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The effects of variable environmental conditions on growth, nutritional state and protein content in cultivated *Saccharina latissima* in Norway

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Marine Coastal Development
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Guri Ellila Brodahl

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Abstract

Cultivation of macroalgae, or seaweed, has been a major industry in Asian countries, predominantly China and Indonesia, for decades. Production of seaweed in Norway is mostly related to research and development (R&D), yet some commercial production exists to this date. *Saccharina latissima* is considered the most promising species for cultivation in Norway due to its ability to grow in a variety of environmental conditions. The aim of this study was to determine differences in growth, internal nitrate (NO_3^--N) and protein content in *S. latissima* under a variable of environmental conditions and assess patterns in growth rate. A specific question was if internal NO_3^--N could describe the nutritional state in the algae under study. Young *S. latissima* sporophytes were deployed at two depths (1-2 m and 8-9 m) at five experimental locations along the Norwegian coast in February of 2017, and sampling of data for growth and chemical analyses occurred during a period of 23 weeks from April to October of 2017. Light and temperature data indicated an earlier seasonal development in the south compared to the north, and a production period reaching over the summer months was found in the north, while loss of biomass occurred during the summer months in the south. The total nitrogen (N) and N:C ratio in *S. latissima* both decreased early in the experimental period and increased in the end of the period, suggesting that these variables interacted. A similar pattern of variation as for total N and N:C ratio was found for the amino acid residues and protein content at all locations except for the northernmost location. The internal NO_3^--N content was higher in the seedlings (that had been kept in the hatchery in nutrient rich deep water) than in the sporophytes that were cultivated in the sea. A positive, yet not significant, relationship between relative growth rate (RGR) and internal NO_3^--N was found. The seasonal variation in internal NO_3^--N contents in the sporophytes were more clear at the two northern locations than at the three further south. Seasonal variations were clear at the northern locations while the experiment started too late for them to be measured in the south, indicating a latitudinal gradient. Factors such as photosynthetic active radiation and supply of nutrients possibly limited the growth due to natural seasonal fluctuations in the seawater, though a clear factor that affected the growth rate at the different locations was difficult to identify. The nitrogen-to-protein conversion factor was found to be 3.9 ± 0.1, irrespective of cultivation depth, and it was suggested to use this factor if protein determination by amino acids is not available. A comparison of the N:C ratio and internal NO_3^--N content revealed the possibility to introduce internal NO_3^--N as a proxy for the nutritional state of *S. latissima* in Norway.

Sammendrag

Oppdrett av tang og tare har i flere tiår vært en stor industri i asiatiske land som Kina og Indonesia. I Norge er produksjonen av tang og tare for det meste produksjon relatert til forskning og utvikling (F&U), selv om det finnes noen kommersielle aktører. *Saccharina latissima* er ansett som den mest lovende arten for produksjon i Norge, grunnet dens evne til å overleve og vokse i et bredt spekter av miljøbetingelser. Dette studiet hadde som mål å fastslå forskjeller i vekst, internt nitrogen i form av nitrat (NO_3^--N) og proteininnhold i *S. latissima* dyrket under forskjellige miljøvilkår, evaluere mønster i vekst og anslå om internt NO_3^--N kan brukes til å beskrive næringsstatusen i den eksperimentelle arten. Unge sporofytter av *S. latissima* ble i februar 2017 plassert ut på to dyp (1-2 m og 8-9 m) hos fem eksperimentelle lokaliteter langs norskekysten, og innsamling av data foregikk i en 23 ukers periode fra april til oktober 2017. Lys- og temperaturdata antydet en tidligere sesongutvikling i sør sammenlignet med i nord, og produksjonsperioden foregikk over sommeren i nord, mens tap av biomasse fant sted i løpet av sommermånedene i sør. Totalt nitrogen (N) og N:C forhold i *S. latissima* hadde en negativ trend i starten av perioden og økte mot slutten, noe som antydet at disse variablene påvirket hverandre. Et lignende mønster ble funnet for aminosyre- og proteininnholdet for alle lokalitetene bortsett fra lokaliteten lengst nord. Den interne konsentrasjonen av NO_3^--N i cellene var høyere i kimplantene (som ble beholdt på laben i rennende næringsrikt dypvann) enn i sporofyttene som ble dyrket i havet hos alle lokalitetene. En positiv, dog ikke signifikant korrelasjon mellom relativ vekstrate (RGR) og intern konsentrasjon av NO_3^--N ble funnet. Den sesongpregede variasjonen i mengden internt NO_3^--N i sporofyttene var klarest hos de to lokalitetene lengst nord. Det ble funnet tydelige sesongvariasjoner på de nordlige lokalitetene mens eksperimentet startet for sent for at disse forskjellene ble registrert i sør, noe som tydet på forskjeller mellom lokalitetene basert på breddegrad. Faktorer som fotosyntetisk aktiv stråling og tilgang til næringsstoffer var mulige begrensninger for vekst grunnet naturlige sesongvariasjoner i havet, og derfor var det ikke mulig å påpeke én faktor som påvirket veksten i *S. latissima* hos alle lokalitetene. Faktoren for å konvertere nitrogen til protein ble estimert til 3.9 ± 0.1, uavhengig av kultiveringsdyp, og denne kan bli anbefalt å bruke dersom estimering av protein på grunnlag av aminosyrer ikke er tilgjengelig. En sammenligning av N:C-forholdet og den interne konsentrasjonen av NO_3^--N gjorde det en tenkbar mulighet å innføre internt NO_3^--N som en mer nøyaktig indikator på næringsstatusen i *S. latissima* i Norge.

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Abbreviations

AA	Amino acid residues
ANOVA	Analysis of variance
APN	Experimental location in Tromsø operated by Akvaplan-niva
ASF	Experimental location in Austevoll operated by Austevoll Seaweed Farm
CTD	Instrument measuring salinity, temperature and depth
DM	Dry matter (% of WW)
FAO	Food and Agriculture Organization of the United Nations
HPLC	High Performance Liquid Chromatography
K_p	Nitrogen-to-protein conversion factor
N	Nitrogen
N:C	Nitrogen-to-carbon ratio
NO_3^-	Nitrate
NO_3^--N	Nitrate-bound nitrogen (mg g^{-1} DM)
NSS	Experimental location in Søgne operated by Norway Seaweed
NTNU	Norwegian University of Science and Technology
PAR	Photosynthetically active radiation (mol m^{-2} d^{-1})
RGR	Relative growth rate (day^{-1})
S. latissima	*Saccharina latissima* (L.) C.E. Lane, C. Mayes, Druehl, et G. W. Saunders (syn. *Laminaria saccharina*)
SAL	Experimental location in Skjerstadfjorden operated by Salten Algae
SES	Experimental location at Frøya operated by Seaweed Energy Solutions
WW	Wet weight

1 Introduction

Macroalgae, more commonly referred to as seaweed, have been a known food resource for decades, especially in Asia (Tseng, 1987). Macroalgae are mainly classified as either Rhodophyceae, Chlorophyceae or Phaeophyceae and the classification is based on their pigmentation; red, green and brown, respectively (McHugh, 2003). The size of adult individuals can range from a few centimeters (which is more commonly observed with respect to red and green algae) to around 20-40 meters (Giant kelp, *Macrocystis pyrifera*) (McHugh, 2003).

According to the Food and Agriculture Organization of the United Nations (FAO), the worldwide production of seaweed was almost 30 million metric tons in 2015, predominantly red and brown seaweed produced in the Asian countries like China and Indonesia (FAO, 2017). The main species cultivated in 2015 was carrageenan producers such as the red algae *Kappaphycus alvarezii* and *Eucheuma* spp., the red agarophytes *Gracilaria* spp. and the two brown algae species *Saccharina japonica* (syn. *Laminaria japonica*) (kombu) and *Undaria pinnatifida* (wakame).

Seaweed can be cultivated in the sea in temperate regions at high-latitude, due to the natural supply of nutrients from deep waters that means they do not have to be "fed" (Olsen, 2011). Seaweed is a versatile resource and can be used for several purposes, such as, among other things, food, feed, energy production and the extraction of chemicals and bioactive compounds (Olafsen et al., 2012; Skjermo et al., 2014).

1.1 *Saccharina latissima*

Saccharina latissima (Linnaeus) C. E. Lane, C. Mayes, Druehl, et G. W. Saunders (syn. *Laminaria saccharina*) is a large and fast growing species of macroalgae. It thrives in rocky coastal ecosystems and grows naturally in the North East Atlantic (Bartsch et al., 2008), thus grow in a broad range of environmental conditions. The experimental species is a component of the coastal ecosystems all along the Norwegian coast (Figure 1.1), and approximately half of the world's natural *S. latissima* forests are found in Norway (Moy et al., 2006). Consequently, *S. latissima* plays an important role in many habitats along the coast of Norway (Christie, 1997; Christie et al., 2009).

In Norway, *S. latissima* is fertile during the period from November to approximately March/April (Andersen, 2013). Its fertility can be verified by looking at the dark, spore producing tissue called sorus (plural: sori) which develops during the course of these months. In mid-Norway, heavy biofouling by bryozoans and other epiphytic species can take place in June and throughout the summer months, causing loss of biomass of *S. latissima* (Handå et al., 2013; Førde et al., 2016).

1.1.1 Geographical- and depth distribution

The geographical distribution of *S. latissima* is determined by the summer isotherm. It grows naturally from Svalbard in the north to Portugal in the south (Lüning et al., 1990), and as such, it is a species of high plasticity. The optimum growth of *S. latissima* has been found in water temperatures between 10 and 15°C (Fortes and Lüning, 1980) and it can survive in both warmer and colder temperatures, which reinforces the statement of plasticity. In Norway the temperatures vary from 2-5°C in the winter to 10-15°C in the summer (Lalli and Parsons, 1997a), but it will vary depending on depth. Figure 1.1 shows that *S. latissima* grows naturally along the whole Norwegian coast.

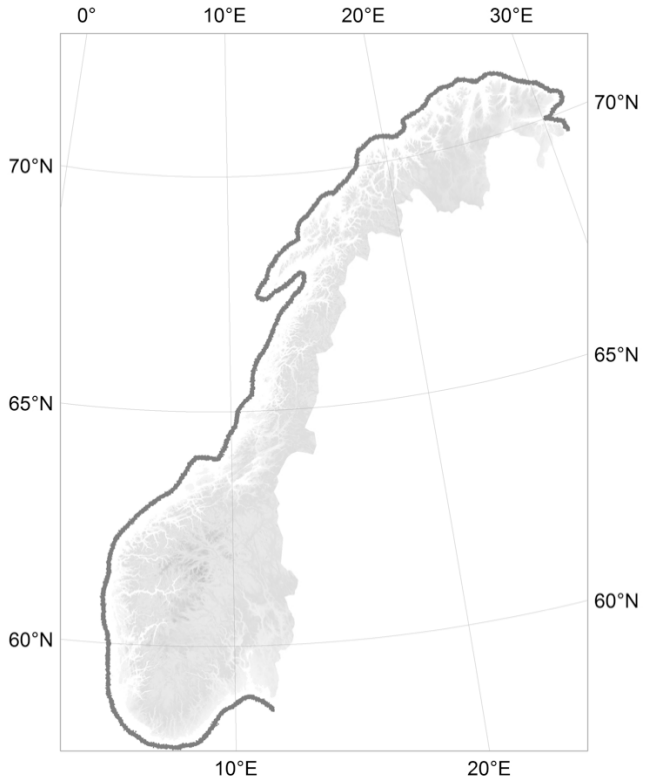

Figure 1.1. The geographical distribution of *S. latissima* in Norway illustrated by the bold line. Data source: Lüning et al. (1990); Statens kartverk (2007).

The major factor when determining depth distribution and productivity of *S. latissima*, is light. Its depth distribution ranges from the intertidal zone and down to 30 m depth (Gerard, 1988). As light gets reduced with depth and photosynthesis rely on the light intensity, the cultivation depth of *S. latissima* has to consider this. Handå et al. (2013) found better growth in cultivated *S. latissima* at 2 and 5 m depth compared to 8 m depth in autumn and winter, while the growth was similar at all depths from February to June in Norway.

1.1.2 Dry matter and ash content

The moisture content in *S. latissima* is rather high. Gerard (1988) showed that the dry matter content of wet mass increased with higher irradiance up to a certain point. This trend was supported by Schiener et al. (2015) and Marinho et al. (2015b), who found that the dry matter content was highest in summer and early autumn in *S. latissima* in temperate areas.

The ash content describes the mineral content in the algae and can be obtained after all organic material has been burnt off. Schiener et al. (2015) found that the ash content in *S. latissima* was on average 31.7 ± 7.6 % of dry matter and that it peaked during the winter months in Denmark.

1.1.3 Nitrogen, amino acids and proteins

Total nitrogen concentration (N) in the tissue of *S. latissima* has been shown to increase relative to ambient NO_3^- concentration (Chapman et al., 1978). An average N content of 1.5 ± 0.5 % of the dry matter was found by Schiener et al. (2015), with a peak value in February/March and lowest values in summer and autumn. Nielsen et al. (2014) found the same pattern of variation and that the average N concentration in the seaweed tissue increased from 4 to 11 m depth. The N contents were inversely related to the temperature, suggesting an effect of seasonality also for N contents. Handå et al. (2013) showed that independent of deployment time for cultivated *S. latissima*, the N content in the tissue decreased during spring and increased again during autumn.

Amino acids

Amino acids are synthesized from inorganic N like nitrate (NO_3^-) that is taken up from the ocean by seaweed (Young et al., 2007). There is a total of 20 amino acids. Nine of them are considered essential for animals, including humans, which means that the body is unable to synthesize them. The remaining 11 amino acids are non-essential, and as such, can be synthesized by animals. Arginine is a non-essential amino acid for humans, but it is found to be essential for fish (Mæhre et al., 2014), consequently, it has to be considered if seaweed has a potential for use in fish feed. According to Holdt and Kraan (2011), the amino acid profiles in *Saccharina* spp. are well suited for use in fish feed.

The total amount of amino acids and their composition in *S. latissima* has been found to change with seasons, and according to Marinho et al. (2015a), this was due to depletion of N in the seaweed tissue rather than an effect of amino acids from epiphytes. The same study showed that all amino acids were present all year around but in different quantities. Sharma et al. (2018) found significantly higher contents of amino acids in *S. latissima* at 8 m depth compared to 3 m depth and the contents varied with season, with the highest values found in August.

Proteins

Proteins and peptides are formed by amino acid residues, and thus, contain high amounts of N. The protein content is generally higher in red and green algae compared to brown algae (Fleurence, 1999; Angell et al., 2016). The protein content in seaweed has been shown to vary between 1.2 % and 44.0 % of dry matter (Holdt and Kraan, 2011), partly depending on the methods used for determination (Mæhre et al., 2018). Higher protein content has been found in the winter than in the summer and early autumn (e.g. Fleurence, 1999; Pangestuti and Kim, 2015; Schiener et al., 2015). The protein content in *S. latissima* has been found to vary between 3 and 26 % of dry matter (Morrissey et al., 2001; Holdt and Kraan, 2011; Pereira, 2011; Schiener et al., 2015) and contents were highest in winter months when the ambient nutrient concentrations were high (Marinho et al., 2015b; Mols-Mortensen et al., 2017). Sharma et al. (2018) found that the protein content was higher at 8 m depth compared to 3 m depth in Central Norway.

Nitrogen-to-protein conversion factor

The most common analytical techniques for estimating contents of proteins are based on the sum of amino acid residues (amino acids after subtracting a molecule of water) or indirect methods such as protein determination based on N content or spectrophotometric methods (Mæhre et al., 2018). A universal nitrogen-to-protein conversion factor of 6.25 is often used to estimate protein from N contents. This factor is representative for proteins and use will assume that all N is believed to be protein-bound. This approach will lead to an overestimation of the protein content in seaweed and other biomass samples due to their high amounts of non-protein N (Lourenço et al., 1998). Therefore, more specific factors are often calculated and used (Jones, 1941). The mean nitrogen-to-protein conversion factor in seaweed has been found to vary between 3.5 ± 0.1 and 6.0 ± 0.2 (Schiener et al., 2015; Angell et al., 2016; Biancarosa et al., 2017; Mæhre et al., 2018).

1.1.4 Internal nitrate (NO_3^-)

Seaweed can take up inorganic ions, such as nitrate (NO_3^-) from the environment, and incorporate it into amino acids and other organic molecules during anabolism. This process consists of three main steps: 1) diffusion of N through the diffusion boundary layer, 2) N is taken up by the cell across the cell membrane and 3) amino acids are synthesized directly from ammonium (NH_4^+) or by reducing NO_3^- or nitrite (NO_2^-) to NH_4^+ by enzymes (Hanisak, 1983). The uptake of NO_3^- in some seaweed has been shown to be active, which can imply that there is considerably higher concentrations in the tissue compared to the surrounding water, and as such, the transport goes against the concentration gradient (Harrison and Druehl, 1982).

Some seaweed can store NO_3^- in their vacuoles when the NO_3^- concentration of the seawater is high and use it for growth when the NO_3^- concentration in the seawater is limited (Chapman et al., 1978). Chapman et al. (1978) also found that photosynthesis in *S. latissima* increased with increasing internal NO_3^- pools. Moreover, Chapman and Craigie (1977) found that the growth of seaweed in temperate seawater increased during spring as a result of the high ambient NO_3^- concentrations during winter, assuming storage of NO_3^- in the tissue, while the growth decreased during the summer months due to depletion of NO_3^- reserves. Ambient NO_3^- concentrations may often show an inverse trend to temperature, starting at high concentrations in winter, it decreases during spring and remains low during summer until it increases again

during autumn and reaching high values during winter months (Young et al., 2007). This trend reflects a seasonality in NO_3^- concentrations in temperate surface seawater. A similar pattern was found by Nielsen et al. (2014) for internal inorganic N (including NH_4^+, NO_3^- and NO_2^-) in a natural population of *S. latissima* in Denmark. The internal inorganic N pools were high during winter and remained low from May to November while temperatures in the ambient water was higher.

1.2 Seasonality in primary production

The light intensity exposed to the sea surface, the temperature and the nutrient availability follows a stronger seasonal pattern at high latitudes than at low latitudes. At around 60°N, there is higher seasonal variation in the solar radiation at sea surface than in the tropics (Lalli and Parsons, 1997a). Above the Arctic Circle, the midnight sun and polar night affect the photosynthetic radiation available for primary producers such as seaweed.

In temperate regions, increasing surface water temperatures and less turbulent seawater during spring cause stratification of the water layers. As a result of such stratification, more nutrients stay in the upper water layers and become available for primary producers, and with that, inducing the spring bloom. As primary production continues, the upper water layers become more oligotrophic as the stratification layer works as a barrier to the upwelling of nutrient rich deep water. As temperature decreases during autumn and winter, the upper seawater layer cools down and the stratification layer weakens. The deep water mixes more easily with the upper water layers, causing vertical dispersion of nutrients in the water column (Black and Dewar, 1949; Lalli and Parsons, 1997a; Lalli and Parsons, 1997b). Because thermal stratification is a temperature-mediated process, there may be changes in the timing and the strength of the stratification along a latitudinal gradient, which substantiates seasonal differences along the Norwegian coast.

1.3 The aim of the study and its objectives

Scientific studies on *S. latissima* in Norwegian waters have been performed previously (e.g., Sjøtun, 1993; Forbord et al., 2012; Handå et al., 2013; Mæhre et al., 2014; Skjermo et al., 2014; Biancarosa et al., 2017; Stévant et al., 2017b; Sharma et al., 2018). This present study performed as part of this thesis takes a large geographical area into consideration. An overall objective was to learn what areas that are most suited for culturing seaweed and also which time periods the chemical composition is at its best with respect to both harvest time and the range of application. In this study, data representative for variable environmental conditions were collected in a regional study with locations distributed along the Norwegian coast, with five locations from Tromsø in the north to Søgne in the south.

Aim of the study

The main aim of this study was to determine if there were differences in growth, internal nitrate-N (NO_3^--N) and protein content in *Saccharina latissima* under variable environmental growth conditions.

Objectives

For this study, three sub-objectives were established:

- To investigate if there were any geographical and seasonal differences in growth and chemical composition in *S. latissima* at cultivating locations characterized by different environmental conditions along the Norwegian coast.
- To assess which abiotic factors and/or cellular compounds that best explain the patterns in growth rate.
- To assess to what extent internal NO_3^--N can be used to describe nutritional state in *S. latissima*.

2 Materials and methods

2.1 Determining the study locations

The geographical differences in growth and chemical content at five experimental locations along the Norwegian coast was investigated during a 23-week sampling period. Samples were taken of algae grown at two depths (1-2 m and 8-9 m). The study locations ranged from Tromsø in the north (69°N) to Søgne in the south (58°N) (Figure 2.1).

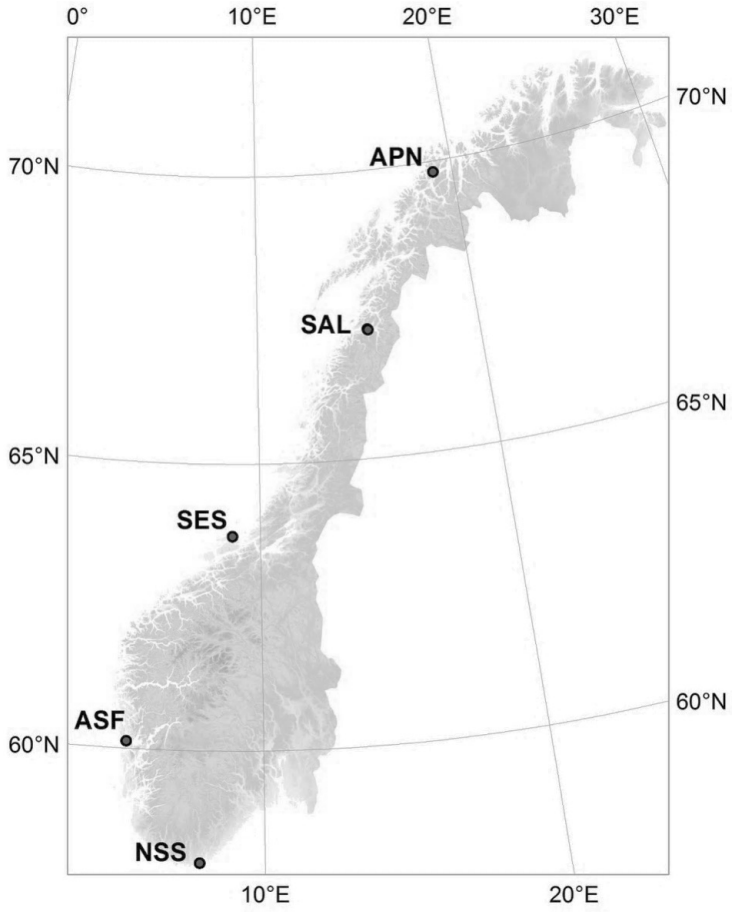

Figure 2.1. The cultivation locations along the Norwegian coast. Data source: Table 2.1; Statens kartverk (2007).

The geographical locations for the seaweed farms, expressed by their coordinates, and their degrees of exposure, are shown in Table 2.1 below. The degrees of exposure of the locations were defined by the placements of the locations in relation to shore. If the position of the locations were close to shore, they were considered sheltered. If further out, but still possible to see land, they were considered semi-sheltered.

Table 2.1. Overview of the locations, with names, their geographical positions and coordinates as well as their evaluated degrees of exposure.

Location	Cultivation location operator	Coordinates	Exposure
APN	Akvaplan-niva (Tromsø)	N69°45.259' E19°02.176'	Sheltered
SAL	Salten Algae (Skjerstadfjorden)	N67°14.190' E14°50.680'	Sheltered
SES	Seaweed Energy Solutions (Frøya)	N63°42.279' E08°52.232'	Semi-sheltered
ASF	Austevoll Seaweed Farm (Austevoll)	N60°08.960' E05°09.264'	Semi-sheltered
NSS	Norway Seaweed (Søgne)	N58°03.325' E07°51.220'	Sheltered

2.2 Production of seedlings and deployment

Wild fertile *S. latissima* sporophytes with sorus were collected in December 2016 (week 50) and stripped for spores. Due to recommendations from the Norwegian Environment Agency, species used for cultivation should be of local genetic origin (Fredriksen and Sjøtun, 2015). The sporophytes were therefore collected from their local area where they later were deployed and cultivated in the sea. The production of *S. latissima* seedlings for all locations was done simultaneously in the hatchery at SINTEF SeaLab in Trondheim, according to the protocol by Forbord et al. (2012); Forbord et al. (2018). Spores were sprayed on a 1.2 mm string and incubated for 7 weeks in the hatchery. A random sample of the seedlings from each of the locations was kept further in the hatchery in running nutrient rich sea water (10°C, 148 µg NO_3^--N L^{-1}, from ~70 m depth) for later analysis of internal NO_3^--N.

The 1.2 mm strings with juvenile *S. latissima* sporophytes were twisted onto two separate marked areas on each of five 10-meter-long thicker ropes (14 mm). Starting at 1 meter from both ends of the rope and reaching 1 meter along the rope, the sporophytes were located at 1-2 m depth and 8-9 m depth when placed vertically in the water (Figure 2.2). The ropes were packed in polystyrene boxes with wet newspapers and cooler bricks before being shipped to the locations where they were initially harvested from (Figure 2.1, Table 2.1). The ropes were deployed at sea by attaching them to the main rope with buoys with approximately 5-6 m

distance between them as shown in Figure 2.2. Deployment was done in early February 2017 at SES, ASF and NSS. At the remaining locations, the ropes were deployed later due to weather conditions or other unexpected events. At APN, deployment of the ropes was done in late February (week 8).

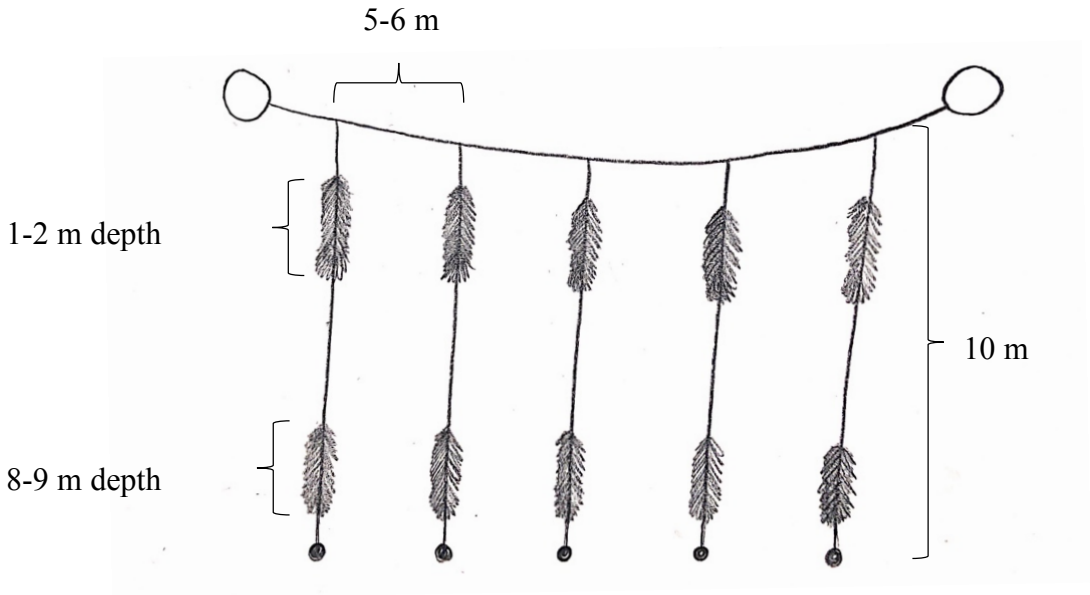

Figure 2.2. Sketch showing the experimental setup with ropes with *S. latissima* from 1-2 m depth and 8-9 m depth. The big circles on top illustrate buoys and the small circles at the bottom end of each rope illustrate weights. The sketch only illustrates a part of the whole setup.

At each experimental location there were separate ropes with light- and temperature loggers attached to the main horizontal rope, logging water temperature and light continuously from 2 and 6 m depth.

2.3 Sampling material

Samplings in 2017 were performed in week 16 (mid-April), 18 (early May), 20 (mid-May), 22 (May/June), 24 (mid-June), 27 (early July), 32 (early August), 36 (early September), a total of 8 samplings. At the northernmost location (APN), one extra sampling in week 39 (September/October) was carried out. The same methodology and sampling protocol was used at all locations. At every sampling, the light and temperature loggers were cleaned to make sure that no biofouling was affecting the light sensors.

Due to unforeseen circumstances, such as rough weather conditions, not all planned samplings were conducted at each location each sampling week. Table 2.2 below shows the dates for the actual samplings during the experimental period.

Table 2.2. Overview of the sampling dates at each cultivation location in 2017.

	APN	SAL	SES	ASF	NSS
Week 16	21.04.17	27.04.17	25.04.17	18.04.17	
Week 18	05.05.17	04.05.17	04.05.17	03.05.17	02.05.17
Week 20	16.05.17	22.05.17	18.05.17	15.05.17	19.05.17
Week 22	31.05.17	30.05.17	31.05.17	*	
Week 24	14.06.17	16.06.17	15.06.17	14.06.17	12.06.17
Week 27	05.07.17	07.07.17	03.07.17	*	
Week 32	09.08.17	18.08.17	09.08.17		
Week 36	05.09.17				*
Week 39	28.09.17				

* Only sampling for chemical analyses were done. Sampling dates are not known.

2.3.1 Growth measurements

Lamina length of ten individuals of *S. latissima* from five ropes at two depths were measured. This resulted in 50 individuals being measured at each depth (n = 50). The same ropes were used for sampling throughout the experiment.

Calculating relative growth rate (RGR)

The relative growth rate per day (RGR day^{-1}) based on length was estimated as:

$$\text{RGR } day^{-1} = \frac{\left(\frac{L_1 - L_0}{T}\right)}{L_0} \quad (1)$$

Where L_0 represents the length in mm at the previous sampling and L_1 represents the length in mm at the present sampling. The time in days since last sampling was represented by T.

2.3.2 Collecting samples for chemical analyses

Ten individuals of *S. latissima* from five ropes at two depths were collected in plastic zip lock bags and placed in cooling bags with cooling bricks. The ten individuals were shaken to remove excess water, mixed and placed together in one bag, resulting in a total of 5 bags (n = 5) at each of the two depths. The samples were stored at -20°C immediately after arriving back to shore until they were shipped frozen to Trondheim at the end of the experimental period (end of September 2017) and stored at -20°C until further analyzed.

2.4 Analyses

In this study both lamina (blade) and stipe (stem-like structure) of *S. latissima* were represented in each sample. Initially, five replicate analyses per depth for all samples were supposed to be performed. Due to time restriction, three replicate analyses per depth for all samples were undertaken in most cases. All samples were stored at -20°C prior to analysis.

2.4.1 Dry matter and ash content

1-2 g of frozen *S. latissima* sample (-20°C) was placed in pre-weighed ceramic crucibles that had been dried and stored in a desiccator to remove any moisture. The samples were heated and kept at 105°C in a Termaks B8133 incubator from Labolytic AS for 24 hours to get the dry matter (DM). To measure the ash content, the samples were burnt at 600°C for 12 hours in muffle furnaces (Hagan Elektroovner AS and CERAMA GL 13). The crucibles with samples were cooled down in a desiccator and weighed. Equation 2 shows the calculation of DM as % of wet weight (WW) and Equation 3 shows the calculation of ash content as % of DM.

(2) $$\%\ DM\ of\ WW = \frac{Dry\ matter\ (g)}{Wet\ weight\ (g)} * 100\ \%$$

(3) $$\%\ ash\ of\ DM = \frac{Ash\ (g)}{Dry\ matter\ (g)} * 100\ \%$$

The DM and ash contents were not analyzed for the seedlings that were kept in the hatchery, so an assumption of 10 % DM was used for these samples, as the average of DM for the actual analyzed samples were approximately 10 %.

2.4.2 Determining total nitrogen content by CN analysis

50-60 g of frozen (-20°C) *S. latissima* was cut in small pieces and placed in small plastic containers and stored at -80°C for approximately 1 hour. The samples were freeze dried (Hetosicc CD 13-2) at -40°C for 48 hours. 50 g of frozen sample resulted in approximately 5 g of freeze dried sample.

In order to measure the total N content in *S. latissima*, approximately 2 mg of freeze dried sample was weighed on a Mettler Toledo UMX2 ultra-microbalance, transferred to 5x9 mm tin capsules, which were packed into small balls on a carbon free metallic plate and placed in a 96 well plate. The samples were stored at -20°C prior to analysis. The analysis of CN was performed on an elemental analyzer (Elementar vario EL cube) with acetanilide as standard by staff at the Norwegian University of Science and technology (NTNU). Total N as % of DM was calculated by using Equation 4. Total N as mg g^{-1} DM was calculated by using Equation 5.

(4) $$\% \, N \; of \; DM = \frac{\mu g \; N}{\mu g \; sample} * 100 \, \%$$

(5) $$\text{mg N } g^{-1} \text{ DM} = \% \, N \; of \; DM * 10$$

2.4.3 Determining internal NO_3^--N content

0.06 g semi-frozen *S. latissima* material (-20°C) from each sample was mixed with 6 mL of distilled water in test tubes. Marbles were put on top of the test tubes to prevent evaporation of the fluid and high pressure. The samples were boiled for 30 minutes, cooled down and filtered into 15 mL plastic tubes using a 0.45 µm polysylfone syringe filter before it was diluted by mixing 0.3 mL of the solution with 9.7 mL distilled water and transferred to new 15 mL plastic

tubes. The tubes were placed in a -20°C freezer until they were further analyzed. Prior to analysis, the tubes were defrosted and shaken. The analysis of internal NO_3^--N was performed by NTNU staff on an autoanalyzer (Flow Solution IV System, I.O. Analytical) according to the Norwegian standard NS 4745. The calculation of NO_3^--N (mg g^{-1} DM) for all the locations was performed as shown in Equation 6:

$$N_{mg} = \frac{IN_N * V * \frac{10\ mL}{1000L} / DM}{1000} \quad (6)$$

Where N_{mg} is mg NO_3^--N g^{-1} DM. IN_N represents the NO_3^--N concentration from the autoanalyzer in µg/L. *V* represents the volume of boiled seaweed solution in mL (6.06 mL). *DM* is the dry matter as decimal digits. When calculating internal NO_3^--N concentration in the seedlings that were kept in the hatchery, *DM* was based on a general assumption of 10 % dry matter because the dry matter of the seedlings was not analyzed.

2.4.4 Determining amino acid content by HPLC

Samples with *S. latissima* were freeze dried prior to analysis (see Section 2.4.2). Freeze dried sample (50-100 mg) was mixed with 2 mL of 6 M HCl containing 4 % mercaptoethanol in a kimax tube. The samples were incubated at 110°C for 24 hours. After 24 hours the samples were cooled down and neutralized to pH 1.5-3.0 by adding approximately 2.l mL of 5 M NaOH. The sample was filtered with a GFC Whatman filter (55 mm ∅) into a 10 mL flask. Diluent (Sodium Diluent Na220, pH 2.2 from Pickering) was used to flush the filter and also used to fill the flask to the 10 mL mark. The samples were diluted 1:1 or 2:1 with diluent. The analysis was performed by High Performance Liquid Chromatography (HPLC) by staff at SINTEF Ocean.

2.4.5 Calculating nitrogen-to-protein conversion factors (K_p)

Equation 7 was used to calculate the specific nitrogen-to-protein conversion factors (K_p) in *S. latissima* according to Mosse (1990):

$$K_p = \frac{AA * 1.1}{N} \quad (7)$$

Where *AA* indicates the sum of amino acid residues in % of DM (the sum of amino acids after subtracting the molecular weight of water). *N* indicates the total N content (% of DM) calculated from Equation 4. The sum of the amino acids was multiplied by 1.1 to correct for the amino acids that were excluded from the HPLC analysis due to destruction during acid hydrolysis (Watanabe et al., 1983; Øie and Olsen, 1997). Calculation of all the factors can be found in Appendix III. The estimated protein content was determined by multiplying total % N of DM with K_p (Appendix III).

2.4.6 Figures and statistical analyses

Shapiro Wilk's test was performed to determine normal distribution. The null hypothesis of this test is that the group is normally distributed, making the groups not considered normally distributed when $p < 0.05$. Independent sample T-test for differences in mean lamina length between depths for each location was used in cases of normal distribution. Mann Whitney U test between depths was used where normal distribution could not be verified. One-way analysis of variance (ANOVA) with post hoc test (Bonferroni) for significant differences in mean lamina length between weeks was used in cases of normal distribution. Kruskal Wallis test with post hoc test (Bonferroni) was used to find significant differences in mean lamina length between groups where no normal distribution was found. Samples were considered statistically different when $p < 0.05$. The statistical analyses were carried out in IBM SPSS Statistics (version 25). The scatterplots were made in IBM SPSS Statistics (version 25). The light and temperature graphs were prepared by Ole Jacob Broch at SINTEF Ocean. The maps were made in Esri® ArcMap™ version 10.5.0.6491. All the rest of the graphs were made in RStudio Version 1.0.143.

3 Results

3.1 Temperature and light data

Figure 3.1 shows the sea temperature at the different locations (Table 2.1) during the experiment. All locations showed an increase in temperature during the course of the season. APN measured the lowest temperatures from May and throughout the rest of the experiment, with a maximum registered temperature at approximately 9-10°C in July/August. ASF and NSS measured the highest temperatures from May and throughout the sampling season, with a maximum temperature at around 17-18°C in July. A maximum temperature of approximately 15°C was measured in August at SES. The largest difference in temperature between depths was found at SAL.

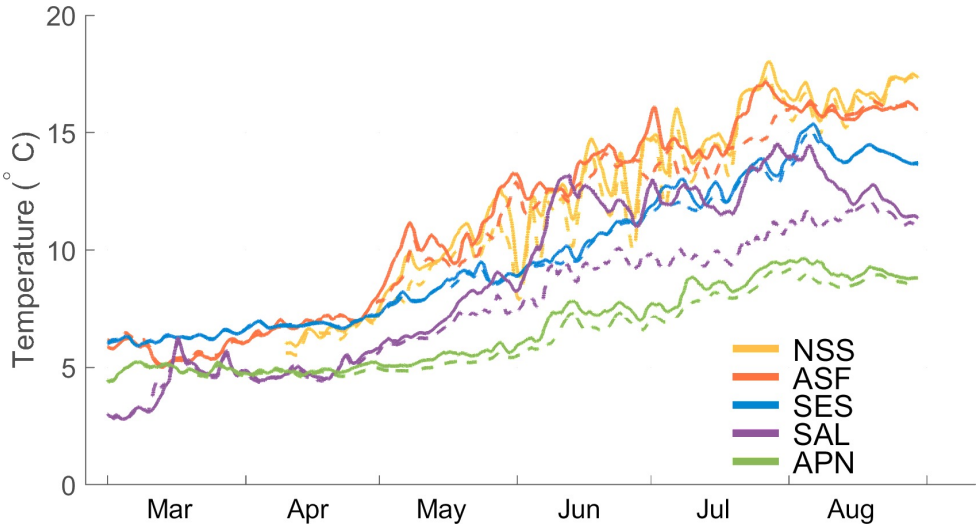

Figure 3.1. Temperature (°C) at the experimental locations from March to August. The solid lines represent the temperatures at 2 m depth, while the dotted lines represent the temperatures at 6 m depth. The lines show a 24 hour running mean.

Figure 3.2 shows the photosynthetic active radiation (PAR) at 2 and 6 m depth for the locations during the season. All locations showed lower PAR at the two depths in the beginning and in the end of the sampling season. Higher PAR was found at 2 m depth compared to 6 m depth at all locations during the whole growth season. The largest difference in PAR between the depths was found at ASF. The two southern locations (ASF and NSS) showed higher PAR early in the season and lower PAR late in the season compared to the three locations further north.

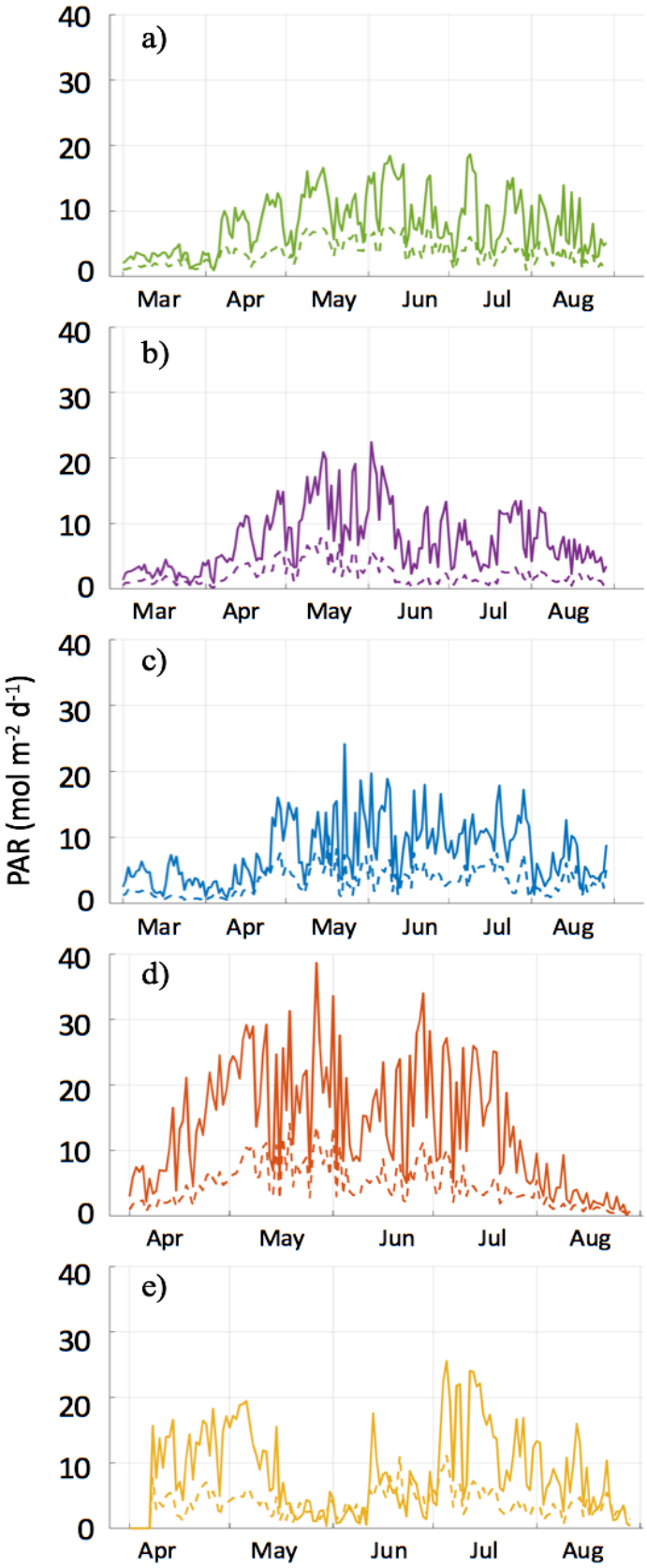

Figure 3.2. Photosynthetic active radiation (PAR) (mol m^{-2} d^{-1}) at a) APN, b) SAL, c) SES, d) ASF and e) NSS from March to August. The solid lines represent the PAR at 2 m depth, while the dotted lines represent the PAR at 6 m depth. The lines show a 24 hour running mean.

3.2 Dry matter and ash content

The dry matter contents of wet weight, calculated from Equation 2, at 1-2 and 8-9 m depths are shown in Figure 3.3. In general, lower dry matter contents in *S. latissima* were found at 8-9 m depth compared to 1-2 m depth. The variation between the locations was higher at 1-2 m depth. The highest percentage of dry matter through the sampling period at 1-2 m depth was found at SAL and SES (Figure 3.3a). At 8-9 m depth, all locations showed an increase in dry matter from start to end of the sampling period (Figure 3.3b). The average dry matter contents of wet weight in *S. latissima* for all locations during the period ranged from 10.4 ± 0.4 % to 23.6 ± 2.6 % at 1-2 m depth and from 8.1 ± 1.6 to 19.2 ± 4.1 at 8-9 m depth (Appendix I, Table I.I).

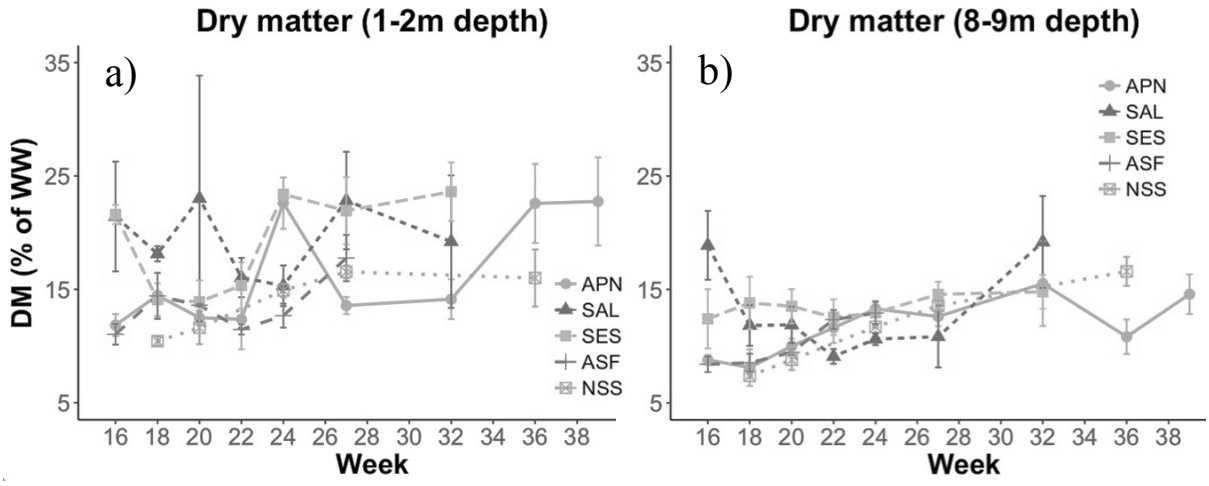

Figure 3.3. The dry matter (% of wet weight) in *S. latissima* at all locations at a) 1-2 m depth and b) 8-9 m depth during weeks 16 (mid-April) to 39 (September/October). Symbols indicate means of 3 replicate measurements (n = 3) except for in b) where n = 2 at ASF and APN in week 18 and n = 1 at APN in week 16. Error bars indicate ± 1SE.

Figure 3.4 shows the ash content as % of dry matter in *S. latissima* at all experimental locations at 1-2 and 8-9 m depth. The ash content of dry matter was calculated from Equation 3 set out in page 13. A more pronounced variation between the locations was generally found at 1-2 m depth *versus* 8-9 m depth, but the overall ash content was higher at 8-9 m depth. No clear differences were found between the locations at 8-9 m depth, but the two southern locations (ASF and NSS) showed a higher ash content in *S. latissima* at the first sampling compared to the other locations, and SAL showed deviation from the rest of the locations in the end of the season at the two depths. The overall mean ash contents of dry matter in *S. latissima* during the

period ranged from 14.6 ± 1.4 % to 42.8 ± 4.1 % at 1-2 m depth and from 28.8 ± 6.1 to 51.9 ± 0.8 at 8-9 m depth (Appendix I, Table I.I).

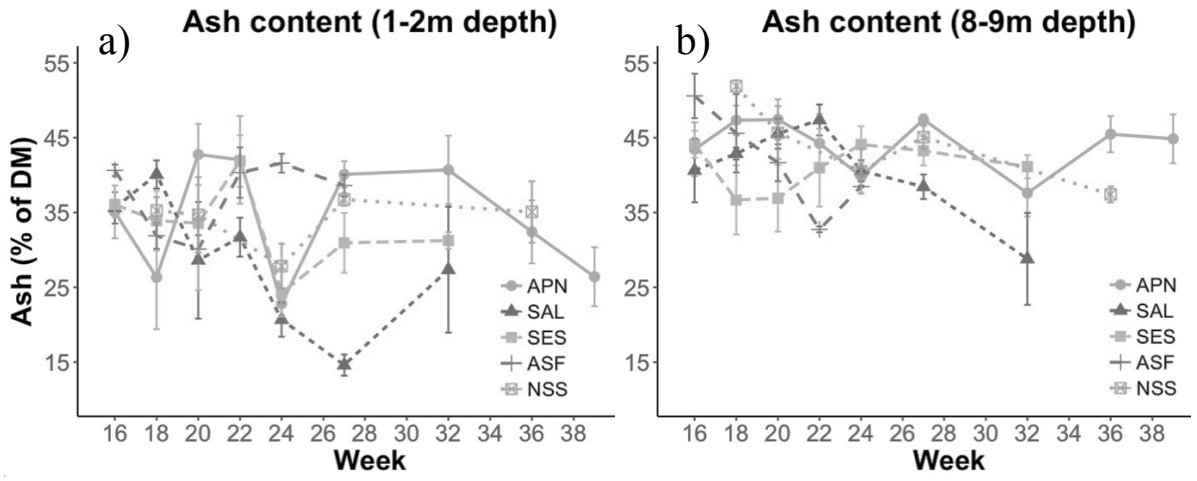

Figure 3.4. The ash content (% of dry matter) in *S. latissima* at all locations at a) 1-2 m depth and b) 8-9 m depth during weeks 16 (mid-April) to 39 (September/October). Symbols indicate means of 3 replicates (n = 3) except for in b) where n = 2 for ASF and APN in week 18 and n = 1 for APN in week 16. Error bars indicate ± 1 SE.

The dry matter contents of wet weight (Figure 3.3) and the ash contents of dry matter (Figure 3.4) showed opposite trends across the locations, suggesting that high dry matter often expressed low ash content in the same samples and low dry matter content often showed high ash content.

3.3 Growth

3.3.1 Growth measured by mean lamina length

Figure 3.5 shows the mean lamina length of *S. latissima* measured in cm for all locations during the experimental period. The general pattern showed the longest individuals at SES during the whole growth season except at the very end of the season, where the length decreased drastically. APN measured the shortest individuals at the first sampling, but the algae increased in length during the season, and the cultivation period was prolonged at this location. Figure 3.6 to Figure 3.10 illustrate visually the growth of *S. latissima* at one early and one late sampling for all locations at the two depths.

The *S. latissima* individuals at 1-2 m depth were significantly longer than at 8-9 m depth at the beginning of the sampling period (Figure 3.6 to Figure 3.10; $p < 0.05$, Appendix I Table I.II), while there were less differences in length between the depths later in the growth season at all locations. The exception was ASF, where significantly longer individuals were found at 1-2 m depth during the whole sampling period ($p < 0.05$, Appendix I Table I.II). Later in the season, significantly longer individuals at 8-9 m depth compared to 1-2 m depth at the two northern locations (APN and SAL) was found ($p < 0.05$, Appendix I Table I.II).

All five locations showed a decrease in mean lamina length in *S. latissima* at the two depths at the end of the season, except for SAL at 8-9 m depth, where the individuals showed an increase in length from week 27 (early July) to week 32 (early August). Pictures of the individuals at SAL are shown in Figure 3.7. Significant differences between weeks at all locations from the earliest samplings to the latest samplings was found ($p < 0.05$, Appendix I Table I.III). The length of the *S. latissima* individuals at the different locations were significantly different from each other in week 22 (mid-May) and week 27 (early July) ($p < 0.05$, Appendix I Table I.IV) when not taking the different depths into consideration.

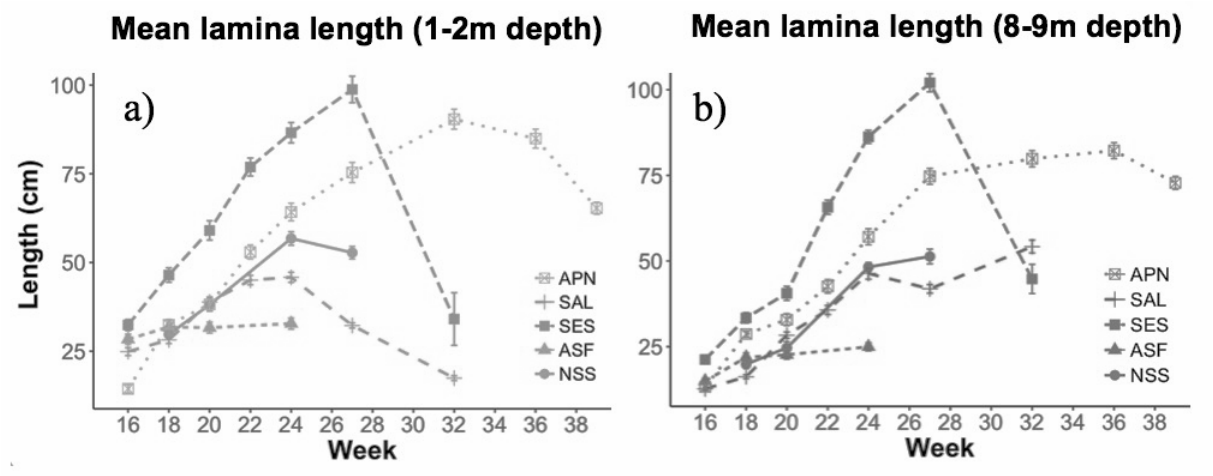

Figure 3.5. The mean lamina length (cm) in *S. latissima* at all the locations at a) 1-2 m depth and b) 8-9 m depth during week 16 (mid-April) to 39 (end of September). Points indicate the mean value of 50 individuals ($n = 50$) except for at SAL in week 16 where $n = 45$ at both depths. $n = 30$ at SES in week 32 at 8-9 m depth, and at 1-2 m depth, $n = 46$, 40, 40, 40, and 10 in week 18, 22, 24, 27 and 32, respectively. At NSS, $n = 49$ in week 20 at 8-9 m depth. Error bars indicate ± 1 SE.

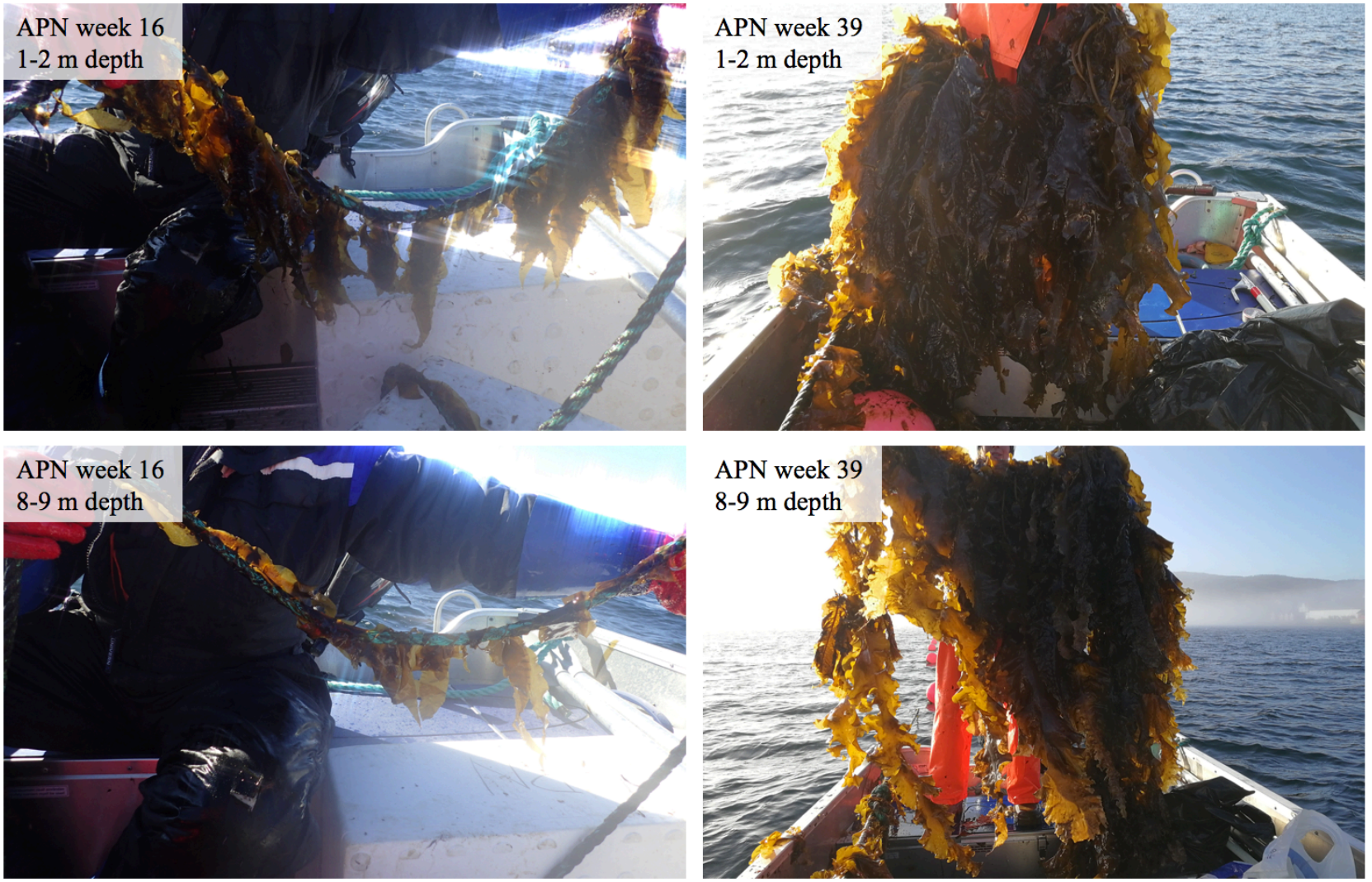

Figure 3.6. Ropes with *S. latissima* at the northernmost location (APN) at the two depths in week 16 (mid-April) and 39 (September/October). Photo: Akvaplan-niva

Figure 3.7. Ropes with *S. latissima* at the second northernmost location (SAL) at the two depths in week 16 (mid-April) and 32 (early August). Photo: Salten Algae

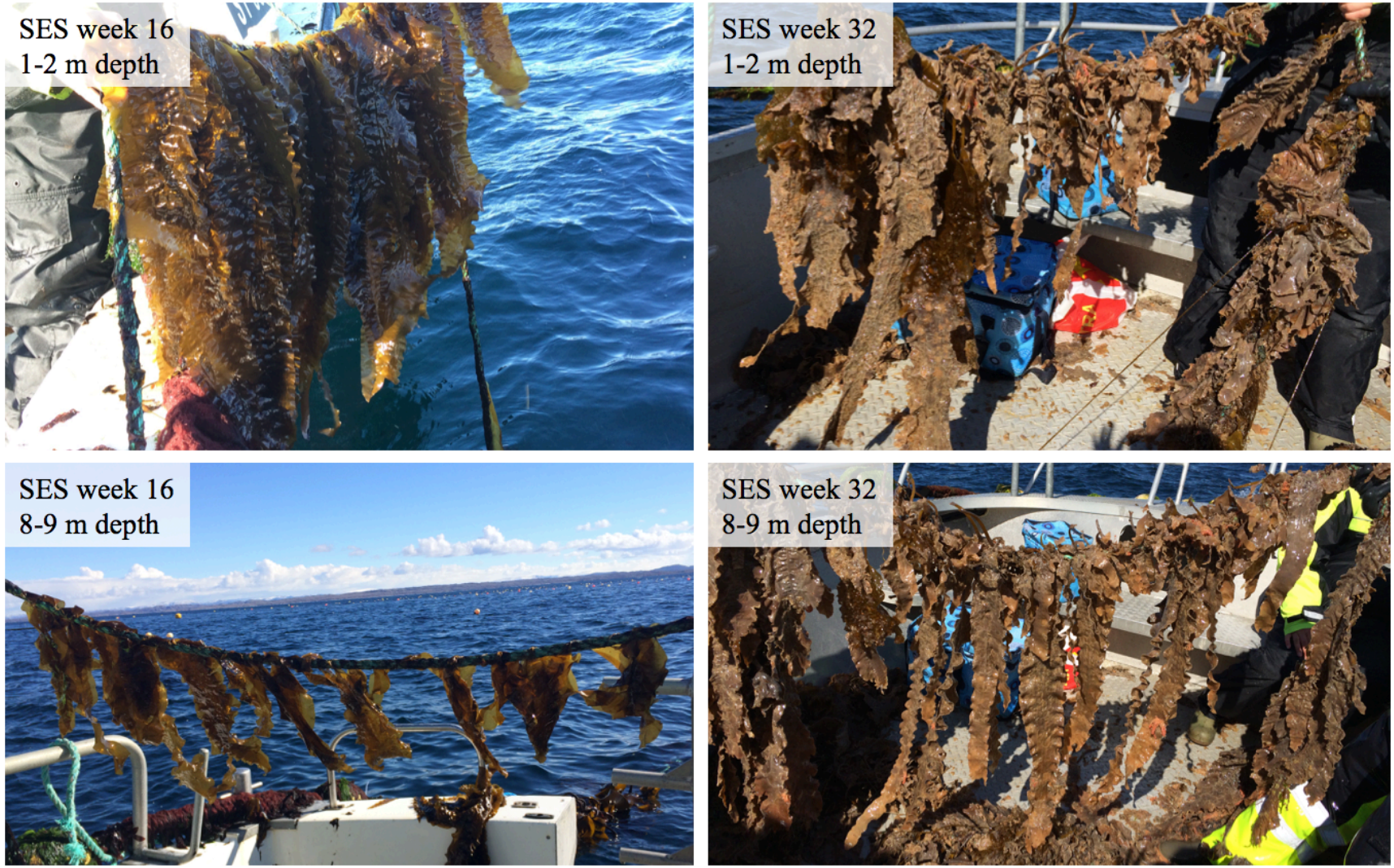

Figure 3.8. Ropes with *S. latissima* at the location in Central Norway (SES) at the two depths in week 16 (mid-April) and 32 (early August). Photo: NTNU/SINTEF/Guri Ellila Brodahl

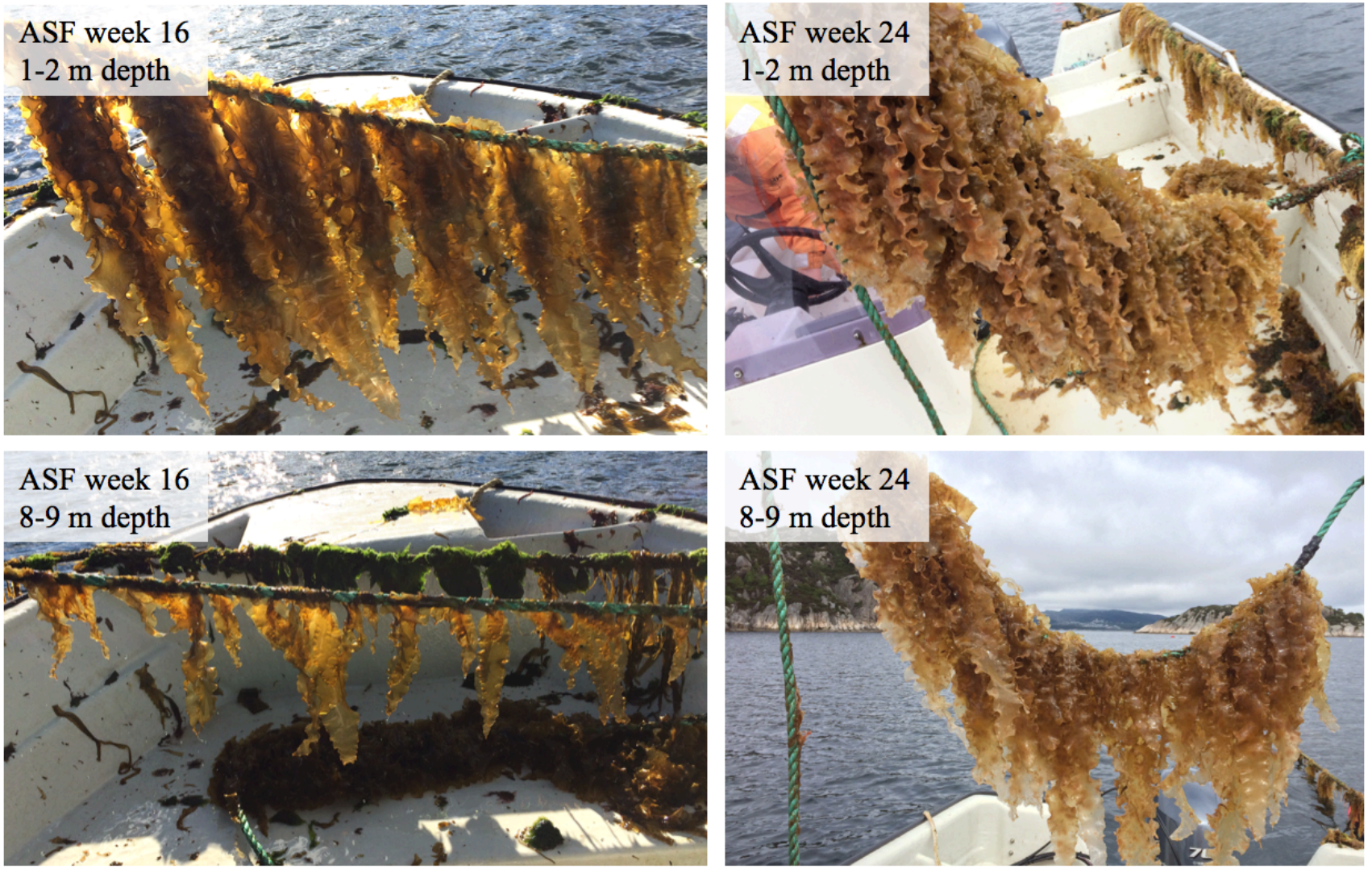

Figure 3.9. Ropes with *S. latissima* at the second southernmost location (ASF) at the two depths in week 16 (mid-April) and 24 (mid-June). Photo: SINTEF/Guri Ellila Brodahl

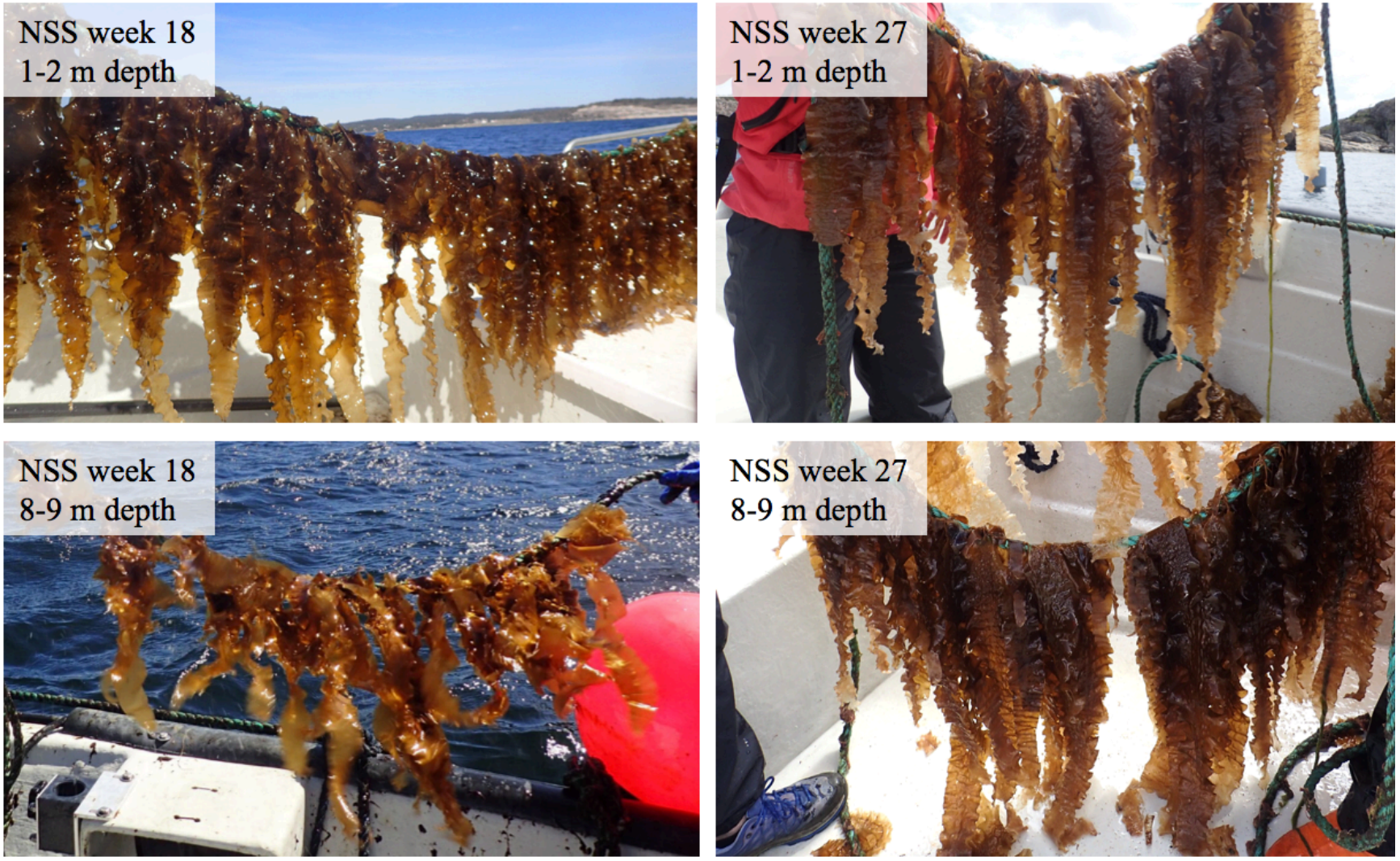

Figure 3.10. Ropes with *S. latissima* at the southernmost location (NSS) at the two depths in week 18 (early May) and 27 (early July). Photo: SINTEF/Norway Seaweed

3.3.2 Relative growth rate

Figure 3.11 shows the relative growth rate per day (RGR day^{-1}) in *S. latissima* calculated from Equation 1 at the two depths. A decreasing trend in RGR with time of the season was found at all locations. SAL showed an increase in RGR at 8-9 m depth at the last sampling. A steady decrease in RGR at the end of the season was found at APN, while the RGR at SES decreased more rapidly at the two depths. A more pronounced variation in RGR between the locations and during the season was found at 8-9 m depth (Figure 3.11b).

The highest RGR in *S. latissima* was registered at SAL at 8-9 m depth between mid-May (week 20) and the turn of the month (week 22) as illustrated in Figure 3.11b. The highest registered RGR at 8-9 m depth was found at the two northern locations (APN and SAL).

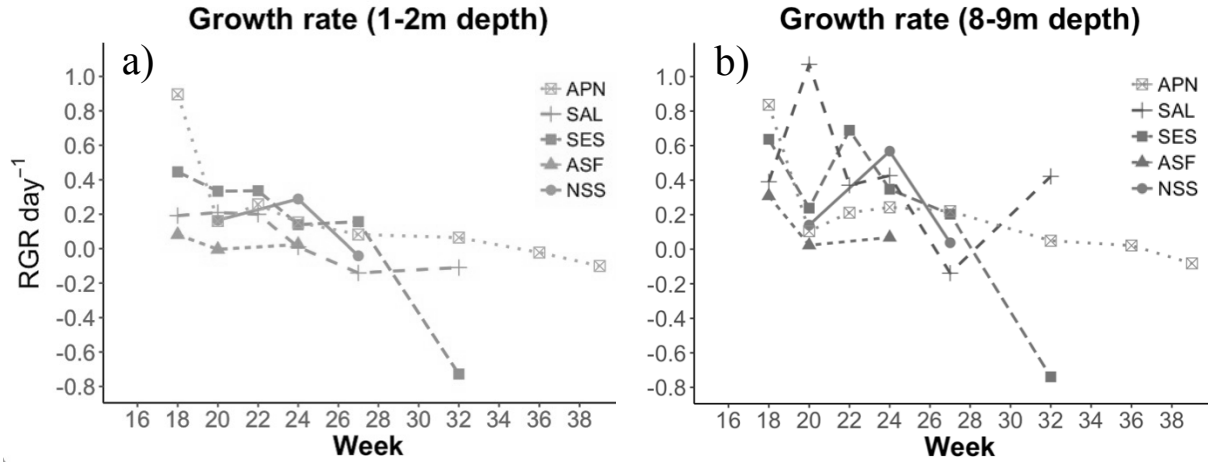

Figure 3.11. The relative growth rate per day (RGR day^{-1}) of *S. latissima* at all the locations at a) 1-2 m depth and b) 8-9 m depth during week 18 (early May) to 39 (end of September). Each symbol represents the mean of the mean of 50 individuals.

3.4 Nitrogen content (N)

Figure 3.12 illustrates the total N content (mg N g^{-1} DM) in *S. latissima* for the two depths at all the locations during the experimental period. The trends were similar across the locations at the two depths. A general finding was the decrease in N contents in the beginning of the sampling season prior to an increase later in the sampling season. The increase in total N was found earlier at the southern locations compared to the locations further north.

Less variation in the N content in *S. latissima* was found at 8-9 m depth compared to at 1-2 m depth. All locations showed a similar pattern in the total N contents at the two depths, with a decrease early in the season followed by an increase in the end of the season, even though each location differed somewhat from each other (Figure 3.12). Overall, less seasonal variation in the total N at the two northern locations was found compared to the two southern locations. Regardless of depth, the highest total N contents of DM in *S. latissima* was found at SES and APN in the beginning of the sampling period while the highest total N contents were found at NSS at the end of the sampling period.

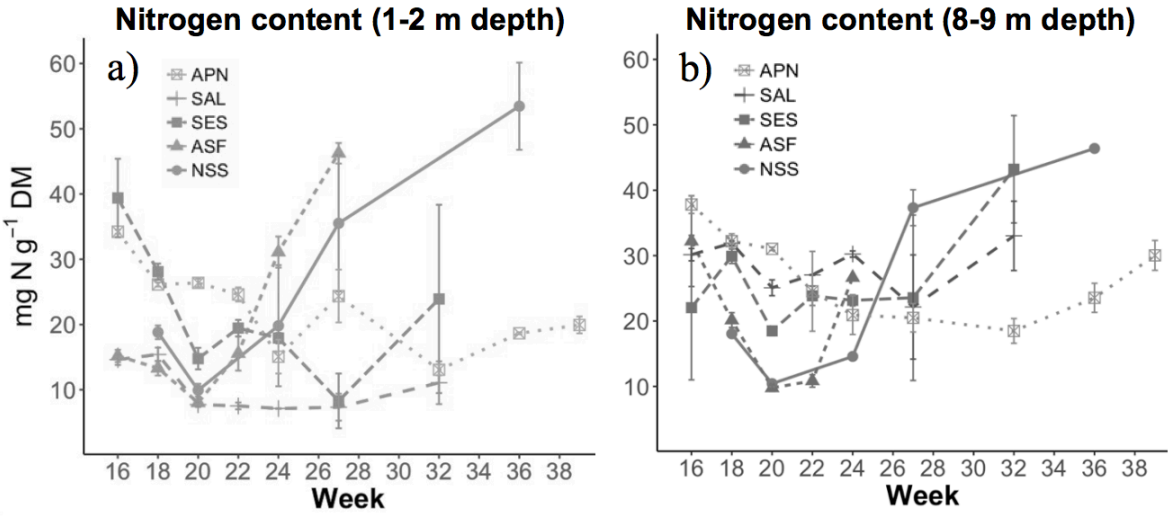

Figure 3.12. The total nitrogen content in *S. latissima* expressed as mg per g dry matter (mg N g^{-1} DM) at all the locations at a) 1-2 m depth and b) 8-9 m depth during week 16 (mid-April) to 39 (end of September). Each symbol represents the mean of 3 replicates (n = 3) except in a) where n = 2 at SES week 27 and 32 and NSS week 36. In b) n = 2 at SES week 16 and 27 and NSS week 24 and 36. Error bars indicate ± 1 SE.

3.4.1 Nitrogen-to-carbon ratio

Figure 3.13 shows the nitrogen-to-carbon ratio (N:C) in *S. latissima* at all the locations at the two depths during the experimental period. The patters of variation were similar to that of the total N, with an earlier increase in the N:C ratio in the south compared to the north. Some minor differences between the total N content and the N:C ratio were found.

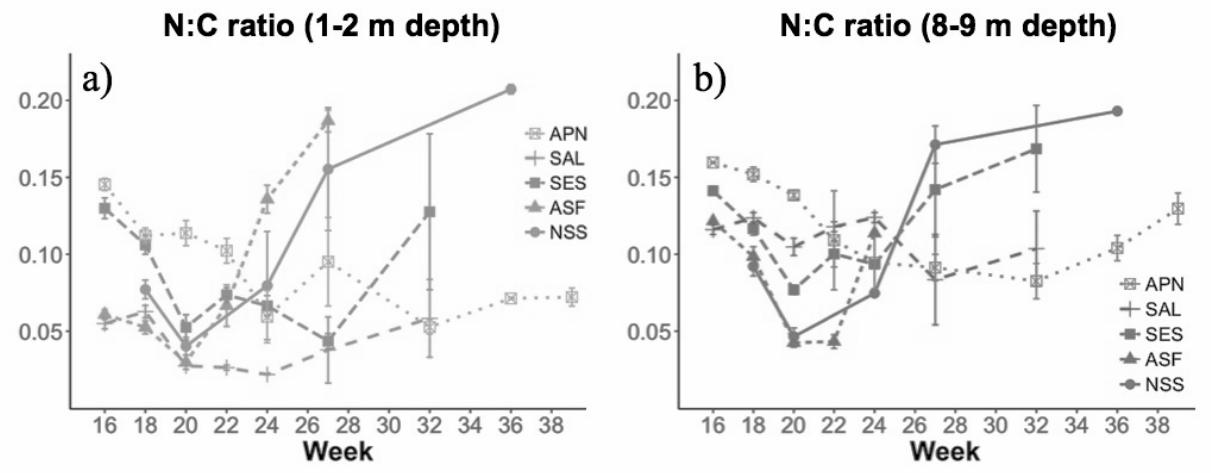

Figure 3.13. Nitrogen-to-carbon ratio (N:C) in *S. latissima* at all the locations at a) 1-2 m depth and b) 8-9 m depth during week 16 (mid-April) to 39 (end of September). Each symbol represents the mean of 3 replicates (n = 3) except in a) where n = 2 at SES week 27 and 32 and NSS week 36. In b) n = 2 at SES week 16 and 27 and NSS week 24 and 36. Error bars indicate ± 1 SE.

3.4.2 Internal NO_3^--N

Figure 3.14 shows the internal nitrate (NO_3^--N) in *S. latissima* calculated from Equation 6 at the two depths at all locations during the sampling period. The internal NO_3^--N in the seaweed tissue indicated a more pronounced seasonal trend at 1-2 m depth than at 8-9 m depth, with a higher concentration in the start of the sampling period and lower at the end. The strongest seasonal pattern of variation in internal NO_3^--N in *S. latissima* was found at APN, SAL and SES, while weaker seasonal variation was found at ASF and NSS. After mid-June (week 24), all locations showed lower concentrations at 1-2 m depth than at 8-9 m depth. The highest variation in NO_3^--N contents between weeks was found at APN, which also showed the highest concentration at both 1-2 and 8-9 m depth, measuring 0.20 ± 0.03 and 0.25 ± 0.07 mg NO_3^--N g^{-1} DM respectively. All locations except SAL and SES showed higher concentrations of NO_3^--N at 1-2 m depth compared to at 8-9 m depth at the first sampling (Figure 3.14b).

The internal NO_3^--N in the *S. latissima* seedlings in the lab showed different values between the locations (grey box, Figure 3.14a). The highest value of 0.28 ± 0.03 mg NO_3^--N g^{-1} DM in the seedlings was found at the southernmost location (NSS). Higher concentration of internal NO_3^--N in the seedlings than in the sporophytes that were growing in the sea was found at all locations. Mean internal NO_3^--N values with standard errors are given in Appendix II.

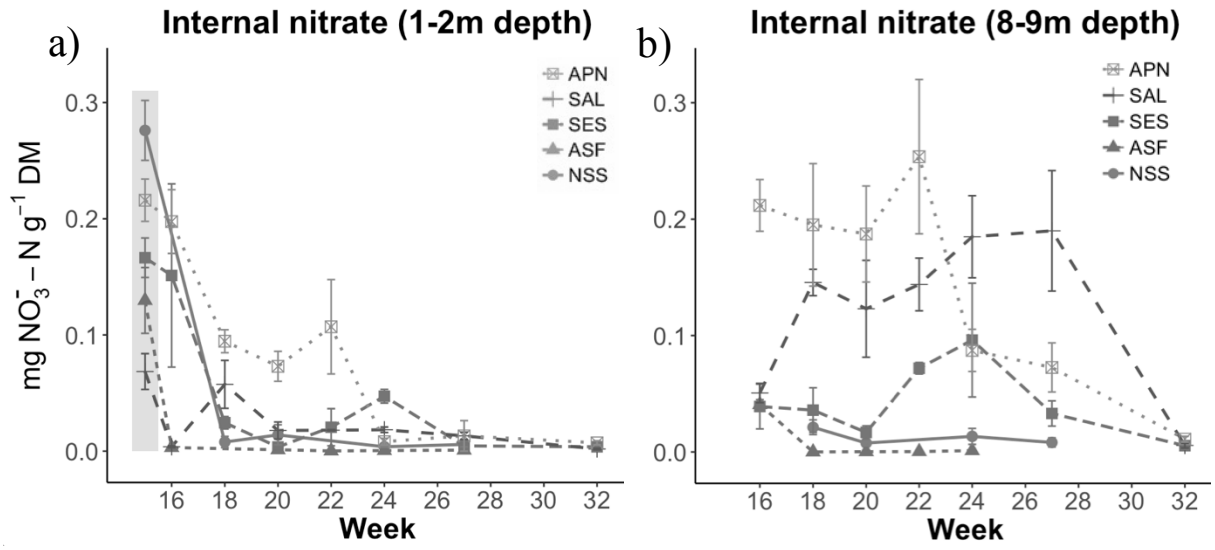

Figure 3.14. The internal NO_3^--N concentration per g dry matter (mg NO_3^--N g^{-1} DM) in *S. latissima* at all locations at a) 1-2 m depth and b) 8-9 m depth during week 16 (mid-April) to 32 (early August). Each symbol represents the mean of 3 parallels (n = 3). Error bars indicate ± 1 SE. The grey box in a) indicate the internal NO_3^--N concentration in the seedlings in the hatchery.

3.5 Relative growth rate as a function of internal NO_3^--N

Figure 3.15 compares relative growth rate (RGR day^{-1}) to internal NO_3^--N concentration (mg g^{-1} DM) in *S. latissima* at all five locations. There was an apparent positive relationship between RGR and internal NO_3^--N concentration, but no significant correlation was found (p = 0.064 - 0.493, Appendix IV). However, a seasonal pattern of variation was found, with a clear shift from the north to the south. At the two southern locations (ASF and NSS), the RGR was at its highest at the lowest registered NO_3^--N concentrations (0.00-0.01 mg NO_3^--N g^{-1} DM). At SES, the values were more spread, but most measurements showed low internal NO_3^--N concentration, and the highest growth rate in *S. latissima* was measured at around 0.05 mg NO_3^--N g^{-1} DM. The RGR did not increase with increasing NO_3^--N concentration at the second northernmost location (SAL), and the same pattern was found at APN, except for a high RGR value at approximately 0.10 mg NO_3^--N g^{-1} DM.

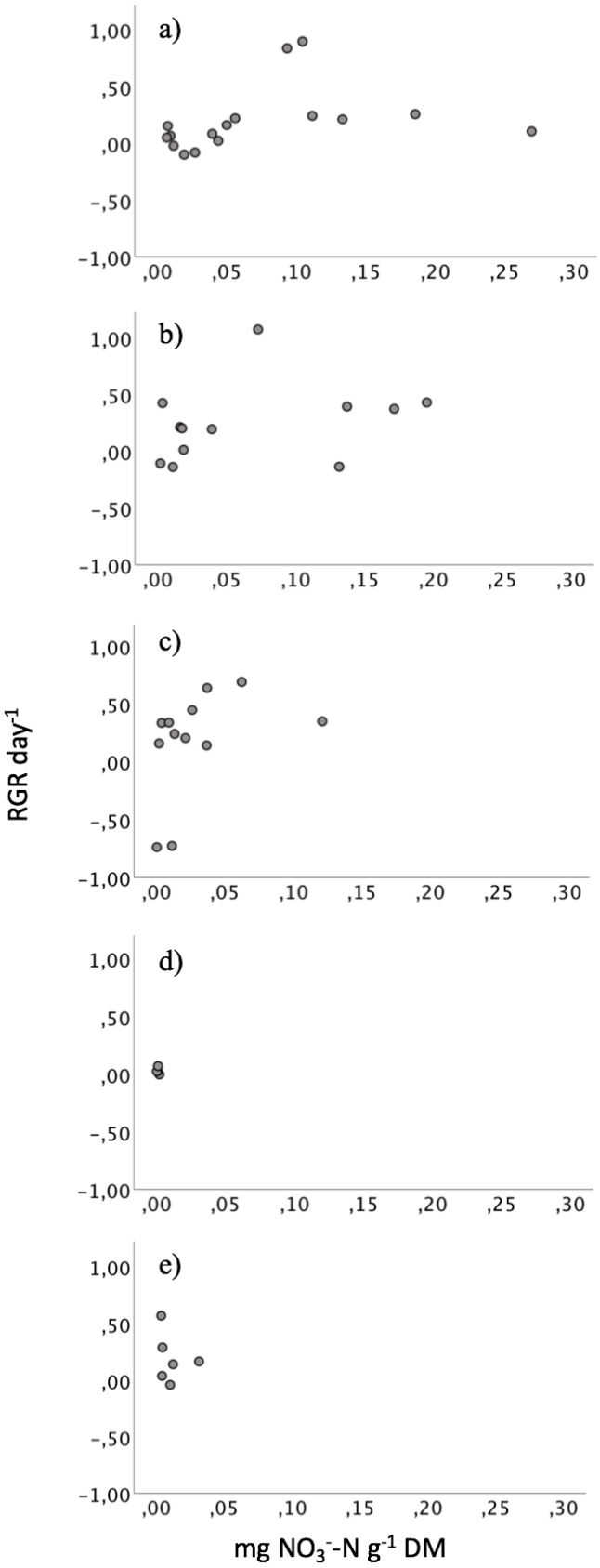

Figure 3.15. The relative growth rate (RGR day^{-1}) plotted as a function of the internal NO_3^--N (mg NO_3^--N g^{-1} DM) in *S. latissima* at a) APN, b) SAL, c) SES, d) ASF and e) NSS. Each point indicates the mean of the two depths.

3.6 N:C ratio as a function of internal NO_3^--N

The internal NO_3^--N concentration (mg g^{-1} DM) as a function of N:C ratio in *S. latissima* at all locations is illustrated in Figure 3.16. A seasonal pattern of variation was found, with a clear shift from north to south. A clear pattern was found at the two northern locations, APN and SAL, with a significant increase in internal NO_3^--N concentration with increasing N:C ratio in *S. latissima* ($p < 0.05$, Appendix IV). A similar pattern was found at the SES location, although weaker and with decreasing internal NO_3^--N concentration with a N:C ratio > 0.1 ($p = 0.032$, Appendix IV). The two southern locations showed no significant correlation between internal NO_3^--N and N:C ratio in *S. latissima*, and the internal NO_3^--N concentration at both locations was low regardless of N:C ratio ($p > 0.05$, Appendix IV).

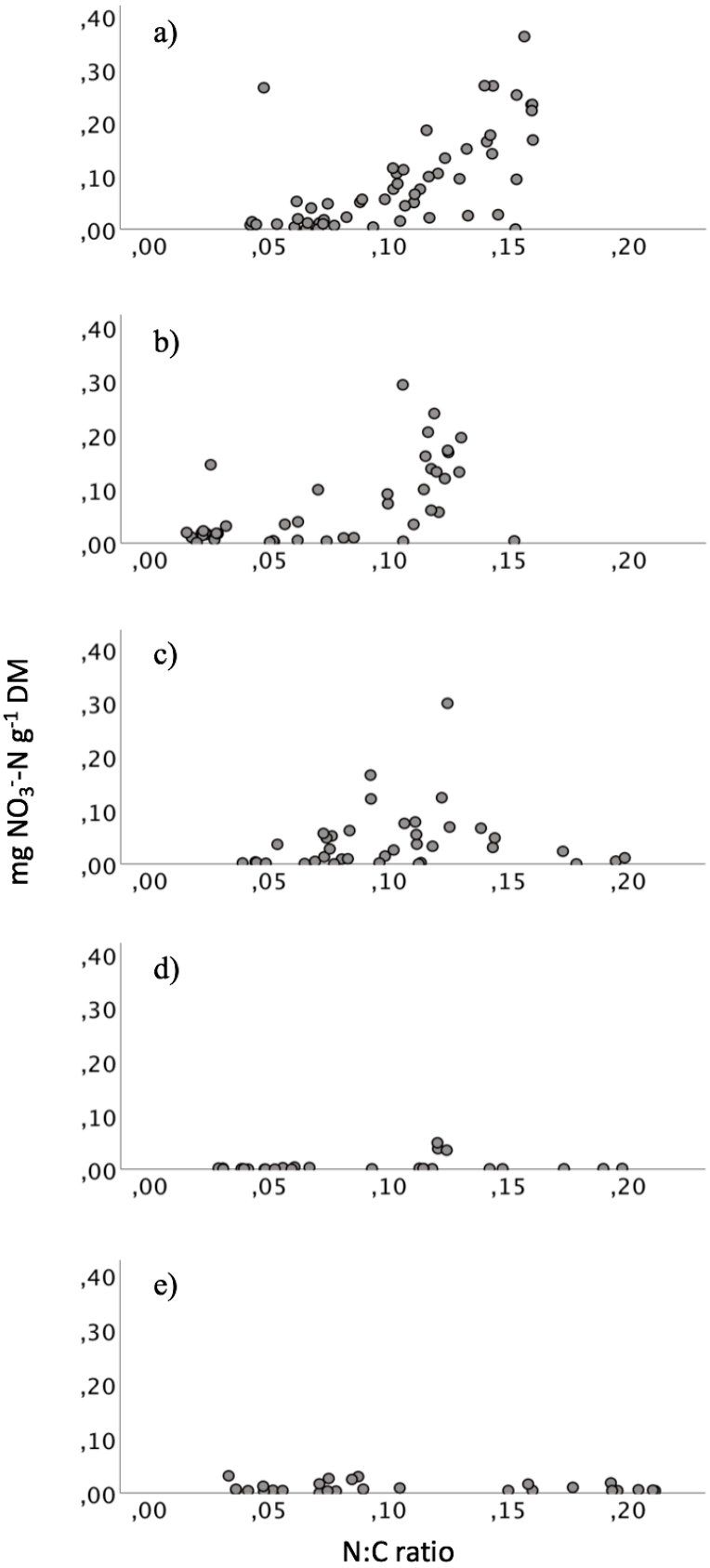

Figure 3.16. The internal NO_3^--N (mg NO_3^--N g^{-1} DM) plotted as a function of the N:C ratio in *S. latissima* at a) APN, b) SAL, c) SES, d) ASF and e) NSS. Each point indicates the mean of the two depths.

3.7 Total amino acid residues

Figure 3.17 illustrates the mean amino acid residues expressed in terms of % of DM in *S. latissima* at the two depths at all locations. Amino acid residues are amino acids minus a water molecule. The general pattern was a decrease in the sum of amino acid residues followed by an increase at ASF and NSS. The content of amino acid residues at APN continued to decrease during the period. The amount of amino acid residues increased earlier at ASF than at SES, and a more rapid increase in amino acid residues was found at 1-2 m depth compared to 8-9 m depth. Higher total content of amino acid residues in *S. latissima* was registered at 8-9 m depth compared to 1-2 m depth at all locations except ASF. The values for the amino acid residues at all locations are listed in Appendix III.

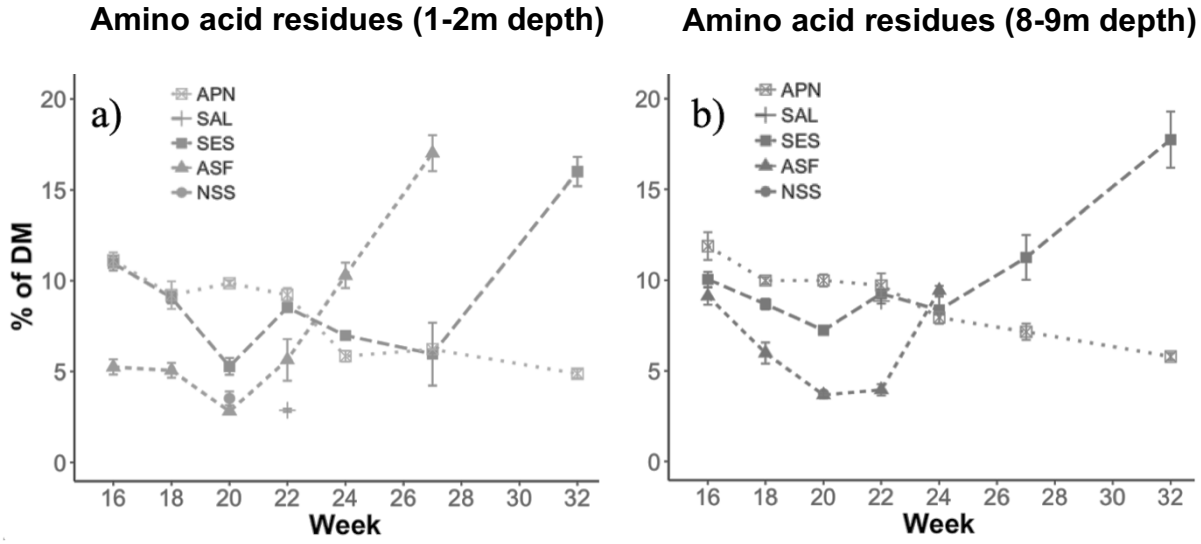

Figure 3.17. Total amino acid residues (% of DM) in *S. latissima* at all locations during week 16-32 (mid-April to early August) at a) 1-2 m depth and b) 8-9 m depth. Each symbol represents the mean of 3 parallels (n = 3) except in a) where n = 2 at SES in week 27 and ASF in week 16 and n = 1 at SES in week 32. In b) n = 2 at APN in week 24, SES in week 16 and 27, ASF in week 18 and NSS in week 20. Error bars indicate ± 1 SE.

3.8 Nitrogen-to-protein conversion factor (K_p)

Specific nitrogen-to-protein conversion factors (K_p) for *S. latissima* were calculated from Equation 7. Table 3.1 shows the seasonal pattern of variation in K_p, with an increasing K_p from mid-April (week 16) to May/June (week 22) followed by a decrease in K_p from May/June to early July (week 27) and an increase from early July to early August (week 32). K_p in *S. latissima* from all locations combined varied from 3.6 ± 0.3 to 4.3 ± 0.2 at 1-2 m and from 3.3 ± 0.2 to 4.3 ± 0.8 at 8-9 m depth, with average values of 3.9 ± 0.1 and 3.8 ± 0.1, respectively. The overall average was 3.9 ± 0.1. The K_p values for all locations are listed in Appendix III. A regression analysis of K_p as a function of time in *S. latissima* showed weak or no significant relationship between the two variables (p = 0.036 - 0.749, Appendix IV).

Table 3.1. Nitrogen-to-protein conversion factors (K_p) at 1-2 m depth, 8-9 m depth, and both depths combined for each week from mid-April (week 16) to early August (week 32).

Depth	Week 16	Week 18	Week 20	Week 22	Week 24	Week 27	Week 32	All weeks
1-2 m	3.6 ± 0.2	3.9 ± 0.2	4.0 ± 0.1	4.3 ± 0.2	4.1 ± 0.2	3.6 ± 0.3	4.0 ± 0.2	3.9 ± 0.1
8-9 m	3.3 ± 0.2	3.3 ± 0.1	4.0 ± 0.1	4.2 ± 0.3	3.7 ± 0.5	3.8 ± 0.2	4.3 ± 0.8	3.8 ± 0.1
Both	3.4 ± 0.1	3.6 ± 0.1	4.0 ± 0.1	4.3 ± 0.2	3.9 ± 0.3	3.7 ± 0.2	4.2 ± 0.4	3.9 ± 0.1

Values are given in mean ± 1 SE.

3.9 Protein content

Figure 3.18 shows the estimated protein contents of *S. latissima* at the two depths at each location. The estimated protein content was calculated by multiplying the % total N contents of DM with the K_p calculated from Equation 7. The overall patterns were similar at the two depths, but more variation between locations and weeks was found at 1-2 m depth compared to 8-9 m depth. APN showed a decrease in protein content in *S. latissima* from the first to the last sampling at the two depths. SES showed a somewhat similar pattern of variation at the two depths, but with more variations at 1-2 m depth than at 8-9 m depths. The protein content at the last sampling at SES was higher than at the first sampling. ASF showed the same pattern of variation at the two depths even though the data from the last sampling at 1-2 m depth was missing. The protein data at SAL and NSS was obtained from one sampling prior to the occurrence of biofouling, in week 22 and 20, respectively. SAL showed differences in protein content between the two depths, with higher protein contents at 8-9 m depth than at 1-2 m depth.

NSS did not show any notable differences between the depths. The values used for calculation of protein content can be found in Appendix III. A regression analysis of RGR as a function of protein content in *S. latissima* showed no significant relationship at any of the locations (p = 0.115 - 0.224) (Appendix IV).

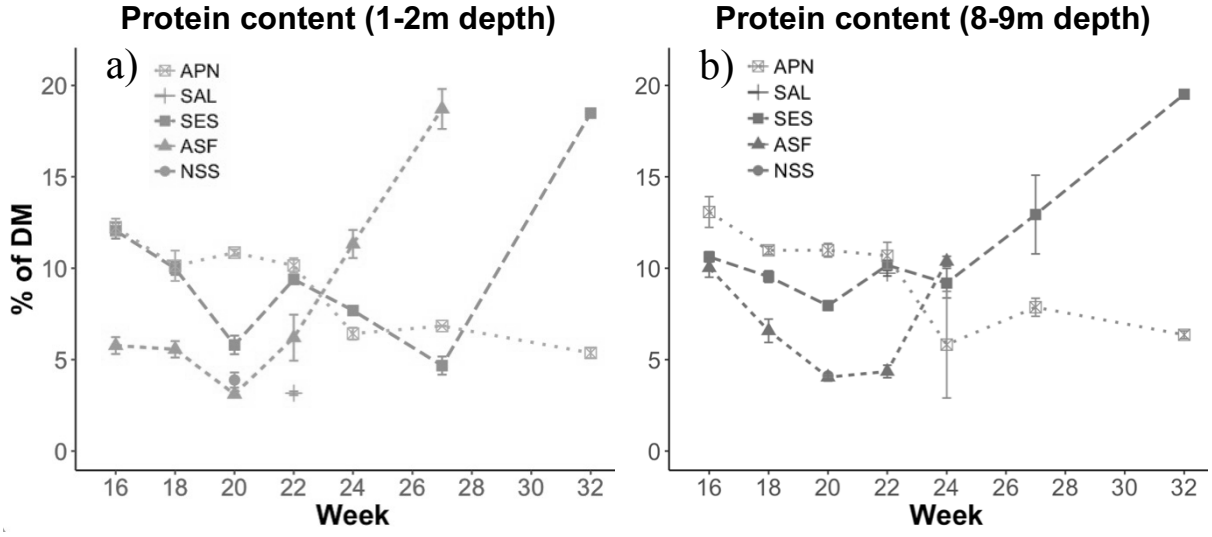

Figure 3.18. The protein content (% of DM) in *S. latissima* at all locations during week 16-32 (mid-April to early August) at a) 1-2 m depth and b) 8-9 m depth. Each symbol represents the mean of 3 parallels (n = 3) except in a) where n = 2 at SES week 27 and ASF week 16, and n = 1 at SES week 32. In b) n = 2 at APN week 24, SES week 16 and 27, ASF week 18 and NSS week 20. Error bars indicate ± 1 SE.

3.10 Protein content as a function of internal NO_3^--N

Figure 3.19 illustrates the estimated protein content (% of DM) as a function of internal NO_3^--N (mg g^{-1} DM) in *S. latissima*. A significant correlation between these two variables was found at APN and SES, with higher protein content at higher internal NO_3^--N concentrations (p < 0.05, Appendix IV). The four points showing the highest protein content values in Figure 3.19b were removed prior to regression analysis due to heavy biofouling (Figure 3.8). At ASF, no significant correlation between protein content and internal NO_3^--N was found (p = 0.818, Appendix IV). Here, protein content ranged from around 0 to 25 % of DM while internal NO_3^--N concentrations were constantly low, and biofouling was observed at this location late in the season (Figure 3.9).

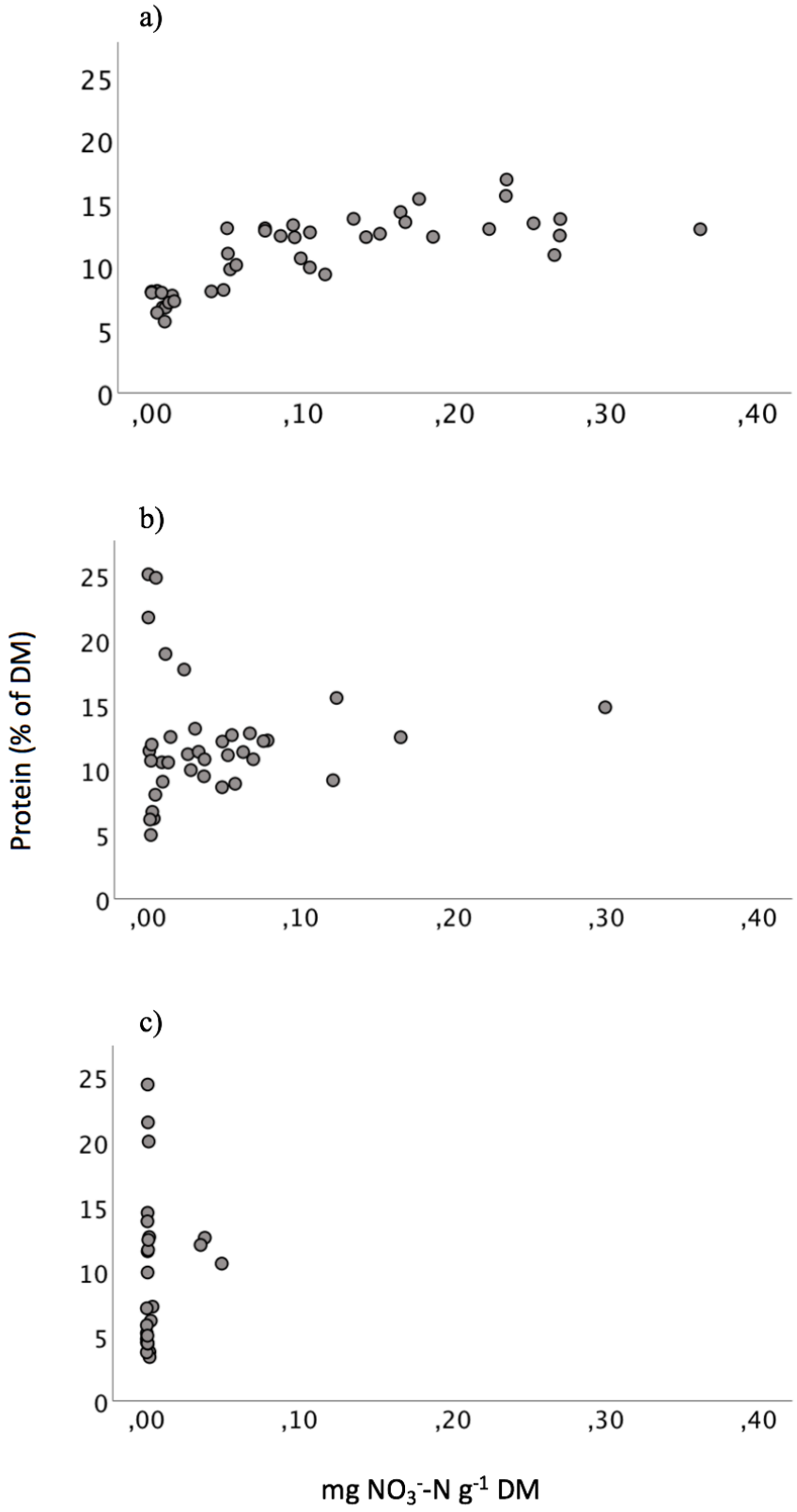

Figure 3.19. Protein content (% of DM) plotted as a function of internal NO_3^--N (mg g^{-1} DM) in *S. latissima* at a) APN, b) SES and c) ASF. Each point indicates the mean of the two depths.

4 Discussion

The temperature increased during the season at all the experimental locations (Figure 3.1), and there was found higher photosynthetic active radiation (PAR) at 1-2 m depth than at 8-9 m depth (Figure 3.2). Higher dry matter (DM) was found at 1-2 m depth (Figure 3.3), while the ash content was highest at 8-9 m depth (Figure 3.4), irrespective of time. The production period was longer in the north than in the south, and losses of biomass was found in the end of the experimental period in the south. A positive relationship, but no significant correlation between internal NO_3^--N and relative growth rate (RGR) was found at the locations (Figure 3.15; Appendix IV). The total nitrogen (N) and N:C ratio showed similar seasonal patterns for all locations with decreasing values early in the experimental period and increasing values in the end of the period, suggesting that these variables interact (Figure 3.12; Figure 3.13). A similar pattern of variation as for total N and N:C ratio was found for the amino acid residues and protein content for all locations except for APN (Figure 3.17; Figure 3.18). Variations through the season, yet no significant relationship with time was found for the nitrogen-to-protein conversion factor (K_p). A stronger seasonal pattern of variation in internal NO_3^--N concentrations was found at the three northern locations (APN, SAL and SES) than at the southern locations (ASF and NSS), and higher concentrations were found at 8-9 m depth (Figure 3.14). The concentrations of internal NO_3^--N correlated significantly with N:C ratio at the three northern locations (APN, SAL and SES) (Figure 3.16; Appendix IV, $p < 0.05$), and with the protein content at the two northern locations (APN and SAL) (Figure 3.19; Appendix IV, $p < 0.05$).

4.1 The effects of latitude and season on growth and chemical content

The temperature and light results were as expected at the given latitudes, with lower temperatures in the beginning of the sampling season, and increasing during the period of the study. The PAR was lower at 8-9 m depth compared to that at 1-2 m, as expected. The sporophytes at 8-9 m depth may also have been affected by shading by individuals at 1-2 m depth if there were weak currents in the area. The PAR loggers were located on a separate rope and did only measure the light where no shading took place.

There were clear differences in mean lamina length between the locations, but no latitudinal pattern was present, which may imply that the growth most likely was more affected by local variations rather than by latitude. RGR did not indicate any clear pattern between locations, but it decreased during the season, as expected. Biofouling occurred earlier in the south than in the north, and at the end of the sampling period, the heavy biofouling had caused great loss of biomasses at ASF and SES (Figure 3.8; Figure 3.9). The loss of biomass and the rapid decrease in RGR occurred at the same time.

Differences in internal NO_3^--N in *S. latissima* and temperature was found between the locations, with the highest concentrations of internal NO_3^--N and lowest temperature at the northern locations (APN and SAL) and the lowest internal NO_3^--N concentrations and highest temperatures at the southern locations (ASF and NSS), suggesting a pattern of variation related to latitude. The results were in accordance with the study conducted by Nielsen et al. (2014), who found that internal nutrients (including NH_4^+, NO_3^- and NO_2^-) showed a variation pattern inverse of temperature. The low concentrations of internal NO_3^--N at the two southern locations (ASF and NSS) during the whole period was presumably a result of the spring bloom occurring prior to the start of the sampling period.

The patterns in N content, amino acid residues, N:C ratio and protein content that were found in *S. latissima* at each of the locations may be better explained by the seasonal variations rather than the geographical positions of the seaweed farms. However, the differences between the locations may be best explained by a latitudinal gradient. There was a decrease in these variables in the spring, which was, disregarding the rapid increase in these variables at SES, ASF and NSS in the end of the season, in accordance with previous studies (Fleurence, 1999; Nielsen et al., 2014; Marinho et al., 2015a; Marinho et al., 2015b; Schiener et al., 2015; Mols-Mortensen et al., 2017). The biofouling appearing earlier in the season further south may also have altered the results late in the season (Figure 3.6; Figure 3.7; Figure 3.8; Figure 3.9; Figure 3.10). Higher amount of proteins, N, N:C ratio and amino acids were found at 8-9 m depth compared to 1-2 m depth at all locations, which may be in relation to lower photosynthetic active radiation and temperature, as well as higher NO_3^- availability at greater depths (Figure 3.14). These results cohere with the findings from Handå et al. (2013), who suggested that *S. latissima* should be cultivated deeper than 5 meters and that the harvest time should be in early summer due to loss of biomass caused by biofouling organisms during summer.

At each of the separate locations, there were seasonal patterns in all variables measured except for the dry matter and ash. The lack of patterns in dry matter and ash could be due to losses of water during defrosting of samples or variations in carbohydrates and proteins. The strongest seasonal variation in all variables was found at the northernmost location (APN). The variations during the season may have been due to random fluctuations because the local environmental conditions varied. Weekly, or even daily samplings could have resulted in a clearer pattern of variations, with reduced high and low peaks that may have been caused by random reasons. Few seasonal variations were found at the southern locations, presuming that the spring bloom already had passed at the respective locations when the sampling period started (Braarud et al., 1958).

4.2 Factors describing the growth patterns at the cultivation locations

A positive relationship, although not significant, between RGR and internal NO_3^--N in *S. latissima* at the experimental locations was found (p = 0.064 - 0.493, Appendix IV). Previous studies have found a clear relationship between NO_3^- concentration in the seaweed tissue and growth rate (Black and Dewar, 1949; Chapman et al., 1978; Jevne, 2015). A possible explanation could be light limitation in the north, while in the south, there was sufficient light, but the waters were presumably more oligotrophic as a result of the spring bloom (Braarud et al., 1958). At SAL, the RGR remained the same regardless of internal NO_3^--N concentrations (Figure 3.15b), suggesting that another factor than N availability limited the growth at this location. SAL was located in a fjord with a narrow opening, where lower salinity in the upper water layers compared to further down in the water column may be assumed (Fagerli et al., 2015). Nielsen et al. (2016) found that N content of *S. latissima* increased with increasing salinity, which, in addition to the reduced PAR at 8-9 m depth, may be an explanation for the big difference in RGR between depths at SAL.

Figure 3.2 and 3.14 illustrate the low PAR and high concentrations of internal NO_3^--N in the beginning of the experimental period at APN, indicating light limitation early in the season. At all locations, the growth at 1-2 m depth was faster than at 8-9 m depth in the beginning of the period, while at the end of the season there were minor differences in growth between the depths (Figure 3.6; Figure 3.7; Figure 3.8, Figure 3.9; Figure 3.10). A likely explanation suggests that light limited the growth prior to the spring bloom at all locations, while the reduced NO_3^- limited

the growth subsequent to the spring bloom. It is plausible that the nutrient limitation appeared later the further north the seaweed farm was located. This is in accordance with the later increase in light and temperature at APN compared to the other locations and also in accordance with the previous studies by Chapman and Craigie (1977), Young et al. (2007) and Mols-Mortensen et al. (2017).

At APN and SES, the total N, N:C ratio, internal NO_3^--N and protein was high at the first sampling where RGR also was high, but all decreased with time during the experimental period. Both locations showed high protein content with high RGR and low protein content with low RGR, but neither APN, SES nor ASF showed significant correlation between RGR and protein content of *S. latissima* (p = 0.115 - 0.224, Appendix IV).

Using the experimental design of the present study, it was not possible to identify the environmental factor that exhibited the strongest influence on RGR of *S. latissima*. However, it seemed that the registration period started subsequent to the spring bloom at the southern locations, while the locations further north experienced limitation in growth by light early in the season. Ambient NO_3^- availability, reflected in the internal NO_3^--N concentration, presumably limited the growth in *S. latissima* earlier in the south than in the north.

4.3 Nitrogen-to-protein conversion factor (K_p) and estimated protein content

The nitrogen-to-protein conversion factor (K_p) in *S. latissima* varied with season, though a weak or no significant positive relationship with time was found (Table 3.1; p = 0.036 - 0.749, Appendix IV), indicating that using one constant factor may overestimate the protein content at one point in time and underestimate it at another. K_p was found to range from 3.6 ± 0.3 to 4.3 ± 0.2 at 1-2 m depth and between 3.3 ± 0.2 and 4.3 ± 0.8 at 8-9 m depth, with an overall average of 3.9 ± 0.1 for both depths (Table 3.1). The K_p found by Sharma et al. (2018) ranged from 4.3 to 4.6 at 3 m depth and from 4.4 to 4.7 at 8 m depth in Central-Norway, and factors of 5 and 5.3 were proposed by Angell et al. (2016) and Schiener et al. (2015), respectively, which were higher than the K_p found in the present study. Because the K_p fluctuated and showed no clear pattern with time, I suggest that protein content is determined by analysis of amino acid residues, as recommended by FAO, if possible (FAO, 2003). However, the mean K_p of 3.9 ± 0.1 is the average of the fluctuations caused by both season and latitude, and may therefore be

used if protein determination by amino acid analysis is not possible, taken into consideration that the protein content may be both under- and overestimated at certain points in time. The K_p of 3.9 ± 0.1 may result in less overestimation of proteins, but maybe also more underestimation, than the higher K_p suggested by previous studies (Schiener et al., 2015; Angell et al., 2016; Sharma et al., 2018).

Sharma et al. (2018) found K_p values that estimated protein contents ranging from 11.7 to 24.3 % in Central-Norway, and higher estimated protein content was found at 8 m depth compared to 3 m depth. Consequently, Sharma et al. (2018) suggested that the higher protein contents were a result of lower PAR. The quantities of estimated protein content in *S. latissima* in the present study ranged from 3.1 ± 1.6 % to 19.5 ± 1.7 % of DM (Figure 3.18; Appendix III). Jevne (2015) found a positive correlation between the protein content in *S. latissima* and the extracellular NO_3^- concentration. In the present study, the protein content was significantly affected by internal NO_3^--N at APN and SES ($p < 0.05$, Appendix IV), suggesting that the results from the present study may have been due to higher internal NO_3^--N early in the season and due to the contribution of amino acids from heavy biofouling late in the experimental period (Figure 3.8; Figure 3.9). Sharma et al. (2018) found higher N contents in August when also epibionts were present. The results from SAL showed higher protein content at 8-9 m depth compared to 1-2 m depth prior to week 22. After week 22, the protein content, N content and amino acid content was higher at 1-2 m depth than 8-9 m depth. These results showed similarities with the study by Nielsen et al. (2016), who found that protein content increased with increasing salinity.

It might be wise to analyze the protein quality of heavy fouled seaweed in such way that possible applications after late harvest are established. If used for human consumption it is important to harvest before the biofouling because it is not desirable to eat after. If used in fish feed, it may be harvested and used when biofouling has occurred.

4.4 Internal NO_3^--N as an indicator of the nutritional status in *S. latissima*

An argument for using the analysis of internal NO_3^--N instead of N:C ratios is the availability of the analysis. The analysis of NO_3^--N is easier to perform compared to a CN analysis, because it can be done manually with reduced need for analytical instruments, while the CN analysis requires an elemental analyzer.

The highest measured internal NO_3^--N value in this experiment (0.28 mg NO_3^--N g^{-1} DM) was lower than the highest value found in a previous study by Jevne (2015), who measured the highest value at 0,69 mg NO_3-N g^{-1} DM in seaweed that had been supplied with nutrient rich water from 100 m depth and stored at low light intensities. All locations exhibited higher concentrations of internal NO_3^--N in the seedlings in the hatchery compared to the individuals growing in the sea (Figure 3.14 and Appendix II), which was in accordance with the findings by Chapman and Craigie (1977) and Chapman et al. (1978), who proposed that *S. latissima* store NO_3^- for later growth.

The access to NO_3^- could be assumed to be limited when N:C ratio was low because N:C currently works as a proxy for the nutritional status of the seaweed. Jevne (2015) found that internal NO_3^- in *S. latissima* was related to external nitrate, making it possible to suggest that internal NO_3^--N may be used as an indicator for the nutritional state of the algae. In the present study, no water samples were taken, and as such, there was no way to relate the concentrations of internal NO_3^--N to the concentrations of NO_3^- in the seawater. However, internal NO_3^--N showed a significant relationship with N:C ratio at APN, SAL and SES (Figure 3.16 and Appendix IV, $p < 0.05$). This correlation suggests that there is a possibility of using internal NO_3^- as a proxy for the nutritional status in the seaweed. It is important to understand that changes in N:C ratio may be due to shifts in C metabolism as well as N metabolism, and therefore, it is of importance to take both C and N into consideration when assessing nutritional state by N:C ratio. Internal NO_3^--N may represent a more robust and accurate proxy for the nutritional status of the seaweed.

4.5 Practical challenges

The period of time lapsing between receiving the ropes at the respective locations and deployment of the ropes in the water, varied from location to location. SES, ASF and NSS deployed shortly after receiving the ropes, while APN stored them in a container in the sea until they were deployed in late February (week 8), which was two weeks after the other locations. According to Stévant et al. (2017a), the chemical content may be altered fast when *S. latissima* is stored in seawater tanks.

Rough weather conditions and other unforeseen circumstances prevented the industry partners from doing samplings in some weeks. The time between the samplings was relatively long and

small variations with time were not always detected. Although the same protocol was followed at all samplings, there may still have been differences in how the samplings were carried out, and high temperatures may have accelerated the breakdown of chemicals and caused some random variations in the results. The low light intensity at NSS in the end of May and in the beginning of June may be explained by biofouling, causing less light to be registered by the sensors.

Due to some analyses requiring very little biomass, more meristem or mature tissue may have dominated the sample, causing the nitrogenous contents to possibly be lower, due to lower uptake rates of N in in mature tissue than in younger tissue (Topinka, 1978; Wheeler and Srivastava, 1984). The statistical tests for some of the chemical analyses were omitted, as results can be misleading when *n* is small (Ghasemi and Zahediasl, 2012). However, the patterns in both growth rate and chemical variables in *S. latissima* were clear.

4.6 Future prospects and further studies

In the future, there will be a need for expansion of the areas where seaweed is cultivated. According to Skjermo et al. (2014), Norway has a big potential as a seaweed producing country. Whereas Norway is already known for its long coastal line, the low summer temperatures and large economic zone makes the area suitable for seaweed cultivation. Stévant et al. (2017b) stated that the most important factor, the commercial market, needs more development. There is a need to understand how to make the seaweed accumulate the desired compounds or produce more of them (Charrier et al., 2017) without being at the sacrifice of the buyers will. The harvest time for *S. latissima* will depend on when the different desired chemical components are present, and these may be shifted with the prospected rise in sea water temperatures (IPCC, 2014).

This study revealed clear seasonal patterns in the north of Norway, while the spring bloom had presumably already passed in the south. Seasonal studies like this should be performed annually and include one whole year and cover the entire coast, to see more clearly the changing variables also in the south. The advantage of *in situ* studies, compared to more controlled experiments, is that such studies reveal the actual production potential, with all environmental variables interacting.

5 Conclusion

There were differences in growth, internal NO_3^--N and protein content in *S. latissima* under the variable cultivation conditions in this study. Differences in mean lamina length between the locations indicated differences in local conditions rather than an effect of latitude, and major losses of biomass was found earlier at the second southernmost location and the location in Central Norway before any of the other locations. However, a later seasonal development in the north compared to the south, indicated an overall latitudinal gradient in the chemical variables. The relative growth rate (RGR) decreased during the season, as expected. Higher quantities contents of N, protein, amino acid residues and N:C ratio were found at 8-9 m depth compared to 1-2 m depth. A decrease in these chemical variables was found during the course of the sampling period, with an increase at the end, suggesting that the results were affected by the presence of biofouling epiphytes at the end of the season.

There was a positive, yet not significant relationship between internal NO_3^--N and RGR. High concentrations of NO_3^--N and low photosynthetic active radiation (PAR) in the beginning of the season in the north, and low NO_3^--N and high PAR in the beginning of the sampling season in the south, suggested that light limited the growth prior to the spring bloom, while the reduced NO_3^- limited the growth subsequent to the spring bloom. This cohered with the expectations that ambient nutrients follow temperature patterns inversely in temperate North-East Atlantic coastal waters.

The three northern locations showed significant correlations between internal NO_3^--N and N:C ratio. Taking into consideration the results from this study, it may be possible to introduce internal NO_3^--N as a more accurate and robust proxy for the nutritional status of *S. latissima* in Norwegian waters.

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Appendix I: Growth, dry matter and ash content

Table I.I. Overview of mean lamina length, RGR (day^{-1}), dry matter and ash content of *S. latissima* at the locations each week at each of the two depths.

Location	Week	Depth	Lamina length	RGR*	Dry matter	Ash content
APN	16	1-2 m	14.4 ± 1.2		11.8 ± 1.0	35.1 ± 3.5
		8-9 m	13.2 ± 0.7		8.8 ± 0.4	43.4 ± 3.6
	18	1-2 m	32.5 ± 1.2	0.90	14.5 ± 2.0	26.3 ± 6.9
		8-9 m	28.7 ± 1.0	0.84	8.1 ± 1.6	47.3 ± 2.0
	20	1-2 m	38.2 ± 1.6	0.16	12.5 ± 0.6	42.8 ± 4.1
		8-9 m	32.9 ± 1.8	0.10	10.0 ± 0.7	47.4 ± 2.7
	22	1-2 m	52.9 ± 2.0	0.26	12.4 ± 2.6	42.0 ± 5.9
		8-9 m	42.6 ± 2.0	0.21	11.6 ± 1.3	44.3 ± 3.9
	24	1-2 m	64.2 ± 2.5	0.15	22.6 ± 2.3	22.8 ± 0.1
		8-9 m	57.1 ± 2.3	0.24	13.3 ± 0.7	39.8 ± 1.3
	27	1-2 m	75.4 ± 2.8	0.08	13.6 ± 0.8	40.1 ± 1.8
		8-9 m	74.7 ± 2.3	0.22	12.6 ± 0.9	47.4 ± 0.7
	32	1-2 m	90.4 ± 2.8	0.06	14.1 ± 1.8	40.7 ± 4.6
		8-9 m	79.9 ± 2.4	0.05	15.5 ± 3.7	37.6 ± 3.1
	36	1-2 m	84.9 ± 2.7	-0.02	22.6 ± 3.5	32.4 ± 4.2
		8-9 m	82.2 ± 2.3	0.02	10.8 ± 1.5	45.5 ± 2.4
	39	1-2 m	65.3 ± 1.7	-0.10	22.7 ± 3.9	26.4 ± 3.9
		8-9 m	72.8 ± 1.9	-0.08	14.6 ± 1.7	44.9 ± 3.3
SAL	16	1-2 m	24.9 ± 1.1		21.4 ± 4.8	35.6 ± 2.1
		8-9 m	12.7 ± 0.5		18.9 ± 3.0	40.6 ± 4.3
	18	1-2 m	28.2 ± 1.0	0.19	18.1 ± 0.7	40.1 ± 1.9
		8-9 m	16.2 ± 0.6	0.39	11.8 ± 1.8	42.9 ± 0.8
	20	1-2 m	38.8 ± 1.5	0.21	23.0 ± 10.8	28.6 ± 7.8
		8-9 m	28.3 ± 0.9	1.07	11.9 ± 1.6	45.5 ± 2.0
	22	1-2 m	45.0 ± 1.3	0.20	16.0 ± 1.7	31.7 ± 2.6
		8-9 m	35.7 ± 1.3	0.37	9.1 ± 0.7	47.4 ± 2.1
	24	1-2 m	45.8 ± 1.4	0.01	15.3 ± 1.8	20.7 ± 2.3
		8-9 m	46.4 ± 1.6	0.43	10.6 ± 0.5	40.6 ± 1.4
	27	1-2 m	32.3 ± 0.7	-0.14	22.8 ± 4.3	14.6 ± 1.4
		8-9 m	41.9 ± 1.3	-0.14	10.8 ± 2.7	38.5 ± 1.6
	32	1-2 m	17.4 ± 0.7	-0.11	19.2 ± 5.8	27.4 ± 8.4
		8-9 m	54.2 ± 1.9	0.42	19.2 ± 4.1	28.8 ± 6.1

SES	16	1-2 m	32.4 ± 1.6		21.6 ± 0.8	36.1 ± 1.7
		8-9 m	21.2 ± 1.2		12.4 ± 2.6	44.1 ± 1.8
	18	1-2 m	45.4 ± 2.0	0.45	14.1 ± 1.4	33.9 ± 4.0
		8-9 m	33.4 ± 1.5	0.64	13.8 ± 2.3	36.7 ± 4.6
	20	1-2 m	59.0 ± 2.7	0.33	13.9 ± 1.9	33.6 ± 8.9
		8-9 m	40.6 ± 2.1	0.24	13.5 ± 1.5	36.9 ± 4.4
	22	1-2 m	76.9 ± 2.5	0.34	15.3 ± 2.0	41.9 ± 3.4
		8-9 m	65.7 ± 2.1	0.69	12.5 ± 1.6	41.0 ± 5.2
	24	1-2 m	86.6 ± 2.9	0.14	23.4 ± 0.4	24.2 ± 3.2
		8-9 m	86.3 ± 1.9	0.35	12.9 ± 1.0	44.1 ± 2.4
	27	1-2 m	98.8 ± 3.7	0.16	21.9 ± 3.0	30.9 ± 4.0
		8-9 m	102.0 ± 2.6	0.20	14.6 ± 1.1	43.3 ± 2.0
	32	1-2 m	34.1 ± 7.4	-0.73	23.6 ± 2.6	31.2 ± 1.1
		8-9 m	44.8 ± 4.3	-0.74	14.8 ± 1.5	41.1 ± 1.6
ASF	16	1-2 m	28.4 ± 1.4		11.0 ± 0.9	40.6 ± 0.8
		8-9 m	15.0 ± 0.8		8.4 ± 0.7	50.6 ± 3.0
	18	1-2 m	31.8 ± 2.0	0.08	14.4 ± 2.0	31.9 ± 1.7
		8-9 m	21.9 ± 1.3	0.31	8.5 ± 0.8	45.6 ± 5.2
	20	1-2 m	31.6 ± 1.5	0.00	13.6 ± 0.1	30.1 ± 1.9
		8-9 m	22.6 ± 1.3	0.02	9.5 ± 0.2	41.7 ± 2.5
	24	1-2 m	2.8 ± 1.6	0.02	11.5 ± 0.4	40.3 ± 3.4
		8-9 m	5.0 ± 1.2	0.07	12.3 ± 0.8	32.7 ± 0.4
	27	1-2 m			12.7 ± 1.1	41.6 ± 1.3
		8-9 m			12.9 ± 1.0	38.5 ± 0.1
NSS	18	1-2 m	29.8 ± 1.1		10.4 ± 0.4	35.3 ± 1.8
		8-9 m	19.8 ± 1.2		7.4 ± 0.1	51.9 ± 0.8
	20	1-2 m	38.1 ± 1.8	0.17	11.6 ± 1.4	34.7 ± 5.0
		8-9 m	24.5 ± 1.8	0.14	8.8 ± 0.9	45.7 ± 3.5
	24	1-2 m	56.8 ± 1.9	0.29	14.9 ± 0.6	27.8 ± 3.0
		8-9 m	48.2 ± 1.3	0.57	11.7 ± 0.1	40.5 ± 3.0
	27	1-2 m	52.8 ± 1.8	-0.04	16.5 ± 0.6	36.7 ± 0.8
		8-9 m	51.3 ± 2.1	0.04	13.6 ± 0.4	45.1 ± 1.6
	36	1-2 m			16.0 ± 2.5	35.1 ± 4.1
		8-9 m			16.6 ± 1.3	37.4 ± 1.1

The mean lamina length is given as mean ± 1 SE. The dry matter is given as mean % of WW ± 1 SE. The ash content is given as mean % of DM ± 1 SE.

* RGR does not show SE because $n = 1$.

Table I.II. Significant differences in mean lamina length of *S. latissima* between 1-2 and 8-9 m depth.

Location	**Week**	***p*-value**
APN	16	0.830
	18	0.052
	20	**0.013**
	22	**0.000** *
	24	**0.033**
	27	0.862 *
	32	**0.005**
	36	0.738
	39	**0.006**
SAL	16	**0.000**
	18	**0.000**
	20	**0.000**
	22	**0.000**
	24	0.904
	27	**0.000** *
	32	**0.000**
SES	16	**0.000** *
	18	**0.000** *
	20	**0.000** *
	22	**0.001**
	24	0.858
	27	0.463 *
	32	0.158
ASF	16	**0.000**
	18	**0.000**
	20	**0.000**
	24	**0.000**
NSS	18	**0.000**
	20	**0.000**
	24	**0.001**
	27	0.606 *

Bold numbers indicate significant differences in mean lamina length of *S. latissima* between depths ($p < 0.05$). Mann Whitney U test was used for most tests except for where stars (*) indicate the use of two-sample T-test. $n \leq 50$.

Table I.III. Statistically significant differences in mean lamina length in *S. latissima* between weeks at each experimental location.

Location	**Week**	**vs. week**	***p*-value**
APN	16	18	**0.003**
		20	**0.000**
		22	**0.000**
		24	**0.000**
		27	**0.000**
		32	**0.000**
		36	**0.000**
		39	**0.000**
	18	20	1.000
		22	**0.000**
		24	**0.000**
		27	**0.000**
		32	**0.000**
		36	**0.000**
		39	**0.000**
	20	22	0.057
		24	**0.000**
		27	**0.000**
		32	**0.000**
		36	**0.000**
		39	**0.000**
	22	24	**0.025**
		27	**0.000 ***
		32	**0.000**
		36	**0.000**
		39	**0.000**
	24	27	**0.005**
		32	**0.000**
		36	**0.000**
		39	0.702
	27	32	1.000
		36	1.000
		39	1.000
	32	36	1.000
		39	**0.010**

	36	39	**0.029**
SAL	16	18	1.000
		20	**0.000**
		22	**0.000**
		24	**0.000**
		27	**0.000**
		32	**0.000**
	18	20	**0.000**
		22	**0.000**
		24	**0.000**
		27	**0.000**
		32	**0.000**
	20	22	**0.006**
		24	**0.000**
		27	1.000
		32	1.000
	22	24	**0.001**
		27	1.000
		32	**0.009**
	24	27	**0.001**
		32	**0.000**
	27	32	1.000
SES	16	18	**0.000** *
		20	**0.000**
		22	**0.000** *
		24	**0.000**
		27	**0.000** *
		32	0.085
	18	20	0.294
		22	**0.000** *
		24	**0.000**
		27	**0.000** *
		32	1.000
	20	22	**0.000**
		24	**0.000**
		27	**0.000**
		32	1.000

	22	24	**0.041**
		27	**0.000 ***
		32	**0.000**
	24	27	1.000
		32	**0.000**
	27	32	**0.000**
ASF	16	18	0.056
		20	**0.003**
		24	**0.000**
	18	20	1.000
		24	1.000
	20	24	1.000
NSS	18	20	0.060
		24	**0.000**
		27	**0.000**
	20	24	**0.000**
		27	**0.000**
	24	27	1.000

This table does not distinguish between depths. Bold numbers indicate significant differences in mean lamina length of *S. latissima* ($p < 0.05$) between weeks. Kruskal Wallis test with post hoc (Bonferroni) was used except for where stars (*) indicate that One-way ANOVA with post hoc (Bonferroni) was used. $n \leq 100$.

Table I.IV. Statistically significant differences in mean lamina length of *S. latissima* between locations each week.

Week	Location	vs. location	*p*-value
16	APN	SAL	**0.000**
		SES	**0.000**
		ASF	**0.000**
	SAL	SES	**0.000**
		ASF	0.471
	SES	ASF	**0.006**
18	APN	SAL	**0.000**
		SES	**0.002**
		ASF	**0.000**
		NSS	**0.000**
	SAL	SES	**0.000**
		ASF	0.113
		NSS	0.415
	SES	ASF	**0.000**
		NSS	**0.000**
	ASF	NSS	1.000
20	APN	SAL	1.000
		SES	**0.000**
		ASF	**0.000**
		NSS	0.276
	SAL	SES	**0.000**
		ASF	**0.000**
		NSS	1.000
	SES	ASF	**0.000**
		NSS	**0.000**
	ASF	NSS	1.000
22	APN	SAL	**0.005**
		SES	**0.000**
	SAL	SES	**0.000**
24	APN	SAL	**0.000**
		SES	**0.000**
		ASF	**0.000**
		NSS	0.137
	SAL	SES	**0.000**
		ASF	**0.000**

		NSS	0.111
	SES	ASF	**0.000**
		NSS	**0.000**
	ASF	NSS	**0.000**
27	APN	SAL	**0.000**
		SES	**0.000**
		NSS	**0.000**
	SAL	SES	**0.000**
		NSS	**0.000**
	SES	NSS	**0.000**
32	APN	SAL	**0.000**
		SES	**0.000**
	SAL	SES	0.559

This table does not distinguish between depths. Bold numbers indicate significant differences in mean lamina length of *S. latissima* ($p < 0.05$) between locations. Kruskal Wallis test with post hoc (Bonferroni) was used. $n \leq 100$.

Appendix II: Internal NO_3^--N

Table II. Internal NO_3^--N values in *S. latissima* at each location, each week at the two depths.

Location	Week	Depth	mg NO_3^--N g^{-1} DM
APN	Seedlings		0.22 ± 0.02
	16	1-2 m	0.20 ± 0.03
		8-9 m	0.21 ± 0.02
	18	1-2 m	0.09 ± 0.01
		8-9 m	0.20 ± 0.05
	20	1-2 m	0.07 ± 0.01
		8-9 m	0.19 ± 0.04
	22	1-2 m	0.11 ± 0.04
		8-9 m	0.25 ± 0.07
	24	1-2 m	0.01 ± 0.00
		8-9 m	0.09 ± 0.02
	27	1-2 m	0.01 ± 0.01
		8-9 m	0.07 ± 0.02
	32	1-2 m	0.01 ± 0.00
		8-9 m	0.01 ± 0.00
	36	1-2 m	0.01 ± 0.01
		8-9 m	0.04 ± 0.01
	39	1-2 m	0.02 ± 0.00
		8-9 m	0.04 ± 0.01
SAL	Seedlings		0.07 ± 0.02
	16	1-2 m	0.00 ± 0.00
		8-9 m	0.05 ± 0.01
	18	1-2 m	0.06 ± 0.03
		8-9 m	0.15 ± 0.01
	20	1-2 m	0.02 ± 0.01
		8-9 m	0.12 ± 0.04
	22	1-2 m	0.02 ± 0.00
		8-9 m	0.14 ± 0.02
	24	1-2 m	0.02 ± 0.00
		8-9 m	0.18 ± 0.04
	27	1-2 m	0.01 ± 0.00
		8-9 m	0.19 ± 0.05
	32	1-2 m	0.00 ± 0.00
		8-9 m	0.01 ± 0.00

SES	Seedlings		0.17 ± 0.02
	16	1-2 m	0.15 ± 0.08
		8-9 m	0.04 ± 0.02
	18	1-2 m	0.02 ± 0.01
		8-9 m	0.04 ± 0.02
	20	1-2 m	0.00 ± 0.00
		8-9 m	0.02 ± 0.01
	22	1-2 m	0.02 ± 0.02
		8-9 m	0.07 ± 0.00
	24	1-2 m	0.05 ± 0.01
		8-9 m	0.10 ± 0.05
	27	1-2 m	0.00 ± 0.00
		8-9 m	0.03 ± 0.01
	32	1-2 m	0.00 ± 0.00
		8-9 m	0.01 ± 0.00
ASF	Seedlings		0.13 ± 0.03
	16	1-2 m	0.00 ± 0.00
		8-9 m	0.04 ± 0.00
	20	1-2 m	0.00 ± 0.00
		8-9 m	0.00 ± 0.00
	22	1-2 m	0.00 ± 0.00
		8-9 m	0.00 ± 0.00
	24	1-2 m	0.00 ± 0.00
		8-9 m	0.00 ± 0.00
	27	1-2 m	0.00 ± 0.00
NSS	Seedlings		0.28 ± 0.03
	18	1-2 m	0.01 ± 0.00
		8-9 m	0.02 ± 0.01
	20	1-2 m	0.01 ± 0.01
		8-9 m	0.01 ± 0.00
	24	1-2 m	0.00 ± 0.00
		8-9 m	0.01 ± 0.01
	27	1-2 m	0.01 ± 0.00
		8-9 m	0.01 ± 0.00
	36	1-2 m	0.01 ± 0.00
		8-9 m	0.01 ± 0.00

Values are given in mean mg NO_3^--N g^{-1} DM ± 1 SE.

Appendix III: Proteins

Table III. Total nitrogen content (N) as % of DM, amino acid residues (AA) as % of DM, nitrogen-to-protein conversion factor (K_p) and protein content in *S. latissima* for each depth at all locations.

Location	Week	Depth	N (% of DM)	AA (% of DM)	K_p	Protein (% of DM)	Protein/N
APN	16	1-2 m	3.4 ± 0.0	11.1 ± 0.4	3.6 ± 0.1	12.2 ± 0.5	3.6 ± 0.1
		8-9 m	3.8 ± 0.1	11.9 ± 0.8	3.5 ± 0.2	13.1 ± 0.8	3.5 ± 0.2
	18	1-2 m	2.6 ± 0.0	9.2 ± 0.8	3.9 ± 0.3	10.1 ± 0.8	3.9 ± 0.3
		8-9 m	3.2 ± 0.1	10.0 ± 0.2	3.4 ± 0.1	11.0 ± 0.2	3.4 ± 0.1
	20	1-2 m	2.6 ± 0.1	9.9 ± 0.2	4.1 ± 0.2	10.9 ± 0.2	4.1 ± 0.2
		8-9 m	3.1 ± 0.0	10.0 ± 0.3	3.5 ± 0.1	11.0 ± 0.4	3.5 ± 0.1
	22	1-2 m	2.5 ± 0.1	9.2 ± 0.4	4.1 ± 0.1	10.1 ± 0.4	4.1 ± 0.1
		8-9 m	2.5 ± 0.6	9.7 ± 0.7	5.0 ± 1.3	10.7 ± 0.7	5.0 ± 1.3
	24	1-2 m	1.5 ± 0.3	5.8 ± 0.3	4.4 ± 0.5	6.4 ± 0.3	4.4 ± 0.5
		8-9 m	2.1 ± 0.3	7.9 ± 0.3	3.1 ± 1.6	5.8 ± 2.9	4.7 ± 0.9
	27	1-2 m	2.4 ± 0.4	6.2 ± 0.0	3.0 ± 0.6	6.8 ± 0.0	3.0 ± 0.6
		8-9 m	2.0 ± 0.2	7.1 ± 0.4	3.9 ± 0.3	7.9 ± 0.5	3.9 ± 0.3
	32	1-2 m	1.3 ± 0.1	4.9 ± 0.3	4.1 ± 0.3	5.4 ± 0.3	4.1 ± 0.3
		8-9 m	1.8 ± 0.2	5.8 ± 0.2	3.5 ± 0.3	6.4 ± 0.2	3.5 ± 0.3
SAL	22	1-2 m	0.8 ± 0.1	2.9 ± 0.1	4.2 ± 0.2	3.2 ± 0.1	4.2 ± 0.2
		8-9 m	2.7 ± 0.0	8.8 ± 0.1	3.6 ± 0.1	9.7 ± 0.1	3.6 ± 0.1
SES	16	1-2 m	3.9 ± 0.6	11.0 ± 0.4	3.2 ± 0.5	12.0 ± 0.4	3.2 ± 0.5
		8-9 m	3.3 ± 0.1	10.0 ± 0.4	3.2 ± 0.0	10.6 ± 0.3	3.2 ± 0.0
	18	1-2 m	2.8 ± 0.1	9.1 ± 0.3	3.6 ± 0.3	10.0 ± 0.4	3.6 ± 0.3
		8-9 m	3.0 ± 0.1	8.7 ± 0.3	3.2 ± 0.1	9.5 ± 0.3	3.2 ± 0.1
	20	1-2 m	1.5 ± 0.2	5.3 ± 0.5	3.9 ± 0.1	5.8 ± 0.5	3.9 ± 0.1
		8-9 m	1.8 ± 0.1	7.2 ± 0.2	4.3 ± 0.3	8.0 ± 0.3	4.3 ± 0.3
	22	1-2 m	1.9 ± 0.1	8.5 ± 0.2	4.9 ± 0.4	9.4 ± 0.2	4.9 ± 0.4
		8-9 m	2.4 ± 0.1	9.2 ± 0.2	4.3 ± 0.2	10.2 ± 0.3	4.3 ± 0.2
	24	1-2 m	1.8 ± 0.1	7.0 ± 0.2	4.3 ± 0.3	7.7 ± 0.2	4.3 ± 0.3
		8-9 m	2.3 ± 0.1	8.3 ± 0.7	4.0 ± 0.4	9.2 ± 0.8	4.0 ± 0.4
	27	1-2 m	1.2 ± 0.1	6.0 ± 1.7	3.8 ± 0.0	4.7 ± 0.5	3.8 ± 0.0
		8-9 m	3.5 ± 0.8	11.3 ± 1.2	3.7 ± 0.2	12.9 ± 2.2	3.7 ± 0.2
	32	1-2 m	3.6 ± 1.4	16.8 ± 0.0	3.7 ± 0.0	18.5 ± 0.0	3.7 ± 0.0
		8-9 m	4.3 ± 0.8	17.7 ± 1.5	5.1 ± 1.5	19.5 ± 1.7	5.1 ± 1.5
ASF	16	1-2 m	1.5 ± 0.1	5.2 ± 0.4	4.0 ± 0.1	5.8 ± 0.5	4.0 ± 0.1
		8-9 m	3.2 ± 0.7	9.1 ± 0.5	3.3 ± 0.5	10.0 ± 0.5	3.3 ± 0.5

	18	1-2 m	1.3 ± 0.1	5.1 ± 0.4	4.2 ± 0.2	5.6 ± 0.4	4.2 ± 0.2
		8-9 m	2.0 ± 0.1	6.0 ± 0.6	3.4 ± 0.6	6.6 ± 0.6	3.4 ± 0.6
	20	1-2 m	0.8 ± 0.0	2.8 ± 0.1	3.9 ± 0.0	3.1 ± 0.1	3.9 ± 0.0
		8-9 m	1.0 ± 0.1	3.7 ± 0.2	4.1 ± 0.1	4.0 ± 0.2	4.1 ± 0.1
	22	1-2 m	1.6 ± 0.3	5.6 ± 1.1	3.9 ± 0.2	6.2 ± 1.3	3.9 ± 0.2
		8-9 m	1.1 ± 0.1	4.0 ± 0.3	4.0 ± 0.2	4.4 ± 0.3	4.0 ± 0.2
	24	1-2 m	3.1 ± 0.2	10.3 ± 0.7	3.6 ± 0.0	11.3 ± 0.8	3.6 ± 0.0
		8-9 m	2.7 ± 0.0	9.4 ± 0.2	3.9 ± 0.1	10.4 ± 0.3	3.9 ± 0.1
	27	1-2 m	4.6 ± 0.2	17.0 ± 1.0	4.1 ± 0.4	18.7 ± 1.1	4.1 ± 0.4
NSS	20	1-2 m	1.0 ± 0.1	3.5 ± 0.4	3.9 ± 0.2	3.9 ± 0.4	3.9 ± 0.2
		8-9 m	1.0 ± 0.0	3.7 ± 0.1	3.8 ± 0.1	4.1 ± 0.1	3.8 ± 0.1
Mean values		1-2 m	2.0 ± 0.1	7.6 ± 0.5	3.9 ± 0.1	8.3 ± 0.5	3.9 ± 0.1
		8-9 m	2.6 ± 0.1	8.7 ± 0.4	3.8 ± 0.1	9.3 ± 0.5	3.9 ± 0.1

Values are given in mean ± 1 SE. K_p for *S. latissima* was calculated from equation 7.

Appendix IV: Regression statistics

Table IV. Regression analyses that shows regression model, values, R^2 values and *p*-values. Bold numbers indicate significant correlation.

Loc.	Regression model	ax	b	ax^2	bx	c	R^2	*p* - value
RGR as a function of internal NO_3^--N								
APN	$y = ax^2 + bx + c$			-24,184	6.431	-0.053	0.306	0.064
SAL	$y = ax^2 + bx + c$			-18.846	4.938	0.053	0.138	0.443
SES [a]	$y = ax^2 + bx + c$			-9.721	1.980	0.274	0.205	0.355
ASF	$y = ax^2 + bx + c$			361.345	-14-727	0.039	0.376	0.493
NSS	$y = ax^2 + bx + c$			1767.413	-65.883	0.503	0.367	0.319
RGR as a function of protein content [b]								
APN	$y = ax^2 + bx + c$			0.480	-0.021	-2.274	0.258	0.224
SES [a]	$y = ax^2 + bx + c$			0.252	-0.014	-0.761	0.327	0.205
ASF	$y = ax^2 + bx + c$			0.192	-0.012	-0.594	0.660	0.115
Internal NO_3^--N as a function of N:C ratio								
APN	$y = ax^2 + bx + c$			20.793	-2.728	0.122	0.472	**0.000**
SAL [a]	$y = ax^2 + bx + c$			15.027	-0.919	0.038	0.527	**0.000**
SES	$y = ax^2 + bx + c$			-11.670	2.864	-0.110	0.178	**0.032**
ASF	$y = ax^2 + bx + c$			-1.985	0.466	-0.015	0.184	0.087
NSS	$y = ax^2 + bx + c$			-0.558	0.113	0.006	0.054	0.512
Protein content as a function of internal NO_3^--N [b]								
APN	$y = ax^2 + bx + c$			-135.882	61.689	7.082	0.751	**0.000**
SES [a]	$y = ax + b$	20.153	9.852				0.202	**0.009**
ASF	$y = ax^2 + bx + c$			-3684.777	211.049	9.329	0.017	0.818
K_p as a function of time [c]								
APN	$y = ax^2 + bx + c$			-0.005	0.234	1.085	0.015	0.749
SES	$y = ax^2 + bx + c$			-0.006	0.366	-0.987	0.178	**0.036**
ASF	$y = ax^2 + bx + c$			-0.005	0.230	1.255	0.049	0.498

[a] Outlier in week 32 was removed due to heavy biofouling.

[b] SAL and NSS were excluded due to protein content analyses from only one sampling date.

[c] SAL and NSS were excluded due to K_p data from only one sampling date