal observation, Fig. 3). There was no notable correlation between tissue age and the photosynthetic parameters in autumn. $P_{max,}$ α and E_k values displayed smaller variations throughout the lamina compared to summer season. E_k followed the same trend as P_{max}, while α showed an inverse response compared to P_{max} (Fig. 8).

Table 5. Coefficient of variation (±CV% of mean value) of P_{max}, α, E_k and NPQ within each individual (#7-9) and combined CV% for autumn season is listed.

Specimen	CV (%) in P_{max}	CV (%) in α	CV (%) in E_k	CV (%) in NPQ
#7	20.5	10.0	35,4	11.2
#8	14.6	14.1	20,1	57.2
#9	7.5	10.2	18,1	49.7
Combined autumn	19.8	12.0	31,6	42.5

P_{max} values showed no significant variation as a function of age (*P*=0.28) in autumn. Small variation in P_{max} with age was observed in *Saccharina latissima* #8 and 9 (10% and 4.3% variation respectively). *S. latissima* 7 showed an increasing P_{max} as a function of age and displayed the highest variation (20.5%) within this season (Fig. 8a-c). Figure 8 (d-f) show that the changes in α with age varied between individuals. α decreased with age in *Saccharina latissima* 7, while in *S. latissima* 8 and 9, α peaked in tissue 3 and 2, respectively. α displayed the smallest variations compared to P_{max} and E_k (CV of 12%) and no significant variations as a function of age were seen (*P*=0.19). The three individuals sampled in autumn showed variable trend of E_k (Fig. 8g-i) with a peak in the oldest tissue in *S. latissima* #7 and in *S. latissima* #8 and 9 highest values were observed in meristem. However, the observed variations with age was not significant (*P*=0.05). Changes in NPQ with increasing tissue age were different in the three individuals; In *S. latissima* #7, NPQ decreased slightly as a function of age (with 11.2% variation), while in individual #8 and 9, which possessed the highest variation of 57.2 and 49.7% variation respectively, NPQ increased from meristem to tissue 2/3 and decreased in tissue 4.

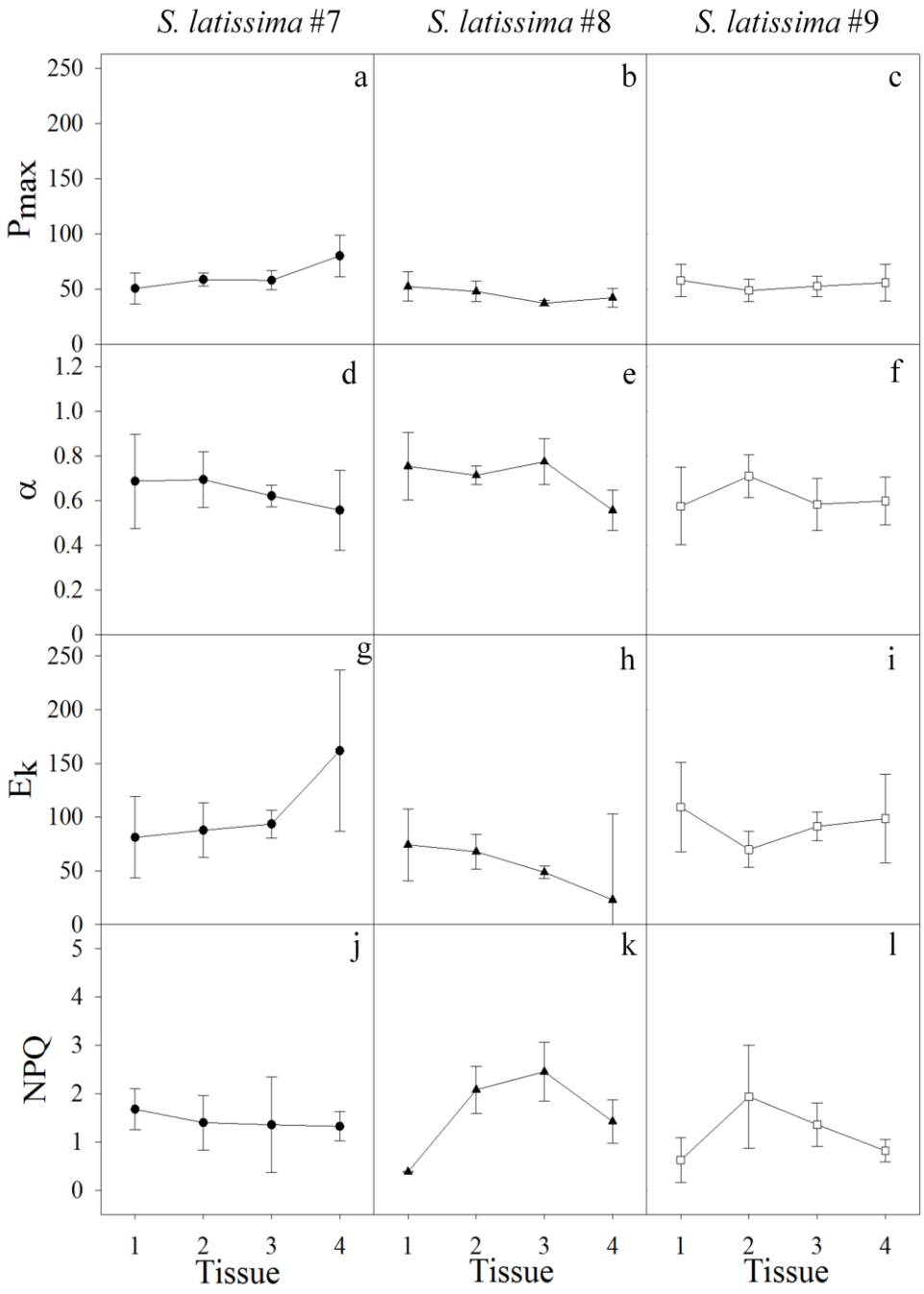

Fig. 8. *Photosynthetic parameters in S. latissima in September, P_{max} (a-c), α (d-f), E_k (g-i) and NPQ (j-l) as a function of lamina age from meristem tissue (tissue 1) to the oldest tissue (tissue 4). Brennebukta, Trondheim.*

Interseasonal variations

Highest variation of P_{max} was observed in summer (CV of 46.7%), and so age had significant effect on P_{max} in summer, contrary to the other seasons (Fig.9). Also meristem tissue had the most stable values between individuals within each season, while P_{max} values in the older tissue varied more between individuals. A multifactor ANOVA test for P_{max} showed that variations in P_{max} were significant between seasons (P=0.00) where lowest values were found in autumn, and interactions between season and age were significant (P=0.01).

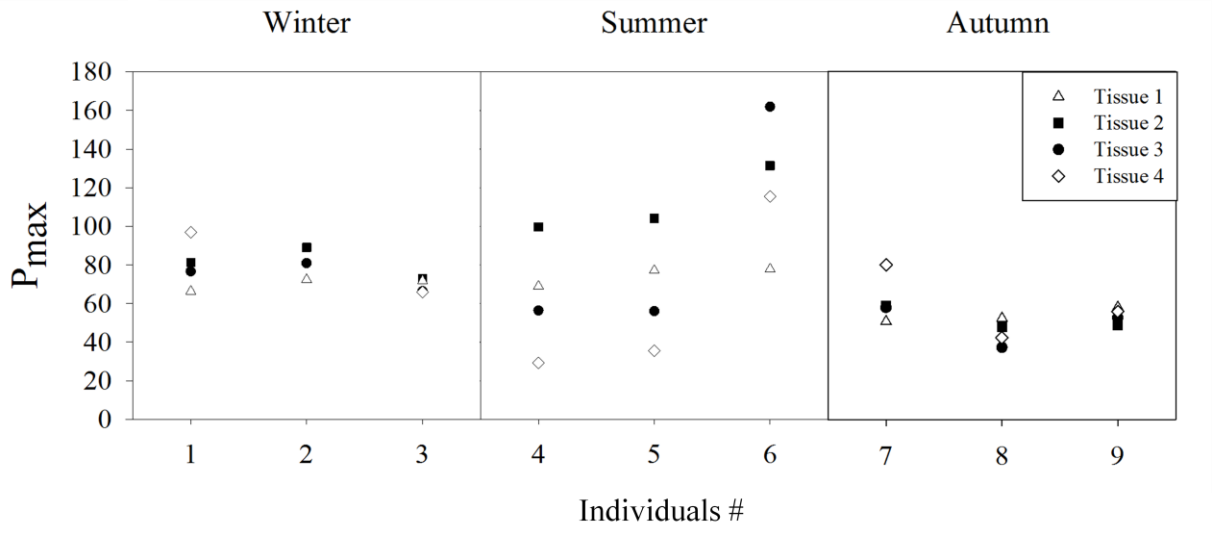

Fig. 9. *Seasonal comparison of P_{max} measured in tissue 1-4 (new tissue 1 and oldest tissue 4) in S. latissima #1-9 sampled in Brennebukta, Trondheim. S. latissima #1-3 sampled in winter (February), S. latissima #4-6 sampled in summer (May) and S. latissima #7-9 sampled in autumn (September).*

The changes in α with increasing age were smallest in winter (6.3% variation) and highest in summer (17.3% variation, Fig. 10). Measurements in meristem tissue were more alike between individuals compared to the older tissue (tissue 2-4) in summer. The highest α were found in kelp sampled in winter, and the lowest in autumn. α varied significantly with seasons (P=0.00), while interactions between season and age was not significant (P=0.30).

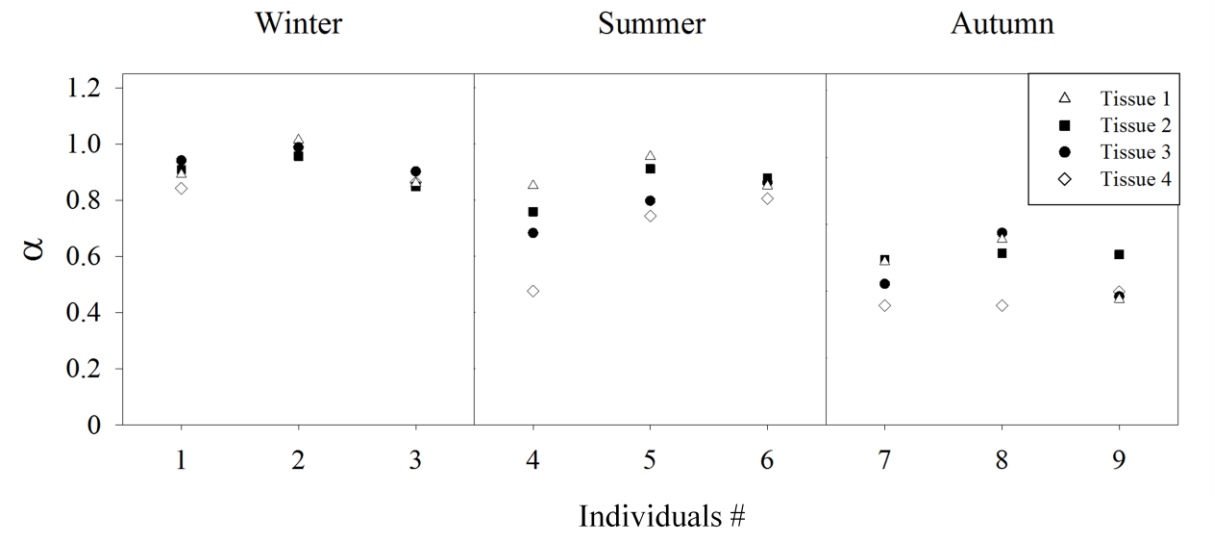

Fig. 10. *Seasonal comparison of α measured in tissue 1-4 (new tissue 1 and oldest tissue 4) in S. latissima #1-9 sampled in Brennebukta, Trondheim. S. latissima #1-3 sampled in winter (March), S. latissima #4-6 sampled in summer (May) and S. latissima #7-9 sampled in autumn (September).*

E_k had a similar trend as P_{max} and the observed trend throughout the lamina varied between individuals sampled in summer and autumn (Fig. 11), while age had a minor effect on E_k in winter. Variations in E_k were significant between seasons (P=0.04). The highest values were observed in summer and interactions between season and age were highly significant (P=0.001).

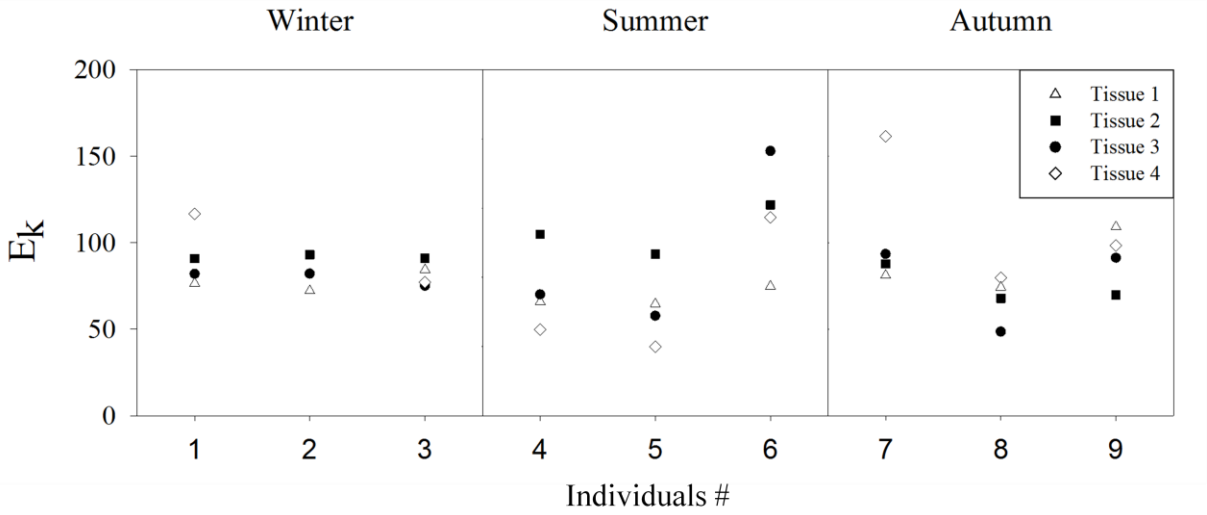

Fig. 11. *Seasonal comparison of E_k measured in tissue 1-4 (new tissue 1 and oldest tissue 4) in S. latissima #1-9 sampled in Brennebukta, Trondheim. S. latissima #1-3 sampled in winter (March), S. latissima #4-6 sampled in summer (May) and S. latissima #7-9 sampled in autumn (September).*

Discussion

Effect of lamina age on photosynthesis

Photosynthetic measurements on lamina tissue of different age revealed great differences in P_{max}, α, and E_k. Increasing age of tissue was only found to significantly influence photosynthetic parameters in summer (May). Results from this study indicate that photosynthetic performance, both across the width and the length of lamina, in a single individual of *Saccharina latissima* is dependent on how the lamina is positioned in the water column, and how the available light is illuminating the different parts of the lamina.

As previously described by Norton et al. (1977), as much as 90% of the incoming light can be absorbed by the canopy layer in a Laminariales forest. Consequently, individuals of *Saccharina latissima* can be exposed to very different light climates depending on an individual's placement within the kelp forest. The light climate can span from a maximum irradiance of 1600 µmol photons $m^{-2}s^{-1}$ (maximum surface irradiance Trondheimsfjorden, midsummer noon, clear day) to < 200 µmol photons $m^{-2}s^{-1}$ for individuals living inside the dense kelp forest (Dean 1985; Valle 2014). A single individual of *S. latissima* will obtain different photoacclimation and photoprotection status throughout the lamina according to the position of the photosynthetic tissue in the water column. The results in this study revealed no uniform trend in how the photosynthetic parameters change throughout the length of the lamina, as should be expected if age had a strong influence on photosynthetic parameters. This indicates that the different lamina tissue in *S. latissima* acclimates to the immediate light environment, and that the different individuals are not positioned in the water the same way, leading to very different light exposure. This is also seen in a study executed by Colombo-Pallotta et al. (2006). They found that a single organism of *Macrocystis pyrifera* showed enhanced photoprotection, highest maximum photosynthetic rate and high light saturation index (high P_{max}, E_k, and NPQ) in surface lamina compared to basal lamina.

Long-term acclimation in response to shifts in light conditions include adjustments of the amount and ratios of light harvesting pigments (LHPs) and alterations of the size of the photosynthetic units (PSU). A photosynthetic unit consists of PSII and PSI and their corresponding light harvesting complexes (Johnsen and Sakshaug 2007; Nymark et al 2009). In HL, number of PSUs is increased and the PSU will contain a high amount of photoprotective carotenoids and low LHP content. In addition, a high rETR is evident in HL

acclimated cells. These adjustments will increase the rate of electron transport relative to light absorption and consequently higher P_{max} and E_k is shown in HL acclimated cells than in LL acclimated cells (Sukenik et al. 1987; Sakshaug et al. 1997, Johnsen and Sakshaug 2007). In winter P_{max} and E_k displayed small variation with tissue age, however there was an increase in P_{max} and E_k from meristem tissue to tissue 2 in all individuals indicating that tissue 2 was acclimated to a higher light climate than meristem tissue. This was especially evident in *Saccharina latissima* 1 and 2. *Saccharina latissima* 1 showed highest P_{max} and E_k in the oldest tissue, suggesting that this tissue was being more exposed to higher irradiances compared to the other parts of the lamina, resulting in different photosynthetic acclimation than in the younger tissue. *S. latissima* 3 showed less variation compared to the other individuals, which might be an effect of the lamina being more evenly illuminated (more horizontal position in the water). It could also be due to the fact that individual #3 was the smallest individual and as a result the different tissues measured was not far away from each other and had not experienced large variation in irradiance. In LL acclimated cells, a higher absorption of light is attained by increasing the size and number of light harvesting antenna (increasing the amount of LHP). The response will increase the chance of photons to be absorbed by the antennae pigments of the photosystems and thus a higher α is evident (Sakshaug et al. 1997; Johnsen and Sakshaug 2007; Nymark et al. 2009). α showed an inverse pattern compared to P_{max} which reinforce the assumption that the oldest tissue in *S. latissima* 1 and tissue 2 in *S. latissima* 2 was acclimated to a higher light than rest of lamina. Variation of α was smallest in *S. latissima* 3, coinciding with smallest variations of P_{max} and E_k.

Enhancement of photoprotection (e.g. NPQ) is a documented response in HL exposed algae (Horton et al. 1996). NPQ reflects short-term acclimation; where violaxanthin is de-epoxidized to zeaxanthin through the xanthophyll cycle, resulting in a larger dissipation of absorbed energy as heat and is triggered by a lowered pH in the thylakoid membrane (Brunet et al. 2011). HL-acclimated cells generally have a low LHP content and a high amount of photoprotective carotenoids; the relationship is inversed for LL-acclimated cells (Nymark et al. 2009). The different individuals sampled in winter showed different NPQ responses throughout the lamina, indicating that photoprotective performance in a single organism of *Saccharina latissima* is dependent on the photo-acclimation status of tissue lamina (e.g. *S. latissima* 1 showed a decreasing NPQ from meristem to tissue 4 with 70.1% variation throughout the tissue indicating high photoprotection in the youngest tissue and lower in the oldest tissue).

Age had no significant effect on the photosynthetic parameters in winter. This could be explained by several factors; the individuals were so small that the measured tissues were not far away from each other (average 9.6cm between measured tissues in winter compared to 18.3cm in the larger individuals sampled in summer) and so there was not a great variation in tissue age. Furthermore, the kelp was sampled from a thread and did not experience a lot of shading from others kelp or by self-shading (as it would in a dense kelp forest). Thus, the different parts of the lamina tissue might not have experienced high variation in irradiance.

In contrast, variation of the photosynthetic parameters with age was highly significant in summer season. The individuals sampled in summer had the longest lamina (69-85cm) compared to the other seasons, and thus the different lamina tissues measured could experience higher variations of irradiances compared to the smaller individuals. In addition, individuals were sampled from a denser kelp forest and consequently different lamina tissue could be positioned through a large gradient of irradiances, from the surface to inside the dense kelp forest.

In summer, *Saccharina latissima* #4 and 5 showed the same response in P_{max} and E_k indicating that the different lamina tissues had similar positions in the water column in the two individuals, while *S. latissima* #6 showed a different trend. However, all individuals showed a peak of P_{max} and E_k in the mid sections of lamina (here tissue 2 and 3). *In situ*, large *S. latissima* is often found standing like an arch, with the meristem and old tissue (tissue 4) at the lowest points, and the mid tissue sections forming the top. The mid sections will as a result be higher in the water column, and sometimes exposed to surface irradiances (e.g. at spring tide), thus more exposed to the incoming light (Fig. 12). α decreased as a function of age throughout the lamina in *S. latissima* #4 and 5. As mentioned by several studies damage to photosynthetic units (especially the D1-protein of PSII) may lower α (Maxwell et al. 1994; Olaizola et al. 1994; Johnsen et al. 2011). The older tissue is usually lost through abrasion and necrosis (especially in long lamina) and in addition, epiphytic growth is typically higher in the oldest tissue, and might explain why α decreases as a function of age in these two individuals (Sjøtun 1993). Changes in NPQ with increasing tissue age varied between individuals, which indicate different short-term photo-acclimation status throughout the lamina. It should be noted that this experiment was not customized to measure NPQ. Dark acclimation of the points measured across the lamina might have been compromised during the light flash measuring F_m in neighboring points. As NPQ calculations rely on F_m measured on

completely dark acclimated cells, each point across the lamina should have been dark acclimated separately before measurement of F_0 and F_m to gain more reliable NPQ values.

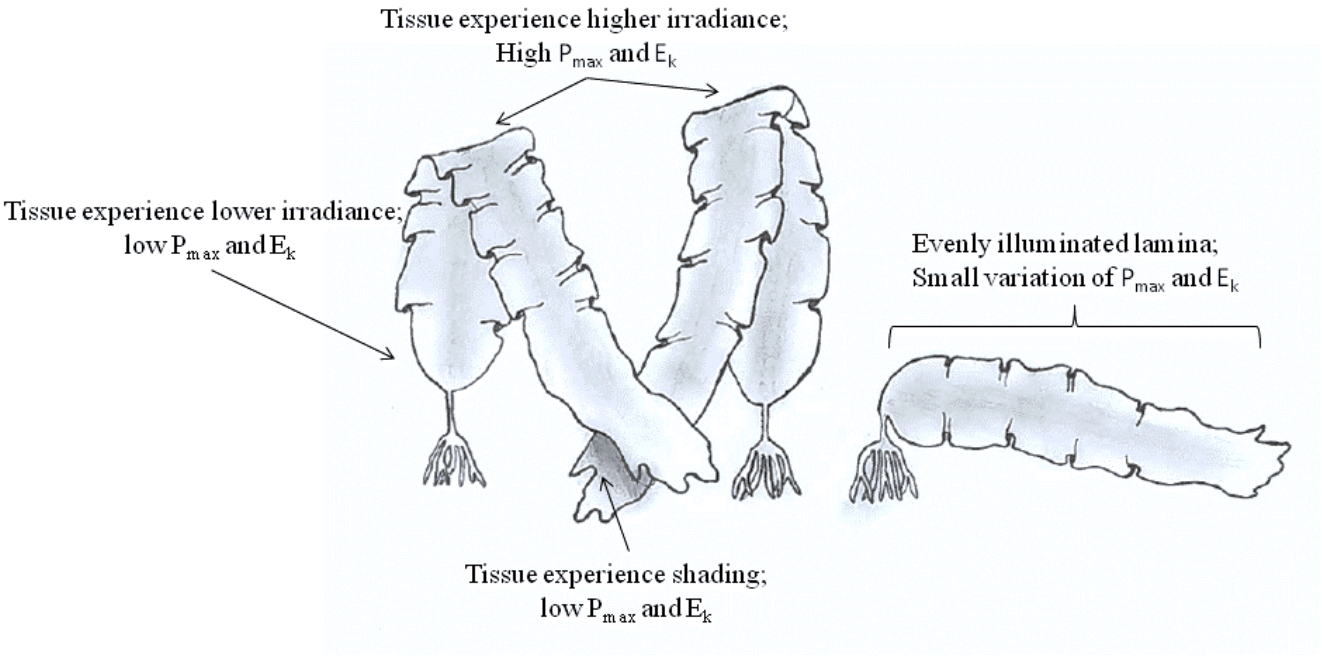

Fig. 12. *Overview of how Saccharina latissima can be positioned in the water column and the resulting photo-acclimation status seen as higher or lower P_{max} and E_k. (Illustration by Ane Cecilie Kvernvik)*

In autumn, variation of the photosynthetic parameters throughout lamina was small compared to summer. One explanation might be that individuals were sampled from a lower density kelp forest and would as a consequence have been more evenly illuminated than the summer individuals. In addition, the individuals were smaller and thus the measured tissues were closer together. The importance of lamina size (and thus the distance between measured tissue) is evident as the specimen with the largest lamina size (*Saccharina latissima* #7 with 66cm) displayed the highest variations in P_{max} (20.5%) compared *to S. latissima* #9 with smallest lamina size (49cm) which displayed 7.5% variation throughout lamina. This relationship is also evident within winter season where the largest individual displayed the highest variation between the different ages. There was no uniform trend in the changes of P_{max}, E_k, α and NPQ throughout the lamina tissue between the autumn individuals. This might be due to different position of the photosynthetic tissue in the water column and reinforces the assumption that photoacclimation and photoprotection is a function of the tissues position in the water, and that each cell in the lamina acclimates to the immediate light climate it is exposed to.

Comparing P_{max}, E_k and α between replicates indicates not only longitudinal but also latitudinal biological variation in lamina tissue in *Saccharina latissima*. The variation might be due to different photo-acclimation status of the tissue. The mid-section of the length of lamina in *Saccharina latissima* is often thicker and more rigid, while the "side" tissue is thin and more flexible. This could lead to differences in light exposure, as the "midrib" will be relatively static, and the side tissue more moving and self-shading. Also, packaging of pigments inside the cells chloroplasts may reduce the absorption efficiency of pigments, which lowers α and may increase E_k. This could be the case in the thicker mid-tissue (Sakshaug et al. 1997). The results in this study support this hypothesis, as the mid-tissue showed higher P_{max} and $E_{k,}$ and lower α than the side tissue (see Figure 13). The tissue position in the water might also be reflected in the oldest tissue where the results show that tissue 1 and 3 had similar values (same side of margin), and thus similar photo-acclimation status.

It should be noted that long-term photo-acclimation time-line is from hours to days, and that some individuals was kept in an outdoor pool in a net over several days (e.g. *Saccharina latissima* #3 for 21 days). This might indicate that the photosynthetic parameters (P_{max}, α and E_k) measured on the different tissues throughout lamina does not reflect *in situ* photo-acclimation and how they were positioned in the water column, but rather to how they were positioned in the net. This could especially be the case for the individuals sampled in winter which were kept in the outdoor pool the longest. The important finding is still that the high biological variation throughout the lamina in *S. latissima* might be caused by the species ability to express different photo-acclimation and photoprotection responses according to the position in the water and the immediate light. The immediate light climate can vary greatly with changes in several different factors (density of kelp forest and lamina movement in waves, absorbance of the canopy, currents, clouds, optical active components in the water and movement of them, focusing by waves etc., Pearcy 1990). By evolving the strategy of functional differentiation of its photosynthetic tissue *Saccharina latissima* became a successful organism in a highly variable environment.

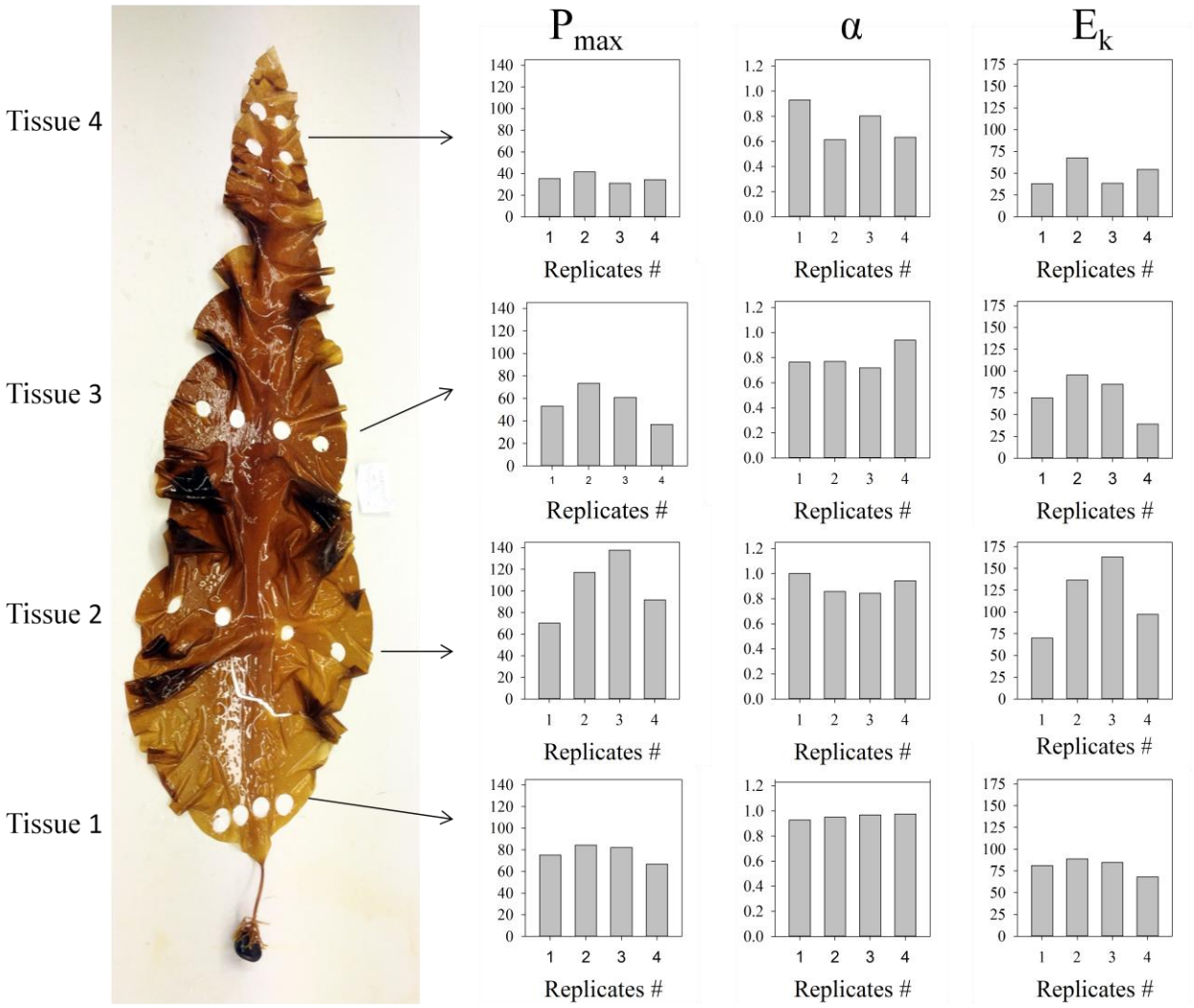

Fig. 13: *Biological variation in photosynthetic parameters in Saccharina latissima #5. P_{max}, α and E_k replicates along lamina indicates longitudinal and latitudinal variation.*

Effect of seasonal differences on photosynthesis

The photosynthetic parameters in *Saccharina latissima* varied seasonally as a response to the varying light climate. Physiological acclimation to changes in light intensity and spectral quality is an important factor determining variations in photosynthetic responses and growth rates (Falkowski 1980).

It is well known that there is a strong seasonal variation in light regime at high latitudes. As seen in Table 2, February is characterized by short days (averagely 8 hours a day), and low solar latitude compared to April and August indicating lower irradiance at the sea surface ($E_{d\text{-}air}$). Valle (2014) found that variations in irradiance close to sea surface in Trondheimfjorden followed the expected solar seasonal variability with low $E_{d\text{-}air}$ in February (<200 µmol m^{-2} s^{-1}) and highest $E_{d\text{-}air}$ values for May-August (>800 µmol m^{-2} s^{-1}). However, this is not

necessarily reflected in subsea irradiance because of concentration of optical active components such as particulate matter, cDOM and microalgae. The results in this study show that P_{max} and E_k were slightly higher in summer compared to winter which is expected due to the irradiance at sea surface which can be up to 4 times higher in summer than winter. As a consequence the individuals sampled in May should be more HL acclimated. Nevertheless, the variation in P_{max} between winter and summer was not significant which indicate that individuals were not experiencing extreme variation in light climate between these seasons. Data of phytoplankton blooms in Trondheimfjorden show that concentrations (cells/liter) were low in February and moderate to high in May. In addition, run-off from Gaula was 4 times higher in May compared to February in 2013. High fresh water run-off is related to high cDOM (terrestrial) and particulate matter concentrations in the water (Kowalczuk et al. 2006). Consequently, more optical active components might have been present in the water in May and thus reduced subsea irradiances. This may explain why these two months have similarly acclimation status, despite higher $E_{d\text{-}air}$ in May. These results indicate that light available for photosynthesis is dependent on the strong season variation in light climate at sea surface together with optical active compounds in the water which reduces irradiance and affect the spectral quality.

In autumn, significantly lower P_{max} and E_k compared to summer was evident which may indicate acclimation to lower light and the fact that growth is arrested in June-November (Andersen 2014). According to Table 2 there is similar average daylength and solar latitude between autumn and summer in addition to number of days with overcast and rainy days, which would make the $E_{d\text{-}air}$ similar between these two months. It was recorded moderate phytoplankton bloom and high run-off from Gaula. In addition, heavy epigrowth (including epiphytes and epifauna) was observed on the individuals sampled in autumn compared to the other seasons. Andersen et al. (2011), showed that shading by epigrowth cover reduce light utilized in photosynthesis from 11-91% and epigrowth shading could explain why individuals sampled in autumn seemed acclimated to lower irradiances than the other seasons. Breakdown of lamina tissue leads to formation of cDOM which is a major determinant of the optical properties of natural waters and it directly affects both the availability and spectral quality of light in the water column (Jerlov 1976). Laminarian kelp grows best during winter-spring when nutrients are readily available, and erosion of blades increases in autumn when nutrients become depleted (Gagné et al. 1982). Additionally, fouling by epigrowth may accelerate erosion and fragmentation of the blades (Dixon et al. 1981; Scheibling et al. 1999).

Consequently, substantial formation of cDOM which absorbs light and reduces photosynthetic active radiation might occur during autumn. The high concentration of optical active components and substantially epiphytic growth might explain the LL acclimation seen as reduction in P_{max} and E_k in autumn.

α varied significantly with seasons with highest α values measured in individuals collected in winter. Algae increase the size and number of light harvesting antenna (which increases the content of LHP) and thus increases photosynthetic light utilization, α, in low light environments like the ones seen in February. Another study shows that the concentration of light harvesting pigments (Chl *a*, Fucoxanthin and Chl *c*) in the same individuals used in this experiment was in fact higher in winter which agrees with the results presented here regarding photosynthetic parameters (Hallerud 2014, personal communication). However, an unexpected result is that α was significant lower in autumn compared to the other seasons. The parameters P_{max} and E_k indicates that the autumn individuals were acclimated to LL environment compared with other seasons, and so these individuals should have increased the size and number of light harvesting antenna, seen as an increase in light harvesting capacity (α). Studies indicate that the number of photosynthetic units may be low in nutrient-deprived cells (Kolber et al. 1988). Chlorophyll a is a nitrogen (N) rich molecule, and it is shown that total N content in brown algae were affected by ambient NO_3 in the water, with highest values in winter, when ambient NO_3 was maximum and declined during summer (Young et al. 2007; Porra et al. 2011). In addition, it is shown that substantial epiphytic growth significantly decreased ammonium uptake of algal tissues (Hurd et al. 2000). Consequently, the individuals sampled in autumn could be nitrogen deprived in Trondheimsfjorden as indicated by Hilstad (2005). This might be one possible reason for the low α.

Both P_{max} and E_k showed significant interaction between age and season (P=0.006 and P=0.005 respectively) which means that the extent of variation of these parameters throughout the lamina is dependent on sampling season. This season influence on age variation, here seen as significant variation with age in summer and not in winter and autumn might be a result of the individuals being larger, and the kelp forest being denser in the summer, leading to larger variations in light climate throughout the length of the lamina. α had the lowest variation throughout the lamina compared to P_{max} and E_k in all seasons and this independent variation of P_{max} and α alters E_k , termed E_k dependent variation (Behrenfeld et al. 2004). The low variation in α and high variation in P_{max} strongly indicates that higher P_{max} are achieved by increasing turnover time for electrons (e.g. increasing electron transfer rate) and

not by increasing the number of PSUs. The transition from low to high irradiances cause changes in the level of activation of key enzymes of the Benson-Calvin cycle to accommodate higher rate of photosynthesis, and when photosynthesis becomes light saturated it becomes limited by the flux through Benson-Calvin pathway (activity of RuBP-carboxylase, Dujardun and Foyer 1989). Nevertheless, several experiments indicate that electron transport rate might over-estimate total photosynthetic rate due to alternative electron cycling pathways (Gilbert et al. 2000). Alternative electron cycling includes cyclic electron flow around PSII and PSI (allows rapid transfer of electrons from P680) and the Mehler reaction (use of O_2 as an electron acceptor leading to formation of H_2O_2) (Mehler 1951; Falkowski et al. 1986; Rumeau et al. 2007). The two latter mechanisms create a proton gradient which can be used for additional ATP synthesis when the rate of Calvin-Benson cycle is limiting for production of ATP through linear electron flow (Suggett et al. 2011). Variation in turnover time for electrons seems to be one of the most important responses in a variable light climate as seen by the changes of P_{max} throughout the lamina (especially in summer season) without large changes in α.

Duration of actinic light exposure – A test

To make sure that *in vivo* Chl *a* fluorescence reached a stable level at each irradiance in the P *vs.* E curves, meristem tissue from *Saccharina latissima* was exposed to increasing actinic irradiance several times in succession (multiple P *vs.* E curves), with increasing duration of each irradiance with each curve. The results are presented in Appendix C. P_{max}, α and E_k was very different when the exposure time for each irradiance was increased from 30 seconds to 1 minute. With illumination time of 3 minutes or more, estimations of P_{max}, E_k and α were the same. This indicates that exposure time of 3 minutes or more to each actinic irradiance might be necessary to get stable *in vivo* Chl *a* fluorescence signal from the tissue. However the experiment was conducted on only one individual of *S. latissima*, and replicates is necessary to get a complete picture of how long the individuals should be exposed to the different actinic light conditions.

Future perspectives

In future studies these findings should be taken into consideration when analyzing net primary production in a single organism of *Saccharina latissima* or the whole kelp forest. Measuring photosynthesis and production in meristem tissue and assume that the rates are representative for the whole length of lamina might not give the exact estimates of these reactions. However if the aim of the study is to compare individuals (e.g. season study) one could measure

meristem tissue as it seems to be the most stable tissue giving similar values of the photosynthetic parameters between individuals. In addition, meristem tissue reflects the present light history through present photo-acclimation status, while the oldest tissue is highly variable depending on the position in the water and might be influenced by former photo-acclimation.

My experiments show that there is need for a more detailed understanding of how tissue position in the water column affects photosynthesis in *Saccharina latissima* as it gives significant variation in photo-acclimation throughout the lamina in a single organism and between organisms. This could be achieved by experiments focused on how different individual is positioned in the water dependent on size of the individuals and environment, and how the position is affected by water currents, waves, other individuals etc. and link this to photosynthetic capacity of the tissue. There is also need for a more detailed understanding of how season affect photosynthesis, where experiments should focus on how *S. latissima* changes photo-acclimation status as light and nutrient availability changes throughout the year.

Conclusion

Photo-acclimation throughout lamina in *Saccharina latissima* investigated in this study strongly indicate to be a function of how the different parts of lamina is exposed to ambient irradiances, which is dependent on lamina position in the water column and closeness to neighboring specimens. In larger individuals this trend seems to be more pronounced as their lamina could be positioned through a large gradient of irradiances (Fig. 12). Variation of photo-acclimation throughout lamina is also dependent on habitat as self-shading and shading by other individuals increase the variation of irradiance the different parts of lamina could experience.

The photosynthetic performance in *Saccharina latissima* was found to be dependent on time of year. This was seen in all the photosynthetic parameter (P_{max}, E_k and α) and it seemed to be highly dependent on temperature, nutrient availability and light climate. Higher P_{max} and E_k were observed in summer indicating HL acclimation, in contrary to autumn where LL acclimation and nutrient depletion was seen as significant lower $P_{max,}$ E_k and α. This indicates high concentration of optical active components and epiphytic growth, which affects both the availability and spectral quality of light utilized in photosynthesis, in the water column.

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Appendix A: Multivariate ANOVA analysis of intraseasonal and interseasonal variations

Intraseasonal variations

Winter

Analysis of Variance for P_{max} - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Specimen	1525,29	2	762,645	4,46	0,0182
B:Age	1201,77	3	400,59	2,34	0,0883
RESIDUAL	6493,17	38	170,873		
TOTAL (CORRECTED)	8991,51	43			

Multiple Range Tests for P_{max} by Specimen

Specimen	*Count*	*LS Mean*	*Homogeneous Groups*
3	16	69,2222	X
1	16	80,2859	X
2	12	83,0157	X

Contrast	*Sig.*	*Difference*
1 - 2		-2,72979
1 - 3	*	11,0637
2 - 3	*	13,7935

* denotes a statistically significant difference.

Analysis of Variance for α - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Specimen	0,0778161	2	0,038908	4,73	0,0146
B:Age	0,0185008	3	0,00616693	0,75	0,5293
RESIDUAL	0,312588	38	0,008226		
TOTAL (CORRECTED)	0,432196	43			

Multiple Range Tests for α by Specimen

Specimen	*Count*	*LS Mean*	*Homogeneous Groups*
3	16	0,867731	X
1	16	0,896	X
2	12	0,976353	X

Contrast	*Sig.*	*Difference*
1 - 2	*	-0,0803531
1 - 3		0,0282687
2 - 3	*	0,108622

* denotes a statistically significant difference.

Analysis of Variance for E_k - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Specimen	741.982	2	370.991	0.81	0.4509
B:Age	2497.25	3	832.416	1.83	0.1590
RESIDUAL	17331.2	38	456.085		
TOTAL (CORRECTED)	20724.0	43			

Multiple Range Tests for E_k by Specimen

Specimen	*Count*	*LS Mean*	*Homogeneous Groups*
3	16	81.9282	X
2	12	85.8403	X
1	16	91.5183	X

Contrast	*Sig.*	*Difference*
1 - 2		5.67796
1 - 3		9.59
2 - 3		3.91204

Summer

Analysis of Variance for P_{max} - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Specimen	33338,9	2	16669,4	17,45	0,0000
B:Age	17784,0	3	5928,0	6,20	0,0014
RESIDUAL	40131,2	42	955,504		
TOTAL (CORRECTED)	91254,1	47			

Multiple Range Tests for P_{max} by Specimen

Specimen	*Count*	*LS Mean*	*Homogeneous Groups*
4	16	63,5613	X
5	16	68,219	X
6	16	121,651	X

Contrast	*Sig.*	*Difference*
4 - 5		-4,65771
4 - 6	*	-58,0895
5 - 6	*	-53,4318

* denotes a statistically significant difference.

Analysis of Variance for α - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Specimen	0,268965	2	0,134483	13,80	0,0000
B:Age	0,308112	3	0,102704	10,54	0,0000
RESIDUAL	0,409179	42	0,00974235		
TOTAL (CORRECTED)	0,986256	47			

Multiple Range Tests for α by Specimen

Specimen	*Count*	*LS Mean*	*Homogeneous Groups*
4	16	0,69225	X
6	16	0,849875	X
5	16	0,852187	X

Contrast	*Sig.*	*Difference*
4 - 5	*	-0,159938
4 - 6	*	-0,157625
5 - 6		0,0023125

* denotes a statistically significant difference.

Analysis of Variance for E_k - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Specimen2	39177.9	2	19589.0	12.93	0.0000
B:Age2	20758.1	3	6919.37	4.57	0.0074
RESIDUAL	63636.3	42	1515.15		
TOTAL (CORRECTED)	123572.	47			

Multiple Range Tests for Ek by Specimen

Specimen2	*Count*	*LS Mean*	*Homogeneous Groups*
5	16	79.8576	X
4	16	90.8164	X
6	16	145.194	X

Contrast	*Sig.*	*Difference*
4 - 5		10.9588
4 - 6	*	-54.3776
5 - 6	*	-65.3364

* denotes a statistically significant difference.

Autumn

Analysis of Variance for P_{max} - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Specimen	2286,27	2	1143,13	6,84	0,0027
B:Age	668,127	3	222,709	1,33	0,2764
RESIDUAL	7015,4	42	167,033		
TOTAL (CORRECTED)	9969,8	47			

Multiple Range Tests for P_{max} by Specimen

Specimen	*Count*	*LS Mean*	*Homogeneous Groups*
8	16	44,9748	X
9	16	53,8379	XX
7	16	61,8732	X

Contrast	*Sig.*	*Difference*
7 - 8	*	16,8984
7 - 9		8,03525
8 - 9		-8,86312

* denotes a statistically significant difference.

Analysis of Variance for α- Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Specimen	0,0586215	2	0,0293107	1,75	0,1856
B:Age	0,119031	3	0,039677	2,37	0,0838
RESIDUAL	0,702061	42	0,0167157		
TOTAL (CORRECTED)	0,879713	47			

Multiple Range Tests for α by Specimen

Specimen	*Count*	*LS Mean*	*Homogeneous Groups*
9	16	0,616875	X
7	16	0,63975	X
8	16	0,69975	X

Contrast	*Sig.*	*Difference*
7 - 8		-0,06
7 - 9		0,022875
8 - 9		0,082875

* denotes a statistically significant difference.

Analysis of Variance for E_k - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Specimen3	12113.4	2	6056.68	4.68	0.0146
B:Age3	10931.7	3	3643.89	2.82	0.0505
RESIDUAL	54315.6	42	1293.23		
TOTAL (CORRECTED)	77360.6	47			

Multiple Range Tests for E_k by Specimen

Specimen3	*Count*	*LS Mean*	*Homogeneous Groups*
8	16	67.6131	X
9	16	92.2276	XX
7	16	106.021	X

Contrast	*Sig.*	*Difference*
7 - 8	*	38.4075
7 - 9		13.793
8 - 9		-24.6145

* denotes a statistically significant difference.

Interseasonal variations

Analysis of Variance for P_{max} - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Season	24734,9	2	12367,4	17,44	0,0000
B:Age	5281,06	3	1760,35	2,48	0,0639
INTERACTIONS					
AB	13713,3	6	2285,55	3,22	0,0056
RESIDUAL	90790,2	128	709,298		
TOTAL (CORRECTED)	134796,	139			

Multiple Range Tests for P_{max} by Season

Season	*Count*	*LS Mean*	*Homogeneous Groups*
3	48	53,562	X
1	44	76,8195	X
2	48	84,477	X

Contrast	*Sig.*	*Difference*
1 - 2		-7,65755
1 - 3	*	23,2575
2 - 3	*	30,915

* denotes a statistically significant difference.

Analysis of Variance for α - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Season	1,47245	2	0,736227	51,52	0,0000
B:Age	0,331429	3	0,110476	7,73	0,0001
INTERACTIONS					
AB	0,105498	6	0,017583	1,23	0,2950
RESIDUAL	1,82923	128	0,0142909		
TOTAL (CORRECTED)	3,84404	139			

Multiple Range Tests for α by Season

Season	*Count*	*LS Mean*	*Homogeneous Groups*
3	48	0,652125	X
2	48	0,798104	X
1	44	0,905488	X

Contrast	*Sig.*	*Difference*
1 - 2	*	0,107383
1 - 3	*	0,253362
2 - 3	*	0,145979

* denotes a statistically significant difference.

Analysis of Variance for E_k - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Season	9895.58	2	4947.79	3.38	0.0371
B:Age	5870.98	3	1956.99	1.34	0.2652
INTERACTIONS					
AB	28668.5	6	4778.09	3.27	0.0051
RESIDUAL	187316.	128	1463.41		
TOTAL (CORRECTED)	232245.	139			

All F-ratios are based on the residual mean square error.

Multiple Range Tests for E*k* by Season

Season	*Count*	*LS Mean*	*LS Sigma*	*Homogeneous Groups*
1	44	86.5025	5.8565	X
3	48	88.6205	5.52157	X
2	48	105.289	5.52157	X

Contrast	*Sig.*	*Difference*	*+/- Limits*
1 - 2	*	-18.7868	15.9263
1 - 3		-2.11795	15.9263
2 - 3	*	16.6689	15.4508

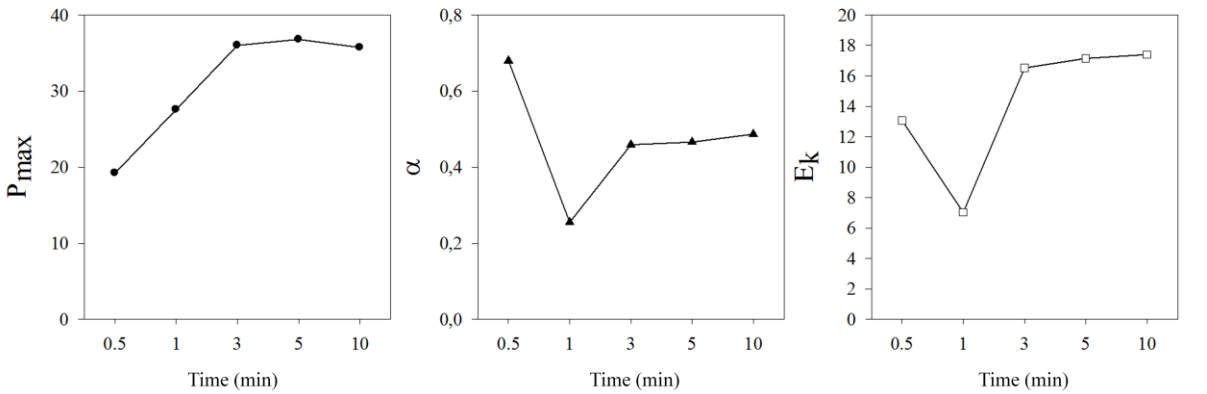
* denotes a statistically significant difference.

Appendix B: Achieved irradiance from experimental setup

Average values of irradiance (µmol photons $m^{-2}s^{-1}$) after sampling in February, May and September.

Irradiance February and May (µmol photons $m^{-2}s^{-1}$)	**Irradiance September (µmol photons $m^{-2}s^{-1}$)**
0.1	0
4.2	7.2
8.1	11.5
13.3	19.1
17.2	23.5
23.3	29.6
27.5	42
46.9	56.2
66.8	56.8
74	76.9
89	88.1
111.6	119.3
138.9	153.4
171.9	182.9
198.1	230.5
252.9	313.2
304.4	441.4

Appendix C: Acclimation to actinic light –A test



The photosynthetic parameters (P_{max}, E_k and α) measured on meristem tissue on one individual of Saccharina latissima. The tissue was acclimated to the different actinic light in the P vs. E curve from 0.5-10 minutes. The parameters stabilized after 3 minutes of acclimation.