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Seasonal and spatial variation in biochemical composition of *Saccharina latissima* during a potential harvesting season for Western Sweden[a]

Abstract: This study monitored the biomass composition of *Saccharina latissima* during a potential harvesting season on the West coast of Sweden, in order to find suitable harvest times for biorefinery purposes. Specimens of *S. latissima* were sampled at three locations in June, August and October and the biomass was analysed for its macromolecular composition, as well as for the content of several specific compounds, e.g. sugars and fatty acids. PERMANOVA analyses showed that there was a significant difference in the biomass composition among time points. The total carbohydrate concentration was lowest in June and peaked at 360 mg g^1 dry weight in August, while the mannitol content was highest, 90 mg g^1, in June and decreased throughout the sampling period. Total protein and fatty acid concentrations were found to be approximately 80 and 3 mg g^1, respectively, with relatively little variation over time. Overall, there was little spatial variation in the macromolecular composition, although the concentration of some specific monosaccharides and fatty acids, as well as the total phenolic content, differed among localities. We discuss the implications of the observed variation in biomass composition of *S. latissima* for future biorefinery purposes.

Keywords: biomass composition; *Saccharina*; seasonal variation; seaweed.

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Introduction

Cultivation and utilization of marine seaweed biomass is still occurring at a relatively small scale in most parts of the world and represents a largely underexploited resource with great potential in a future, biobased society. Today, cultivation is largely restricted to Asia and, in 2012, this region accounted for more than 96% of the 23.8 million tons of seaweed produced worldwide (FAO 2014). The present use of seaweeds includes human foods (e.g. hydrocolloid ingredients), animal feed, fertilizers and cosmetics, of which food products and extraction of hydrocolloids are the most important (Bartsch et al. 2008, Buchholz et al. 2012). In addition, seaweeds are a potential source of new compounds with pharmaceutical applications, such as antiviral, antibiotic, antitumor, anticancer or anti-inflammatory compounds (Holdt and Kraan 2011).

Stimulated by the global demand for new sources of biomass, there is a growing interest in expanding seaweed cultivation in Europe. Seaweeds can have high growth rates, with pilot cultivation in Scottish waters suggesting that annual yields of the kelp *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl *et* G.W. Saunders (formerly *Laminaria saccharina*, Lane et al. 2006) can reach 100–200 ton wet weight ha[1] (Hughes et al. 2012, Sanderson et al. 2012).

Kelp species, such as *S. latissima*, typically contain high amounts of carbohydrates, which can undergo pronounced seasonal fluctuations (Holdt and Kraan 2011). Alginate, a structural component of the cell wall and intracellular matrix is the most abundant carbohydrate and can account for up to 40% dry weight (dw) in some species (Horn et al. 1999), with the highest content

reported in early spring and the lowest in autumn (Black 1950, Schiener et al. 2015). Structural cellulose has been reported at concentrations of around 10% dw with only minor seasonal variations (Schiener et al. 2015). On the other hand, the levels of the storage carbohydrates laminarin and mannitol in *Laminaria* sp. varies considerably between 5–32 and 2–25% dw, respectively, with lowest levels reported in winter/spring (Black 1950, Adams et al. 2011, Schiener et al. 2015).

Other components of kelp biomass also display seasonal variations. Ash content in *Laminaria digitata* (Hudson) J.V. Lamouroux and *S. latissima* can reach up to 32% dw during the winter months, but is considerably lower in the summer (Adams et al. 2011, Schiener et al. 2015). A similar pattern is found for proteins, with concentrations around 10% dw in winter and 6% dw in summer (Schiener et al. 2015). Lipid content in kelp is also low, ranging from <1 to 4.5% dw, with the fatty acids (FA) being predominantly unsaturated (Holdt and Kraan 2011, Miyashita et al. 2013). According to Haug and Jensen (1954), the lipid content in *L. digitata* and *S. latissima* reaches the maximum concentration during the winter.

Other characteristic components of brown algae are the polyphenolic compounds, phlorotannins, and the oxygenated carotenoid, fucoxanthin. Due to their antioxidative activity, phlorotannins are interesting for industrial applications as additives in functional foods, cosmetics and pharmaceuticals (Li et al. 2009). Fucoxanthin has received significant attention for its remarkable biological properties, in particular in relation to human health, owing to the unusual allenic bond structure and oxygenic functional groups (Peng et al. 2011). Several extrinsic factors are known to affect the concentration of phlorotannins and fucoxanthin in brown algae, including UV-B radiation, irradiance, nutrient availability and herbivory (Pavia et al. 1997, Pavia and Toth 2000), contributing to seasonal variations in concentration (Schiener et al. 2015).

Given the significant variation in the chemical composition of kelp species among seasons and localities, it is important to have area-specific knowledge about seasonal variation in biomass composition if algal compounds are to be industrially exploited. In this study, we investigate seasonal and spatial variation of the biochemical composition of *S. latissima* during a potential harvesting season for Western Sweden. Seasonal fluctuations in the biochemical composition of *S. latissima* need to be considered to optimize the time of harvest, depending on what components are of highest importance or value. This study is part of a larger initiative with the goals of establishing fundamental knowledge for optimal cultivation of kelp biomass in Swedish waters, and to evaluate the potential to biorefine seaweed biomass into food, feed, biobased materials and bioenergy.

Materials and methods

Harvesting of seaweed and sample preparation

Samples of *Saccharina latissima* (Linnaeus) C.E.Lane, C.Mayes, Druehl *et* G.W.Saunders were harvested by SCUBA diving from three localities during June–October 2012 in the archipelago of Bohuslän, on the Swedish west coast (Figure 1). The localities were Vattenholmen (VH, 58.8750° N, 11.1048° E), Ulvillarna (UL, 58.8233° N, 11.0695° E) and Ursholmen (UR, 58.8354° N, 10.9903° E). Samples were taken from a depth of approximately 2–3 m and temporarily stored at -20°C within 8 h of collection. After the last collection date, the blades (with stipes removed) were individually ground in a mortar with liquid nitrogen and subsequently stored at -20°C or -80°C. The algae were freeze-dried and ground into a fine powder with a mortar and stored again at -20 or -80°C before analytical work was done, for which exact weights were recorded for each test. Biomass pre-stored at -80°C was used for analysis of fatty acids (FA), polyphenols and pigments; all other analytical methods were done on material stored at -20°C. Freeze-dried biomass was noted to have absorbed moisture during storage (i.e. was highly hygroscopic) and thus the moisture content of the freeze-dried material was determined to allow correction of sample weights. Seaweed biomass stored at -80°C had a dry weight of 930–950 mg g^1 and material stored at -20°C had a dry weight of 890–940 mg g^1 (Supplemental Table S1).

Moisture and ash content analyses

The moisture content of homogenized wet biomass was determined on 1.4–5 g of material that was dried at 105°C for 19 h. The moisture content of dry biomass was measured on approximately 110 mg of material in a moisture balance (HA 300, Precisa, Dietikon, Switzerland). For ash content determination, 70–85 mg dry biomass were dried again at 105°C overnight. After determining the weight, the samples were combusted in a furnace at 550°C for 3 h. The oven was cooled to about 300°C before the samples were transferred to a desiccator and cooled at room temperature for at least 2 h before weighing.

Figure 1: Location of seaweed sampling sites Vattenholmen (VH), Ulvillarna (UL) and Ursholmen (UR) on the Swedish west coast (59° N, 11° E). Map data: Google, DigitalGlobe.

Carbohydrate extraction and analyses

A two-step hydrolysis was performed for the extraction of total monosaccharides, based on the method of Dallies et al. (1998). Dry biomass (performed in duplicate, with two amounts of biomass, aproximately 5 and 10 mg) was mixed with 0.75 ml of 72% H_2SO_4 and 0.25 mg glass beads (0.5 mm) and homogenized (Fastprep-24, MP Biomedicals, Santa Ana, CA, USA) for 8×40 s at 6.5 m s^{-1} (cooled on ice between runs). Samples were transferred to 10 ml glass centrifuge tubes and another 0.25 ml of 72% H_2SO_4 was added and incubated at room temperature for 3 h. After incubation, tubes were placed on ice and 9 ml milliQ water added, yielding a concentration of approximately 1 mM H_2SO_4. A second incubation was performed for 4 h at 100°C. Tubes were cooled to room temperature before filtration (0.2 μm pore size, nylon filters) and storage at -20°C until analysis. Quantification of monosaccharides was performed using high-performance anion exchange chromatography (Xiros and Olsson 2014), using external standards curves for glucose, galactose, xylose, mannose, and fucose between 0.1 and 100 mg l^{-1} (Sigma-Aldrich, St. Louis, MO, USA).

Mannitol was extracted according to the CuSO$_4$ method of Obluchinskaya (2008) but scaled down ten times, thus using approximately 300 mg of dry biomass. Solubilized mannitol was detected by HPLC (Koppram et al. 2012) using an external mannitol standard curve between 0.3 and 10 g l^{-1} (Fluka Analytical, Buchs, Switzerland). Laminarin was extracted from 15–17 mg of dry biomass and hydrolysed by boiling in 2.5 ml of 0.5 M H_2SO_4 for 5 h (Iwao et al. 2008). Samples were neutralized using 5 M NaOH and centrifuged at 5000 *g* for 15 min. Released glucose was quantified using a commercial enzymatic assay kit (R-Biopharm GmbH, Darmstadt, Germany).

Fatty acid extraction and analysis

The lipids were extracted and fatty acids (FA) transesterified using the "in house two-step transesterification with KOH" of Cavonius et al. (2014). Approximately 400 mg of dry biomass, 50 μl of C17:0 internal standard (1.02 g l^{-1} in toluene), and in the extraction, 0.5% pyrogallol as antioxidant and 1 g of KOH pellets were used.

After separation of the organic phase, the remaining aqueous phase was washed once (2 ml of toluene) and the organic extracts were pooled. For analysis of methylated FA (FAME), 1 ml of the organic phase was evaporated under N_2 atmosphere and then re-suspended in 0.5 ml of iso-octane (2,2,4-trimethylpentane) and measured by GC (HP, 5890, Agilent, Santa Clara, CA, USA), with a polar column (Agilent J&W DB®-WAX), oven temp at 100–250°C, injector at 250°C, He & H_2 (50/50) as carrier-gas with flame ionization detection at 275°C. Quantification was done by relating peak areas of individual FA to the internal standard (heptadeconoic acid, C17:0, Sigma-Aldrich).

Protein extraction and analysis

Protein was extracted from dry biomass and quantified according to Lowry et al. (1951). Approximately 25 mg of samples in 500 µl of denaturation solution (2% sodium dodecyl sulfate, 1 mM dithiothreitol) and 0.2 g of 0.5-mm glass beads were homogenized (Fastprep-24, MP Biomedicals) 8 times for 40 s at 6.5 m s^{-1} (cooled on ice between runs). The suspensions were boiled 3 times for 5 min with vortexing in between. The tubes were centrifuged for 20 min at 12,000 g and 4°C and the supernatant (protein extracts) were stored at -20°C for later analysis. For the Lowry analysis the *DC*™ protein assay (BioRad, Hercules, CA, USA) was used, with 0–1.35 g l^{-1} bovine serum albumin in denaturation buffer as standards and the modified reagent A′ (20 µl reagent S+1 ml reagent A). Absorbance at 750 nm was measured in a microplate reader (FLUOstar Omega, BMG LABTECH, Ortenberg, Germany).

Acetone extraction and analyses of phenolic compounds and pigments

For the acetone extraction, approximately 0.25 g of dry biomass was suspended in 0.75 ml of milliQ water and homogenized (FastPrep-24, MP Biomedicals) 8 times with 0.2 g glass beads (0.5 mm) at speed of 6.5 m s^{-1} for 40 s. Subsequently, 0.75 ml milliQ water and 3.5 ml acetone were added to create a 70:30 acetone:water ratio for the extraction. The samples were incubated in a rotating wheel overnight at room temperature and centrifuged at 3000 g for 5 min. The supernatants were transferred to glass vials and stored at -80°C until analysis of phenolics and pigments.

Phenolic compounds were analysed by the Folin-Ciocalteu method using phloroglucinol (the monomer of brown algal polyphenolics, i.e. phlorotannins) at 20–100 mg l^{1} for the standard curve. For analysis, 100 µl of sample or standard was mixed with 50 µl of 10% Folin-Ciocalteu reagent (Sigma-Aldrich) and 50 µl of 7.5% $NaHCO_3$ directly in a microplate well. The absorbance at 765 nm was determined in a plate reader (Safire2, Tecan, Männedorf, Switzerland) with three measuring cycles with 5-s shaking steps between measurements.

Pigment quantification was conducted according to the following equations for chlorophyll *a* and *c* (Jeffrey and Humphrey 1975) and for total carotenoids (Parsons et al. 1984):

$$\text{Chl } a = (11.43 \cdot A_{664}) - (0.40 \cdot A_{630})$$

$$\text{Chl } c = (-3.80 \cdot A_{664}) + (24.88 \cdot A_{630})$$

$$\text{Total carotenoids} = (7 \cdot A_{480}) - (1.49 \cdot A_{510})$$

Absorbance was measured at 480, 510, 630 and 664 nm using a 1-cm quartz cuvette (DU800 spectrophotometer, Beckman Coulter, Brea, CA, USA) and blanked against 70% acetone in deionized water. Samples with absorbances above 0.9 were diluted in 70% acetone. The absorbance maxima for these pigments were not found to differ significantly when using 70% acetone compared to 90% acetone (for which the equations were originally derived) and it was decided that these equations were suitable for quantification.

Statistical analysis

For statistical analysis, the chemical composition of *Saccharina latissima* was divided into three groups, which were analyzed separately. The groups were: (1) major chemical compound groups consisting of ash, water content and total levels of proteins, carbohydrates, carotenoids and phenolics; (2) sugar composition consisting of laminarin, mannitol, fucose, galactose, glucose, mannose and xylose; (3) 13 different fatty acids (5 further FA were analyzed, but excluded from the statistical analysis due to highly sporadic occurrence). In addition to individual FA, two separate analyses were also performed for total fatty acids (TFA), and saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). TFA was analyzed separately since it was not possible to analyze this group together with the other major compound groups due to unbalanced sampling. Seasonal and spatial variation in these compound groups was analyzed using permutational multivariate analysis of variance (PERMANOVA; Primer-E, Ivybridge, UK) (Anderson 2001). As

data was obtained from only one locality (VH) in October, two separate PERMANOVA analyses were conducted: 1-factor PERMANOVA (factor season, fixed) for data from VH for all seasons and 2-factor PERMANOVA (factor season, fixed, and factor locality, random) for June and August for all localities. Significant factors were further analyzed using PERMANOVA pairwise comparisons and Monte Carlo (MC) tests were included to obtain more reliable p-values in cases with few possible permutations (Anderson et al. 2008). Tests were carried out using 9999 unrestricted permutations. The analyses were accompanied with PERMDISP to determine possible reasons for the rejection of the null hypotheses as PERMANOVA can be sensitive to sample dispersion (Anderson et al. 2006). Similarities among samples and importance of individual chemical compounds were visualized and evaluated using a canonical analysis of principal coordinates (CAP) (Anderson and Willis 2003), a constrained ordination method. PERMANOVA and CAP analyses were based on Euclidian distance measures, using unit variance scaled data.

Results

The composition (i.e. macromolecules along with polyphenols, pigments, individual sugars, and FA) of *Saccharina latissima* was investigated for individual blades harvested at three different dates and three locations (VH, UL and UR) during the period June–October 2012 and is reported in the following section.

Content of macromolcules and possible high-value products in *S. latissima*

The content of macromolecules, representing the main biochemical groups (carbohydrates, proteins, FA and ash) and possible high-value products (phenolic compounds and pigments) is presented in Table 1.

Carbohydrates (calculated as the sum of total monosaccharides and mannitol) were the major constituent of the biomass, ranging from 200 to 440 mg g^{-1} dw. Ash, representing minerals and metal ions also constituted a major portion of the biomass (140–320 mg g^{-1} dw). The content of total proteins was rather low in these samples, ranging from 30 to 110 mg g^{-1} dw, whereas mean values of total FA were also low and varied from 1.7 to 3.9 mg g^{-1} dw for all samples (Table 1). The phenolic compounds were found in the range 4.3–11.9 mg g^{-1} dw.

The pigments, chlorophyll *a* and *c,* and total carotenoids were found at concentrations of 0.12–0.58, 0.07–0.38, and 0.10–0.45 mg g^{-1} dw, respectively. Since, the main carotenoid in brown algae, such as *S. latissima*, is fucoxanthin (Haugan and Liaaenjensen 1994, Andersen et al. 2013), which is a potential high-value product, only the total carotenoid data were used in the statistical analysis described below. FA were also excluded from the main PERMANOVA analysis since these were only determined for half of the samples and instead included in the corresponding analysis on the FA profiles described below.

PERMANOVA showed that there was a significant difference (p=0.0021–0.0321) in the composition of the major chemical compound groups in *S. latissima* between all seasons at VH (Supplemental Table S2). The

Table 1: Water content of wet biomass (mg g^{-1} wet weight, mean±SD, n=6) and cellular content of freeze-dried biomass (mg g^{-1} dw biomass, mean±SD, n=6) of *Saccharina latissima* harvested at three locations between June and October, 2012.

	Vattenholmen			Ulvillarna		Ursholmen	
	5 June	**8 August**	**23 October**	**5 June**	**8 August**	**5 June**	**8 August**
Water content (mg g^{-1} wet weight)	780±50	640±100	640±50	750±30	700±30	680±60	700±10
Ash (mg g^{-1} dw)	210±30	140±30	200±20	330±20	180±30	270±20	200±20
Total carbohydrates[a] (mg g^{-1} dw)	310±50	440±80	380±40	200±40	380±60	260±40	350±40
Total proteins (mg g^{-1} dw)	80±30	110±40	80±40	30±10	90±10	60±20	90±30
Total fatty acids[b] (mg g^{-1} dw)	2.8±0.3	1.7±0.5	3.9±1.8	2.5±0.3	2.5±0.6	2.1±0.6	2.8±0.2
Phenolics[c] (mg g^{-1} dw)	11.9±3.8	8.1±2.0	8.2±1.6	8.3±0.7	4.8±1.2	10.5±2.2	4.3±1.4
Total carotenoids[c] (mg g^{-1} dw)	0.10±0.01	0.25±0.13	0.39±0.17	0.45±0.1	0.27±0.1	0.26±0.04	0.24±0.04
Chlorophyll *a*[c] (mg g^{-1} dw)	0.12±0.03	0.32±0.18	0.37±0.24	0.58±0.1	0.37±0.1	0.29±0.07	0.34±0.06
Chlorophyll *c*[c] (mg g^{-1} dw)	0.07±0.03	0.15±0.08	0.19±0.14	0.38±0.1	0.25±0.1	0.22±0.09	0.20±0.07

[a]Total carbohydrates were calculated as the sum of mannitol and monosaccharides (weights were adjusted for loss of water when polymerized into polysaccharides).
[b]n=3 for total fatty acid determination.
[c]Phenolic compounds and pigments were in 70% acetone extracts.

analysis including all localities showed a significant (p=0.0005) interaction between season and locality, although pairwise tests showed consistent differences (p=0.002–0.0025) in the composition of major chemical compound groups between June and August at all localities (Supplemental Table S3). Subsequent PERMDISP analysis showed that the seasonal differences were not partially explained by heterogeneity in sample dispersion (Tables S2 and S3). Consistent with PERMANOVA, CAP revealed significant differences between all seasons (Figure 2A; separation along first axis for UL and UR samples and separation along both first and second axis for VH samples). June samples were characterized in general by a high content of ash, water, and phenolic compounds. June samples also showed clear differences between localities with the highest content of ash and lowest content of total phenolics at UL and vice versa at VH. Samples collected in August were more homogenous than those in June and were characterized by a high content of proteins and carbohydrates. Seasonal comparisons for VH showed that October samples were characterized by an intermediate content of protein, carbohydrate and ash compared to June and August samples. No consistent seasonal change was found for total carotenoid levels (Table 1).

Sugar composition of *S. latissima*

The determination of the monosaccharide composition showed that glucose was the dominant monomer, constituting about 70–95% of the total monosaccharides, depending on time and location of harvest (Table 2). Fucose was detected as the second largest monosaccharide constituent, reaching up to 19% of total monosaccharides, and galactose, mannose and xylose were minor constituents (<10% of total monosaccharides). Laminarin is the most important glucose-containing polysaccharide

in *S. latissima*, and was present at varying levels in the range of 40–390 mg g^{-1} dw. The non-laminarin glucose (i.e. other glucans) could be calculated as the difference
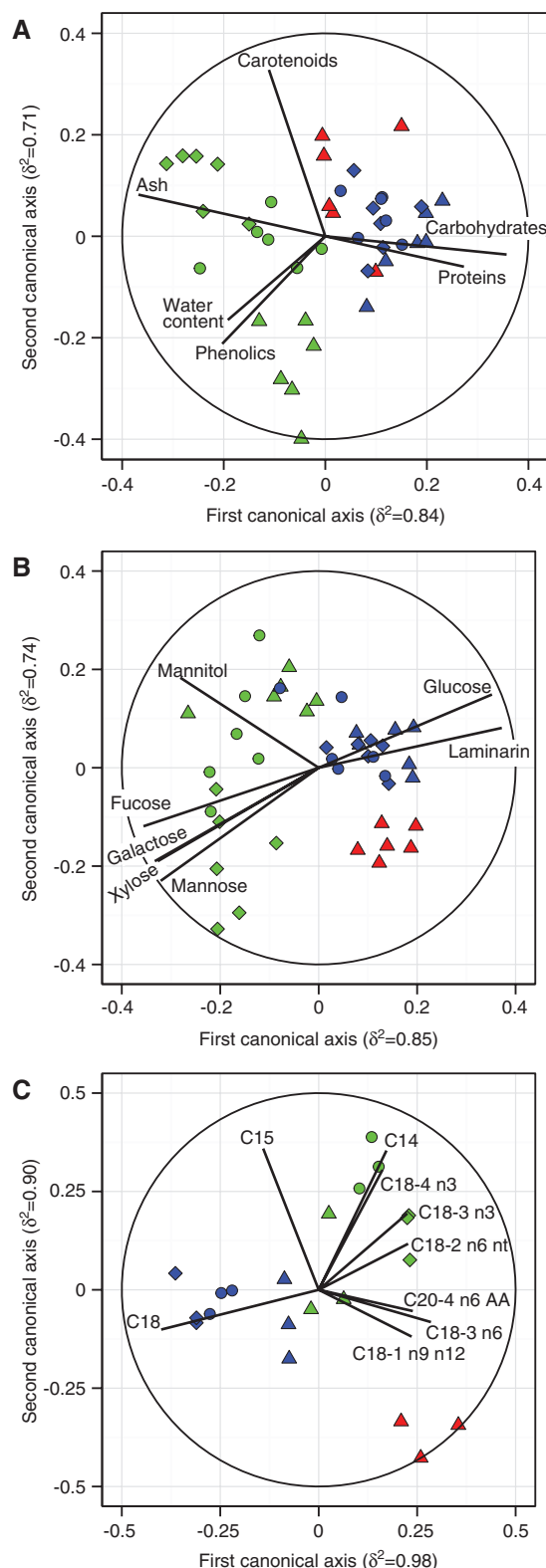

Figure 2: Biplots of canonical analysis of principal coordinates analysis on biomass composition data for *Saccharina latissima* sampled during different seasons at three localities. A. Total carbohydrates, total proteins, ash, phenolic compounds, total carotenoids and water in wet biomass. B. Sugar composition. C. Fatty acid composition. Vectors represent individual compounds significantly correlated to canonical axes and indicate the general increase for quantities of individual compounds. The proximity of each component line to the edge of the vector circle indicates the strength of the correlations. Different symbols indicate locality: circles, Ursholmen; diamonds, Ulvillarna; triangles, Vattenholmen. Colors indicate season: green, June; blue, August; red, October.

Table 2: Mannitol and laminarin content (mg g^{-1} dry weight, mean±SD, n=6) and distribution of monosaccharides after a total hydrolysis of biomass (%, mean±SD, n=6) of *Saccharina latissima* harvested at three locations between June and October, 2012.

	Vattenholmen			Ulvillarna		Ursholmen	
	5 June	**8 August**	**23 October**	**5 June**	**8 August**	**5 June**	**8 August**
Mannitol (mg g^{-1} dw)	94±9	63±14	46±7	77±15	54±20	97±8	76±26
Laminarin[a] (mg g^{-1} dw)	160±50	390±90	270±70	40±30	300±70	70±50	230±40
Monosaccharide composition (% of total monosaccharides)							
Glucose	86±7	95±2	90±2	70±6	92±3	76±9	91±3
Fucose	9±5	3±1	5±1	19±4	5±2	16±6	6±2
Galactose	3.1±1.5	1.3±0.3	2.6±0.8	6.4±1.1	1.6±0.6	4.6±1.6	2.0±0.6
Mannose	0.9±0.5	0.4±0.1	0.9±0.3	2.5±0.5	0.6±0.1	1.5±0.6	0.6±0.2
Xylose	0.8±0.4	0.3±0.1	0.8±0.2	2.1±0.4	0.5±0.2	1.7±0.7	0.7±0.2

[a]Weight adjusted for loss of water during polymerization of the polysaccharide.

between total glucose and the laminarin content (adjusted for loss of water during polymerization of polysaccharides). For most of the time points and locations there were no significant differences between the glucose and laminarin contents, except for the locations UL and UR in June, for which the contents of other glucans were 46±6 mg g^{-1} dw for UL and 52±7 mg g^{-1} dw for UR (means±standard deviations). The sugar alcohol mannitol was in the range 46–97 mg g^{-1} dw.

PERMANOVA showed that there was a significant difference (p=0.0013–0.0021) in the sugar composition of *S. latissima* between all seasons at VH (Supplemental Table S4). The analysis including all localities showed a significant interaction (p=0.017) between season and locality, although pairwise tests showed consistent differences (p=0.0023–0.0054) in the sugar composition between June and August at all localities (Supplemental Table S5). Subsequent PERMDISP analysis showed that the seasonal differences were not partially explained by heterogeneity in sample dispersion. Further analysis using CAP also showed significant differences between all seasons (Figure 2B; separation along first axis for UL and UR samples, and separation along both first and second axis for VH samples). June samples were characterized by high contents of fucose, galactose, mannitol, mannose and xylose. June samples also showed clear differences between localities with the highest content of, for example, mannitol at UR and VH, and the highest content of mannose at UL. Samples collected in August were in comparison more homogenous and were characterized by high contents of glucose and laminarin. Seasonal comparisons for VH showed that October samples were characterized by a low mannitol content and intermediate contents of glucose and laminarin, compared with June and August samples (Figure 2B).

Fatty acid composition of *S. latissima*

The distribution of FA showed that average levels of SFA and PUFA were higher than levels of MUFA (Table 3). Among the SFA, C16, palmitic acid largely dominated, with average values up to 25% of TFA. Levels of C14, myristic acid, were ranked second (7.8–11.3%), followed by C18, stearic acid (1.1–3.7%). Regarding MUFAs, C18:1 (oleic acid) dominated, with relative amounts up to 18%. C16:1 was ranked second (3.6–7.2%), followed by C20:1, which was only detected in August samples from UL and UR (1.15% and 1.02%, respectively). Among the PUFA detected, C18:2 n-6 (linoleic acid), C20:4 n-6 (arachidonic acid) and C20:5 n-3 (eicosapentaenoic acid, EPA) were found in relatively large quantities among all samples (6.9–9.7%, 7.8–11.8% and 6.3–9.1%, respectively). The n-3 FA docosahexaenoic acid (C22:6 n-3, DHA) was detected only in August samples from all three locations, but particularly in UL and UR samples (8.0 and 8.5%, respectively). VH samples had significantly lower relative DHA levels (0.9%).

Using total FA data for June and August for all localities in the statistical analysis resulted in a significant interaction (p=0.0137) between season and locality, but pairwise tests on the interaction showed no differences between June and August at UL and UR (Supplemental Table S7). PERMANOVA showed that there was no significant differences in the TFA levels among seasons at VH (Supplemental Table S6). However, in contradiction to the analysis using data from VH for all seasons, the pairwise tests showed a significant difference (p=0.0196) in TFA levels between June and August samples. Subsequent PERMDISP analysis showed that the difference between June and August at VH was not partially explained by heterogeneity in sample dispersion.

Table 3: Distribution of fatty acids (FA,% of total fatty acids, mean±SD, n=3) of *Saccharina latissima* harvested at three locations between June and October, 2012.

FA (% of Total FA)	Vattenholmen			Ulvillarna		Ursholmen	
	5 June	8 August	23 October	5 June	8 August	5 June	8 August
C14	9.6±2.8	8.8±0.5	7.5±1.6	11.0±1.0	7.8±2.1	11.3±0.3	8.3±0.4
C15	0.51±0.11	0.61±0.03	0.35±0.10	0.55±0.02	0.57±0.12	0.67±0.06	0.54±0.02
C16	21.0±6.0	21.8±1.5	22.1±1.5	22.0±1.8	22.1±2.4	25.0±4.9	21.7±0.8
C18	1.10±0.29	1.60±0.49	1.53±0.98	0.90±0.06	3.71±0.57	1.25±0.74	3.59±0.04
C20	0.76±0.16	0.97±0.08	0.86±0.10	0.71±0.05	0.81±0.13	0.78±0.18	0.76±0.04
C22	ND	ND	ND	ND	0.7±0.2	ND	0.3±0.2
Total SFA	33.0±9.3	33.8±1.8	32.3±1.0	35.1±2.9	35.7±4.6	39.0±6.2	35.2±0.4
C16:1 n7	3.6±2.6	6.0±0.6	7.2±3.2	5.5±0.8	5.4±0.9	7.0±1.6	4.8±0.5
C18:1[a]	13.8±3.8	15.7±1.5	18.9±7.8	15.0±1.1	12.7±1.4	15.7±3.8	11.7±0.8
C20:1 n15	ND	ND	ND	ND	1.15±0.31	ND	1.02±0.04
Total MUFA	17.6±5.3	21.8±1.8	26.2±4.8	20.4±0.7	19.3±1.9	22.7±5.4	17.6±1.2
C18:2 n6ct	7.7±1.8	9.7±1.0	8.3±0.8	8.5±1.4	7.0±0.5	9.6±0.7	6.9±0.3
C18:3 n6	1.18±0.35	1.66±0.54	1.75±0.11	1.57±0.36	1.07±0.08	1.51±0.17	1.11±0.19
C18:3 n3	2.6±0.6	3.3±0.6	3.2±0.3	3.3±0.4	2.6±0.3	4.0±0.6	2.7±0.3
C18:4 n3	4.16±0.90	4.75±0.85	4.33±0.68	5.76±0.67	4.30±0.75	7.09±1.67	4.49±0.07
C20:2 n6	ND	ND	ND	ND	0.73±0.15	ND	0.73±0.02
C20:3 n6	0.39±0.06	ND	0.65±0.25	ND	ND	ND	ND
C20:4 n6 AA	7.9±1.6	12.4±1.5	11.8±2.0	10.5±0.7	8.7±1.3	11.4±2.3	7.8±0.2
C20:5 n3 EPA	6.3±1.2	8.2±1.2	7.9±1.1	8.0±0.6	7.1±0.8	9.1±1.9	7.1±0.4
C22:6n3 DHA	ND	0.9±0.9	ND	ND	8.0±2.7	ND	8.5±0.5
Total PUFA	30.2±6.1	40.9±3.5	37.8±3.8	37.7±3.5	39.5±3.1	42.9±7.4	39.4±0.2

Unidentified peaks represented 3.8–7.9% and small identified peaks represented ≤0.62% of the total peak area. ND, Not detected; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AA, arachidonic acid; EPA, Eicosapentaenoic acid; DHA, docosahexaenoic acid.
[a]The sum of 18:1 types, n7, n9,n12.

No seasonal differences in SFA, MUFA and PUFA content were found for *S. latissima* sampled from VH in June, August and October (Supplemental Table S8). Similarly, no seasonal differences were found when June and August samples from all localities were analyzed together (Supplemental Table S9).

PERMANOVA showed no significant differences in individual FA composition (only those detected at all locations and time points were included) of *S. latissima* among seasons at VH (Supplemental Table S10). The analysis including all localities showed a significant interaction (p=0.0019) between season and locality, and pairwise tests showed significant differences in individual FA composition between June and August at UL and UR, but not at VH (Supplemental Table S11). Subsequent PERMDISP analysis showed that the seasonal differences observed for UL and UR were not partially explained by heterogeneity in sample dispersion. Further analysis using CAP also showed significant differences between June and August for samples from UL and UR (Figure 2C; separation along both first and second axis), which is mainly attributed to the higher content of C14, C18:4 n3, C18:3 n3 and C18:2

n6 FA in June, and higher content of C18 FA in August. In addition, CAP analysis indicated that samples from VH in October were different from those in June and August for all localities, characterized by low content of C15 FA (Figure 2C).

Discussion

We found considerable seasonal variations in several of the investigated chemical components of *Saccharina latissima*, which suggests different harvest times for cultivated algae on the Swedish west coast, depending on which components are of greatest interest.

The dominant component of *S. latissima* are carbohydrates, for which the concentration changed significantly over time with highest levels up to 44.5% of the dry weight biomass in August, excluding alginates. Laminarin was the main contributor to the high carbohydrate concentrations in August; this is consistent with the high glucose content also found at this time point. The concentration

of mannitol was generally lower in August than in June, which could be an effect of phosphorus limitation, as this has been reported to cause transiently lower mannitol production (Lee 2008). The lower concentrations of both mannitol and laminarin in October than in August highlight the effect of low irradiance and the utilization of carbon storage products during winter months for new growth (Henley and Dunton 1995, Belseth 2012). If the main purpose for the harvested biomass is production of bioethanol, the most suitable time of harvest would be in August, since the laminarin can be considered the most important substrate for fermentation (Adams et al. 2009).

The protein concentrations also peaked in August, with concentrations up to 110 mg g^1 dry weight. This may appear low compared to other protein crops, such as soya beans (up to 40%) but, considering the high productivity of macroalgae and that the protein can be extracted from the whole biomass, the theoretical protein yield per production area can exceed that of soy. This may be of great interest in a biorefinery context, and should be considered when deciding on harvesting time. The increase in protein concentration from June to August (Table 1) conflicts with some previous reports of a higher protein content in *S. latissima* by the end of May than in August (Black 1950, Schiener et al. 2015). It has, however, been found previously that the nitrogen content of the algal biomass increases throughout the summer, which could correspond to a higher protein content (Tønder 2014). Significant changes in other biochemical groups, e.g. storage carbohydrates, will obviously influence the concentration of other components (measured as their relative contribution to total biomass), especially those of relatively low concentration such as proteins. The combined content of mannitol and laminarin in the Swedish *S. latissima* individuals in August was around 30% lower than that reported from Norway and Scotland (Tønder 2014, Schiener et al. 2015), which could be an explanation of why the relative protein content appears higher in the Swedish algae at this time of year.

Seaweeds usually contain low levels of lipids, varying from 9.2 to 50 mg total lipids g^1 dw (Sánchez-Machado et al. 2004, Dawczynski et al. 2007, Schmid et al. 2014) and the TFA levels we report (1.7–3.9 mg TFA g^1 dw) were even lower than that. From a biorefinery perspective, the lipid fraction could still be of interest since it contains relatively high-value products, of which the PUFAs EPA and DHA are the most important. In previous studies, EPA was found at 10.8 and 1.8% of total FA (in June and November, respectively) in *S. latissima* from the Irish west coast (Schmid et al. 2014), at 8.6% (July) in *Laminaria ochroleuca* (Bachelot de la Pylaie) from the Iberian coast

(Sánchez-Machado et al. 2004) and at 16.2% in *Laminaria* sp. from China, Japan and Korea (Dawczynski et al. 2007). In all of these studies no DHA was found. Our data on *S. latissima* from the Swedish west coast are thus unique in that they indicated up to 8.5% DHA in August. The combination of DHA and EPA increases the attractiveness of utilising this resource as a functional food product. The European Food Safety Authority has recently permitted several beneficial health claims to be associated with the consumption of products containing EPA and DHA, including three claims for DHA alone, and three for EPA and DHA together (EFSA 2015). Among these is the claim that EPA and DHA together help to maintain normal heart function, but a product claiming this benefit is required to contain at least 40 mg EPA+DHA per 100 g. In August 100 g of dried *S. latissima* biomass from Ursholmen was just above this limit (42.1 mg (100 g)$^{-1}$ dw).

Pigments and polyphenols are the other high-value compound groups that can be extracted from *S. latissima*. The highest concentrations of phenolic compounds in our study were observed in the June samples, and were up to 11.9 mg g^1 dw biomass. This is in agreement with Schiener et al. (2015), who found the lowest levels of phenolics in March, and the highest between May and July. The amounts of phenolic compounds extractable in acetonitrile:water:formic acid in their study (on average 0.41%) were at the lower end of those found in our study (0.43–1.2%) when extracting with 70% acetone. Since acetone is a better solvent for food applications, these findings are positive for industrial applications. Phlorotannins are known to comprise the major part of the phenolic compounds in kelps and have earlier been reported in the range 1–5% dw (Toth and Pavia 2002, Angela and Katrin 2012). They are oligomers of phloroglucinol (Shibata et al. 2004) and have been particularly highlighted due to their antioxidative properties in *in vitro* tests and in food products (Wang et al. 2010).

The range of carotenoid contents for the different sites (0.10–0.45 mg g^1 dw) are comparable to those reported for *S. latissima* collected in Norway (~0.5 mg g^1 dw; Andersen et al. 2013). Schmid and Stengel (2015) reported the combined fucoxanthin and β-carotene content of *S. latissima* to be 0.2–0.3 mg g dw^{-1}, which was significantly lower than other *Laminaria* species collected from the same site (~0.8–0.9 mg g^1 dw). *Saccharina latissima* is reported to have a lower pigment content, particularly carotenoids, under warmer conditions (Andersen et al. 2013, Hallerud 2014), but no consistent trend was seen between time points for sites in our study. This could be due to variation between individual fronds sampled from each site in physiological status (age and thickness), which affects

pigment content in several kelp species (Belseth 2012, Hallerud 2014). In addition, individual macroalgae acclimate to their ambient light regime (quantity and quality) by adjusting frond pigment content, possibly reflecting differences in their immediate environment (Hallerud 2014). This may also explain the variation seen between the replicate blades taken from each site as well as variation between sites, not just for pigment content, but also for carbohydrate and FA content and profile. This highlights the importance of careful sampling as well as the heterogeneity that is to be expected if commercial exploitation of these compounds is undertaken.

A large fraction of *S. latissima* biomass consists of moisture and ash. The highest ash concentration we determined (32% dw) was similar to the average ash values reported by others, ~31% (Obluchinskaya 2008, Schiener et al. 2015). Schiener et al. (2015) found the highest moisture and ash content during the winter months, while we did not sample during the winter, but still found highest ash and moisture in June rather than October. The ash fraction probably represents mainly minerals, many of which are associated with the amino acid glutamate in brown seaweed and may be of interest as salt (sodium chloride) replacers (Lopez-Lopez et al. 2009).

The total weight of the compounds analysed within this study accounted for 60–70% of the total algal dry weight. There were no significant differences among locations, apart from Ulvillarna in June when the total weight was significantly higher than in the other two locations (71±6% versus a mean value of 61±5% at UR and VH). We did not include alginate in our analyses and, since it is reported to constitute 15–25% of dry weight *S. latissima* biomass (Black 1950, Schiener et al. 2015), it may account for a large part of the missing 30–40%. In addition, the lipid analysis only covered the FA, meaning that all other lipid components are not accounted for; however, this would not affect the sum of weights substantially, since the total lipid fraction is so small.

Here we have highlighted how different biochemical groups vary across potential harvest time points. The results suggest that for carbohydrate- or protein-rich biomass, August is the preferred time of harvest. However, several other factors influence when the optimal harvest time may be, not least the effect of bryozoan growth on kelp fronds. The main species causing fouling in Northern latitudes has been identified as the colonial species *Membranipora membranacea* (Linnaeus), which can develop calcified mats across kelp fronds (Handå et al. 2013, Tønder 2014). The effect of biofouling from other species could result in a significant decrease in growth, negative effects on biochemical composition from an industrial point of view and, in worst case scenarios, defoliation of kelp beds and loss of biomass (Handå et al. 2013, Tønder 2014). Bryozoan colonization coincides with increased temperatures and can become a significant problem in late summer, reaching 80–100% coverage of frond area. This may be correlated with reduced pigment content and photosynthetic rate (Hurd et al. 2000) and could consequently reduce carbohydrate formation (Handå et al. 2013, Tønder 2014). Alternatively, the concentration of these compounds could be simply diluted by the presence of the bryozoans, which can make up 50% of the biomass. Further work is required to evaluate the possible effects of epiphytic colonization of cultivated kelp in Western Sweden and the effects on algal biochemistry. These effects could have significant impacts on possible seaweed biorefinery processes, and harvesting should be timed to maximise product composition, and to avoid epiphytism, which suggests that a flexible or responsive strategy may be required.

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Graphical abstract

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Tony Werner, Linnea Qvirist,
Joshua J. Mayers, Henrik Pavia,
Ingrid Undeland and Eva Albers
**Seasonal and spatial variation
in biochemical composition of
Saccharina latissima during a
potential harvesting season for
Western Sweden**

Research article: The chemical
composition of *Saccharina latissima*
from Western Sweden was distinctly
different between June, August
and October, with levels of several
components varying but levels of
total protein and fatty acids rather
constant; little spatial variation was
found.