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Eco-physiology of the Arctic kelp *Laminaria solidungula*

- using divers, Remotely Operated Vehicle and Pulse Amplitude Modulated fluorometry

Elen Belseth

Marine Coastal Development
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Supervisor: Geir Johnsen, IBI
Co-supervisor: Jørgen Berge, UNIS

Norwegian University of Science and Technology
Department of Biology

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Elen Belseth

Front page: Collection of *Laminaria solidungula* at Tommelen in August 2009. Photo: Geir Johnsen.

Abstract

This study of the Arctic kelp species *Laminaria solidungula* (J. Agardh) comprises its eco-physiology, morphology, habitat, photosynthetic performance, chemical- and pigment composition. Differences due to age of tissue (1-3 years) in *L. solidungula* and differences compared to four other kelp species living in Svalbard waters was investigated.

Laminaria solidungula thrives best in between other kelp species on hard bottom substrate, and is characterized by distinct lamina parts with brighter areas of fertile tissue and a disk formed haptera. The fertile tissue seems to need ~3 years to mature, before the zoospores are released.

The results did not show any uniform alteration of tissue content due to age in *L. solidungula.* 1 year old tissue had a higher operational quantum yield of chlorophyll a fluorescence than older tissue. Ambient light was only high enough to saturate photosynthesis in 3 year old tissue. The photosynthetic parameters (maximum utilization coefficient (α), maximum photosynthetic rate (P_{max}) og light saturation index (E_k)) reveled higher P_{max} in 2 year old tissue than 1 year old tissue collected in August indicating that *L. solidungula* need time (years) to mature its chloroplasts before it can utilize the available light efficiently. Results from the specimen collected in May indicates an acclimation to higher irradiances during the summer season. There was no overall alteration in pigment composition in older than younger tissue of *L. solidungula* (mean values), but great biological differences (e.g Fucoxanthin:Chlorophyll a ratio (weight:weight) with mean =0.77 and CV%=55) between specimens.

In comparison to four other kelp species living in Svalbard waters, photosynthetic parameters of *L. solidungula* did not emphasize the species as specially adapted to low light conditions. Time-series of photosynthetic characteristics showed diurnal trends with highest quantum yield of photosynthetic charge separation during nighttime for all species investigated (close to the theoretical limit of 0.8 for macroalgae). *Laminaria solidungula* had the lowest quantum yield values indicating lower utilization of photons available, compared to meristem tissue of the other species examined. Lowest values of rETR was detected in *A. esculenta.* The pigment composition was similar in all five species investigated eliminating *L. solidungula* as a blue-green light specialist. *Laminaria solidungula* did not fulfill all aspects of a low light adapted algae characterized by high α, low P_{max} and low E_k. The results for meristem tissue of *L. solidungula* gave a high α, high P_{max} and high E_k. This study indicates that *A. esculenta* is the species best adapted to the low-light climate and low temperatures in the Arctic.

The light saturation indexes for all five species investigated are also used to explain an overall zonation among the common kelp species in Svalbard waters with *L. solidungula* as the shallowest growing species and *A. esculenta* as the deepest growing species, when examining meristem tissue.

Sammendrag

Denne studien av den arktiske tarearten *Laminaria solidungula* (J. Agardh) sammenfatter dens økofysiologi, morfologi, habitat, fotosyntetiske parametere, cellekjemi samt pigment sammensetning. Det er sett på forskjeller mellom årsvev innad i arten samt forskjeller i nylig syntetisert (meristem) vev i forhold til fire andre arter på Svalbard.

Laminaria solidungula trives best mellom andre tarearter på hardbunn. Arten har tydelige markerte årsvev med et lysere område med fertilt vev og et diskformet hapter. Det fertile vevet ser ut bruke tre år på å danne modne zoosporer, som så blir frigjort fra 3 års vevet.

Resultatene viste ingen enhetlig degradering av vevet med økende alder hos *L. solidungula.* Ved sammenligning av de tre årsvevene, ga ett års vevet et høyere kvante utbytte av klorofyll a fluorescens fra fotosystem II enn eldre vev. Naturlig lys var kun sterkt nok til å mette fotosyntese i 3 års vevet. Resultater for fotosyntetiske parametere (maksimum lysutnyttelses koeffisient (α), fotosyntetisk rate (P_{max}) og lysmetnings indeks (E_k)) ga en høyere P_{max} i 2 års vev enn i 1 års vev, og kan indikere at vevet til *L. solidungula* trenger tid på å modne sine fotosyntetiske organeller (=kloroplaster). Resultater fra individet fra mai indikerer akklimatisering til høyere irradianser i løpet av sommersesongen. Det var ingen endring i pigmentkomposisjon med økende alder, men de biologiske forskjellene mellom individene var store (f.eks Fucoxanthin:Klorofyll a ratio (vekt:vekt) med gj.snitt = 0,77 og CV = 55 %).

Sammenlignet med fire andre arter som lever rundt Svalbard, fremhever ikke de fotosyntetiske målingene gjort på *L. solidungula* arten som spesielt adaptert til lavlys forhold. Tidsserier på fotosyntesemålinger viste døgnvariasjon med høyest kvante utbytte av klorofyll a fluorescens fra fotosystem II om natten for alle artene (nær teoretisk grense på 0,8 for makroalger). Sammenligning mellom meristem vev hos alle artene viser at *L. solidungula* har lavest kvante utbytte av klorofyll a fluorescens fra fotosystem II, og indikerer at meristem vev hos denne arten utnytter tilgjengelige fotoner mindre effektivt enn de andre artene. Laveste verdier for rETR ble funnet hos *A. esculenta.* Pigmentsammensetningen i meristem vevet hos de 5 undersøkte artene var lik, noe som eliminerer *L. solidungula* som den eneste blå-grønne lys spesialist innen brunalger i Arktis. *Laminaria solidungula* oppfylte ikke alle punktene for lav lys adapterte arter, karakterisert av høy α, lav P_{max} og dermed lav E_k. Resultatet fra målingene av fotosyntese parameterne for meristem vev hos *L. solidungula* ga en høy α, høy P_{max} og høy E_k. Denne studien indikerer at *A. esculenta* er den arten som best er tilpasset det lave lysmiljøet og den lave temperaturen i Arktis.

Lysmetningsindeksen (E_k) er i tillegg brukt til å forklare en mulig sonering av de vanligste tareartene på Svalbard, med *L. solidungula* som den arten som lever grunnest, og *A. esculenta* som den dypest voksende arten når ser på meristem vev.

Contents

1 Introduction

Laminaria solidungula (J. Agard) is an endemic high Arctic species specially adapted for low-light conditions and low temperatures (Dunton et al., 1982). At these latitudes (> 75˚N), ice and snow cover have a strong limiting effect on the amount of light (intensity, spectral) that penetrates down into the water column (Sakshaug et al., 2009). Hence, in areas with annual ice cover light and nutrient conditions may limit algal photosynthesis and growth caused by light limitation (Lüning et al., 1990). *Laminaria solidungula* has a unique adaptation towards such conditions that enables it to utilize the light for photosynthesis only for a short period of time (down to just a few days per year of positive photosynthesis), and then use the stored energy for heterotrophic growth during winter-spring darkness (Schmitz and Lobban, 1976). For definitions of adaptation and acclimation, see section 1.9.

To my knowledge there has been few studies concerning *L. solidungula* in Svalbard waters. Earlier studies have been focusing on pigment accumulation and biochemical defense systems (Aguilera et al., 2002) and sensitivity of photosynthesis to ultraviolet radiation (Bischof et al., 2002, Aguilera et al., 1999, Bischof et al., 2000, Michler et al., 2002, Roleda et al., 2006). Photosynthetic studies on *L. solidungula* has mainly been performed on basis of the ^{14}C-method (Dunton and Schell, 1986), but some recently studies have been using a Diving-PAM (Karsten, 2007).

1.1 Distribution and habitat

Laminaria solidungula is a common species in the west and east coast of Greenland, in the Canadian high Arctic and has infrequently been detected at different location in Svalbard waters, from Isfjorden and northwards on Spitsbergen (Kjellman, 1883, Svendsen, 1959). There is no record of discovery of *L. solidungula* south of Isfjorden (Weslawski et al., 1993, Muller et al., 2009), and distribution is limited by the demand of low temperature and short day lengths for reproduction (Wiencke, 2007). The species is depth limited to the lower sub-littoral zone (2-20 m, (Svendsen, 1959, Wiencke et al., 2004), and occurs on stony and rocky bottoms. Species like *Laminaria digitata* (Hudson) Lamouroux 1813 (Kjellmann, 1883) and *Saccharina latissima* (Linnaeus) Lane, Mayes, Druehl et Saunders 2006 (Lane et al., 2006) often dominate the sub-littoral zone at Svalbard and are together with *Alaria esculenta* (L) Greville (Kjellmann, 1883) dominating species at Svalbard (Carlsen et al., 2007, Svendsen, 1959). These are also the species compared to *L. solidungula* in this thesis, and in addition S*accorhiza dermatodea* (de la Pylie) J. Agardh (Kjellmann, 1883).

1.2 Morphology

Specimens of *L. solidungula* normally range between 50-110 cm in length (Svendsen, 1959, Dunton et al., 1982), but longer specimens have been observed (e.g. several meters in the Eastern Canadian Arctic and Siberia)(Henley and Dunton, 1995)(and references herein). The lamina has distinct annual blade segments of different size which is attached for at least three growth seasons. The shape are often characterized as hart- or circular shaped for both 1 and 2 year old tissue (Henley and Dunton, 1995). Henley and Dunton (1995) investigated the length of stipes in dark- and light-held thalli, and found the length to be highly variable and significantly greater in dark-held thalli. They concluded that it was a developmental response to raise the blade towards light. The haptera is disk formed (Dunton et al., 1982) and fertile tissue is often seen both in 1 and 2 year old tissue and released during late spring/summer when the algal tissue reaches 3 year. When sori are released is not commonly clarified. Henley and Dunton (1995) concluded that sori was released by late January, while Hooper (1984) proposed that spore release do not occur before spring.

1.3 Cell chemistry

Laminaria solidungula depends on stored energy reserves for growth during winter season, when irradiance is at a minimum and the nutrient level in the sea reaches a maximum. Earlier studies (Chapman and Lindley, 1980) revealed that the species accumulates carbon reserves during summer, when irradiances are high enough to saturate photosynthesis. These reserves are then exploited during the low-light period from November to April, and the species experience carbon depletion of ~25-30 % when reaching spring (Dunton and Schell, 1986). Previous publications also indicates that *Laminariales spp* are capable of storing nitrogen in large quantities in periods of high [N] in the water, i.e in winter darkness (Hilstad, 2005, Chapman and Craigie, 1977, Hanisak, 1983, Gevaert et al., 2001). By measuring the algaes content of organic bonded carbon (POC) and nitrogen (PON) the ratio of N:C can be calculated. This ratio can be used to evaluate the algaes physiological status by demonstrating seasonal variations in growth and storing (Hilstad, 2005). Dunton and Schell (1986) found that basal blade had highest content of N and highest N:C ration between February and April. A study by Gordillo et al (2006) concluded that Arctic seaweed are not N-limited in the summer, after examining twenty-one different species of macroalgae, including *L. solidungula* in July.

1.4 Light, nutrient and temperature limitations

In addition to constant low light, macroalgae inhabiting the lower sub-littoral zone in Arctic waters are exposed to strong seasonal changes in the light conditions (Sakshaug et al., 2009, Wiencke, 2007, Kirst and Wiencke, 1995). The species comprising Phaeophyta has a lower growth limit at ~0.6-1.2 % of surface irradiances (<1 E_{PAR}) and are characterized by high maximum utilization coefficient (α) and low light saturation index (E_k, 14-52 µmol photons $m^{-2}s^{-1}$ (Wiencke et al., 1993, Dunton and Jodwalis, 1988). The solar elevation mid-summer in Ny Ålesund (79˚N) is only 34˚ (Sakshaug et al., 2009) and the annual solar radiation at 80˚N is 30-50% less than in temperate to tropical regions (Lüning et al., 1990). During a year, there are large seasonal differences in E_{PAR} reaching the macroalgae. Measurements of annual E_{PAR} in Stefansson Sound Boulder Patch, Alaskan Beaufort Sea, ranged from max 5 µmol photons $m^{-2}s^{-1}$ during winter ice-covered periods, to ~200 µmol photons $m^{-2}s^{-1}$ during summer open-sea periods (Dunton, 1990). During the ice-covered period (~9 months), the light received represented <10% of total annual E_{PAR} available for the macroalgae (Dunton, 1990).

Secondly, water temperature has an impact on photosynthesis. Polar species are not as strongly adapted to ambient temperatures as Antarctic species due to different cold-water history (Crame, 1993). In the Arctic, water temperature seldom exceed 5 ˚C and highest photosynthetic rate was found to be at ~20 ˚C (Healey, 1972). Highest growth rates of Arctic macroalgae have been achieved at 10-15 ˚C (Wiencke et al., 1994). Temperature demands of *L. solidungula* has not yet been investigated (Wiencke, 2007).

A third issue for Arctic species are nutrient content in the seawater. The growth season of *L. solidungula* is from November to late April, with a peak between late February and late April (Dunton et al., 1982) when the concentrations of nutrients in the Arctic water is at a maximum (Aguilera et al., 2002) close to sea-ice break-up when spring phytoplanktonblooms occure ((Dunton et al., 1982, Chapman and Lindley, 1980). The low nutrient content in the Arctic waters during summer have been concluded to be growth limiting for macroalgaes.

In addition, salinity can also have an effect on Arctic species. The salinity in Svalbard waters usually account for ~34 psu, but can be as low as 15-20 psu during spring ice-melting and inflow of melt-water. A stratification can be evident in the upper 20 meters of the water column (Karsten et al., 2003, Karsten, 2007). A study of salinity effects on photosynthetic efficiency in brown macroalgae species from Svalbard, reveled that *L. solidungula* and *S. dermatodea* are more influenced by variance in salinity than *L. digitata, A. esculenta* and *S. latissima* (Karsten, 2007).

1.5 Photosynthesis measured by Chlorophyll a fluorescence kinetics

Chlorophyll (Chl) a fluorescence is the decay process of a photon from an excited state back to ground state, where the difference in energy is lost as a visible photon (Owens, 1991) with peak at wavelength 685 nm (Govindjee, 1995). The main pathway for the excitation energy is photochemistry, while other processes in addition to fluorescence are thermal emission and decay via triplet excited state (Owens, 1991). Chl a fluorescence amounts to ~1-5 % of the total light absorbed and is a light-harvesting and utilization indicator in living organisms. Photochemistry amounts to 25-30 % and about 70% is lost as heat (Falkowski and Raven, 1997). The fluorescence signal is extremely rich in information, but might be difficult to interpret (Govindjee, 1995). The relationship between fluorescence and photochemistry in utilization of light energy, makes it possible to use fluorescence measurements as a non-invasive, real-time insight into variation in biomass, ecophysiology and photosynthetic rates in living organisms, such as macroalgae (Nedbal and Koblízek, 2006)

When algal tissue is acclimated in darkness, the chl a fluorescence emitted from PSII is minimal and all functional RC_{PSII} are open and initial fluorescence (F_o, *re. units*) can be measured. When photosynthesis is saturated, the fluorescence signal will increase until all RC_{PSII} are closed, and maximum (3-5 %)(Johnsen et al., 2011) fluorescence (F_m, *re. units*) is obtained. When the algal tissue has been exposed to light, subsequent F_o and F_m are denoted F'_o and F'_m.

The measurements of F_o and F_m can be used to calculate the maximum quantum yield of Chl a fluorescence in darkness (Φ_{PSII}, *re.units*) (Genty et al., 1989, Hancke et al., 2008), which is the ratio between total numbers of photons emitted from a light source and the number of photons absorbed by the photosynthetic reaction centers of PSII (RC_{PSII}). More exactly, Φ_{PSII} is the fraction of light that is absorbed and utilized by RC_{PSII} and re-emitted as fluorescence (Parson and Nagarajan, 2003). Φ_{PSII} denotes the fraction of open (low light conditions, minimum photosynthetic rate, F_o) or closed (saturation light conditions, maximum photosynthetic rate, F_m) RC_{PSII}. Highest theoretical value of Φ_{PSII} (max 3-5%) in macroalgal tissue is 0.8 since some RC will be out of order or in process of being restored (Ralph and Gademann, 2005)

The relative electron transfer rate (rETR) from PSII to PSI gives a relative value which describes how fast PSII can recieve and utilize the light of each irradiances (E_{PAR}). Ideally the value of rETR follows the curve of E_{PAR}, and can be used as an indication on the gross photosynthetic rate in eg macroalgal tissue (Hancke et al., 2008). The calculation of relative ETR is useful when the absorption of light in the algae is unknown and are done by the equation:

$$rETR = \Phi_{PSII} \times E \quad (1)$$

The photosynthetic rate is related to irradiance and characterized with 3 parameters. The maximum light utilization coefficient (α) describes the linear rise in photosynthetic rate with increasing E_{PAR}, max photosynthetic rate (P_{max}) where the photosynthetic rate reaches a light saturation level, and the light saturation index (E_k, units µmol photons $m^{-2}s^{-1}$) which is P_{max}/α . The high values of α in a species indicates a better utilization of low irradiances. These parameter definitions and denotations was suggested by Sakshaug et al (1997). The relative photosynthetic irradiance curve (P vs E curve) can be prepared by changes of rETR as a function of E_{PAR}, and can be used to look at the photoacclimational state of the algae. The photosynthetic parameters can be calculated using Webb's (1974) non-linear least-square procedure (Sakshaug et al., 1997). The definition of P vs E curves ideally refer to the photoacclimational state of the algae at the moment of sampling. Further in this thesis, the term "P vs E " indicates measurements over a period of 24 hours (except for the *L. solidungula* collected in Ny Ålesund in January 2010).

Earlier values of E_k reported for Antarctic and Arctic species range between 10-170 µmol photons $m^{-2}s^{-1}$ (Gomez et al., 2009), which are lower than values seen for temperate species (Kirk, 1994) with Antarctic species in the range below 100 µmol photons $m^{-2}s^{-1}$. Dunton and Jodwalis (1988) found that summer open-water saturation levels for *L. solidungula* in ambient light was 38 µmol photons $m^{-2}s^{-1}$.

1.6 Pigments

Two types of pigments are situated in the light harvesting complexes of photosyntethically active organisms. Photosyntethic pigments (all chlorophylls and photosynthetic carotenoids) are used for light harvesting, and transport of excitation energy from the sun to the RC of PSII and PSI (Brunet et al., 2011, Johnsen et al., 2011), while photoprotective pigments (PPC, non-photosynthetic carotenoids) do not transfer absorbed energy to the RC. The organism uses their pigment composition to cope with a fluctuating light environment. The organisms ability to harvest light is dependent on the size, number, morphology and distribution of chloroplasts in the cell and also the light climate surrounding them (Johnsen et al., 2011).

1.7 The Diving-PAM

In the past decades, the pulse-amplitude-modulated (PAM) fluorescence method has been used to gain information on the photosynthetic activity of macroalgae. Earlier studies of *L. solidungula* was based on the ^{14}C method, which is a labor intensive method dependent on various changing conditions, and therefore, an inaccurate method for modeling primary production (Hancke et al., 2008)(and references herein).

The Diving-PAM is suppose to be a non-invasive method of measuring photosythesis on living organism in the ocean.

1.8 Aim of thesis – study questions

Hypothesis 1
Laminaria solidungula is able to growth heterotrophically at low irradiances and in dark (polar night) with functional chloroplasts

Prediction: We expect *L. solidungula* to be able to start synthesizing a new tissue and chloroplasts during the polar night without being exposed to ambient or actinic light.

Approach: photosynthetic measurements on a newly synthesized specimen/tissue of *L. solidungula* during polar night, analyzes of pigment composition and in addition analyses of POC and PON.

Hypothesis 2
There are distinct differences due to age of tissue in *L. solidungula* regarding nutrient content, photosynthetic characteristics and pigment composition.

Prediction: We expect photosynthetic rate and pigment composition will be lower as a function of age in *L. solidungula.*

Approach: Diurnal time-series of photosynthetic characteristics in 1-3 year tissue of *L. solidungula,* analyses of pigment composition in the different year tissues and in addition analyses of POC and PON.

Hypothesis 3
Laminaria solidungula exhibits specific adaptations to survive and grow in a high Artic light climate in terms of its photosynthetic characteristics and pigment composition.

Prediction: Compared to the four other species (*L. digitata, Saccharina latissima, Alaria esculenta* and *Sacchoriza dermatodea)* examined, we expect *L. solidungula* to exhibit more extreme photosynthetic characteristics of α, P_{max} and E_k and in addition a pigment composition that utilizes the blue-green light better relatively to the other species examined.

Approach: Diurnal time-series of photosynthetic measurements conducted on meristem tissue of the 5 different species using a Diving-PAM, analyses of pigment composition using a HPLC and in addition analyses of POC and PON.

In addition to these hypothesis, photos of all collected specimens of *L. solidungula* from both Tommelen (August 2009) and Rossøya (September 2009) together with photos of the habitat taken while SCUBA-diving will be analyzed.

1.9 Definitions and terms used

1.9.1 Definitions

Adaptation:	Indicates evolutional long-term adjustments of genes in a given species capable to live in a given environment (e.g. in cold and in low light habitats)(Brunet et al., 2011).
Acclimation:	Short-term adjustments (seconds to weeks) in living organisms to minimize effects on variations in key environmental variables such as e.g. light regime and growth rate (Brunet et al., 2011).
Chromophytes :	Chl c-containing algae
Fertile tissue:	Specialized tissue (area) on lamina in the macroalgae where zoospores develop.
Heterotrophic growth:	The process when an algae assimilates surplus material and store the compounds as reserve material and reallocate it to growth zone during periods of high nutrient content in the water (Chapman and Lindley, 1980).
Meristem tissue:	Tissue in the section between stipes and lamina where new lamina tissue is formed.
POC:	Particulate organic carbon
PON:	Particulate organic nitrogen
Photosynthetic parameters	(α, P_{max} og E_k)
Sori:	Mature zoospores of macroalgae
1 year old tissue:	The year-tissue closest to stipes (starting with the meristem), which has been growing during the last year (growth season). The term "year old tissue" refers to each distinct lamina section.
2 year old tissue:	Distinct marked tissue closest to 1 year old tissue.
3 year old tissue:	Oldest distinct tissue area where fertile area usually have been released.

1 year old macroalgae:	Specimen with only one distinct lamina part. The term "year old macroalgae" refers to the age of the specimen and if it has 1, 2 or 3 distinct lamina sections.

1.9.2 Terms

E	Irradiance (µmol photons $m^{-2}s^{-1}$)
PAR	Photosynthetically Active Radiation (400-700 nm)
PSII	Photosystem II
PPC	Photoprotective carotenoids
F_o	Dark-adapted initial fluorescence
F'_o	Light acclimated initial fluorescence
F_m	Dark-adapted maximum fluorescence
F'_m	Light acclimated maximum fluorescence
Φ_{PSII}	Maximum quantum yield of Photosystem II
Φ'_{PSII}	Operational quantum yield of Photosystem II
rETR	Relative electron transport rate
α:	Max utilization coefficient
$P_{max:}$	Max photosynthetic rate
E_k:	Light saturation index

2 Material and Methods

2.1 Study area

The field sampling was carried out during three cruises in 2009 and 2010 at four different location at Svalbard, with different types of habitat (Figure 1, Table 1). For 2009 location 1. Tommeløyene (Tommelen) in Hinlopenstredet, 2. Rossøya, north of Nordaustlandet and 3. Ny Ålesund (NÅ) in Kongsfjorden were visited. For 2010, site 3 was re-visited and site 4. Smeerenburgfjorden was visited.

Figure 1: Map of Svalbard displaying the four sampling locations (positions in Table 1). 1. Tommeløyene, Hinlopenstredet, 2. Rossøya, north of Nordaustlandet, 3. Ny Ålesund, Kongsfjorden and 4. Smeerenburgfjorden. Figure from www #1.

Table 1: Geographical position and names of the 4 sampling sites at Svalbard during 2009 and 2010. In addition, the total number of *L. solidungula* collected at each site and at which depth.

Site	Latitude	Longitude	Name of location	Date of sampling	No. of specimens (*L. sol.*)	Depth (m)
1	79˚33´N	18˚44´E	Tommelen	28.08.2009	52	3-5
2	80˚49´N	20˚18´E	Rossøya	02.09.2009	9	10-17
3	78˚55´N	11˚56´E	Ny Ålesund	05.09.2010 / 21.01.2010	0/1	15
4	79˚41´N	11˚09´E	Smeerenburgfjorden	21.05.2010	1	3-4

2.2 Collection and handling of samples

During the cruises in 2009 and 2010, a total of 63 specimens of the macroalgae *L. solidungula* were collected by SCUBA-diving to max depth of 17 m except for the

specimen of *L. solidungula* from NÅ (site 3) which was collected by a VideoRay Pro III S ROV (see below section [VideoRay Pro III S ROV] for more details (Table 1, Figure 1, Figure 2 A). In addition, specimens of 4 other macroalgae species was collected; *Alaria esculenta* (site 3), *Laminaria digitata* (site 1)*, Saccorhiza dermatodea* (site 1) and *Saccharina latissima* (site 2, Figure 1). Each of the specimens was marked with number, date and location (Figure 2 B). All specimens where kept in water (at in situ temperature of ~0.5-1.0˚C) and darkness before and during handling. Five specimens of *L. solidungula* with 3 year old tissue (collected at Tommelen), where selected and 3-5 disks (3.1 cm in diameter) where cut from each growth zone (Figure 2 C). Disks was also cut out from the specimen of *L. solidungula* collected in Smeerenburgfjorden in May 2010 (1 and 2 year old tissue plus fertile tissue). All algal tissue (disks, hole plants and remnants) were packed in aluminum foil and marked with number, tissue age, date and location and then frozen (at -20˚C). The specimens of *A. esculenta*, *L. digitata, S. dermatodea* and *S. latissima* were kept whole when frozen. The samples were brought back to Trondhjem Biologiske Stasjon (TBS) for chemical analyses.

2.3 VideoRay Pro III S Remotely Operated Vehicle

Considering safety and time limitation due to low temperatures, ice and darkness during polar night, a Remotely Operated Vehicle (ROV) was used instead of SCUBA-divers to collect the specimen of *L. solidungula* in Ny Ålesund in January 2010. The ROV in use was a VideoRay Pro III S ROV (Figure 2 D) equipped with a forward facing WDCC-6300 CCD color video camera and a simple sample collector arm in addition to depth gauge, compass and forward facing halogen video lights. The VideoRay Pro III S ROV has a total of three thrusters; for movement, differential drive propulsion and depth control. This results in exclusively forward and backward movements. The ROV is depth rated to 152 m and has ~76 m of umbilical to provide power and, carries both command and return signals from the ROV itself and the camera mounted on the vehicle (Jun and Clark, 2006).

2.4 Photosynthetic measurements

2.4.1 PAM-measurements

Chl a fluorescence kinetics was measured using a Diving-PAM (Walz, GmbH, Effeltrich, Germany, Figure 2 E) equipped with a 1.5 m long optical fiber for photosynthetic measurements. A 2π cosine corrected Diving_LS PAM irradiance sensor (Heinz Walz, GmbH, Germany) was used to measure the ambient E_{PAR}. The measurements were conducted by illuminating each tissue with a red "probe light" from LED (peak at 655 nm) at <0.15 µmol photons m^{-2} s^{-1} (0.6 kHz) to avoid actinic activity. Probe light was transmitted through the optical fiber at a distance of ~5 mm from the tissue to obtain F_o/F'_o signal of 300-500 mV. The same optical fiber was used to obtain a light saturation pulse (halogen lamp) at 4000 µmol photons m^{-2} s^{-1}, with duration of 1.0 sec, at a frequency of 20 kHz. This light pulse closed all the RC_{PSII}. The data was transferred from the Diving-PAM to a computer (using Walz Diving-PAM "WinControl" software) through a RS 232 interface as an ASCII-file and stored as a text-file. Charging power (Mascot 12 V, Norway) was used for the battery level. Excel and SigmaPlot were used to process the data. The main settings used on the Diving-PAM during experiment III are quoted in Table 2, and some of the menus are explained below.

Table 2: User-settings for the Diving-PAM during experiment III. Menu-point 3-5 was used when measuring F_o and F_o'. Menu-point 46-47 was used when measuring F_m and F_m'.

Menu	Type of menu	Settings
3	Measuring light	On
4	Measuring probe light (F_o / F'_o)	Low (measuring frequency 0.6 kHz)
	Saturating light (F_m / F'_m)	High (measuring frequency 20 kHz)
5	ML-burst	Off
6	Light average 15 second	On
7	External light-sensor	On
28	Repetition clock	On
29	Clock-item	Sat-pulse
30	Clock-time	5 minutes
34	Battery	12 V
46	Saturation- light width	1.0 sec
47	Saturation-light intensity	10 (4000 µmol photons m^{-2} s^{-1})
48	Damping	2
49	Gain	2
50	Measuring-light intensity (red LED_{650nm})	8 (0.15 µmol photons m^{-2} s^{-1})

Charging power was used for the battery level (menu 34) to avoid drops below 11.0 V, which would result in errors in lamp output intensity and spectral characteristics. The saturation-light width was increased to 1.0 sec from standard 0.8 sec due to change of lamp. The replacement lamp (custom made) had thicker filament and some adjustment in the standard settings was required.

Experiment I and II:

Measurements of dark acclimated algal tissue was conducted on the shore of Tommelen to obtain F_o of the algal tissue. All collected specimens where stored in a bucket of water with a lid prior to the experiment to avoid direct sunlight and to keep the tissue as dark acclimated as possible. Six 3 year old specimens of *L. solidungula* was selected and measurements of Φ_{PSII} was conducted. Each distinct year tissue was measured by hand-holding the optical fiber close to the tissue (~5 mm). After measuring Φ_{PSII}, the six specimens were incubated in ambient E_{PAR} for 2 minutes and re-measured for Φ'_{PSII}. The results provided an overview of the basic values for non-stressed tissue and how the tissue reacted to bright light. Intraspecific differences and differences due to age was also displayed. These experiments gave an insight to the biological variations between the specimens and differences due to age of tissue. Based on these results, the further use of one specimen of each year tissue of *L. solidungula*, and one specimen of each of the other kelp species investigated when conducting high resolution (5 min between measurements for 24 h) time-series (24 h measurements of each age) are justifiable.

Experiment III:

Diurnal time-series measurements of F'_o and F'_m were conducted on top deck of R/V Jan Mayen (Figure 2 F). One specimen of each species was selected and fastened in a sample holder and placed in a tray with seawater (Figure 2G). An analog thermometer was used to keep track of the water temperature. Measurements were conducted every 5 minute for a period of ~24 hours (total of ~250 measurements for each tissue). For *L. solidungula*, three different year tissues were measured, for *A. esculenta, L. digitata, S. dermatodea* and *S. latissima* only meristem tissue was measured. Relative ETR was calculated and P vs E curves prepared. During these experiments the ambient light was not enough to close all RC_{PSII} of the algal tissue. To obtain saturated photosynthesis an external light source was deployed (slide projector; Leica Pradovit P2002, 250 W (24V) halogen lamp) for ~1 hour each evening (~23:00-00:00).

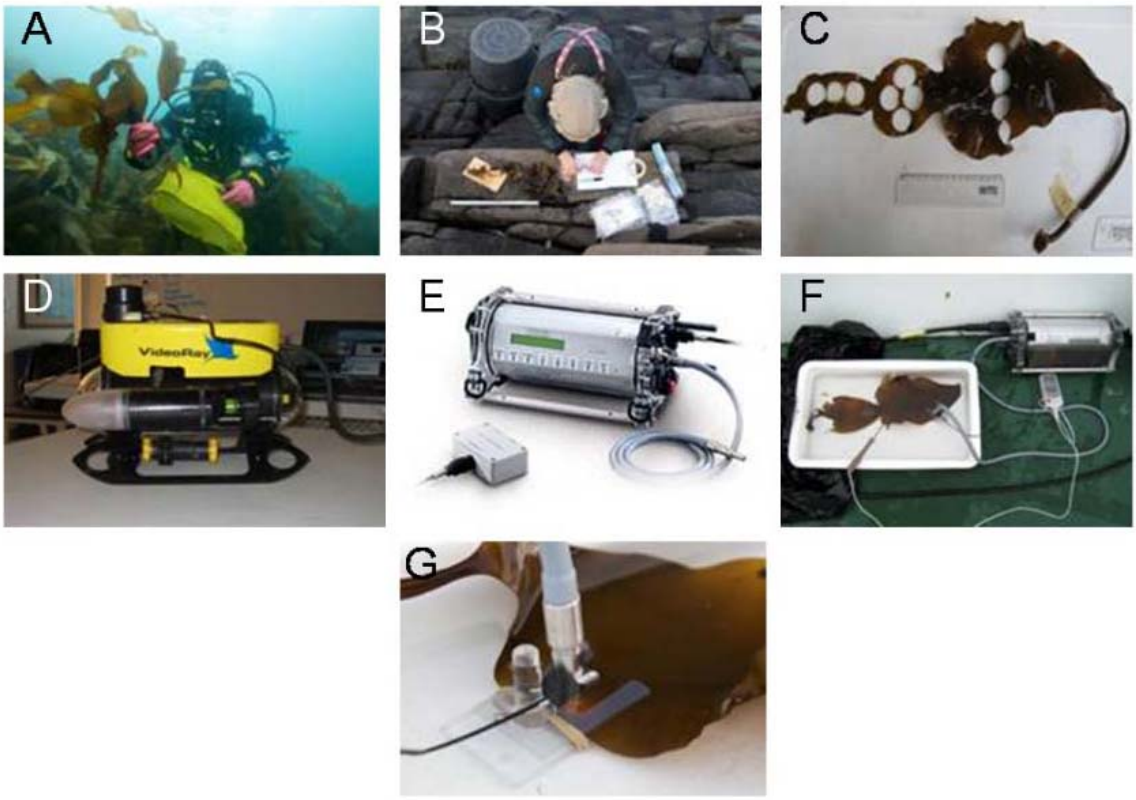

Figure 2: A: Collection of *L. solidungula* at Tommelen. Photo: Geir Johnsen. B: Processing the collected macroalgae specimens at Tommelen. Photo: Geir Johnsen. C: Specimen of *L. solidungula* with disks cut out. Photo: Elen Belseth. D: The VideoRay Pro III S ROV. Photo: Elen Belseth. E: The Diving-PAM. Figure from www #2. F: Photosynthetic measurement conducted on top deck of R/V Jan Mayen. Photo: Elen Belseth. G: The external photometer was placed on the side of the macroalgae sample holder. Photo: Geir Johnsen.

2.4.2 Photosynthesis versus irradiance curves, curve fitting.

A nonlinear least-square procedure and the equation by Webb et al (1974; eq. 2) was used to fit the P vs E data in SigmaPlot (SigmaPlot 2001 for windows version 7.0, SPSS Inc.). The photosynthetic parameters; P_{max}, α and E_k was calculated for each year tissue of *L. solidungula* and the four other macroalgae species collected. The E_k parameter was calculated as P_{max}/α.

$$P = P_{\max}\left[1 - \exp\left(\frac{-\alpha \cdot E}{P_{\max}}\right)\right] \qquad (2)$$

2.5 Pigment analyses using high performance liquid chromatography (HPLC)

Pigments were separated using a Hewlett Packard 1100 Series HPLC system. Analyses was carried out on the five specimens of *L. solidungula* with three distinct year tissue collected at Tommelen in August 2009, one specimen of each of the four other macroalgae species collected in August and September 2009, the specimen of *L. solidungula* collected in Ny Ålesund in January 2010 and the specimen of *L. solidungula* collected in Smeerenburgfjorden in May 2010. To separate the pigments in the tissue, half a disk of each algal tissue was cleaned for possible epifauna and water with a paper cloth and extracted in 5 ml methanol in glass vials. The vials was shaken for ~2 sec in a Vortex mixer to mix the pigment in the solution evenly, bubbled with N_2 gas to avoid oxidizing of pigments and stored in a freezer (-4˚C) over night. The samples were subsequently refiltered using a RC 0.2 µm filter (25 mm Syringe Filter w/0.2 µm cellulose Acetate Membrane, VWR International) and ~1.5 ml transferred to dark colored HPLC vial and placed in the HPLC machine. Agilent Chemstation was used to analyze the chromatographs and determine the pigments. The absorbance of each pigment was measured at four different wavelengths (420, 440, 450 and 460 nm). In this study the 440 nm wavelength was used, since peaks of all the pigment groups in the kelp species investigated was shown. To identify the pigments, both retention time and absorption properties was used and compared to a HPLC library and reference literature (Jeffrey, 1997). The spectra from the library often gave <100% match, which can be due to partly separation of pigments, degradation, low signal to noise ratio (low consentration) or instrumental noise (e.g. dark current). For a more precise identification, the band ratio of the pigments was calculated (Jeffrey, 1997). When identified, the pigments retention time and area at 440 nm was transferred to Excel and quantified using equation 3.

$$\mu g / L = \frac{area \times Rsf \times V_e \times 1000}{V_i} \quad (3)$$

Area: area of pigment peak from HPLC at 440 nm
Rsf: response factor calculated by calibration at 440 nm
V_e: extraction volume, (5) ml methanol used to extract discs of tissue
V_i: injection volume, (77) µl extract injected in HPLC

Pigment composition (concentration and speciation) in all year tissues and all macroalgae species was analyzed. To be able to compare the different amount of the pigments according to year tissue and among the different species, the pigment:Chl *a* ratio was calculated.

Chl c_1 and Chl c_2 are often difficult to separate due to resembling wavelength of the peak of absorbance, and also retention time. The two pigments therefore often co-eluate. In analyzes where they formed two distinct peaks, they were separated and where they co-eluted, the peak was identified as Chl c_2 (Rodriguez et al., 2006).

2.6 POC / PON analyses

Tissue from the same specimens as analyzed for pigments in the HPLC was dried and analyzed for particulate organic carbon (POC) and nitrogen (PON) on a Costech elemental combustion System (CHNS-O, Costech ECS model 4010, Serienr: 260610079, Costech Analytical Technologies Inc., Valencia, CA, USA). Cups of aluminum foil were made and burned in a ceramic oven at 450 ˚C for 3 hours to eliminate sources of error concerning carbon. Elimination of redundant water from thawing was carried out on all tissue of the macroalgae species, then weighed (wet weight), placed in the aluminum cups and dried in a electrical oven (Termaks) at 60 ˚C for 48 hours. When removed from the electrical oven (Termaks), dry weight was determined and ~3.5 mg of tissue transferred to tin capsules (5-9 mm, 3 parallels), wrapped and placed in marked wells on a tray. The tray was stored in a freezer (-4˚C) in pending of POC and PON analyses. 5 empty tin capsules was weighed and added in the tray to check for traces of nitrogen. The dry weight of 3.5 mg emerged from the fact that the instrument can not handle results of more than 2.0 mg carbon, and the knowledge of carbon content in the macroalgae species between ~25-40 % of dry weight (Henley and Dunton, 1997). The percentage share of POC and PON due to dry weight and the N:C ratio of the algal tissue was determined for all samples.

2.7 Determination of length of stipes and lamina

The length of stipes and lamina of *L. solidungula* was determined from the pictures taken. A scalebar was added together with the specimens in the pictures. The scalebar was measured, scaled to real length in cm, and used to stipulate the length of stipes and lamina. 5 specimen (#2-5 and #7) did not have a scalebar in the pictures and was excluded from the analyzes. The specimens was divided into 1-, 2- and 3 year old algae by counting the number of distinct lamina sections. Mean values, SD and CV% was calculated for all specimens.

2.8 Statistical measurements

Standard deviation (SD) and variation coefficient (CV) was calculated for all mean values for both pigment concentration ratios and length of stipes and lamina, using eq. 3 and eq. 4.

$$SD = \sqrt{\frac{\sum\left(\mathrm{X} - \overline{\mathrm{X}}^2\right)}{n-1}} \tag{4}$$

SD = standard deviation
n = Number of replicates
X = measurement in focus (pigment concentration ratio / length of stipes or lamina)

$$CV = \frac{SD}{\overline{X}} x100\% \quad (5)$$

CV = Variation coefficient of mean value (+/- mean %)

X = the mean of measurement in focus (pigment concentration ratio / length of stipes or lamina)

The CV is the SD of the mean represented as a percent of the mean, and gives a indication of the dispersion of the data. The statistical analyses was done in Sigmaplot (Sigmaplot 2001 for Windows version 7.0, SPSS Inc., 2001) and Statgraphics (Statgraphics Centurion XVI, version 16.1.11, StatPoint Technologies, Inc.).

To determine any statistically significant ($P < 0.05$) differences between the means of the different age of *L. solidungula,* statistical tests was applied. To test if there was a significant difference between two or several of the age groups, a one-way between-groups analyses of variance (one-way ANOVA) was applied (testing both length of stipes/lamina and pigment ratios), followed by a Multiple Range Test (MRT). The one-way ANOVA test will reveal a difference between two or several of the samples tested, but not which groups. The MRT was therefore applied afterwards to determine which of the groups differ. Statistical significance concerning length of stipes and lamina was only determined for the specimens from Tommelen since the sample size from Rossøya was rather small, and a test on that material is not reliable. The sample size for 1 year old specimens from Tommelen was also rather small to be used in any conclusions, but the results will be an indication of possible differences. To test if both age and specimen number had a significant effect on pigment ratio, a two-way between-groups ANOVA (two-way ANOVA) was applied in addition to a one-way ANOVA..

3 Results

3.1 Habitat

The specimens of *L. solidungula* were found on steep bedrock slopes at Tommelen and Rossøya at 5-30 m depth (Figure 3). In contrast, the habitat in Kongsfjorden and Smeerenburgfjorden was characterized by soft-bottom (sand and silt) with occasionally rocks and pebbles for attachment of the macroalgae. At both Tommelen and Rossøya *L. solidungula* were "hidden" in between other kelp species like *S. latissima, A. esculenta* and *L. digitata.*

Figure 3: The macroalgae habitat at the two different locations A) Tommelen and B) Rossøya.

3.2 Morphology of *L. solidungula*

Morphological differences of the 61 specimens of *L. solidungula* are shown in Figure 5-7. No visible differences between morphological features and sampling sites was possible to detect from the pictures. The species was characterized by having low visual cover of epifauna and flora, except for one specimen (#46) with epifauna growth on both stipes and lamina (Figure 6).

Haptera
The haptera was more or less disk shaped with a diameter ranging between 1-3 cm for all specimens collected on Tommelen and Rossøya (Figure 15, discussion). The haptera was not visible looking at *in situ* photos.

Stipes
The length of stipes of the collected specimens ranged from 4.0 cm (1 year old specimen) to 42.2 cm (3 year old specimen, Table 3 Figure 5-7. The age of stipes was defined by the age of lamina. The mean stipes length for 1, 2 and 3 year old algae from Tommelen and Rossøya was 13.9-17.2-21.0 cm and --13.9-17.5 cm, respectively (Figure 4, Table 3). The length of stipes in the different age groups were not statistically different.

Lamina

The length of lamina ranged from 15 cm (1 year old specimen) to 76 cm (2 year old specimen, Table 3, Figure 4). The mean lamina length for 1, 2 and 3 year old algae from Tommelen and Rossøya was 26.0-38.6-52.3 cm and --39.3-42.5 cm, respectively (Table 3). Some of the 1 year old part of lamina was heart-shaped, while other was circle formed or elongated (Figure 5-7). The transition sections between the different year tissue differed in width and the edge of the lamina was either smooth or fringed. One-way ANOVA showed a significant difference in length of lamina due to age ($F_{2,44}$ = 13.25, P = 0.00) of *L. solidungula* from Tommelen and the Multiple Range Test showed that the mean for all three age groups was significantly different.

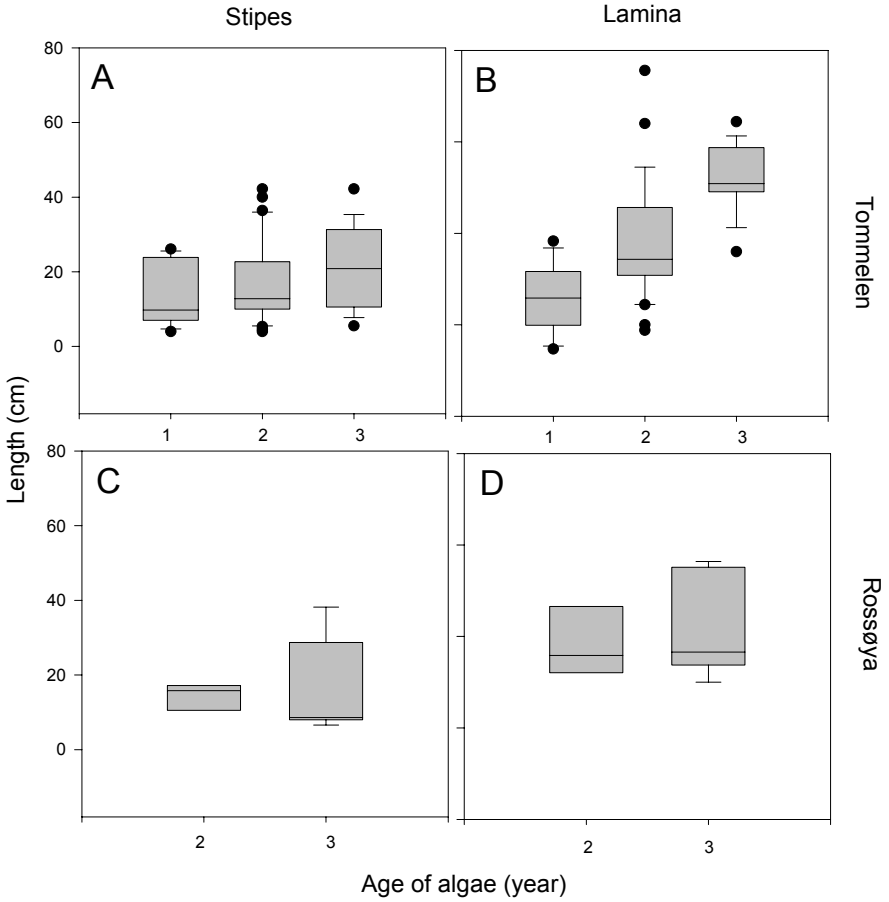

Figure 4: Box plot showing A) length of stipes for 1 (N=8), 2 (N=29) and 3 (N=15) year old tissue of *L. solidungula* collected at Tommelen, B) length of lamina for 1, 2 and 3 year old tissue collected at Tommelen C) length of stipes of 2 (N=4) and 3 (N=5) year old tissue of *L. solidungula* collected at Rossøya (August/September 2009) and D) length of lamina for 2 and 3 year old tissue of *L. solidungula* collected at Rossøya. There was only a statistically significant difference between all age groups for lamina in specimens collected at Tommelen. The boxes shows the interquartile range, whiskers shows the 10th and 90th percentile, circles shows the extreme values and line show the median length of stipes and lamina.

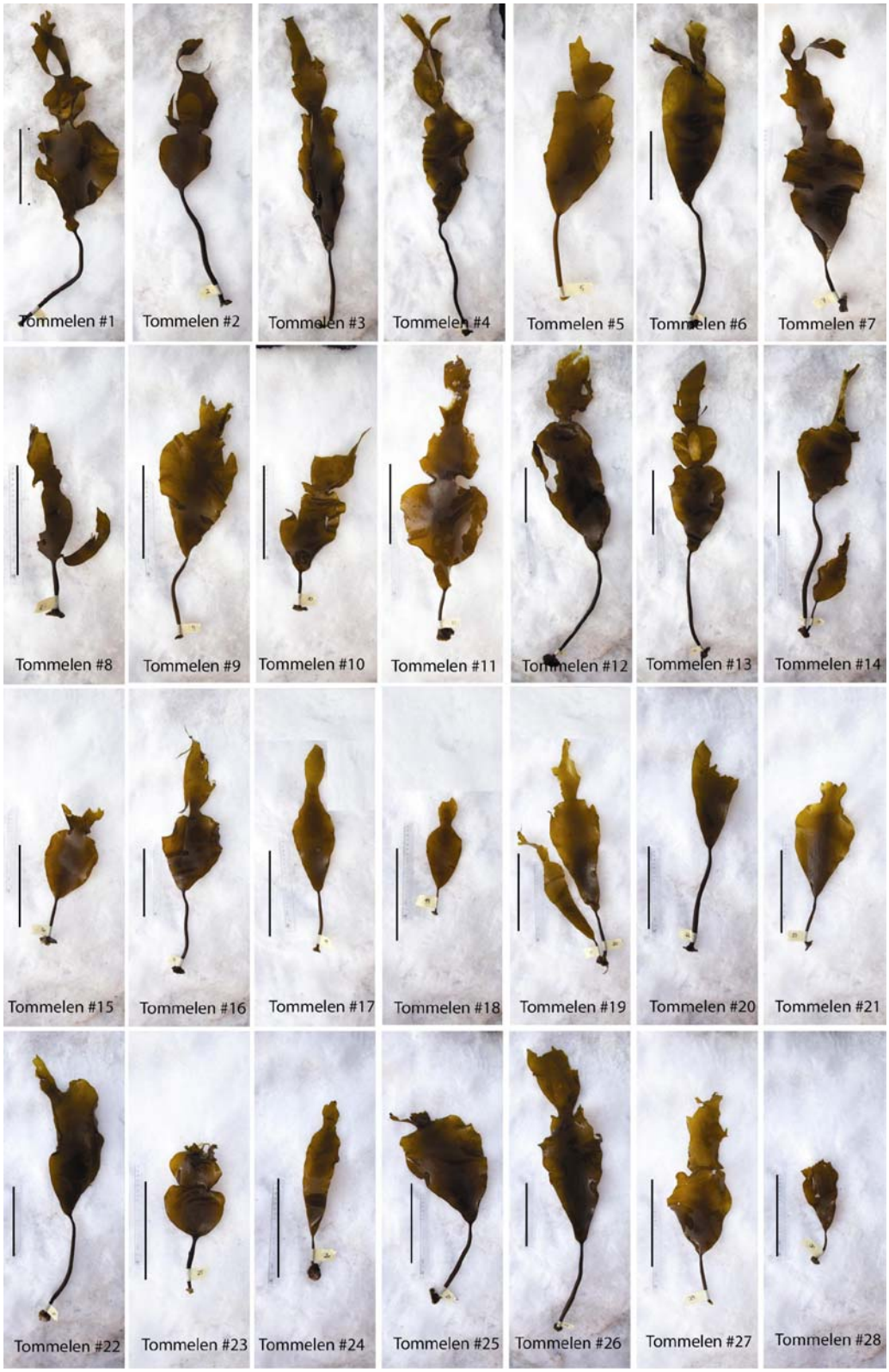

Figure 5: Photos of specimen #1-28 of *L. solidungula* collected at Tommelen in August 2009. Sampling site and number of specimen are noted on each picture. Scalebar equals 20 cm.

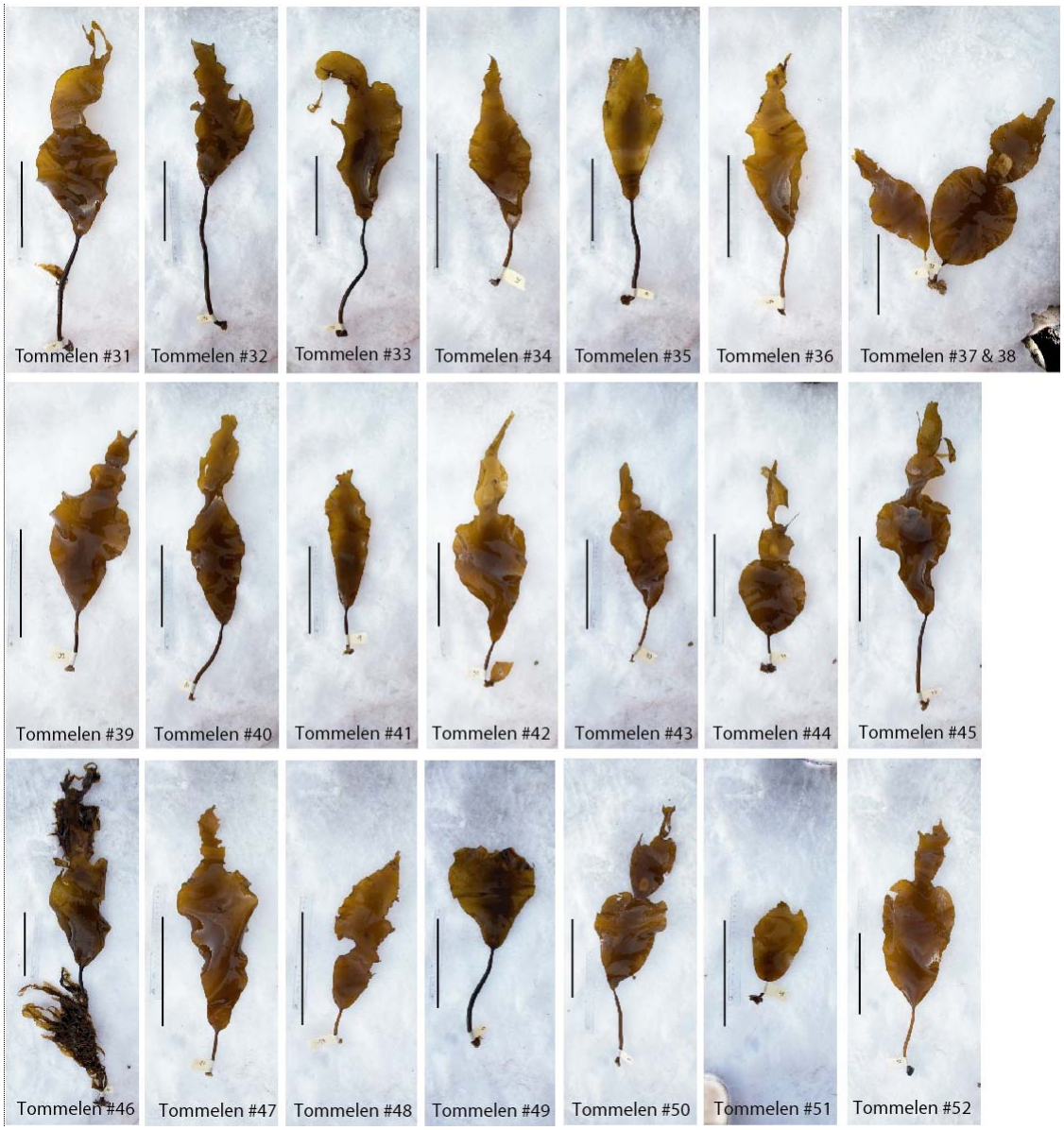

Figure 6: Photos of specimen #29-52 of *L. solidungula* collected at Tommelen in August 2009. Sampling site and number of specimen are noted on each picture. Scalebar equals 20 cm.

Figure 7: Photos of specimen #1-9 of *L. solidungula* collected at Rossøya in September 2009. Sampling site and number of specimen are noted on each picture. Scalebar equals 15 cm.

Table 3: Morphological data of *L. solidungula.* Statistics summarizes the length of stipes and lamina of specimens sampled from Tommelen and Rossøya (August and September 2009). The specimens were divided into 1, 2 and 3 year old algae by counting number of distinct lamina parts (Figure 5-7). Age of stipes was defined by the age of lamina.

	Sample size	Min length (cm)	Max length (cm)	Mean (cm)	SD	CV (%)
Tommelen						
Stipes	52	4.0	42.2	17.6	11.0	62.6
1 year old	8	4.0	26.1	13.9	9.1	65.3
2 year old	27	4.0	42.2	17.2	11.1	64.6
3 year old	12	5.5	42.2	21.0	11.8	56.5
Lamina	52	14.7	75.6	39.9	14.1	35.4
1 year old	8	14.7	38.3	26.0	7.9	30.6
2 year old	27	18.8	75.6	38.6	13.3	34.4
3 year old	12	36.0	64.4	52.3	8.0	15.2
Rossøya						
Stipes	9	5.3	38.2	15.9	10.6	66.9
2 year old	4	5.3	18.5	13.9	5.8	42.1
3 year old	5	6.6	38.2	17.5	13.9	79.5
Lamina	9	30.0	56.4	41.1	10.9	26.6
2 year old	4	30.8	54.7	39.3	10.7	27.3
3 year old	5	30.0	56.4	42.5	12.1	28.5

Fertile tissue

Fertile tissue was only visible in some of the specimens of *L. solidungula* (observed from photos, *in situ* and collected specimens), and not in all year tissues. The fertile tissue was elongated and lance shaped in 1 year old tissue and reduced in size resembling a spade formed area in 2 year old tissue (Figure 8). In 3 year old tissue the fertile tissue had been released in most of the specimens and shape was more oval (Figure 5-7).

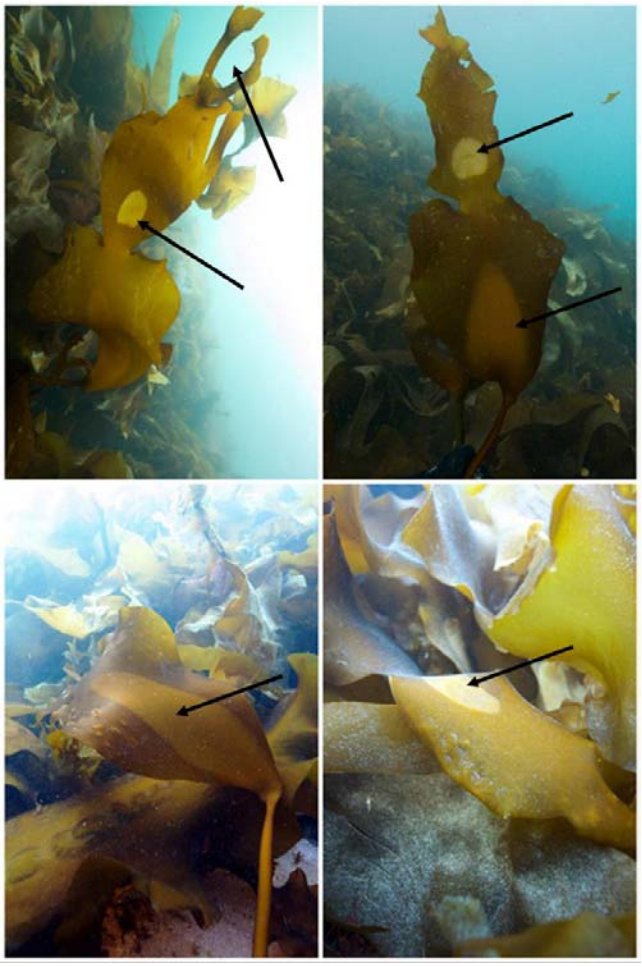

Figure 8: Areas with fertile tissue in 1, 2 and 3 year old parts of *L. solidungula*. Fertile tissue in 3 year old parts was usually released (top left picture). The *in situ* photos was taken during collection of specimens at Tommelen in August and September 2009 by SCUBA-diving. Arrows indicates fertile tissue in 1-3 year old lamina. Photo: Geir Johnsen.

3.3 POC and PON content in kelp tissue

*Intraspecific differences (*L. solidungula)

The wet:dry (w:w) ratio was higher in older tissue than younger tissue in *L. solidungula*. The mean ratio for 1, 2 and 3 year old tissue was 3.4 (CV 19 %), 6.1 (CV 16 %) and 7.0 (CV 12 %), respectively.

The N:C ratio (at:at) was highest in 3 year old tissue in *L. solidungula* (Table 4). The values for the different tissue was 0.03, 0.04 and 0.05 for 1, 2 and 3 year old tissue, respectively. The mean values for POC decreased with age, with a content of ~40 % carbon in 1 year old tissue, ~33% in 2 year old tissue and ~30 % for 3 year old tissue (Table 4). The content of PON showed an opposite trend than POC. The content of nitrogen for 1, 2 and 3 year old tissue was 1.2 %, 1.4 % and 1.8 % of dry weight (DW), respectively. The content of PON increased with a higher percentage share than the content of PON decreased. The specimen of *L. solidungula* collected in May 2010 had a higher N:C ratio, and lower amount of POC than the mean ratio for the

specimens collected in August 2009. The specimen collected in January 2010 had a ratio in between the samples from August and May.

Table 4: N- and C content in *L. solidungula*. Statistics summarizes the content of POC (µg pr DW) and PON (µg pr DW) together with the N:C ratio in different age collected at Tommelen in August 2009.

Age of tissue	POC (µg pr DW)				PON (µg pr DW)				N:C (at:at)
	Mean (n=5)	SD	CV %	% POC	Mean (n=5)	SD	CV %	% PON	
1 year	0.40	0.01	3	40	0.01	0.002	17	1.2	0.03
2 year	0.33	0.05	15	33	0.01	0.004	27	1.4	0.04
3 year	0.30	0.04	13	30	0.02	0.004	21	1.8	0.05

Interspecific differences (all 5 kelp species)

Compared with the other macroalgae species collected during August 2009, the meristem tissue of *L. solidungula* had the lowest N:C ratio (0.03, Table 5), with the highest content of POC (0.40 µg pr DW) and lowest content of PON (0.01 µg pr DW). *Sacchoriza dermatodea* had the highest N:C ratio (0.06) and also the highest content of N (0.02 µg pr DW). The N:C content of *L. digitata, S. latissima* and *A. esculenta* was identical (~0.04 µg pr DW).

Table 5: N- and C content in 5 species of kelp. Statistics summarizes the content of POC (µg pr DW), PON (µg pr DW) and N:C ratio (at:at) in meristem tissue (5 species) and the fertile tissue of *L. solidungula* collected during 2009 and 2010.

Macroalgae specie	POC (µg pr DW)	PON (µg pr DW)	N:C (at:at)
Meristem tissue			
L. solidungula (Aug 2009)	0.40	0.01	0.03
L. solidungula (Jan 2010)	0.33	0.02	0.06
L. solidungula (May 2010)	0.30	0.03	0.08
L. digitata (Aug 2009)	0.38	0.02	0.04
S. latissima (Aug 2009)	0.36	0.01	0.04
A. esculenta (Aug 2009)	0.39	0.02	0.04
S. dermatodea (Aug 2009)	0.36	0.02	0.06
formative tissue			
L. solidungula (May 2010)	0.24	0.02	0.06

3.4 Photosynthetic characteristics

3.4.1 Low vs high acclimated tissue of *L. solidungula*

Experiments I and II were conducted straight after collection of the kelp to compare the photoacclimation status between specimens of *L. solidungula* collected at Tommelen. The results provided an overview of the basic characteristics for non-stressed tissue and also insight into how the tissue reacts to high irradiances (>40 µmol photons m^{-2} s^{-1}). Intraspecific differences and differences due to age was also shown. The two experiments indicated biological variation between the same age of tissue of *L. solidungula*.

In experiment I, Φ_{PSII} for 1 year old tissue was close to the theoretical limit of 0.8 for all six specimens (Figure 9) (Ralph & Gademann, 2005). 3 year old tissue had a lower mean Φ_{PSII} than 2 year old tissue, except for specimen #13 which had a higher Φ_{PSII} for 3 year old tissue (0.38) than for 2 year old tissue (0.28). The same trend was seen in experiment II for Φ'_{PSII}, with higher values for 1 year old tissue than for older tissue, but with values ~50% of the values for Φ_{PSII}. There was less distinct differences between 2 year old and 3 year old tissue compared to 1 year old tissue when exposed to light. In addition to the displayed differences due to age, there was differences between the same age group in the different specimens. The CV% gives an indication for how large the range within where expected values of measurements for the average *L. solidungula* will be found, e.g. the range for Φ_{PSII} in 1, 2 and 3 year old tissue are ±7%, ±35% and ±21%, respectively. The range was higher for all year tissues for Φ'_{PSII} (Table 6). There was a larger difference between the values of 2 year old tissue than the two other age groups.

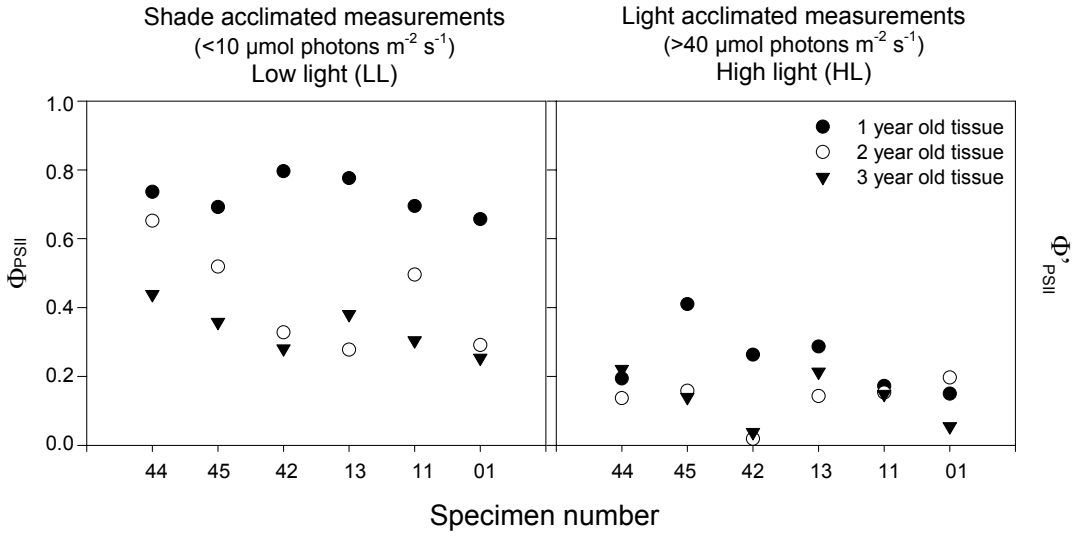

Figure 9: Measurements of Φ_{PSII} and Φ'_{PSII} in six specimens of *L. solidungula* collected at Tommelen in August 2009 (experiment I and II). PAM measurements were conducted on the shore, right after collection of the macroalgae and indicates biological differences due to age in the species and a lowering of yield when exposed to higher irradiances. E < 10 µmol photons $m^{-2}s^{-1}$ for LL acclimated tissue and E > 40 µmol photons $m^{-2}s^{-1}$ for HL acclimated tissue.

Relative ETR vs E_{PAR} gave different values for LL and HL acclimated tissue (Figure 10). In LL acclimated tissue, mean value for the different year tissues was rather low with 1.9, 1.9 and 1.3 for 1, 2 and 3 year old tissue, respectively, which gave high CV% for the different age groups (Figure 10, Table 6). In HL acclimated tissue the mean values of rETR for the different year tissue increased with a factor of >10 to 24.4, 14.2 and 12.1 for 1, 2 and 3 year old tissue, respectively (Figure 10, Table 6). There was a larger difference between the values of 2 year old tissue than the two other age groups.

Table 6: Values of Φ_{PSII}, Φ_{PSII} and rETR for *L. solidungula*. Statistics for calculated Φ_{PSII}, Φ_{PSII} and rETR values from experiment I and II.

	1 year old tissue			2 year old tissue			3 year old tissue		
	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%
Shade acclimated (LL)									
Φ_{PSII}	0.73	0.05	7	0.43	0.15	35	0.34	0.07	21
rETR	1.9	2.3	127	1.9	1.9	98	1.3	1.0	76
Light acclimated (HL)									
Φ'_{PSII}	0.25	0.10	39	0.13	0.06	45	0.14	0.08	57
rETR	24.4	5.1	21	14.2	9.6	68	12.1	6.3	52

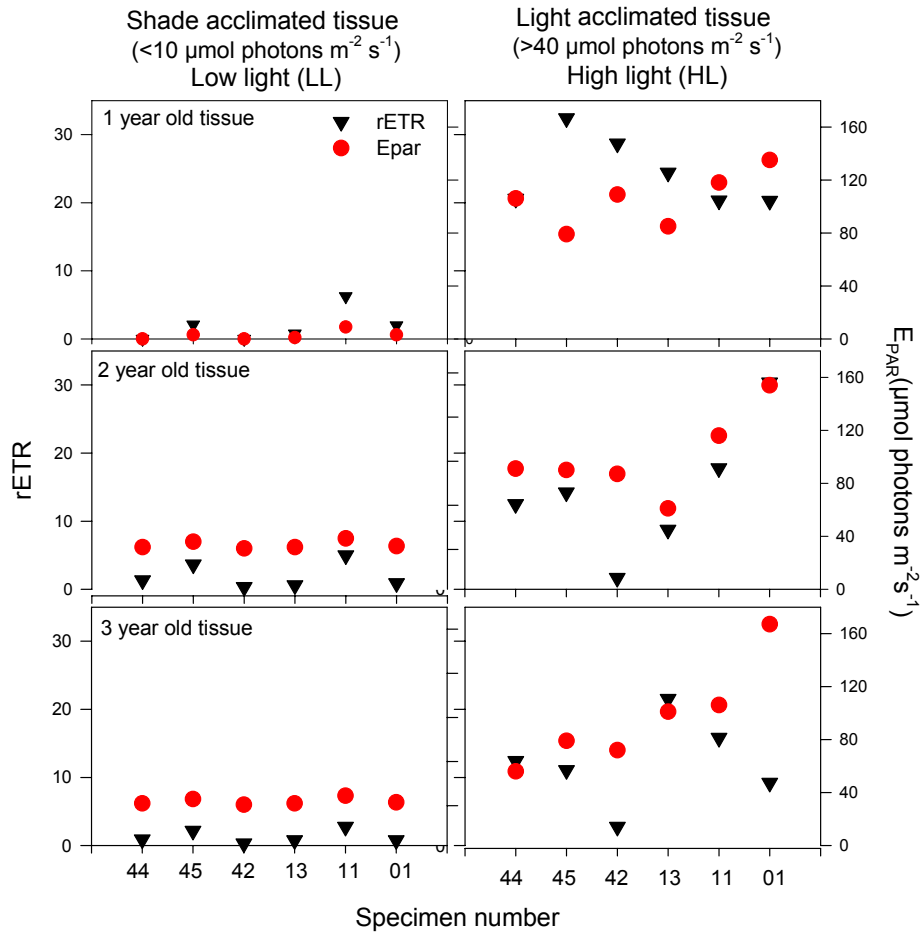

Figure 10: Calculated values of rETR for LL and HL acclimated tissue of *L. solidungula* collected at Tommelen in August 2009 and the rETRs correlation to E_{PAR} (experiment I and II) indicating higher fluctuation between the specimens when exposed to higher irradiances. The rETR value decreased slightly from 1 to 2 year old tissue, while 2 and 3 year old tissue was similar. In the cases where the value of rETR is not visible, the mark is over written by the value of E_{PAR} (applies to both graphs of 1 year old tissue).

3.4.2 F'_o and F'_m vs time

The minimum (F_o/F'_o) and maximum (F_m/F'_m) level of Φ_{PSII}/Φ'_{PSII} relates to the opening and closing of the RC_{PSII} and gives information of the photosynthetic efficiency of the macroalgae. F'_o and F'_m (experiment III) vs time displayed diurnal alterations which was closely related to E_{PAR} (max value 190 µmol photons m^{-2} s^{-1}) for all tissues measured (Figure 11, Figure 14 - Appendix).

The persistent overcast during this experiment resulted in approximately the same values of E_{PAR} throughout the days of measurements, with a maximum of 190 µmol photons m^{-2} s^{-1} between 00:00-18:00; September 7, 2009. During night time (00:00-05:00) E_{PAR} was plotted as zero in the figures even though some light was presence (light sensor not sensitive enough to detect difference in E < 1 µmol photons m^{-2} s^{-1}).

Intraspecific differences (L. solidungula)

There was small changes in F'_o throughout the 24 hour period of measurements for the three year tissue investigated. F'_m values were highest during evening and night-time for all three age groups. 1 year old tissue of *L. solidungula* had a lower F'_v value than 2 year old tissue. The fluorescence signal in 2 year old tissue was highest, while the signal was lowest in 1 year old tissue. There was some variation in E_{PAR} during morning and evening of the three days of measurements on the different age tissue of *L. solidungula.*

Interspecific differences (all 5 species)

There was small changes in F'_o throughout the 24 hour period of measurements for all the macroalgae species investigated. F'_m values were highest during evening and night-time for all species. *Alaria esculenta* obtained highest values (22:00-23:00). F'_v values was high during nighttime (F'_o and F'_m furthest apart) and low during daytime, indicating diurnal changes in the fraction of open and closed RC_{PSII}. Compared to *L. digitata*, *S. latissima, A. esculenta* and *S. dermatodea*, F'_o and F'_m of *L. solidungula* was lower for all year tissues.

3.4.3 Φ'_{PSII} vs time

Φ_{PSII} is the fraction of light that is absorbed and utilized by RC_{PSII} and re-emitted as fluorescence. Measurements of Φ'_{PSII} (experiment III) showed a close correlation to E_{PAR} (Figure 11, Figure 14 - Appendix).

Intraspecific differences (L. solidungula)

Max Φ'_{PSII} in *L. solidungula* decreased with age of tissue and was 0.76, 0.66 and 0.53 for 1, 2 and 3 year old tissue, respectively (Figure 11). Most max levels of Φ'_{PSII} in *L. solidungula* was reached during late evening (22:00-23:00), with an exception of 2 year old tissue of *L. solidungula*, where max level was attended in the morning (07:00). This might be due to an error measurement. Regarding the period of higher actinic irradiances during the evening, 2 and 3 year old tissues of *L. solidungula* reached almost the same level as prior to exposure of high irradiances.

Interspecific differences (all 5 species)

All max values of Φ'_{PSII} for *S. latissima*, *A. esculenta*, *L. digitata* and *S. dermatodea* was close to the theoretical max level of Φ'_{PSII} in macroalgae (0.8, Figure 14 - Appendix) and higher than max Φ'_{PSII} of meristem tissue of *L. solidungula*. Regarding the period of higher actinic irradiances during the evening for each species, meristem tissue of *S. dermatodea* reached almost the same level as prior to exposure of high irradiances. Φ'_{PSII} lower than 0.1 was only reached in *A. esculenta* (~10:30-11:15, September 7, 2009).

3.4.4 rETR vs time

The rETR indicates the efficiency of PSII in the algae and usually follows E_{PAR}. The rETR of all tissues measured followed the curve of ambient E_{PAR} throughout the measuring period, with lowest values during night and reaching a plateau during morning and midday (06:00-12:00, Figure 11, Figure 14 - Appendix).

Intraspecific differences (L. solidungula)

The ambient light was strong enough to saturate photosynthesis for 3 year old tissue in *L. solidungula,* but not for 1 and 2 year old tissue. The additional use of actinic light, saturated photosynthesis in the two youngest tissues of the species. Maximum rETR was highest in 1 year old tissue in *L. solidungula,* and the values was 42, 40 and 14 for 1, 2 and 3 year old tissue, respectively.

Interspecific differences (all 5 species)

The rise in rETR during morning was rapid in all macroalgae species investigated, in contrast to a more steady decline in the evening. For *A. esculenta,* maximum value (15) was the lowest value for meristem tissue of all species investigated. The maximum value for the other species was mainly below 40.

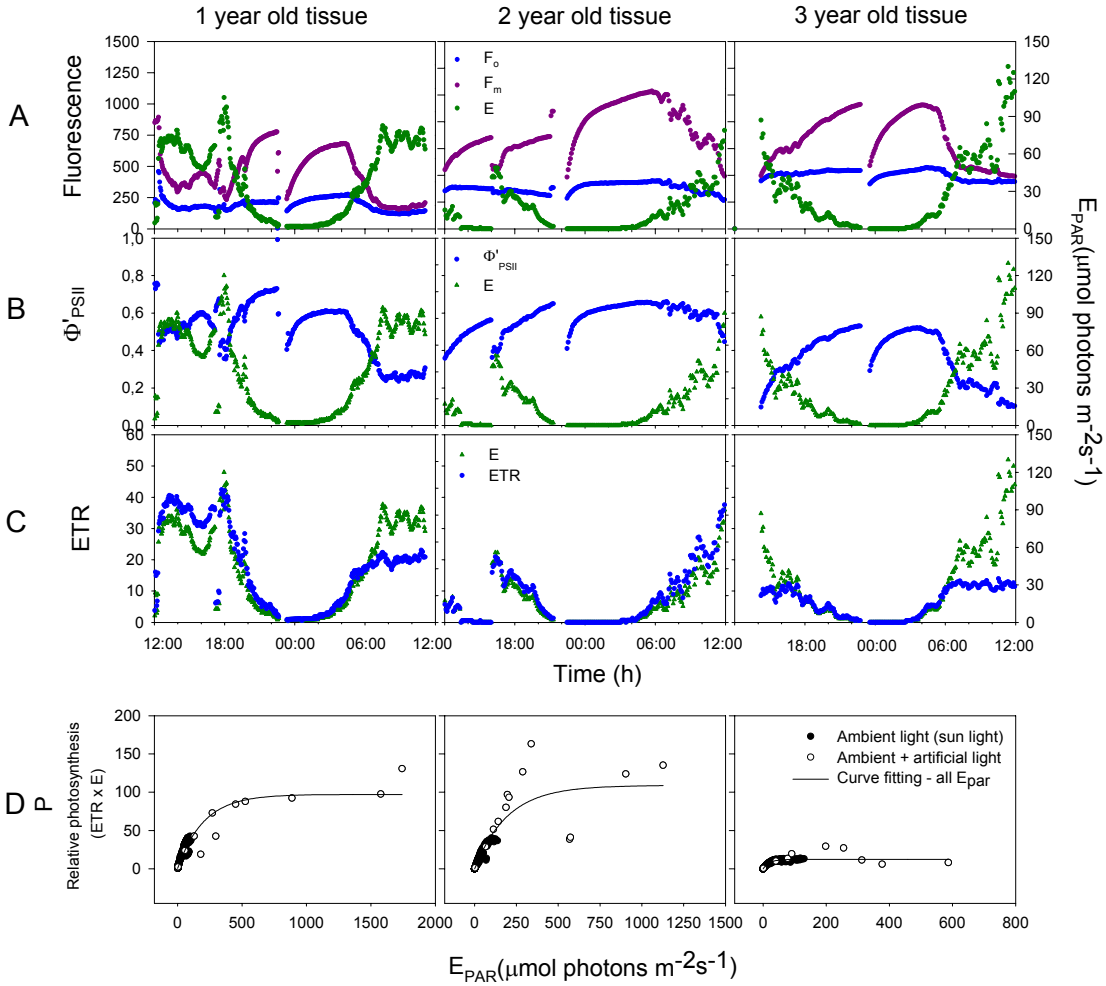

Figure 11: Time-series measurements of A) fluorescence (F'_o, F'_m), B) Φ'_{PSII}, C) ETR (Φ'_{PSII} x E_{PAR}) and D) P vs E curves for *L. solidungula* (1, 2 and 3 year old tissue) collected at Tommelen in August 2009. The graphs displays a normal variation of opening and closing of RC_{PSII}, indicating a healthy macoalgae. The ETR x E displays that 2 year old tissue of *L. solidungula* tolerate higher irradiances than 1 and 3 year old tissue.

3.4.5 P vs E curves

The P vs E curves demonstrates changes in the photosynthetic parameters as a function of irradiance. These photosynthetic parameters give an insight to how well the algae utilizes the available light and how high irradiances they tolerate (photoacclimation).

Intraspecific differences (L. solidungula)

The calculated P_{max} values in the different algal tissue of *L. solidungula* was 97, 109 and 12 for 1, 2 and 3 year old tissue, respectively (Table 7), while α did not show any significant variations due to age. E_k was highest in 1 year old tissue and the values was 202, 196 and 20 µmol photons $m^{-2}s^{-1}$ in 1, 2 and 3 year old tissue, respectively (Figure 11, Table 7).

The specimen collected in Ny Ålesund in January 2010 had a similar α (0.6) as the specimens collected at Tommelen in August 2009 (all year tissues), while the values

of P_{max} (48) and E_k (81 µmol photons $m^{-2}s^{-1}$) was in between the values for 2 and 3 year old tissue from Tommelen. When conducting the measurements it was observed that the tissue reacted slowly to the actinic light (Table 7, Figure 12).

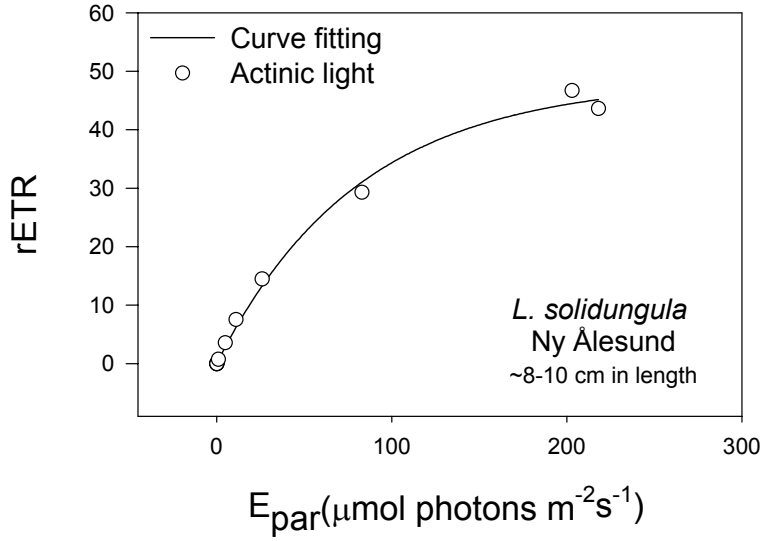

Figure 12: P vs E curve for *L. solidungula* collected in the polar night at Ny Ålesund in January 2010. The specimen was ~8-10 cm in length. Age was estimated to two months, and we assume this kelp specimen have not experienced actinic light prior to our experiment. The tissue responded rather slowly to exposure of actinic light, and gave a small α.

The α for 1 year old tissue of *L. solidungula* collected in Smeerenburgfjorden in May 2010 was the same as for *L. solidungula* collected at Tommelen in 2009, while α for 2 year old tissue was half this value. Both P_{max} and E_k for 1 and 2 year old tissue was lower than 2 year old tissue collected at Tommelen in August 2009. Regarding the parameters for the fertile tissue measured in the specimen from Smeerenburgfjorden in May 2010, α was only 0.03, P_{max} equaled 10 and E_k equaled 328. The curve was displayed as a straight line (Figure 13).

Interspecific differences (all 5 species)

Regarding interspecific differences in the meristem tissue of all the macroalgae species investigated, α was generally higher (0.6-1.1) than in meristem tissue of *L. solidungula*, while both P_{max} (12-65) and E_k (10-113 µmol photons $m^{-2}s^{-1}$) values was lower. *Alaria esculenta* had the highest α (1.1) and the lowest E_k value (10 µmol photons $m^{-2}s^{-1}$) of all the species investigated.

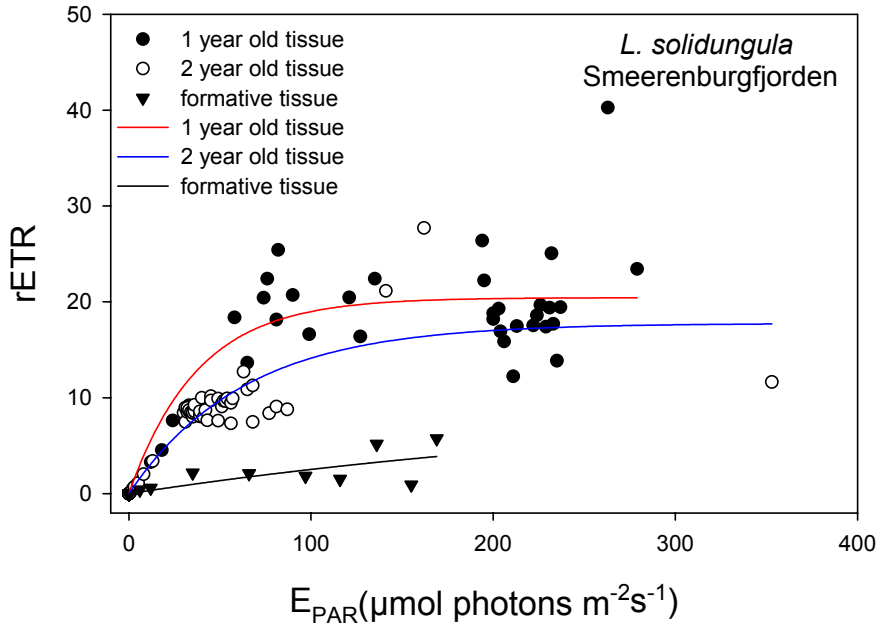

Figure 13: P vs E curves of 1 and 2 year old tissue together with fertile tissue of the specimen of *L. solidungula* collected in Smeerenburgfjorden in May 2010. There was clear differences in photosynthetic parameters in the different tissues of this specimen, both in α, P_{max} and E_k (Table 7). Fertile tissue appear as a straight line.

Table 7: Photosynthetic parameters for all macroalgae species investigated during 2009 and 2010 (24 h time-series with ~250 measurements for each tissue). The values of E used in the calculations was provided by both ambient and artificial light. P_{max} and E_k values of *L. solidungula* was highest in August. All other species investigated had lower values for P_{max} and E_k and higher values for α than meristem tissue of *L. solidungula.*

Specie/ tissue type	P_{max}	α	E_k*
L. solidungula, Tommelen, 1 year tissue	97	0.5	202
L. solidungula, Tommelen, 2 year tissue	109	0.6	196
L. solidungula, Tommelen, 3 year tissue	12	0.6	20
L. solidungula, Ny Ålesund	48	0.6	81
L. solidungula, Smeerenburgfjorden, 1 year tissue	20	0.6	38
L. solidungula, Smeerenburgfjorden, 2 year tissue	18	0.3	63
L. solidungula, Smeerenburgfjorden, fertile tissue	10	0.03	328
L. digitata	65	0.6	113
S. dermatodea	51	0.8	61
S. latissima	38	1.0	38
A. esculenta	12	1.1	10

*Unit for E_k are µmol photons $m^{-2}s^{-1}$.

3.5 Pigments

Intraspecific differences (L. solidungula)

The major light harvesting pigments detected in *L. solidungula* were Chl *a*, Chl c_1, Chl c_2 and Fucoxanthin (Fuco, Table 8). In addition, Violaxanthin (Viola), Zeaxanthin (Zea, four specimens, all year tissues), Neoxanthin (Neo, one specimen, 1 year old tissue, trace amount) and Beta-beta carotene (one specimen, 1 year old tissue, trace amount) was detected. The mean value of pigment:Chl a ratio in *L. solidungula* showed no variation in Chl c_1, Chl c_2 and Viola due to age. The amount of Chl c_2 was rather high (~20 % of chl a) for all year tissues. The Fuco:Chl a ratio was higher in 1 year old tissue than 3 year old tissue (Table 6). One-way ANOVA showed no significant difference in mean pigments ratios due to age of *L. solidungula* but the test showed a significant effect of specimen number of Chl c ($c_1 + c_2$, $P = 0.008$), Fuco ($P = 0.046$) and Viola ($P = 0.0002$). No significant effect due to specimen number (all age groups) concerning Pheophytin a (Pheo, degraded Chl a) was detected ($P = 0.171$).

In the specimen of *L. solidungula* collected in Smeerenburgfjorden in May 2010, only Chl a, Chl c_2, Fuco and Pheo a was detected (Table 9). The ratio of all pigments was rather high, specially Chl c_2 (0.73) and Pheo a (1.22). The ratios increased in 2 year old tissue (Appendix). The pigment composition in fertile tissue was the same as in 1 and 2 year old tissue, and the pigment:Chl a ratios higher than 2 year old tissue (Appendix).

Degraded pigments like Pheo a (from Chl *a*) was also isolated. The ratio increased with age in *L. solidungula,* in the order 0.06, 0.23 and 0.22 in 1, 2 and 3 year old tissue (Table 8), respectively. The amount of Pheo a detected in *L. solidungula* from Smeerenburgfjorden was high (1.22) and reflects high values of both Chl c_2 and Fuco compared to the specimens collected in August 2009 and January 2010.

Table 8: Mean values of pigment:Chl a (w:w) ratio for the different year tissue of *L. solidungula* collected at Tommelen in August 2009. "-" indicates no pigment detected. Trace amount of Neo and Beta-beta carotene is not listed in the table.

	Chl c_1	Chl c_2	Fuco (Fucoderivates)	Viola	Zea	Pheo a	Chl b	Lut
1 year tissue	0.04	0.20	0.77 (0.06)	0.04	0.01	0.06	0.01	-
2 year tissue	0.04	0.22	0.72 (0.07)	0.04	0.02	0.23	0.02	0.01
3 year tissue	0.04	0.21	0.66 (0.06)	0.04	-	0.22	0.11	0.03

Interspecific differences (all 5 species)

Laminaria solidungula had mainly the same pigment composition (Table 9) as the other kelp species investigated, except for Zea, Neo and Beta-beta Carotene which was exclusively detected in *L. solidungula*. The ratio of Chl c_2:Chl a and Fuco:chl a was higher in all species except *S. dermatodea* compared to meristem tissue of *L. solidungula*.

Regarding degraded pigments like Pheo a, *L. solidungula* had the lowest Pheo a:Chl a ratio compared to the other species investigated (Table 9), except for *S. dermatodea*,

where Pheo a was not present. The Pheo a:Chl a ratio in *A. esculenta* was rather high (0.36).

Table 9: Pigment:Chl a ratio for the different macroalgae species collected at Svalbard in 2009 and 2010. All values are from meristem tissue. "-" indicates no pigment detected.

	Chl c_1	Chl c_2	Fuco (Fucoderivates)	Viola	Zea	Phe a	Chl b	Lut
L. solidungula (Aug)	0.04	0.20	0.77 (0.06)	0.04	0.01	0.06	0.01	-
L. solidungula (Jan)	0.15	0.15	1.76	-	-	0.70	-	-
L. solidungula (May)	-	0.73	0.91	-	-	1.22	-	-
L. digitata	0.11	0.30	0.78 (0.02)	0.02	-	0.17	-	-
S. dermatodea	-	0.15	0.40	0.03	-	-	-	-
S. latissima	-	0.33	0.88 (0.02)	0.02	-	0.07	-	-
A. esculenta	-	0.37	1.06 (0.03)	-	-	0.36	-	-

3.5.1 *L. solidungula* from the polar night

The specimen of *L. solidungula* collected in Ny Ålesund in January 2010, contained all the main pigments as the specimens collected in August 2009 and May 2010, except Viola and Chl c_1 (see section [3.5 Pigment analysis using high performance liquid chromatography (HPLC)]). The ratios of Fuco:Chl a (1.76), Chl c_2:chl a (0.73) and Pheo a:chl a (1.22) was high compared to the specimens collected at Tommelen in August 2009.

3.5.2 Epi-growth on *L. solidungula*

Trace amounts of Chl b were found in all five specimens of *L. solidungula* collected at Tommelen, and trace amounts of Lutein (Lut) in three of the specimens, but never in 1 year old tissue (Table 8). The amount of both pigments increased due to age, Lut only from 2 to 3 year old tissue. The high ratio of Pheo a in some specimens might also be due to epi-growth.

4 Discussion

The results from this study will be discussed with focus on a) habitat, morphology and ecology in *L. solidungula* and b) Ecophysiology: photosynthesis and pigment composition, herein differences due to age (intraspecific) and between the 5 species collected (interspecific).

4.1 Habitat, morphology and ecology in *L. solidungula*

4.1.1 Habitat

Laminaria solidungula thrives on hard bottom substrate between other kelp species totally dominated in numbers/biomass by *S. latissima, A. esculenta og L.digitata*.

Zonation of perennial macroalgae in the Arctic are often absent due to ice scraping in shallow areas (0-3 m depth, own observations) (AB-323, 2000-2010, Gulliksen et al., 2009), from 3-30 m depth perennial algae such as kelp species struggle for light and substrate (AB-323, 2000-2010) and deeper than 30 m light limitation is the major environmental variable limiting kelp growth (AB-323, 2000-2010). A short season for establishment of new specimens might also be an explanation. Some literature also present the Arctic sub-littoral zone characterized by defined belts of organisms (Gomez et al., 2009, Lüning et al., 1990). Few studies on zonation in Svalbard waters have been published and none of these from Hinlopenstredet nor Rossøya (Wiencke et al., 2004, Florczyk and Latala, 1989)(and references therein). Observations when SCUBA-diving at Tommelen and Rossøya, gave an indication of a belt of kelp with different species, where the five species investigated in this study was collected. Some of the species, e.g. *L. digitata* was found both in shallow areas (3-5 m depth) and further down (10-17 m depth), and was seen as two different morphotypes. The morphotype growing in shallowest areas, had short and truncated lamina, while the morphotype growing further down, had longer lamina parts which were not truncated. *Alaria esculenta* was the species observed deepest of the five specimen investigated. *Laminaria solidungula* was always located in between (often covered by) the other kelp species. The only way to find *L. solidungula* was to dive in between the other kelp species and look for the characteristic morphological feature like areas of fertile tissue, shape of lamina and haptera described in Figure 15 and Figure 16.

These observations have been based on SCUBA-diving and ROV (Jan 2010) with corresponding analysis of photo and video. The usefulness of an ROV is usable, particularly when environmental conditions sets limits for human divers like polar night, low temperature and ice cover. The ROV technologies makes science during winter possible. In addition, the VideoRay Pro III S ROV is small, easy to handle and low cost (~$10,000). Future advances in underwater vehicle navigation systems will

further improve the value of scientific data collected with these platforms and making re-visits possible.

4.1.2 Morphology

It was not possible to distinguish specimens of *L. solidungula* from one location to another based on morphological characteristics. One explanation for this might be that the species is sheltered among the other kelp species on the location of growth. The kelp forest shelter will thou be the basis for differences in growth conditions and thus morphology in *L. solidungula.*

One-way ANOVA analysis only reveled a significant difference due to age in lamina, indicating that the growth of stipes is not related to the growth of lamina. One possible explanation might be that the macroalgae species funnels all energy into growth of stipes in the first part of the first growth season, diminish stipes growth during later growth seasons (year 2 and 3) and reallocate energy to sustain growth of lamina. This might be to ensure that they can reach high enough in between the other macroalgae species, and be able to harvest available light when the growth of lamina starts. In earlier studies, the focus has been on growth of lamina in *L. solidungula* (Chapman and Lindley, 1980, Dunton, 1990), and not on growth of stipes. The Multiple Range Test for lamina showed a significant difference between the mean of all age groups, indicating that the lamina, in contrast to the stipes, have a distinct growth during each growth season.

Based on analyses of photos of *L. solidungula* collected at both Tommelen and Rossøya in August 2009, I will suggest a schematic drawing on the morphological appearance of *L. solidungula* (Figure 15). In comparison to the sketches commonly known of *L. solidungula* (see www #3) (Dunton and Schell, 1986) this one can be regarded as representative for the population on Svalbard.

4.1.3 Fertile tissue

Intact fertile tissue region in *L. solidungula* was visible in both 1 and 2 year old lamina. In 3 year old lamina the area of fertile tissue was released, except in two specimens (#44 and #45). Most possible explanation for the visible fertile tissue regions in all year tissues of *L. solidungula* may be that the zoospores need longer maturation time due to lower temperatures, i.e. 3 years in contrast to kelp species in temperate regions which only need one year to develop mature zoospores, and therefore starts the maturation at an early life stage (Bartsch et al., 2008). The fertile tissue seems to be released during July- September.

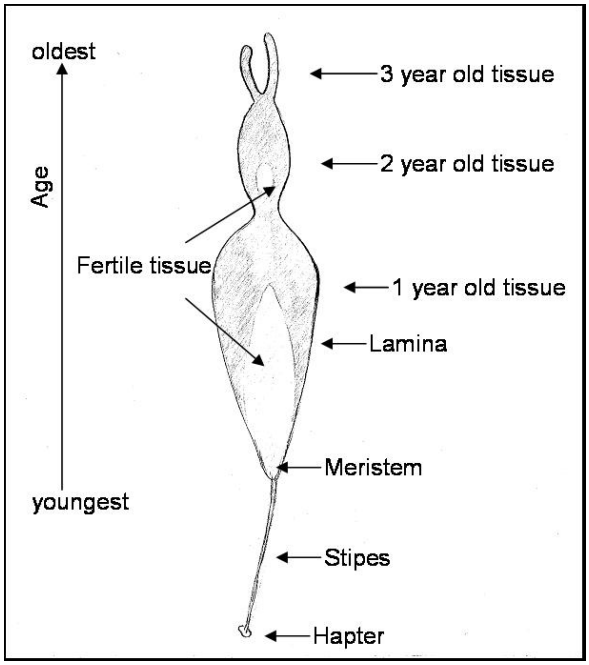

Figure 15: Schematic drawing of the morphology of *L. solidungula* in Svalbard waters based on Figure 5-7, n = 61 specimens. Fertile tissue in 1 year old tissue is immature (lance formed), in 2 year old tissue intermediate mature (spade formed) and in 3 year old tissue mature (oval formed) and released from the algae.

4.1.4 Epifauna growth

Epiflora and fauna was only visible on one specimen (# 46, 2 and 3 year old tissue, specimen not analyzed further) of *L. solidungula* (of total 63) and might indicate that *L. solidungula* secrete bio-active repellent compounds that reduces epifaunal growth or the absent of herbivore gracers in the Arctic that prefer *L. solidungula* as a host. Dunton et al (1982) reported that the species *Amicula* was feeding on the lamina and stipes of *L. solidungula* in the Alaskan Beaufort Sea. Another explanation for the small amount of epifauna, is the time of sampling. Carlsen et al (2007) found that epifauna on macroalgae was strongly coupled with season and water column processes, with higher abundance of epifauna on macroalgae during spring phytoplankton bloom than during autumn, when the salinity was lower due to freshwater run-off and sedimentation and low presence of microfauna. This makes august a less suitable time to investigate epifauna on macroalgae species. Lippert et al (2001) concluded in their study that abiotic factors such as water motion and sedimentation together with biotic factors like structural characteristics (e.g. thallus), lifetime, growth rate and interaction between animals and algae influences the attractiveness of the macroalge as a host.

The results from the pigment extraction detected both Chl b and Lut in almost all specimens which indicates the presence of microscopic epigrowth of Chl b-containing microalgae, i.e. chlorophytes, euglenophytes and prasinophytes (Johnsen and Sakshaug, 2007) also seen in kelp from the Norwegian coast (Forbord, 2004), or that

the algae was in bad physiological condition. Lut and Chl b were never detected in 1 year old tissue. The trace amount of Neo detected in one specimen of *L. solidungula* (3 year old tissue) and the elevated amount of Pheo a in several of the specimens (2 and 3 year old tissue) might also be due to epiflora growth.

4.2 Heterotrophic growth in *L. solidungula*

The collection of a newly formed specimen of *L. solidungula* (lamina ~8-10 cm long) from under ice in Ny Ålesund (January 2010), indicates that the species is able to grow heterotrophically. The ability to translocate stored energy was early discovered in the *Lamiariales* (Schmitz and Lobban, 1976) and was proposed as applicable for *L. solidungula* by Dunton et al (1982). Based on size, morphology and the fact that the growth season for *L. solidungula* extend from November until late April (Dunton et al., 1982), we estimate the specimen from Ny Ålesund to be ~two months old and due to polar night at the time of sampling, only exposed to actinic light during our measurements. This do fit with the displayed P vs E curve and the slow reaction to actinic irradiances compared to the specimens collected at Tommelen (August 2009), which might indicate immature tissue (Figure 12). Another age proposal, where the results from the measured length of stipes and lamina are interpretated, is that the specimen was in its second growth season - the first season allocated to growth of stipes, and the second season for growth of lamina. Still, lamina will be immature and react slowly to actinic irradiances and fit the P vs E curves (Figure 12) .

Since this was the only specimen collected during the polar night in this study, interpretation of the results is difficult. Differences between this specimen and the specimens collected at Tommelen might just as well be a result of spatial, temporal or seasonal differences. More work and several specimens must be investigated before a conclusion can be drawn. Questions in mind for further investigation might be if the low α reveled by the P vs E curve can be due to non-active D1 proteins in PSII (Stitt and Hurry, 2002, Allen and Ort, 2001) or immature chloroplasts (Valle, 2005), if the pigment composition with high Fuco and Chl c_2:Chla a indicate that this is a specimen in it's first growth season, or if the high ratio are due to a high amount of degraded Chl a pigments.

Typically, when diving along the Norwegian coast during wintertime, the meristem of kelp is characteristically and easily seen as bright yellow-orange-brown colored tissue (in contrast to dark brown surrounding tissue of stipes and older lamina) due to high Fuco:Chl a ratio (personal observations)(Forbord, 2004). S. Forbord (2004) found that new specimens of *L. saccharina* had up to 17 times the concentration of pigments than new tissue in old specimens, which could be a reason for the results from the pigment analysis for the specimen.

4.3 Time-series measurements

Time-series of photosynthetic characteristics (F_o, F_m, Φ'_{PSII} and rETR) can be used to elucidate biological differences in a given kelp species as a function of age, and also for one species in comparison to other species. When interpreting the results, a generalization about photosynthetic characteristics needs to be done carefully since a tissue with different age and organelle distribution (such as chloroplasts) will react differently to key-environmental variables such as irradiance (Valle, 2005). The purpose of experiment I and II was to illustrate these differences. Experiment I and II was then followed by time-series measurements following one specimen with diurnal time-series measurements for 1, 2 and 3 year old tissue (experiment III). These measurements was in addition compared with other kelp species (measurements on meristem tissue only).

Experiment I & II

Measurements of photosynthetic performance (experiment I and II) was carried out with several specimens (n=6) of *L. solidungula* (Figure 9 and Figure 10) to illustrate differences between specimens with 1 (meristem), 2 and 3 year old tissue.

The Φ_{PSII} and Φ'_{PSII} showed intra specific differences between the specimens of *L. solidungula,* and demonstrated that 2 year old tissue had lower quantum yield in ambient light than 3 year old tissue and indicated that 3 year old tissue was still photosynthetic active.

The differences in quantum yield are most possible due to biological variation between the specimens in thickness of tissue, number and development stage of chloroplasts and difficulty of measuring accurate E_{PAR} (Valle, 2005). The rETR for HL acclimated tissue displayed a greater difference of output signal among the specimens than for LL acclimated cells. The range of variation (CV%) from these figures (Figure 10) can be interpretated in the photosynthetic measurements and give an indication of the variation we need to take into consideration when interpretating the results from the time-series.

4.4 Intraspecific differences in *L. solidungula*

Cell chemistry (POC and PON)

The N:C ratio can give an indication of the yearly variation in nutrient status of macroalgal tissue, and therefore also the physiological status of the macroalgae (Hilstad, 2005). Inorganic nitrogen and phosphate have frequently been suggested to limit macroalgal growth in coastal areas (Hanisak, 1978) and *L. solidungula* is one of many kelp species that have evolved physiological responses, such as storing nutrients-rich compounds when nutrient supply is high during winter darkness, to avoid nutrient competition with other phototrophic organisms (Bartsch et al., 2008).

Earlier studies indicates that the nutrient content (N:C ratio) of macroalgae vary between a ratio of 0.025-0.20, were the critical values indicating N-depletion is between 0.06-0.10 (Niell, 1976, Hanisak, 1983, Hilstad, 2005) and that the ratio can be as high as 0.125 (C:N = 8:1) during springtime and as low as 0.02 (C:N = 50:1) during summer (Rinde et al., 1998).

The higher wet:dry ratio in 3 year old tissue may indicate that older tissue has a higher content of water. Older tissue are usually ticker (if not worn by e.g. current and other harsh conditions), more hollow and have the possibility of storing more water and thus more nutrient.

The nutrient content in 3 year old tissue was higher than in 1 year old tissue of *L. solidungula* (Table 4), which was not expected prior to the experiment since we assumed that old tissue was worn out and had started to decay. The values of POC gave a lower content in 3 year old tissue than in 1 year old tissue, while the content of PON was slightly higher.

Considering seasonal variation, this study do not have the basis to make any conclusions, but the results from the specimens collected in January and May indicates that the content of POC was lower in May than in August while the content of PON was higher in May than in August. This is in agreement with results of Dunton & Jodwalis (1988) for *L. solidungula*, and Hilstad (2005) for *S. latissima,* among others. This is also in agreement with the findings of Dunton and Schell (1986) whom discovered that ~25 % of the carbon in *L. solidungula* was depleted during the dark season (from November to late April) due to growth. Regarding the content of PON, the amount of N (NO_3^- and NO_2^-) in arctic seawater raise during winter (Chapman and Lindley, 1980) and the macroalgae will thus have a higher uptake during winter months and hence the content will increase from autumn towards spring. The results of N:C ratio in this study indicates N-depletion for all age groups of *L. solidungula* in August, with all values in the critical low zone (0.03-0.095).

The specimen from Ny Ålesund did not show a high N:C value as expected from these earlier studies which might be due to the age of the specimens (first growth season) and no performance of autotrophic activity yet.

Time-series of PAM data

The time-series of fluorescence measurements in this study indicated that all age tissues of *L. solidungula* was in a healthy condition. The diurnal variation in fluorescence signal indicated the opening of RC_{PSII} in low light and the closing of RC_{PSII} in high light for all age groups (Figure 11). The Φ'_{PSII} for all age groups followed the expected trend of highest values when E_{PAR} was low and opposite. When displaying the rETR, the diurnal progress followed the curve of E_{PAR} indicating a good utilization of ambient light.

Photochemical quenching of fluorescence (PQ)

Photochemical quenching is the process when excitation energy is used for photochemical reactions and thereby lowering of Φ_{PSII}. The mechanism is related to the fraction of open and closed reaction centers in PSII. The measurements of F_o/F'_o and F_m/ F'_m can be used to study the Φ'_{PSII}.

Max Φ'_{PSII} (1 year 0.76 vs 3 year 0.53) in *L. solidungula* was lower in older tissue, relative to younger tissue, and may reflect that the effectiveness of photon-utilization by RC_{PSII} was decreasing with age. The time-series also indicates differences in different year tissue regarding recovery from exposure to high irradiances ($E_{PAR} > 200$ µmol photons $m^{-2}s^{-1}$). Only 2 and 3 year old tissue was reaching the same values of Φ_{PSII} when re-introduced to darkness after a period of high irradiances.

Regarding the specimen from Ny Ålesund in the polar night (January 2010), one explanation for the displayed curve can be that chloroplasts was not fully developed during the first growth season, and do not respond as 2 and 3 year old tissue do. The rETR gives an indication on how fast the PSII is absorbing and utilizing light with each E_{PAR}. When the rETR curve follows the curve of E_{PAR}, it indicates that the tissue utilizes available light effectively.

Non-photochemical quenching of fluorescence (NPQ)

Non-photochemical quenching includes all the mechanisms that lower the fluorescence emission (k_F) and increases the non-radiative dissipation (k_N) except for photochemistry. The three main such mechanisms are the energy-dependent fluorescence quenching (Brunet et al., 2011), state II-I transition quenching (qT) and photoinhibitory quenching (Krause and Weis, 1991)

From the results of this study, it is clear that qE is the major NPQ mechanism (Figure 11). High E_{PAR} leads to more electrons transported through RC_{PSII} and more H^+ transported into the thylakoid lumen and thus, a decrease of the lumen pH to values of 4.5-6.5. This is a negative feedback response which lowers the fluorescence emission from the algal tissue (Brunet et al., 2011). The mechanism of qT does not occur in the phyla Phaeophyta but mainly in rhodo- and chlorophytes (Brunet et al., 2011) and can therefore be disregarded. Regarding qI, the amount of photoprotective pigments (PPC;

Viola and Zea) detected in both *L. solidungula* (4-6 % of Chl a) are too low to be of any significance (Table 8). Some of the Zea detected in *L. solidungula* might have been de-epoxidated to Viola due to handling when packing the samples, but the amount would still be considered to small to have any impact. In addition, the light was not high enough at any time during the experiments that it would be regarded as damaging to D1 proteins of PSII (Allen and Ort, 2001, Stitt and Hurry, 2002).

Photosynthetic parameters

The photoacclimational state of a "low light adapted" polar (Antarctic and Arctic) kelp are characterized by LL acclimation properties, i.e. high α, low P_{max} and low E_k (Gomez et al., 2009). A change in E_k is affected by a rapid change in α or P_{max} (time frame from seconds to days) or both (Brunet et al., 2011). While P_{max} is the cause for variation in E_k for the different year tissues of *L. solidungula* (steady α) in this study, both α and P_{max} causes the difference in E_k values for the four other kelp species investigated (Table 7)(Behrenfeld et al., 2004, AB-323, 2000-2010).

Regarding the photosynthetic parameters due to age, 1 and 2 year old tissue was rather similar concerning α and E_k, but with a slightly higher P_{max} value for 2 year old tissue. The tissue utilizes lower irradiances but do also tolerate high irradiances up to ~1700 µmol photons $m^{-2}s^{-1}$. The 3 year old tissue, on the other hand, utilizes low irradiances well, but was rapidly saturated by photons (E_k = 20 µmol photons $m^{-2}s^{-1}$), typically for moderate LL acclimated tissue (Gomez et al., 2009). The specimens of *L. solidungula* collected in August, was collected at the brightest time of year. It might be possible that they are able to acclimate to the high irradiances during summer, and therefore gave a high value for E_k in August. The variation in E_k during August for the hole specimen was 20-200 µmol photons $m^{-2}s^{-1}$.

The specimen of *L. solidungula* from Smeerenburgfjorden (May 2010, Figure 13) showed a clear difference in α and E_k between 1 and 2 year old tissue compared to the specimens from August. These differences in photosynthetic parameters might be due to season variations in e.g. light availability, silt and freshwater run-off. The photosynthetic rate of the fertile tissue displayed a straight line and might be explained by a tissue with few chloroplast (Valle, 2005).

Pigment composition

Pigment composition in living organisms are species-dependent and reflects the environmental light history.

Overall, the pigment composition in *L. solidungula* did not alter due to age of tissue as expected (Henley and Dunton, 1995), but there was biological variation among the specimens (n = 63) and seasons. Explanations to these biological differences might be differences in tissue thickness, which results in uneven chloroplast density per area unit, differential growth and maturation across lamina or uneven light exposure on lamina (Valle, 2005), or due to depth differences and hence light availability (Borum

et al., 2002). The specimens in this study were collected at depths between ~5 and 17 meters.

The pigment composition in *L. solidungula* (Tommelen, Aug 2009) consisted mainly of the light harvesting pigments Fuco (77 % of Chl a, 1 year old tissue, Table 8), Chl c_1 and c_2 (25 % of chl a) and a small fraction of PPC (Zea, 1-2 % of Chl a). Some fraction of Zea may have been de-epoxidated back to Viola (4 % of Chl a) while collecting and packing the samples in low light.

Calculating the mean for the five specimens of *L. solidungula* (Table 12, Appendix) a small decrease in Fuco relative to Chl a with age was present, while the other light harvesting pigments (Chl a, c_1 and c_2 and Viola) was more or less constant. The sum of all light harvesting pigments did not change with age. There is a common opinion that the pigment concentration increase with age and maturation of the thallus (Dring, 1982). Forbord (2004) discovered that the amount of Fuco:Chl a in *S. latissima*, increased with decreasing irradiances, also seen for *L. Solidungula* in this study. Comparing pigment composition for meristem tissue of *L. solidungula* collected in different seasons (Table 9), highest concentration of Fuco:Chl a was displayed in the specimen from January and lowest in the specimens from August. The amount of Pheo a was increasing due to age which are naturally and expected. Some of the detected Pheo a can also be connected to epifauna.

The content of Chl c_2 was rather high (~20% of Chl a) and higher than expected. The amount was higher than in most chromophyte algae, but might be due to co-elution of the two chlorophylls c_1 and c_2. In chromophytes generally Chl c_1 and c_2 occurs in approximately equal proportions (Larkum, 2003). In my results there was an askew distribution of the different types of Chl c (~5 % Chl c_1 and ~20 % of Chl c_2 for all year tissues of *L. solidungula*).

The amount of Pheo a was higher in older tissue than younger as expected. See below section for more details regarding Phaeo a.

4.5 Interspecific differences (5 kelp species)

Cell chemistry (POC and PON)

Comparing the N:C ratio of meristem tissue of all species examined, *L. solidungula* had the lowest N:C ratio (0.03) and *S. dermatodea* the highest ratio (0.06), but no evident differences when looking at the amount of POC and PON indicating that *L. solidungula* store nutrients at a higher quantity. All species had ratios in the critical range indicating N-depletion (Niell, 1976, Hanisak, 1983, Hilstad, 2005). These results are opposite to the results from a study done by Gordillo et al (2006) during July 2002. They concluded that Arctic seaweeds (collected in July), including *L. solidungula*, are to be regarded as not N-limited during summer. To be able to draw any conclusion, more specimens of the 4 other species should have been analyzed.

Time-series - Interspecific differences (all 5 species)

The time-series of fluorescence measurements in this study also indicated that meristem tissue for all kelp species collected in addition to *L. solidungula* was in a healthy condition, with a normal diurnal trend of opening and closing of RC_{PSII} in relation to raise and fall in ambient light. The Φ'_{PSII} for all species followed the expected trend of highest values when E_{PAR} was low and opposite. When displaying the rETR, the diurnal progress followed the curve of E_{PAR} indicating a good utilization of ambient light. The most conspicuous differences between the species was that *A. esculenta* reached a plateau of rETR = ~10 from 06:00 and during late morning (Figure 14 – Appendix), even though the irradiances was still raising. The tissue seems to be saturated with light, and do not utilize the higher irradiances. This trend was also seen in 3 year old tissue of *L. solidungula.*

Photochemical quenching of fluorescence (PQ)

Comparing the parameters of meristem tissue of L. solidungula with meristem tissue of the other macroalgae species investigated, three of the other species gained a higher Φ_{PSII} than L. solidungula. Laminaria digitata was the macroalgae species with the highest value (0.8). Only meristem tissue of S. dermatodea seemed to recover completely after the period of elevated irradiances.

Photosynthetic parameters

The photosynthetic parameters and corresponding pigment composition for L. solidungula do not indicate that the species is extremely adapted to low light during the Arctic summer. Typically LL acclimated kelp are characterized by E_k of 14-52 µmol photons $m^{-2}s^{-1}$ measured in August (Dunton and Jodwalis, 1988, Wiencke et al., 1993). The specimens of L. solidungula from Tommelen (August 2009) fulfill the point of high α values (0.5) for meristem tissue, but compared to the other species (α =0.6-1.1), the α value was not extremely high. The light saturation index in meristem tissue of L. solidungula (E_k = 202 µmol photons $m^{-2}s^{-1}$) was the highest of all 5 species examined. In comparison, A. esculenta had an E_k of 10µmol photons $m^{-2}s^{-1}$ (Figure 11 and Figure 14 - Appendix, Table 7)(Kirst and Wiencke, 1995). These findings are in agreement with both Henley & Dunton (1997) and Kirst & Wiencke (1995). Our results are thou in disagreement with the findings of Dunton & Jodwalis (1988) which carried out photosynthetic measurements (based on ^{14}C-method) in the late 1980's on L. solidungula in the Alaskan High Arctic (Stefanson Sound). In they're study, P_{max} and E_k in meristem tissue was lower than both 1 and 2 year old tissue, while α showed an opposite result. They concluded that the relative low E_k (38 µmol photons $m^{-2}s^{-1}$) and the higher α (value) was distinct adaptations in L. solidungula compared to other kelp species species.

In this study, *A. esculenta* had an α (1.1) more than twice the α (0.5) of *L. solidungula* (meristem tissue, Tommelen). The E_k was lower by a factor of 20, which are in the

range of E_k-values of Antarctic species, typically ranging between 11-100 µmol photons $m^{-2}s^{-1}$(Wiencke et al., 1993, Gomez et al., 2009). The differences in both α and E_k might be due to different sampling location rather than only differences between species. *Laminaria solidungula* seems to tolerate extreme shade conditions growing under the canopy, but thrives also in higher irradiances. *Alaria esculenta* on the other hand, seems to be more capable of utilizing low E_{PAR}, and therefore better adapted to the low light climate in Svalbard waters.

Pigment composition

Generally, the pigment composition (in ratio to Chl a) was similar in all kelp species investigated. The concentration of Fuco:Chl a were expected to give highest values in *L. solidungula*, but gave an opposite result. The similar pigment composition in the investigated species eliminate the hypothesis that *L. solidungula* has a different pigment composition that utilizes the available blue-green light better than other species growing in Svalbard waters and that the species is a blue/green light specialist with a higher ratio of Fuco than Chl a (Volent et al., 2007, Johnsen et al., 2009). This conclusion is in agreement with the findings of Henley and Dunton (1995) whom investigated *L. solidungula* in comparison to *S. latissima*.

The amount of Pheo a was various among the species collected. A lower concentration of Chl a (partly caused by degradation to Pheo a) gave a higher ratio of the light harvesting pigments which might be due to natural decay or methodological artifacts. A summation of Pheo a and Chl a and using the new calculated value to gain pigment ratios, the different pigments ratios seems to coincide between all species investigated. These high ratios of Pheo a can also be explained by a more rapid degradation of chlorophylls than carotenoids (Fuco) due to a less stable molecular structure (Nelson, 1993). There is no clear interpretation of the occurrence of Pheo a, whether Chl a is degraded due to age, season, light, handling during experiment or such. This study can not make any concluding remarks concerning this.

4.6 Diurnal trends in P vs E parameters

Regarding the measurements of 1 and 2 year old tissue of *L. solidungula* (Figure 11, P vs E curves) and meristem tissue of *S. dermatodea* and *S. latissima* (Figure 14 - Appendix, P vs E curves) there are evidence of different photosynthetic characteristics during the 24 hours the time-series were conducted. If dividing the dataset into three distinct periods (midday (12:00-20:00), evening/night (20:00-04:00) and morning (04:00-11:00)), there would be displayed differences in the photosynthetic efficiency in these periods. The same trend was discovered by Edwards and Kim (2010) whom investigated diurnal patterns in the giant kelp *Macrocystis pyrifera*, with higher photosynthetic efficiency during morning than mid-day and evening. This points out that it is important to measure time-series throughout the hole day and not only one

time during the day and make conclusions from those data. If only ambient light are considered in the P vs E graphs, the E_k will give roughly the same value as registered by Dunton and Jodwalis (1988) for 1 year old tissue (38µmol photons $m^{-2}s^{-1}$).

4.7 Future perspectives

There has been many publications on biology of marine benthic algae, but the main focus have been on temperate algae and not algae from polar regions (Wiencke and Clayton, 2009). *Laminaria solidungula* is one of the species where more research is needed, especially during wintertime. As this study indicated that *A. esculenta* seemed better adapted to the high Arctic light climate than *L. solidungula*, it would be interesting investigating both these species at a more molecular level (e.g. functional genetics studies) (Nymark et al., 2009), to elucidate the mechanisms for these kelp species growing at high latitudes. Other interesting aspects to work with are *L. solidungulas* ability to avoid epi-growth, fertile tissue as a function of maturation (year), zoospores and how new zoospores accumulate nutrients for growth during winter.

5 Conclusions

We conclude that there is no significant morphological difference between *L. solidungula* from the two sampling sites Tommelen and Rossøya. Regarding the low visible amount of epi-growth on *L. solidungula*, this needs more investigation and we can only speculate if it indicates secretion of bio-active repellent compounds or low abundance due to season variation.

The lower N:C ratio in meristem tissue of *L. solidungula* compared to meristem tissue of the other kelp species in August displays a higher content of POC and lower content of PON in *L. solidungula* and verifies earlier indications that *L. solidungula* is capable of storing energy for heterotrophic growth during low-light periods.

Regarding alterations due to age in *L. solidungula* the different results did not show any uniform alteration of tissue content and/or degradation when the tissue got older. There was a higher nitrogen to carbon ratio in 3 year old tissue than 1 year old tissue indicating that older tissue is still physiologically active. There was an evident decrease of photosynthetic rate ($P_{max,\ 1\ year\ old\ tissue}$ =97 vs $P_{max,\ 3\ year\ old\ tissue}$ = 12) with age but no overall change in pigment composition in older versus younger tissue when looking at mean values. Still there were differences between specimens (not significant).

In comparison to the four other kelp species, *L. solidungula* did not show any specific characteristics that emphasizes the species as specially adapted to the low light climate of the Arctic. Meristem tissue of *L. solidungula* had the lowest N:C in August, but the content of POC and PON was rather similar to the meristem tissue of the other species. The diurnal time-series of F'_o, F'_m, Φ'_{PSII} and ETR did not point out any great differences in photosynthetic characteristics among the five species, though *A. esculenta* reached a plateau of ETR=~10 during mid day indicating saturated photosynthesis at lower irradiances than the other species examined. When comparing meristem tissue between the species, a high E_k (202 µmol photons $m^{-2}s^{-1}$) was observed in *L. solidungula* relative to meristem tissue in the other species and indicates a better ability of acclimation to higher irradiances. *Alaria esculenta* was the species with lowest value of E_k (=10 µmol photons $m^{-2}s^{-1}$). The pigment composition was similar in all five macroalgae species, which eliminates *L. solidungula* as the "only" blue-green light specialist of the kelp species.

All the results from this study taken together, *L. solidungula* does not fulfill all aspects of a LL adapted species (with high α, low P_{max} and low E_k). *A. esculenta* do fulfill these aspects and, hence, the species best adapted to the high Arctic light climate in this study.

The different E_k values from meristem tissue of the kelp species examined may be caused by altered light conditions. The light requirements defined by E_k can be used to explain patterns of zonation (Gomez et al., 2009). The results from this study

indicates a division in the upper 30 meters of the sub-littoral zone in the Arctic, 1) Ice-scraping zone (only annual algae), 2) zone 3-30 m (euphotic zone with string competition for light and substrate) and 3) zone below 30 m depth with light limitation for kelp (red calcareous algae is commonly observed >30 m, own observations)

A higher E_k (*L. digitata*; E_k = 113 µmol photons $m^{-2}s^{-1}$) indicates that the kelp species can handle higher irradiances before saturation of photosynthesis take place and thus thrives at shallower depth than a species with lower E_k (e.g. *A. esculenta*; E_k = 10 µmol photons $m^{-2}s^{-1}$). A sketch of zonation is given in Figure 16, and displays *L. solidungula* as the shallowest growing species of all five species investigated, when

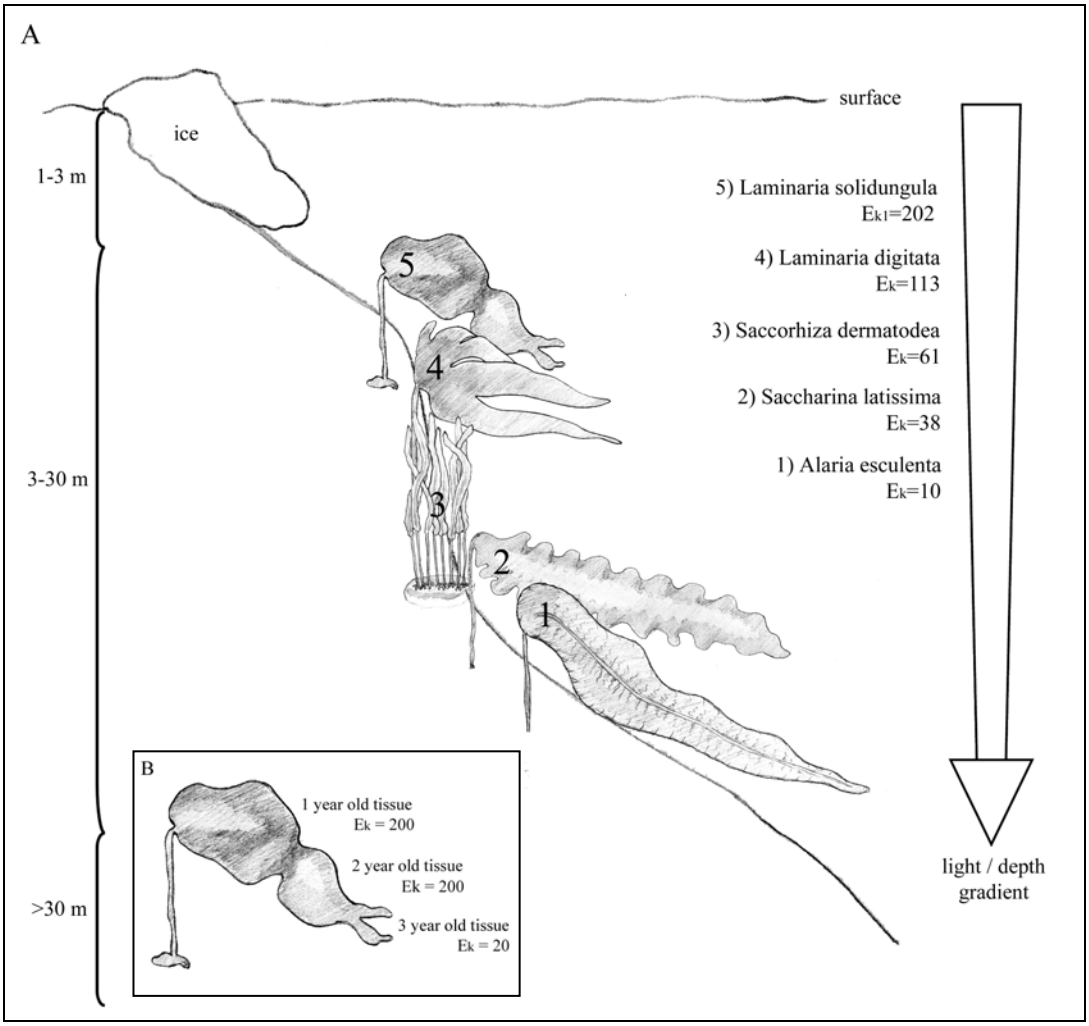

Figure 16: A) Sketch of a possible zonation of macroalgae species in Svalbard waters, where *L. solidungula* are displayed as the shallowest growing species due to a high E_k and *A. esulenta* as the deepest growing species with a low E_k. The sub-littoral zone can be divided into three sections 1) Ice-scraping zone 2) zone 3-30 m and 3) zone below 30 m depth. B) Sketch of *L. solidungula* with E_k values for the different year tissue. Unit for E_k is µmol photons $m^{-2}s^{-1}$.

comparing meristem tissue. If 2 and 3 year old tissue of *L. solidungula* is taken into consideration, the zonation picture is changing due to the species low E_k in 3 year old tissue (20 µmol photons $m^{-2}s^{-1}$). This study show that *L. solidungula* has a unique ability to acclimated to the surrounding light climate. When SCUBA-diving at the different sampling sites, zonation was also evident, even though there was sections where such division seemed absent.

6 References

6.1 Litterature

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#3 **http://www.iopan.gda.pl/~wiktor/macroalgae/sol.html.** Institute of Oceanology Polish Academy of Science. Laminaria Solidungula**.** Copyright (C) by IOPAS 2006. Accessed February 2012.

7 APPENDIX

Table 10: Length of stipes, lamina and the lamina:stipes ratio of *L. solidungula* collected at Tommelen in August 2009. Specimen #2-5 plus #7 did not have a scalebar in the picture and are not included in the table.

Specimen no	Length stipes	Length lamina	Lamina: stipes	Age of algae
1	31.7	60.0	1.9	3
6	34.5	54.5	1.6	2
8	10.0	24.4	2.4	2
9	16.8	33.5	2.0	2
10	7.7	33.5	4.3	2
11	10.8	58.5	5.4	3
12	42.2	75.6	1.8	2
13	32.4	59.0	1.8	3
14	40.0	45.7	1.1	2
15	9.5	22.9	2.4	1
16	10.8	24.6	2.3	2
17	23.6	45.5	1.9	2
18	14.3	34.3	2.4	2
19	5.3	20.0	3.8	2
20	4.6	44.6	9.7	2
21	12.3	33.8	2.8	2
22	24.4	26.7	1.1	1
23	11.9	26.7	2.3	2
24	29.1	49.1	1.7	2
25	10.0	18.8	1.9	2
26	6.3	27.5	4.4	2
27	20.0	30.8	1.5	2
28	28.0	64.0	2.3	2
29	11.4	37.1	3.3	2
30	7.7	16.9	2.2	1
31	26.7	51.7	1.9	3
32	36.4	38.2	1.1	2
33	30.9	49.1	1.6	3
34	10.0	31.3	3.1	2
35	26.1	38.3	1.5	1
36	15.7	37.1	2.4	2
37	9.1	30.9	3.4	2
38	5.5	49.1	9.0	3
39	10.7	36.0	3.4	3
40	20.0	54.5	2.7	2
41	10.0	33.3	3.3	1
42	10.4	57.4	5.5	3
43	12.8	33.6	2.6	2
44	8.7	43.5	5.0	3
45	21.7	50.0	2.3	3
46	42.2	64.4	1.5	3
47	4.0	42.7	10.7	2
48	6.3	30.0	4.8	1
49	23.3	25.0	1.1	1
50	20.0	49.1	2.5	3

Specimen no	Length stipes	Length lamina	Lamina: stipes	Age of algae
51	4.0	14.7	3.7	1
52	16.4	49.1	3,0	2

Table 11: Length of stipes, lamina and the lamina:stipes ratio in *L. solidungula* collected at Rossøya in September 2009.

Specimen no	Length stipes	Length lamina	Lamina: stipes	Age of algae
1	8.6	35.3	4.1	3
2	15.8	30.8	2.0	2
3	18.5	54.7	3.0	2
4	8.5	30.0	3.5	3
5	38.2	56.4	1.5	3
6	25.6	54.7	2.1	3
7	15.9	38.4	2.4	2
8	5.3	33.3	6.3	2
9	6.6	36.6	5.6	3

Table 12: Mean values, SD and CV % of pigments for all year tissue of *L. solidungula* collected at Tommelen in August 2009. Squares denoted "-" indicates no pigment detected. Na indicates only one value for the tissue and mean values can not be calculated.

Pigments	1 year old tissue			2 year old tissue			3 year old tissue		
	Mean	SD	CV %	Mean	SD	CV %	Mean	SD	CV %
Chl c2	0.20	0.06	28	0.22	0.06	26	0.21	0.04	20
Chl c1	0.04	0.06	145*	0.04	0.06	137*	0.04	0.05	137*
Fuco	0.77	0.42	55	0.72	0.23	32	0.66	0.13	19
Neo	-	-	-	-	-	-	Na	Na	Na
Viola	0.04	0.01	30	0.04	0.02	35	0.04	0.01	38
Fuco-derivat	0.06	0.08	125*	0.07	0.06	80	0.06	0.04	65
Zea	0.01	0.01	158*	0.02	0.02	142*	Na	Na	Na
Beta-beta carotene	Na	Na	Na	-	-	-	-	-	-
Lut	-	-	-	0.01	0.004	77	0.03	0.02	87
Chl b	0.01	0.01	116*	0.02	0.01	74	0.11	0.08	79
Chl a	1.00	-	-	1.00	-	-	1.00	-	-
Pheo a	0.06	0.03	51	0.23	0.18	78	0.22	0.13	58

*when trace amount as in chl c_1, chl b, neo and zea CV% will be relatively high.

Table 13: Wet weight (WW), dry weight (DW) and and wet:dry ratio of the macro algal tissue collected. Mean values, SD and CV % for the collected samples analyzed for POC and PON.

Specie/ tissue type	Macro algal tissue			POC			PON		
	Wet weigth (g)	Dry weight (g)	Wet:dry weigth	Mean	SD	CV%	Mean	SD	CV%
L. solidungula # 01, 1-year tissue	0.3816	0.1179	3.24	0.40138	0.00076	0.19	0.00938	0.00017	1.80
L. solidungula # 01, 2-year tissue	0.4176	0.0872	4.79	0.37410	0.00245	0.66	0.01034	0.00010	0.94
L. solidungula # 01, 3-year tissue	0.3964	0.0667	5.94	0.33546	0.00348	1.04	0.01710	0.00031	1.80
L. solidungula # 11, 1-year tissue	0.2745	0.0848	3.24	0.40795	0.00127	0.31	0.01046	0.00036	3.42
L. solidungula # 11, 2-year tissue	0.3733	0.0606	6.16	0.34549	0.00520	1.51	0.01723	0.00036	2.08
L. solidungula # 11, 3-year tissue	0.4774	0.0607	7.86	0.23587	0.00647	2.74	0.02229	0.00076	3.42
L. solidungula # 13, 1-year tissue	0.6928	0.2562	2.70	0.41593	0.00094	0.23	0.01245	0.00035	2.82
L. solidungula # 13, 2-year tissue	0.5553	0.0737	7.53	0.24040	0.01322	5.50	0.01137	0.00050	4.36
L. solidungula # 13, 3-year tissue	0.5549	0.0727	7.63	0.28817	0.01105	3.84	0.01222	0.00093	7.59
L. solidungula # 42, 1-year tissue	0.3252	0.0725	4.49	0.38203	0.00248	0.65	0.01419	0.00051	3.57
L. solidungula # 42, 2-year tissue	0.3492	0.0599	5.83	0.33270	0.01265	3.80	0.01927	0.00185	9.62
L. solidungula # 42, 3-year tissue	0.2803	0.0453	6.19	0.32647	0.00898	2.75	0.01852	0.00105	5.68
L. solidungula # 45, 1-year tissue	0.9634	0.2867	3.36	0.40280	0.00761	1.89	0.01363	0.00029	2.12
L. solidungula # 45, 2-year tissue	0.4438	0.0745	5.96	0.34049	0.00973	2.86	0.01342	0.00062	4.60
L. solidungula # 45, 3-year tissue	0.4169	0.0580	7.19	0.29364	0.02453	8.36	0.01790	0.00190	10.6
L. solidungula Ny Ålesund	0.2059	0.0475	4.33	0.32994	0.01522	4.61	0.02270	0.00181	7.97
L. digitata	0.3216	0.0881	3.65	0.38306	0.00413	1.08	0.01618	0.00036	2.21
S. latissima	0.3073	0.0952	3.23	0.35902	0.00048	0.13	0.01423	0.00127	8.89
A. esculenta	0.2634	0.0662	3.98	0.38604	0.00803	2.08	0.01519	0.00099	6.53
S. dermatodea	0.2444	0.0529	4.62	0.36393	0.01110	3.05	0.02383	0.00104	4.38
L. solidungula Smeerenburgfjorden, 1-year tissue	0.1097	0.0165	6.65	0.30246	0.01302	4.30	0.02881	0.00131	4.54
L. solidungula Smeerenburgfjorden, 2-year tissue	0.2966	0.0467	6.35	0.28230	0.00798	2.83	0.02006	0.00042	2.08
L. solidungula Smeerenburgfjorden, 2-year formative tissue	0.4085	0.0518	7.89	0.23866	0.01598	6.70	0.01589	0.00129	8.12
L. solidungula # 50, 2-year tissue	0.2306	0.0375	6.15	0.33212	0.00585	1.76	0.01483	0.00056	3.77

Table 14: Pigment composition normalized to Chl a (µg pigment / µg Chl a) in different year tissue of *L. solidungula*, and meristem tissue of *L. digitata*, *S. dermatodea*, *S. latissima* and *A. esculenta.* Trace amount of Neoxanthin (0.04) was found in 3 year old tissue of *L. solidungula* #45 (not shown in table). "-" = not detected.

Specie/ tissue type	Chl c_1*	Chl c_2	Fuco (Fucoderivates)	Viola	Zea	Beta-beta carotene	Phaeoph a	Chl b	Lutein
L. solidungula, Tommelen									
L. solidungula # 01 - 1 year tissue	-	0.15	0.46	0.05	0.01	0.01	0.04	-	-
2 year tissue	-	0.18	0.51 (0.03)	0.05	-	-	0.04	0.01	-
3 year tissue	-	0.18	0.50 (0.03)	0.05	-	-	0.06	0.07	0.02
L. solidungula # 11 - 1 year tissue	-	0.17	0.52 (0.02)	0.06	-	-	0.03	-	-
2 year tissue	-	0.19	0.49 (0.03)	0.05	-	-	0.07	0.01	-
3 year tissue	-	0.21	0.56 (0.03)	0.04	-	-	0.13	0.01	-
L. solidungula # 13 - 1 year tissue	0.14	0.28	1.43 (0.19)	0.03	-	-	0.07	0.01	-
2 year tissue	0.11	0.19	0.82 (0.13)	0.03	0.03	-	0.35	0.02	0.01
3 year tissue	0.09	0.20	0.72 (0.10)	0.02	0.05	-	0.23	0.07	0.01
L. solidungula # 42 - 1 year tissue	-	0.16	0.48 (0.01)	0.05	-	-	0.05	-	-
2 year tissue	-	0.25	0.71 (0.04)	0.06	0.01	-	0.23	0.02	0.01
3 year tissue	-	0.29	0.80 (0.03)	0.06	0.01	-	0.36	0.15	0.04
L. solidungula # 45 - 1 year tissue	0.08	0.23	0.96 (0.10)	0.03	-	-	0.10	0.01	-
2 year tissue	0.10	0.31	1.05 (0.14)	0.03	0.01	-	0.47	0.04	0.01
3 year tissue	0.09	0.20	0.70 (0.09)	0.03	-	-	0.31	0.22	0.06
L. solidungula # 50 - 2 year tissue	-	2.05	3.17	-	-	-	1.99	0.21	-
L. solidungula, Smeerenburgfjorden									
1 year tissue	-	0.73	0.91 (0.03)	-	-		1.22	-	-
2 year tissue	-	1.11	1.15 (0.08)	-	-		2.10	-	-
2 year fertile tissue		1.40	1.62	-	-		2.31	-	-
L. solidungula Ny Ålesund	0.15	0.45	1.76	-	-		0.70	-	-
L. digitata	0.11	0.30	0.78 (0.02)	0.02	-		0.17	-	-
S. dermatodea	-	0.15	0.40	0.03	-		-	-	-
S. latissima	-	0.33	0.88 (0.02)	0.02	-		0.07	-	-
A. esculenta	-	0.37	1.06 (0.03)	-	-		0.36	-	-

* Chl c_1 often co-elute with Chl c_2

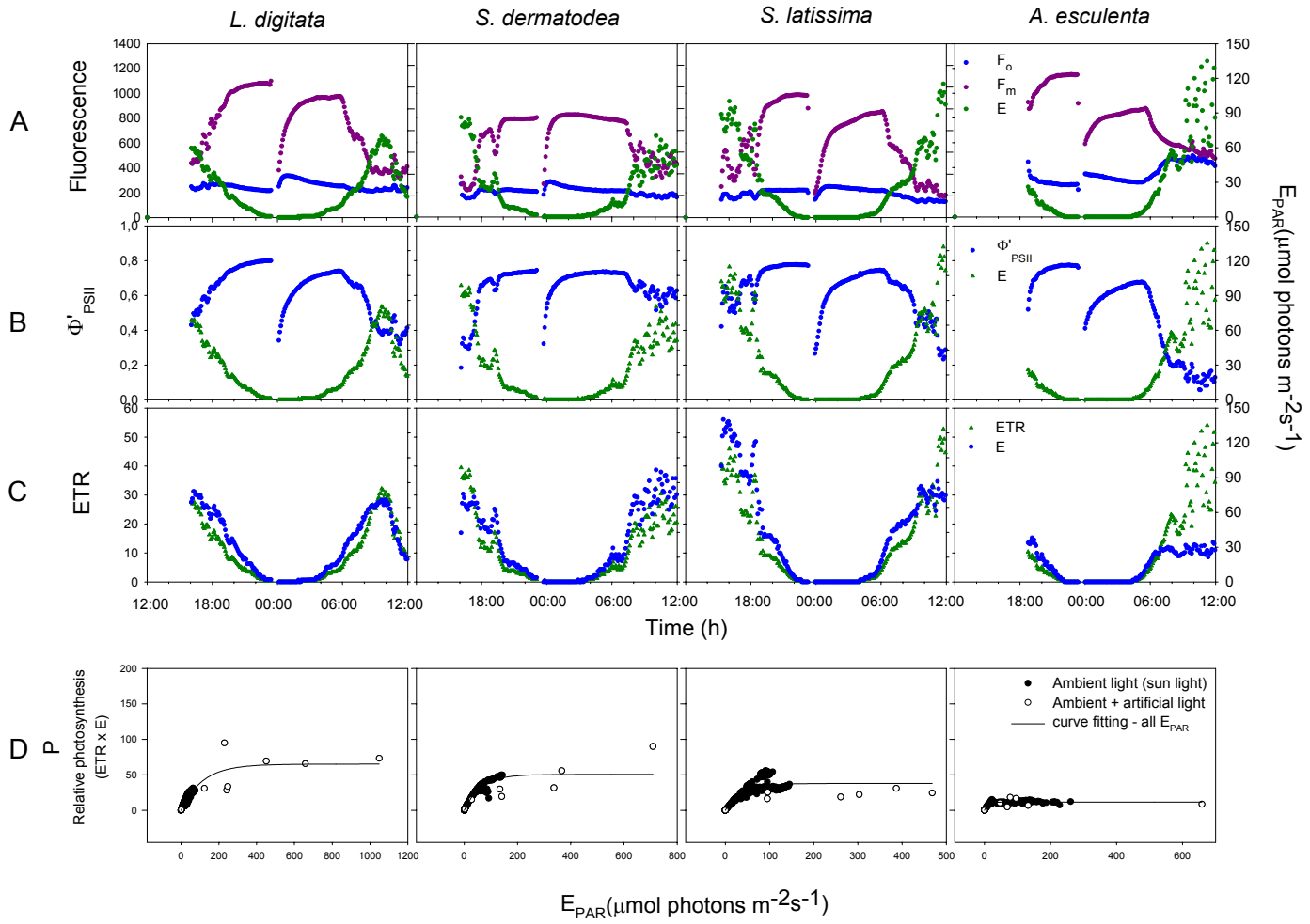

Figure 14: Time-series measurements of A) fluorescence (F'_o, F'_m), B) Φ'_{PSII}, C) rETR (Φ'_{PSII} x E_{PAR}) and D) P vs E curves for meristem tissue of *L. digitata*, *S. dermatodea*, *S. latissima* and *A. esculenta* collected in August and September 2009. The graphs displays a normal variation of opening and closing of RC_{PSII}, indicationg healthy macoalgae tissue.