Composition of *Laminaria digitata* biomass during a potential harvesting season

Degree project

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Abstract

Emissions from fossil fuels and a change of land use are two of the main contributors to climate change and thereby threatens our health and well-being. Finding new sources of materials for production of fuels, food and commodities are of high importance. Biomass from plants and photosynthetic organisms is a potential solution, and algae is one type of organisms under investigation today.

The interest in brown macroalgae, seaweeds, has increased in recent years, as they contain carbohydrates that can be converted into fuels as well as nutritional substances. Seaweed has advantages from having the ocean as its natural habitat, thereby not requiring freshwater or agricultural lands. They also require milder processing conditions compared to terrestrial biomass sources. The composition in seaweeds varies with the seasons and environmental conditions. The potential of seaweed biomass is determined by the composition, and thereby knowledge of the variations between seasons is crucial to determining the optimal harvesting time, which was the aim of this project.

The project was performed using samples of the brown seaweed *Laminaria digitata* collected from three different locations in Kosterhavet National Park on the Swedish West Coast in June, August and October. Samples were analyzed for compositional variations in carbohydrates and elemental content. *Laminaria digitata* was found to contain high amounts of carbohydrates ranging between 34 and 55 % of the dry weight (excluding one of the major carbohydrates, alginate). The elemental components carbon, hydrogen, nitrogen and sulphur ranged between 34.5-38.5, 4.9-5.55, 1.09-2.34 and 1.24-1.60 % respectively. Proteins estimated from the nitrogen content were 5-12 % of dry weight.

Mannitol and protein content showed the hypothesized seasonal fluctuations, both with peaking values in autumn, while the remaining carbohydrates did not show these. The variations between plants collected at the same site and month where high and to some extent confounded the hypothesized seasonal variations. Seasonal variations in the total carbohydrate content showed no consistent trends between sites, nor where there any considerable variations within the sites. Thus, a conclusion of the optimal harvesting season should be drawn based on size of the plants as well as fouling. Harvest in spring was concluded to be optimal.

Laminaria digitata biomassas sammansättning under en potentiell skördesäsong

Sammanfattning

Utsläpp från fossila bränslen samt en förändring i hur vi använder mark är två utav de största bidragande faktorerna till klimatförändringar och hotar därmed vår hälsa och välmående. Att hitta nya källor till material för att framställa bränsle och andra varor är därför en nödvändighet. Biomassa från växter och fotosyntetiserande organismer är en potentiell lösning och inom denna kategori är alger någonting som det forskas på idag.

Intresset för bruna makroalger, tång, har ökat de senaste åren. De innehåller höga halter av kolhydrater som kan omvandlas till bränslen, samt näringsämnen som gör dem passande som föda. Fördelar med tång är att deras naturliga miljö är havet, de kräver därför varken färskvatten eller jordbruksytor. För dem räcker det även med mildare processförhållanden.

Potentialen hos tångbiomassa avgörs av dess komposition och denna varierar beroende på säsong och miljöfaktorer. Vetskap om hur kompositionen varierar är därför viktigt för att bestämma en optimal skördesäsong, vilket var syftet med denna studie.

Prover av den bruna algen *Laminaria digitata* som användes för projektet hade samlats in från tre olika platser i Kosterhavets nationalpark, Sverige, under de tre månaderna juni, augusti samt oktober. Proverna analyserades för variationer i sammansättning av kolhydrater samt dess innehåll av huvudsakliga grundämnen. *Laminaria digitata* visade sig innehålla höga halter av kolhydrater med värden mellan 34 och 55 % av torrvikten (där alginat, en av de kolhydrater som förekommer i hög halt, ej har kvantifierats). Kol, väte, kväve samt svavel mättes vara respektive 34.5–38.5, 4.9-5.55, 1.09-2.34 och 1.24-1.60 % av torrvikten. Proteininehållet beräknades från kväveinnehållet och låg mellan 5 och 12 % av torrvikten.

Mannitol samt proteininnehåll visade de förväntade säsongsförhållandena, bägge med högsta värden under hösten, medan resterande kolhydrater inte visade dessa. Variationen i komposition mellan prov från plantor samlade på samma plats och månad var stor och dolde sannolikt förväntade säsongsförändringar till viss del. Variation i totala kolhydratinnehållet mellan säsonger var inte konsekvent mellan platserna, inte heller var det särskilt stora variationer inom en plats. En slutsats om optimal skördesäsong drogs därav baserat på storlek på plantorna samt med hänsyn till bakterier som minkar sjögräsets kommersiella värde. Skörd under våren beslutades vara optimalt.

Contents

1. Introduction

One of the largest threats to today's society is climate change, which might result in our health and well-being being threatened. Two large factors that contributes to climate change are CO_2-emissions from fossil fuels and a change of land use (Pires J C M 2017). There is a constant search for new solutions, new material sources and processes to achieve sustainable production of energy and commodities. A solution might be found in the biomass of various plants and photosynthetic organisms, which can be converted into energy or used as food. Examples of biomass for these purposes are lignocellulosic materials, food crops and algae. (EESI 2018).

In recent years, brown macroalgae, seaweeds, have received an increased interest as a resource of biomass. Historically, seaweeds have been utilized for food and food additives, animal feeds, fertilizers and cosmetics. Brown algae contain high amounts of carbohydrates that can be converted into biofuels (Manns D 2014), as well as minerals, vitamins, proteins and lipids making brown algae suitable as a food resource (Arasaki S 1983).

Seaweeds are fast growing with oceans as their natural habitat. Cultivation of seaweeds will therefore not require fertilizer nor fresh water, and neither will they be competing with agricultural lands needed for food crops. The processing conditions of seaweed biomass require relatively mild conditions compared to lignocellulosic biomass, meaning less acidic conditions and temperatures as well as shorter reaction time (van Hal J W 2014). Remaining challenges in the area of seaweed utilization lies in developing cost-effective ways of growing, harvesting, transporting and processing substantial amounts of seaweed (Kraan S 2013).

The composition of seaweeds varies by environmental conditions and season. Brown algae in colder climates store carbohydrates during summer as an energy source for winter. Protein content will be higher in winter since it accumulates nitrogen that the algae in summer needs for tissue growth. The composition of the algae determines its potential as a biomass for biofuel production and other applications, and thereby knowledge of how the composition varies over a potential harvest season is crucial to optimize the attained value (D'Este M 2017).

In this research, the brown seaweed *Laminaria digitata* is investigated. Samples from three different locations in Kosterhavet National Park on the Swedish West Coast were used that had been collected at three different seasons and analyzed for compositional variations in

carbohydrates and elemental content. By comparing the differences in composition between seasons, the optimal harvesting season was evaluated.

2. Background

2.1 Seaweeds

Algae are photoautotrophic organisms and exist in many different forms ranging from unicellular organisms to large kelps and comprise thousands of different species. Multicellular marine algae are called seaweeds and mainly inhabit shallow coastal areas (Fuller M 2004). Seaweeds are classified into groups by their color. *Laminaria digitata* belongs to the *Laminariales*, which is a subgroup of brown algae. *Laminariales* consists of some of the largest and most complex brown algae, called kelp. Kelp is a term used for brown seaweeds that usually have a long, tough stalk with a broad frond divided into strips. They can be found in the northern pacific, north and south Atlantic (Sahoo D 2015).

Seaweeds have for a long time been utilized by humans, starting as a source for food. In recent years, the interest in seaweed has increased due to its wide range of applications and functionality in society. Today, seaweeds are utilized as food and food additives, animal feeds, medicine, fertilizers, cosmetics and for energy production (Sahoo D 2015), which are discussed below.

Food: The high nutritional value in brown algae makes them attractive as a food source. They contain protein, carbohydrates, amino acids, minerals, vitamins etc. Seaweeds can be consumed raw or cooked and are today used in a variety of food products (Sahoo D 2015).

Fertilizer: Brown algae is rich in micro- and macronutrients, growth hormones and vitamins. Properties that makes it suitable as a fertilizer. Countries like China, France, Ireland and Japan utilize seaweeds as a fertilizer (Murakami K 2011). Seaweed fertilizers have been suggested superior to chemical fertilizer due to the high level of organic matter that aids in retaining moisture and minerals in the upper soil level available to the roots (Singh D P 2009).

Biofuel: Biomass from kelps can be used to produce biofuels (mainly ethanol and methane) from carbon dioxide and water. The interest in using algae for biofuel production has increased due to the decreasing availability and increasing cost of fossil fuels (Sahoo D 2015). Research has shown that seaweed is a promising biomass for biofuel production, though still relatively unexplored (Wei N 2013).

2.2 Composition of *Laminaria digitata*

The biomass of *Laminaria digitata* contains a variety of different substances. Carbohydrates make up a significant part, along with proteins and ash. It contains a wide range of different saccharides that have been measured to reach up to a total of 90 % of the dry weight (Manns D. 2017). The saccharides consist of mono- and polysaccharides.

Monosaccharides

Mannitol is a sugar alcohol containing six carbon atoms. It is used for reserves as well as a primary photosynthetic product (Iwamoto K 2005). Mannitol might also play a role as an osmolyte (Dittami S M 2011).

Polysaccharides

Brown algae have a storage carbohydrate called laminarin. Laminarin consists of glucose units liked together by β-1,3-linkages and occasional β-1,6-linked branches. The levels of laminarin in brown seaweed vary strongly by season (Adams J M M 2011).

Another type of polysaccharides in brown seaweeds is fucoidans. They are part of the cell wall of brown seaweed, which protect them from the environment. Fucoidans are mainly composed of sulphated L-fucose with less than 10 % of xylose, mannose and galactose combined (Holdt S L 2011) (Ale M T 2011).

Alginate is the major component in the cell walls of brown seaweeds. It consists of a chain of α-L-guluronic acid and β-D-mannuronic acid, linked together by 1-4-glucosidic bonds. The ratio between mannuronic and guluronic acid in the alginate differs depending on harvesting season and site (Aarstad O A 2012) (Rhein-Knudsen N 2015). The cell walls also contain cellulose (Manns D. 2017).

Ash and protein content

The biomass of *Laminaria digitata* can consist of up to 32 % ash (dry weight, dw). A significant amount of the ash consists of the ions potassium, sodium, magnesium and calcium (Schiener P 2015). Protein content in brown seaweeds is generally between 3 to 15 % of the dry weight (Schiener P 2015).

2.3 Seasonal fluctuations

The amounts of different saccharides in *Laminaria digitata* vary depending on season, age and environmental conditions (Ito K 1989) (Murakami K 2011). The levels of laminarin are strongly affected by season, due to its utilization as reserves in winter time (Manns D. 2017). Previous research has shown that laminarin levels are almost non-existing in spring, and maximum levels are detected in autumn (Jensen A 1956).

Mannitol is also used as a reserve substance, and thereby also undergoes seasonal fluctuations (Manns D. 2017). Research by Dittami *et al.* shows that the levels of mannitol in brown algae are affected by the salinity of the environment. A decrease in mannitol under hyposaline stress and increase during hypersaline stress was measured, suggesting a role of mannitol as an osmolyte (Dittami S M 2011).

Protein and ash content are both higher during winter time (Schiener P 2015). Possibly due to the decreased amounts of carbohydrates that have been used up by the plant, increasing the percental amounts of protein and ash.

2.4 Methods theory

2.4.1 Hydrolysis of *Laminaria digitata*

To measure the carbohydrate content in the biomass, the glucosidic bonds between the sugar units must be broken through hydrolysis to obtain single monosaccharides to be measured. The glucosidic bonds are sensitive to acidic conditions and thus by adding acid and lowering the pH the polysaccharides can be hydrolysed. In this case, sulfuric acid was used and hydrolysis was performed with two different methods. One method was used for the total quantification of the carbohydrate content by ion chromatography and a second for the laminarin and mannitol content by high performance liquid chromatography. Whereas the first method breaks the laminarin chain as well as cellulose, fucoidan and alginate into its monosaccharide units, the second method, using weaker acid, only breaks down the laminarin chains. The first method was performed according to Manns D *et al*. (Manns D 2014). Released monosaccharides can be analyzed by different chromatographic techniques due to their differences in polarity, pKa-values, size and structure.

2.4.2 Ion-exchange chromatography, HPAEC-PAD

Ion-exchange chromatography, IC, separates molecules based on their attraction towards charged sites on the stationary phase. IC can be performed either through cation- or anion-exchange, whereas the anion exchange method is used for separating anions and cation exchange for cations. Analytes are attracted to opposite charged functional groups on the stationary phase and must thereby be ionic or polar in order to be separated. By altering the flow of eluents containing ions or difference in pH, one can control the elution of the analytes. Increasing the concentration of ions in the eluent will bring competition for the charged sites and analytes will leave the stationary phase and elute. Change in pH affects the charge of the molecules and thereby their affinity towards the stationary phase.

High performance anion exchange chromatography with pulsed amperometric detection, HPAEC-PAD, is a form of anion exchange chromatography. Carbohydrates are generally not anionic at pH 7, and so, in this technique a high pH is used to turn the carbohydrates into their oxyanions. The stationary phase thereby needs to be resistant to mobile phases of high pH-values. Monosaccharides are structurally similar to each other and in order to separate them a stationary phase providing high resolution is required.

Detection of carbohydrates at low concentrations requires high sensitivity. Refractive index is not a very sensitive method and UV would require derivatization of the sugars in order for them to absorb UV-light. In pulsed amperometric detection (PAD), a gold working electrode in highly alkaline solution is used to oxidize the carbohydrates, resulting in generation of a current. A series of potentials are applied to the electrode whereas the first potential is for detection and the remaining ones clean the oxidation products from the carbohydrates of the electrode. HPAEC-PAD is suitable for separating and detecting all the monosaccharides derived from *Laminaria digitata.*

2.4.3 High pressure liquid chromatography, HPLC

HPLC separates compounds based on two principles; the affinity of the analyte to the stationary phase and the analytes partitioning between the mobile and stationary phases. The stationary phase consists of fine particles of the adsorbent material to achieve a surface area as large as possible. In order to use this technique for analyzing a specific compound, the analyte must bind

to the stationary phase to a certain degree. The sample containing the analytes are transported through the column packed with the stationary phase, using a mobile phase whose polarity can vary between polar, less polar and non-polar, depending on the analyte.

There are two main configurations of HPLC, reversed and normal phase. In a normal phase separation, the stationary phase is polar while the mobile phase is less polar. When the analytes travel through the column, they will be attracted to the polar stationary phase and bind to its surface. The analytes in the sample as well as the mobile phase bind to the stationary phase. The higher the ability a solvent has to bind to the stationary phase, the greater its eluent strength. A more polar mobile phase will be a stronger competitor of the binding sites and the analytes will elute faster. In a reversed phase, the process will be basically reversed. There we have a non-polar stationary phase and a more polar mobile phase. This means that as the polarity of the mobile phase decreases the higher its attraction to the stationary phase will be, and the greater its eluent strength.

Retention time and separation of the analytes is controlled mainly by choosing the polarity of the mobile phase. Basically, the eluent strength will increase gradually as the mobile phase is made more similar to the stationary phase. HPLC will be used only to measure the amount of laminarin and mannitol in the seaweed.

2.4.4 Elemental analysis

Elemental analysis is a method for determining the amount of carbon, nitrogen, sulphur and hydrogen in organic materials. In this study, the Elementar vario MICRO cube was used. The weighed-out sample falls into a reaction chamber which contains an excess of oxygen where it is combusted at 990 °C. Carbon will be converted into carbon dioxide, Hydrogen to water, nitrogen to nitrogen gas and oxides, and sulphur to sulphur dioxides. Other elements present that will be converted into oxides, as well as some of the principal elements not of interest, are removed by absorbents.

Inert gas transports the combustion products out of the combustion oven and passes them over heated copper (about 600 ℃). The copper removes any remaining oxygen and converts the

nitrogen oxides to nitrogen gas. Gases are then transported through the absorbent traps which removes everything but water, carbon dioxide, sulphur dioxide and nitrogen.

Gases are then separated by gas chromatography (GC). This method differs from HPLC in a few ways; the mobile phase in GC consists of an inert gas and not a liquid; the stationary phase can be either a liquid or solid; elution is controlled with temperature; the compounds concentration in the gas phase is a function of the vapor pressure of the gas. Factors that influence separation are as follows:

- Vapor pressure: the lower the boiling point, the higher the vapor pressure resulting in shorter retention time since the compound will stay longer in the gas phase.
- Interaction with stationary phase: The compounds will interact with the stationary phase to a degree depending on the polarity of the compound as well as of the stationary phase. The more similar a compound is to the stationary phase, the higher the adsorption and the longer the retention time.
- Flow rate of the carrier gas and temperature: increasing the temperature will result in a larger concentration of analytes in the gaseous phase, meaning shorter retention times. A temperature too high will result in poor separation since all the components will stay in the mobile phase. Temperature gradients can be used. Higher flow rates will decrease retention times but also provide less time for compounds to interact with the stationary phase, resulting in poor separation. Length of the column and amount of injected sample will have an effect on the separation as well.

Detection is performed with a thermal conductivity detector, TCD. This detector senses a change in the thermal conductivity inside the column as an analyte gas passes through, the difference in thermal conductivity between carrier gas and analyte gas results in a response.

3. Materials and Method

3.1 Sample collection and preparation

Samples of *Laminaria digitata* were previously collected at three different locations in Kosterhavet National Park on the Swedish West Coast in the year 2012. The three locations were Ulvillarna (UL), Ursholmen (UR) and Yttre Vattenholmen (VH). From each location, samples from six different plants were collected in the months June, August and October, respectively. Within 8 hours the biomass was stored at -20 °C until the final sampling date. When all samples were collected, the algae was crushed and homogenized in liquid nitrogen using a mortal, and stored at -20 °C. Before any extractions or analyses were performed, the algae were freeze dried and ground into an even finer powder. The freeze-dried biomass was put into Eppendorf tubes with screw lids and two metal beads (5 mm diameter) were added to each tube. They were then run in a TissueLyser (Qiagen) at maximum frequency for one minute.

3.2 Hydrolysis of biomass

For IC quantification:

The hydrolysis was performed according to Manns *et al.* (Manns D 2014) as follows; 30 (±2,5) mg of dried biomass was weighed. 1 ml of 72 % sulfuric acid was added per 100 mg of biomass. The tubes where incubated at 30 °C for 1 hour, being vortexed every 15 minutes. The samples were diluted with mQ-water to 0.36 weight %, and then autoclaved at 120 °C for 40 min. The solutions were cooled down to 4 °C. The tubes were centrifuged for 1 minute and supernatant was diluted with internal standard solution of 20 g/l fructose. The samples were filtered through 2 µm PDFE filters into IC glass vials. Triplicates of each sample were hydrolyzed.

For HPLC quantification:

The hydrolysis was performed according to Veide Vilg *et al.* (Veide Vilg J 2015) as follows; 15 (±2.5) mg of dried algae was weighed. 0.167 ml of 0.5 M sulfuric acid was added per mg of algae. Tubes where heated to 100 °C in a thermomixer for 5 hours, vortexed at 500 rpm. Samples where cooled and then centrifuged for 5 minutes at 5000 xg and 4 °C. The supernatant was transferred into a new tube and kept in a freezer until analysis. Triplicates of each sample were hydrolyzed.

3.3 Quantification of total carbohydrate content by IC

Seven standards containing all of the monosaccharides existing in the hydrolyzed samples of *Laminaria digitata* were prepared. Samples were run on an ICS-3000 IC (Dionex, USA), using four different eluents consisting of mQ water(A), 300 mM NaOH (B), 85 mM sodium acetate 100 mM NaOH solution (C). Eluents were degassed with helium gas for 20 minutes. The column was run at a flow rate of 1.0 ml/min for pump 1, and 0.5 ml/min for pump 2. Column temperature was set to 30 °C with multi-step gradients as shown in Table 1. Triplicates of each sample were run.

Retention time (min)	Eluent A (%)	Eluent B (%)	Eluent C (%)
-12	0	40	40
-7	0	40	40
-6	100	0	0
27	100	0	0
27	33.3	66.7	0
37	33.3	66.7	0

Table 1: Gradients in the ion chromatograph program. Eluent (A) is mQ water, (B) is 300 mM NaOH and (C) is a solution of 85 mM sodium acetate and 100 mM NaOH. Injection occurs at time 0.

3.4 Quantification of laminarin and mannitol content by HPLC

This procedure was performed according to Koppram *et al.* (Koppram R 2012). Seven standards containing mannitol and glucose where prepared. Hydrolyzed samples were run on the UltiMate 3000 HPLC (Dionex, USA) with an eluent consisting of 5 mM H_2SO_4, the temperature in the column was kept at 80°C and the flow at 0.8 ml/min. Analytes were detected with an RI-detector. Injection volume of the sample was set to 20 µl. Triplicates of each sample were run.

3.5 Elemental analysis

Samples were run as singles, 4 (±1.0) mg of sample was weighed in a tin boat and folded into a packet. Each sample was run in the Elementar vario MICRO cube (Elementar, Germany) using

an inert gas consisting of helium and separated in a gas chromatograph. Detection was performed with a thermal conductivity detector. Protein content was estimated from the nitrogen values, using a nitrogen-to-protein factor of 5 according to Angell *et al.* (Angell R A 2016)

4. Results and discussion

4.1 Carbohydrate content

All saccharides except mannose and the components of alginate were quantified. The elemental composition was measured, and protein content was evaluated by using a nitrogen-to-protein factor. Standard deviations were calculated between the sample replicates as well as the plants collected at one site and month.

4.1.1 Mannitol

Mannitol content ranged between 9.80 and 20.64 % of dw, with higher values in June and August and lowest values occurring in October. The content measured in June was relatively constant between sites, while values in August fluctuate (Figure 1). The specific numbers are given in the Appendix table 2. These seasonal variations were in accordance with a study performed by Jensen *et al.* who also found that the mannitol content is higher in summer than in autumn (Jensen A 1956). In their research, they found the mannitol content to lay between 4 and 20 % of dw over the year. Due to mannitol being a primary photosynthetic product in *Laminaria digitata*, a higher content during summer is logical. Mannitol's potential role as an osmolyte would mean that the amount present is affected by the salinity. Some seasonal fluctuations might therefore be caused by changes in salinity with an increase in salinity resulting in an increase in mannitol content.

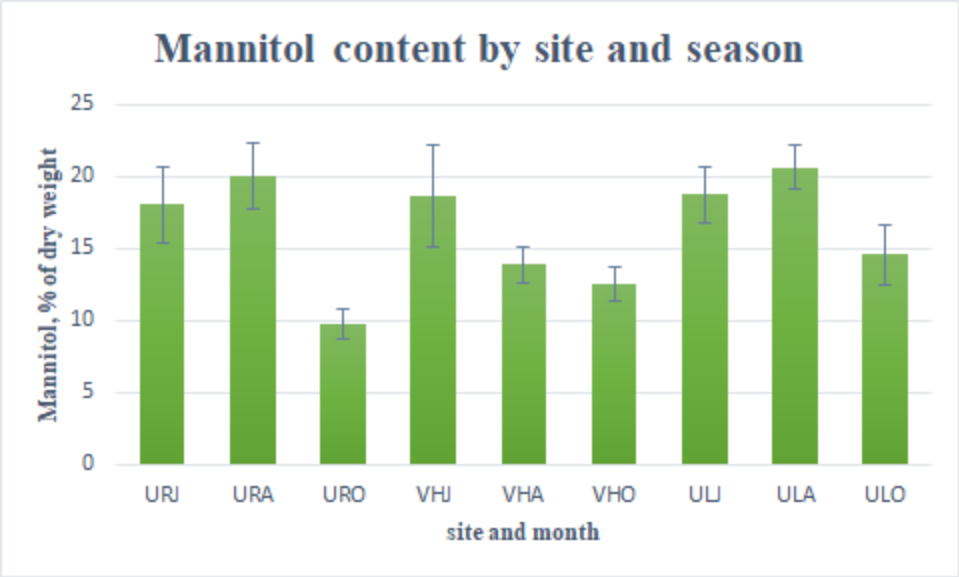

Figure 1: Mannitol content by site and season. UR = Ursholmen, VH = Vattenholmen, UL = Ulvillarna, J = June, A = August, and O = October. Samples from six plants were collected at each site and season and three replicates of each sample were run. Averages of the replicates were calculated, and the error bars show the standard deviation between these averages.

4.1.2 Xylose, Galactose and Mannose

Xylose, galactose and mannose were detected in all samples, although the levels of mannose were too low for quantification. Both xylose and galactose were quantified, though interpretation had to be made carefully as they came close to the quantification limit.

Results from the IC showed a variation in xylose levels between about 0.38 and 0.65 % of dw. Each site showed similar seasonal variations where the levels were slightly higher in October and slightly lower in June, with August somewhere in between (Figure 2, the specific numbers are given in the Appendix table 2), However, the differences were so small that a conclusion is hard to draw. Xylose content was generally higher at Ulvillarna.

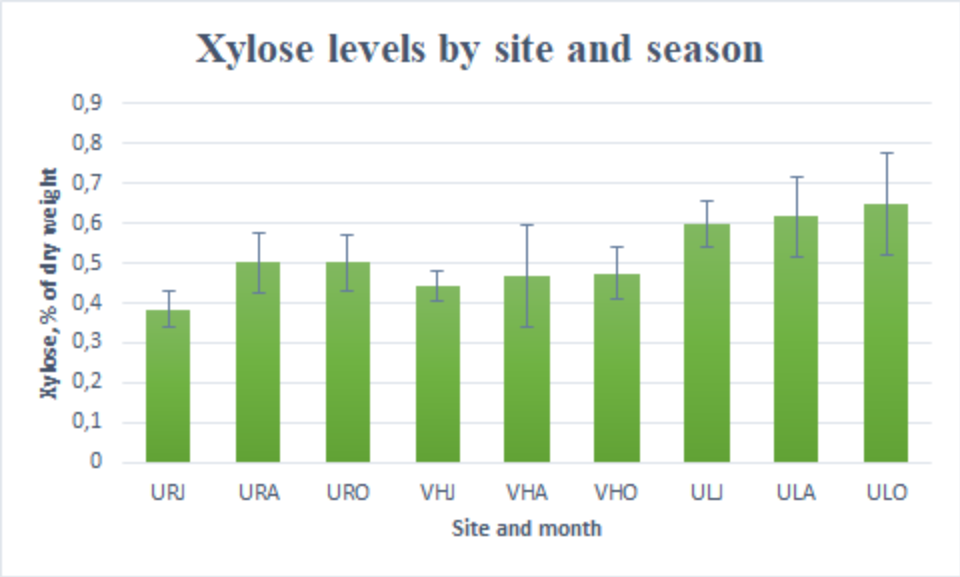

Figure 2: Xylose levels by site and season. UR = Ursholmen, VH = Vattenholmen, UL = Ulvillarna, J = June, A = August, and O = October. Samples from six plants were collected at each site and season and three replicates of each sample were run. Averages of the replicates were calculated, and the error bars show the standard deviation between these averages.

The levels of galactose ranged from 0.66 to 0.94 % of dw. Like xylose levels, the highest contents were found in October with small variations between seasons as well as the different sites. The highest values were measured outside Ulvillarna (Figure 3, the specific numbers are given in the Appendix table 2).

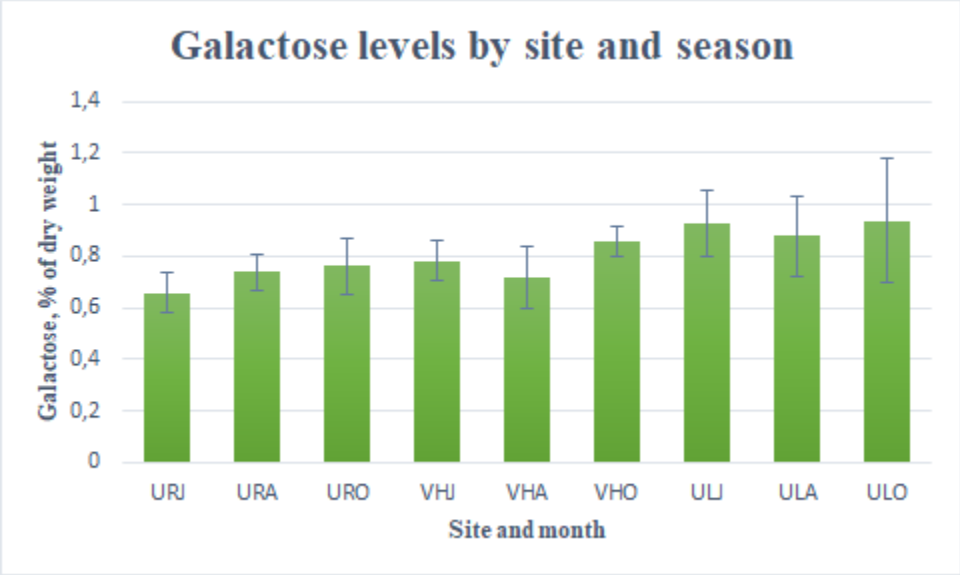

Figure 3: Galactose levels by site and season. UR = Ursholmen, VH = Vattenholmen, UL = Ulvillarna, J = June, A = August, and O = October. Samples from six plants were collected at each site and season and three replicates of each sample were run. Averages of the replicates were calculated, and the error bars show the standard deviation between these averages.

4.1.3 Fucose/Fucoidan

Fucose content was measured to be between 3.71 and 4.95 % of dw. (Figure 4, the specific numbers are given in the Appendix table 2). Bruhn *et al.* (Bruhn A 2017) showed that the levels of fucose are linear to the amount of fucoidan in the seaweed. No consistent seasonal fluctuations in fucose content between sites could be observed from the results, indicating that fucoidan levels were quite stable over seasons. Comparing the sites, the levels of fucose were slightly higher outside Ulvillarna, the same pattern shown by xylose and galactose contents. Considering that fucose, xylose and galactose are all part of the polysaccharide fucoidan, a conclusion might be drawn that fucoidan levels were higher outside Ulvillarna. Though, consideration should be taken to the high variation in both xylose, galactose and fucose content between plants at Ulvillarna.

The reason as to why Ulvillarna showed such a large variation between plants collected at the same season might be due to several factors such as age, disease, growing depth and so on. There is a possibility of differences in genetic expression but proving such requires complicated tests where all parameters must be taken into account (Under section *4.2* a large variation between the plants collected outside Ulvillarna in June is discussed). The fact that these variations between

plants occured during all three collection months in Ulvillarna indicates that the reason for the differences have a connection to the site. Possibly, the environmental conditions vary depending on where the plants were located outside Ulvillarna.

Fucoidan is a cell wall component protecting the plant from the environment, the generally higher content at Ulvillarna might be due to harsher environmental conditions. Fucoidan can constitute up to 10 % of the dw. (Obluchinskaya E D 2008) (Holdt S L 2011). Mannose has not been quantified but considering that the mannose constitutes such a small percentage of the biomass, it will have a minimal effect on the percentage. Glucuronic acid was not quantified due to insufficient peak separation but like mannose, it constitutes a small percentage of the biomass. The fucoidan can thereby still be estimated and was calculated to range between 5.00 and 6.48 % of dw. (Figure 5).

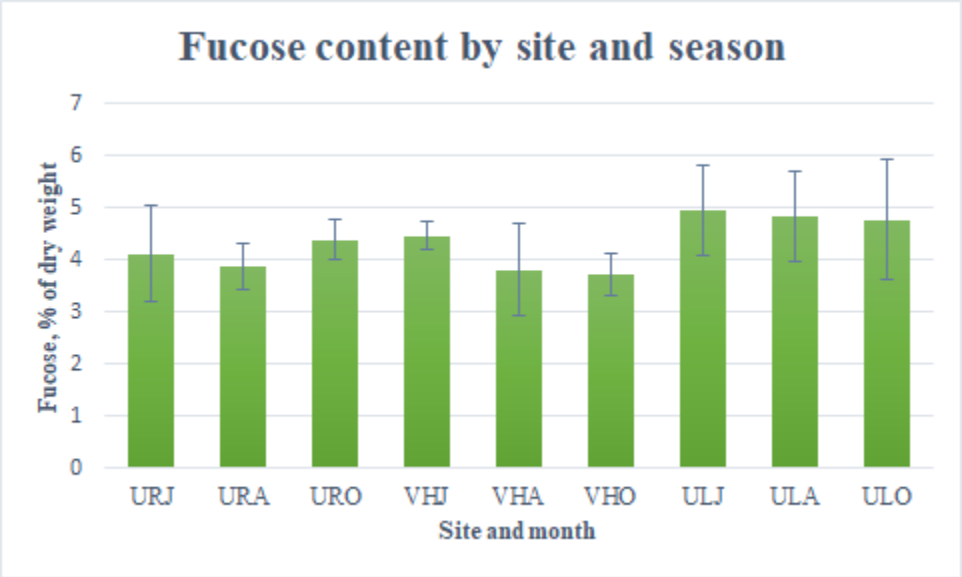

Figure 4: Fucose content by site and season. UR = Ursholmen, VH = Vattenholmen, UL = Ulvillarna, J = June, A = August, and O = October. Samples from six plants were collected at each site and season and three replicates of each sample were run. Averages of the replicates were calculated, and the error bars show the standard deviation between these averages.

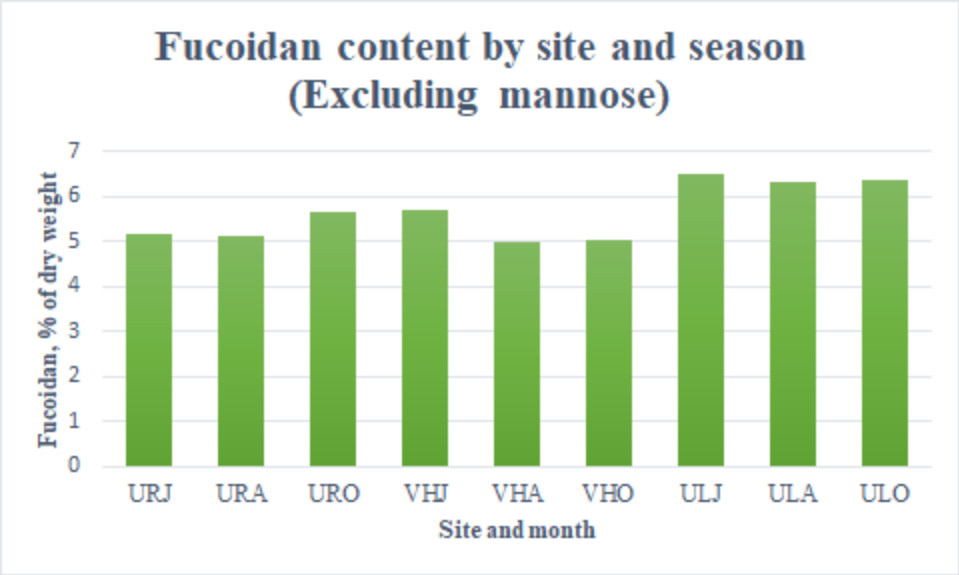

Figure 5: Estimated fucoidan content by site and season. Calculated by addition of galactose and xylose. UR = Ursholmen, VH = Vattenholmen, UL = Ulvillarna, J = June, A = August, and O = October.

4.1.4 Laminarin content

The amounts of laminarin ranged between 13.0 and 41.0 % of dw. (Figure 6, the specific numbers are given in the Appendix table 2). The values did not show any similar trends in seasonal variations between the different sites. Previous studies have shown low values in spring and peaking values in autumn (Jensen A 1956). Research by Adams *et al*. (Adams J M M 2011) also showed that concentrations of laminarin where low or absent in spring, but unlike Jensen *et al*., their research showed a peak of laminarin content in July. There was a considerable variation between the plants collected at each site, which means that the seasonal fluctuations hypothesized might have been confounded by genetic variations. A difference between sites could be observed for samples from Ulvillarna, which seemed to contain less laminarin than the other sites.

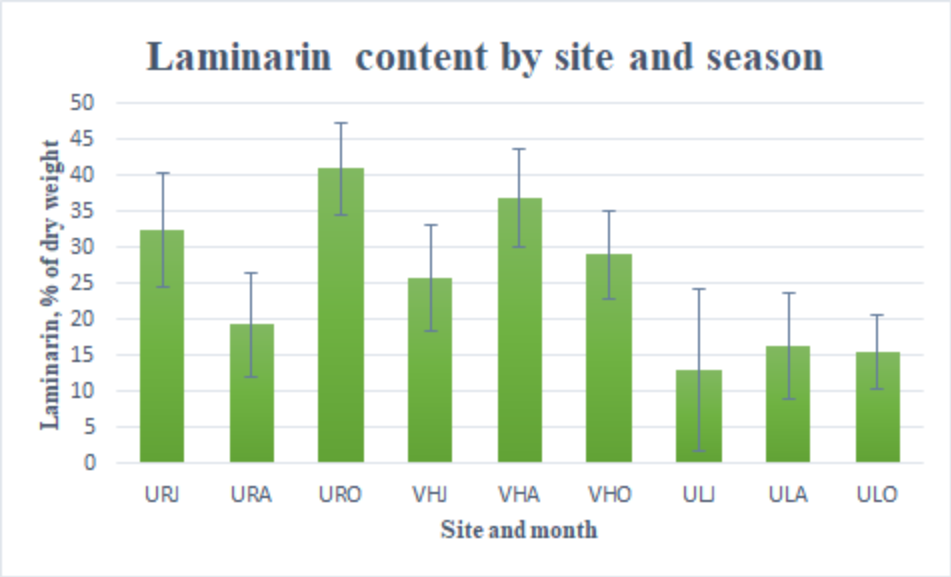

Figure 6: Laminarin content by site and season. UR = Ursholmen, VH = Vattenholmen, UL = Ulvillarna, J = June, A = August, and O = October. Samples from six plants were collected at each site and season and three replicates of each sample were run. Averages of the replicates were calculated, and the error bars show the standard deviation between these averages.

The largest standard deviation between plants collected at one site and season where observed outside Ulvillarna in June where the concentration of laminarin ranged between 4.1 and 30.6 % of dw. (Figure 7, the specific numbers are given in the Appendix table 2). One explanation might be that plant 1, 3, 4 and 6 were young and utilize energy for growth, while plant 2 and 5 were older and stored the glucose for the winter. It is also possible that plant 2 and 5 were a different species of seaweed called *Laminaria hyperborea*. The *L. hyperborea* and *digitata* species are very similar in appearance and can easily be confused when small. Of course, it could also be that it is plant 1, 3, 4 and 6 that are of the *L. hyperborea* species. The laminarin content in plant 2 and 5 were more in accordance with the values measured at the other two sites in June, while plant 1, 3, 4 and 6 agreed with each other. Therefore, it is hard to motivate leaving any samples out of the calculations, and hence they were all accounted for in the average calucation.

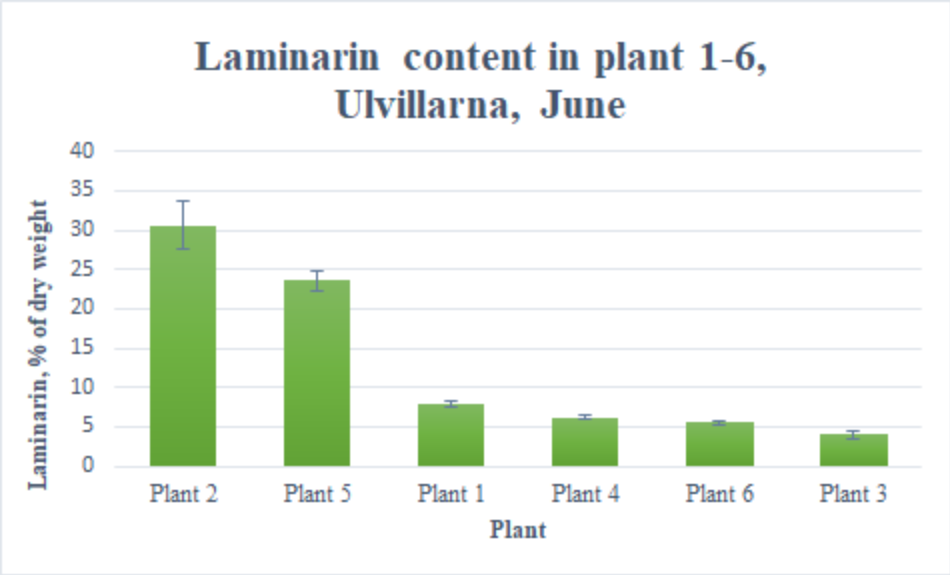

Figure 7: Laminarin content in plant 1-6, Ulvillarna in June. The error bars show the standard deviation between three replicates.

4.1.5 Glucose

The total glucose content ranged between 3.3 and 36.0 % of dw. (Figure 8, the specific numbers are given in the Appendix table 2). The seasonal fluctuations followed the same pattern as the laminarin content, implying that the amount of cellulose was consistent. Research by Schiener *et al*. (Schiener P 2015) has shown that the cellulose content is stable over the year. The mean values of total glucose content measured by ion chromatography (Figure 8) was generally lower than, although within the standard deviation of, the mean values of glucose from the laminarin measured by high performance liquid chromatography (Figure 9). Statistically, this indicates that there were no difference between the values. Determining cellulose content is therefore not possible.

The only samples showing higher or equally high mean values were those collected outside Ulvillarna. The potentially lower content measured by IC can be explained by differences in the methods as well as a low cellulose content that has a low impact on the total. Total hydrolysis is a harsh method, possibly degrading part of the saccharides to HMF and furfural. The IC samples that showed values that were higher than or equal to those from HPLC possibly contained more cellulose, although this cannot be argued with certainty considering the differences between the plants at these sites and months. Just as with the laminarin content, comparison between sites showed lower levels of glucose outside Ulvillarna (Figure 10).

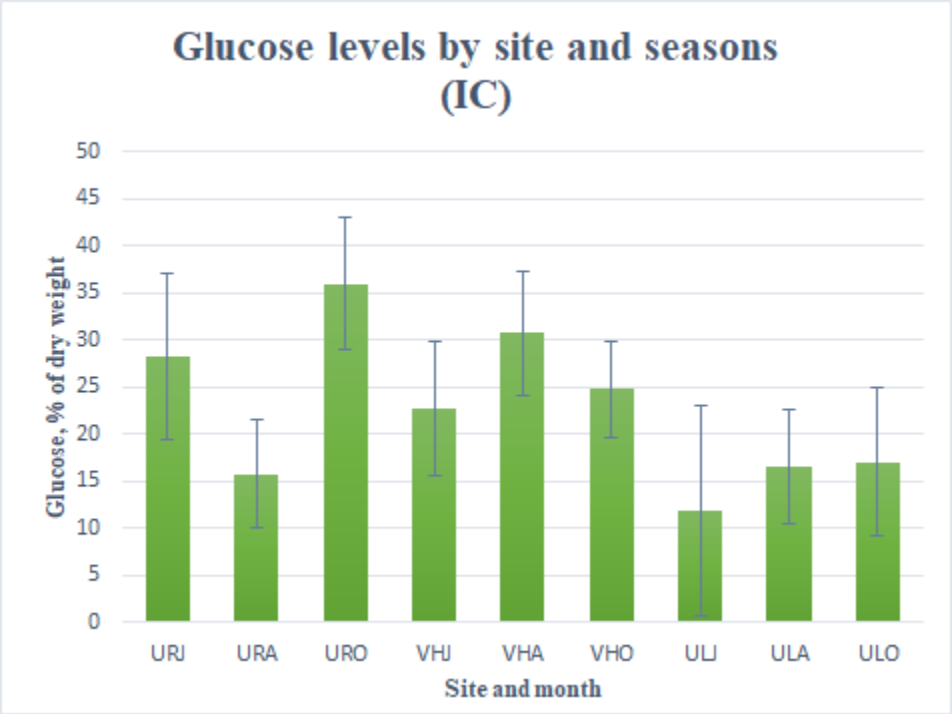

Figure 8: Glucose levels by site and season (IC). UR = Ursholmen, VH = Vattenholmen, UL = Ulvillarna, J = June, A = August, and O = October. Samples from six plants were collected at each site and season and three replicates of each sample were run. Averages of the replicates were calculated, and the error bars show the standard deviation between these averages.

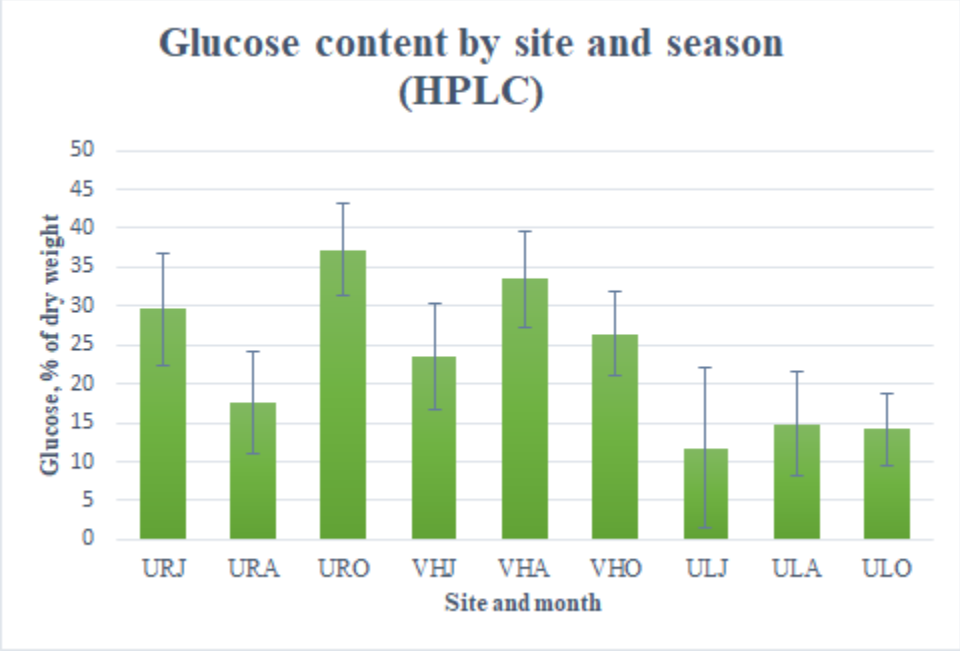

Figure 9: Glucose content by site and season (HPLC). UR = Ursholmen, VH = Vattenholmen, UL = Ulvillarna, J = June, A = August, and O = October. Samples from Six plants were collected at each site and season and three replicates of each sample were run. Averages of the replicates were calculated, and the error bars show the standard deviation between these averages.

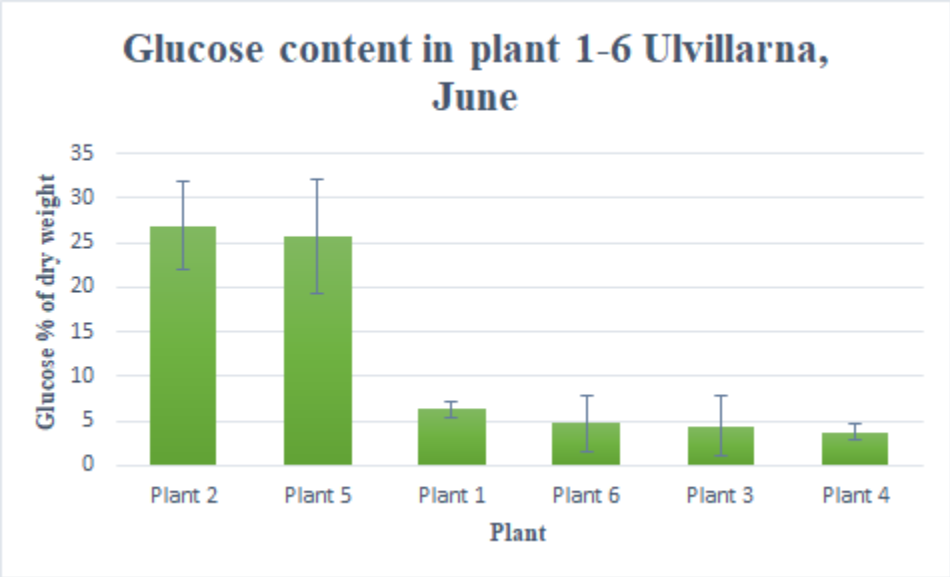

Figure 10: glucose content in plant 1-6, Ulvillarna in June. The error bars show the standard deviation between three replicates.

4.2 Elemental content

One of the samples were ran as duplicates, and no considerable variation was observed. The elemental content showed no drastic variation between seasons or sites (Figure 11, the specific numbers are given in the Appendix table 3). Nitrogen values fluctuated but remained low with values between 1.09 and 2.34 % of dw. Highest amounts were measured outside Ulvillarna in October.

The carbon content ranged between 34.5 and 38.5 % of dw with highest value outside Ursholmen in October and lowest outside Ulvillarna in October. Research by Schiener *et al.* showed values between 27.3 ± 2.8 and 36.4 ± 1.7 % of dw during August and October respectively (Schiener P 2015). Sulphur content varied from 1.24 and 1.60 % of dw with higher values at Ulvillarna than the other sites. Sulphur is a component in the fucoidan and the levels measured show the same pattern as fucoidan levels. Hydrogen content ranged between 4.90 and 5.55 % of dw.

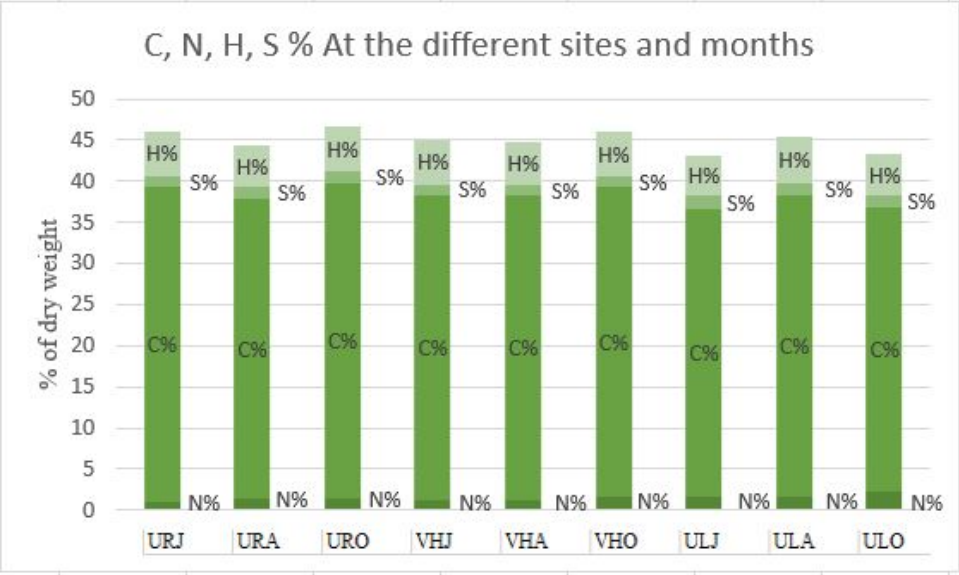

Figure 11: Elemental content at the different sites and months. UR = Ursholmen, VH = Vattenholmen, UL = Ulvillarna, J = June, A = August, and O = October.

4.3 Protein content

The protein content can be calculated using a universal nitrogen-to-protein conversion factor of 5 determined for seaweeds by Angell *et al*. (Angell R A 2016). Protein accounted for 5-12 % of the dw with the highest content measured in October with no considerable variations between June and August (Figure 12). Chapman suggested that the reason for nitrogen build-up during winter is to sustain the rapid growth rate into the summer months (Chapman A R O 1977). Ulvillarna in October showed considerably higher protein content than the other sites and months. This might be due to the plants having been younger and growing as well as contained less storage nutrients.

Previously documented amounts of protein ranged between 3 to 21% (Indergaard M 1991). In a study performed by Manns *et al.*, analyzing samples of *Laminaria digitata* collected in the Danish North Sea, the amount of proteins peaks in March with 15 % of dw (Manns D. 2017). Lowest values were seen in July and August ranging between 2.3 and 8.4 % depending on site. In this study, they used a nitrogen-to-protein factor of 4, hence resulting in lower values than would be achieved with a protein factor of 5.

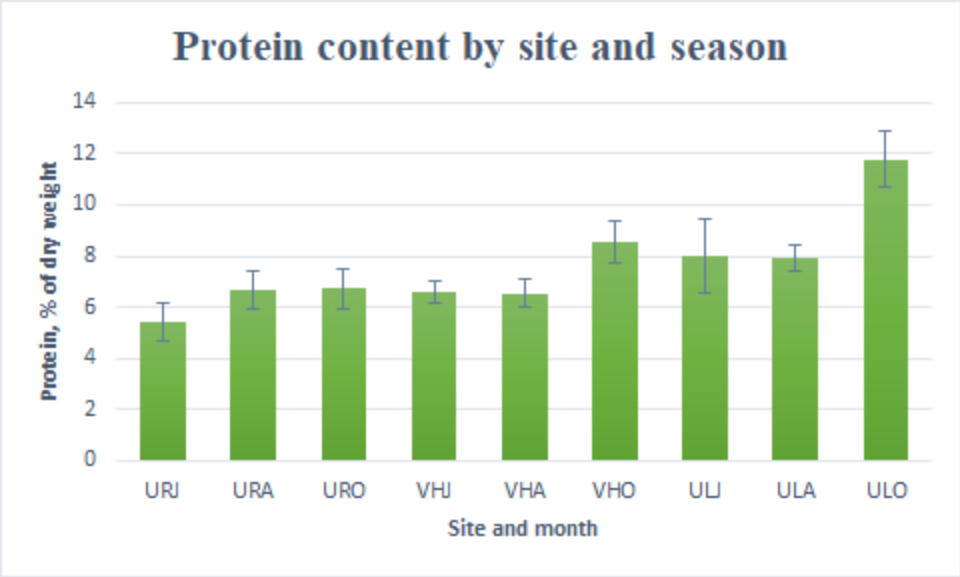

Figure 12: Protein content by site and season. UR = Ursholmen, VH = Vattenholmen, UL = Ulvillarna, J = June, A = August, and O = October. Samples from Six plants were collected at each site and season, the error bars show the standard deviation between these.

The largest variation between individuals was observed between the plants outside Ulvillarna in June (Figure 13), the same plants that showed high variations in carbohydrate content, whereas the plants containing lowest values of carbohydrates showed highest values of proteins. A lower amount of carbohydrates would increase the percental amount of proteins in the plant and might explain these variations, or the variations might be due to factors such as environmental conditions depending on where the plant grows, the age of the plant, diseases etc. where age could be a relevant factor considering nitrogen build-up is suggested to sustain the rapid growth rate and younger plants would grow faster.

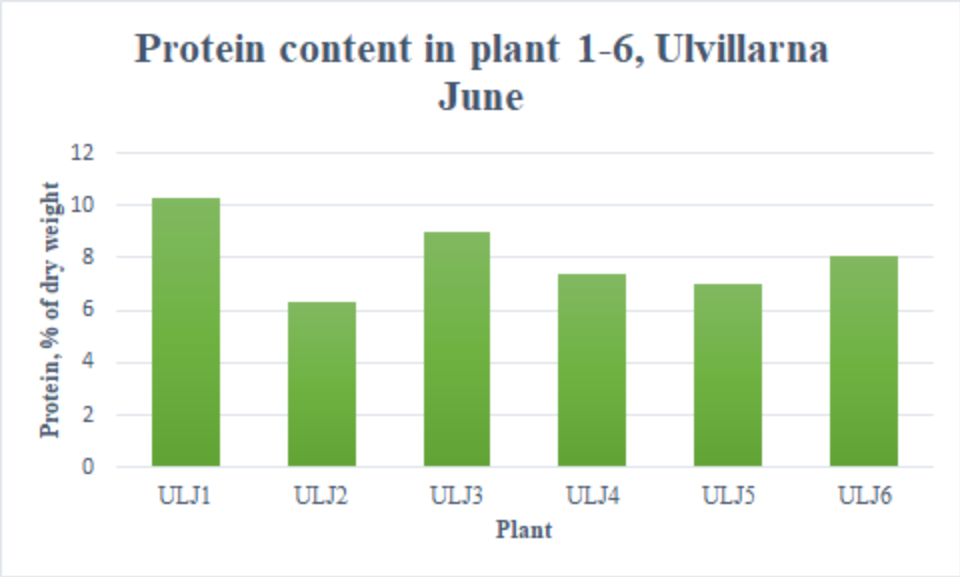

Figure 13: Protein content in plant 1-6, Ulvillarna June. UL = Ulvillarna, J = June.

4.4 Total carbohydrate content

The total of the carbohydrates quantified in this study reached highest values outside Ursholmen in June and August as well as Vattenholmen in August. Lowest values were observed outside Ulvillarna (Figure 14). Alginate, which was not quantified, may constitute 17 to 45 % of the dw of various brown algae (Jensen A 1956) (Holdt S L 2011). The amount of alginate in the seaweed has been shown relatively constant through seasons (Manns D 2014). Comparing the carbohydrate content between the sites, the lowest values were observed outside Ulvillarna with a mean value of 38.0 % of dw, while Ursholmen measured 49.4 % and Vattenholmen 48.1 % of dw. Ulvillarna had a lower laminarin/glucose content than the other sites but showed higher values of the other carbohydrates as well as the protein. A decrease in the laminarin values will increase the percentage of the other components in the seaweed. Low laminarin values as well as high nitrogen/protein indicate that these were growing individuals.

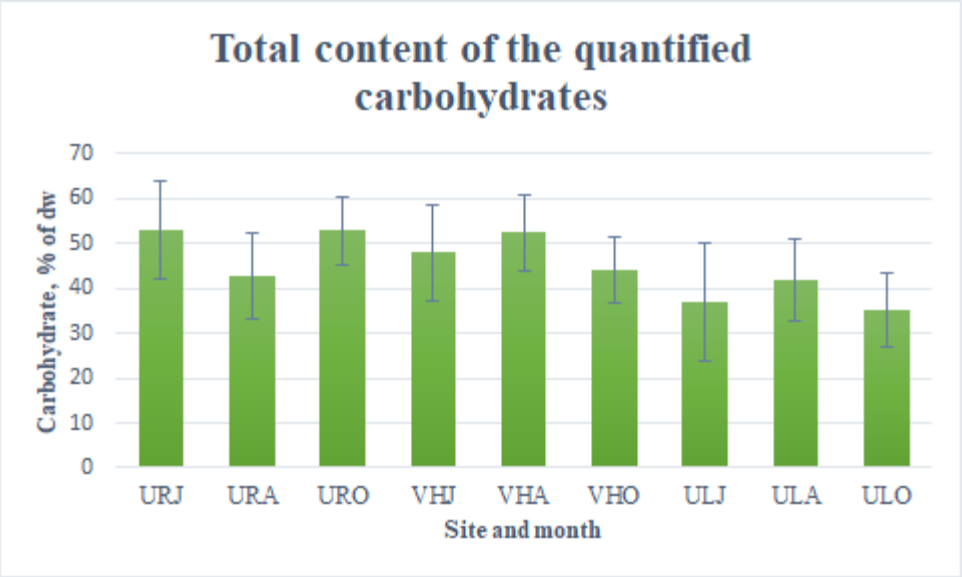

Figure 14: Total content of the quantified carbohydrates. UR = Ursholmen, VH = Vattenholmen, UL = Ulvillarna, J = June, A = August, and O = October. Samples from Six plants were collected at each site and season and three replicates of each sample were run. Averages of the replicates were calculated, and the error bars show the standard deviation between these averages.

4.5 Total measured content

Addition of all the components measured cover between 52 and 65 % of the dry weight (Figure 15). With previously measured values of alginate between 17 and 45 % of the dry weight, at least 69 to 82 % of the dry weight of biomass should be covered. This leaves a maximum of 18-31 % of the biomass to be accounted for. The remaining biomass should contain phenolic substances (phlorotannins), lipids (small amounts) and ash. Ash can compose up to 32 % of the dw with highest values during winter months which decline in to autumn (Adams J M M 2011) (Schiener P 2015).

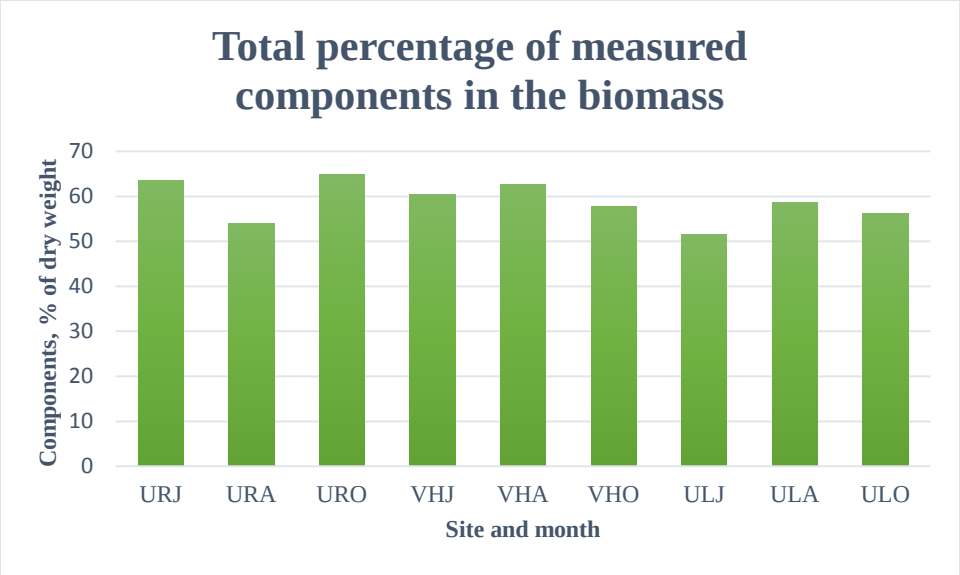

Figure 15: Percentage of the dry weight of biomass of all the measured components added together.

5. Conclusion

The amounts of saccharides measured were within the range of previously documented values. The hypothesized seasonal fluctuations could be observed for mannitol as well as protein content. The results indicated that the content of fucoidan and cellulose were stable over the seasons. The laminarin and glucose did not show any consistent seasonal variations between the different sites, likely due to other sources of variations confounding them. Standard deviation between the different plants collected at the same site and month were generally high. These differences might be caused by several factors such as variations in environmental conditions within a site, age differences between the plants or possibly diseases.

Ulvillarna showed lower amounts of total carbohydrates. Still, the conclusion cannot be drawn that Ursholmen or Vattenholmen are the optimal harvesting sites considering that the amount of alginate was not quantified. However, alginate just like fucoidan and cellulose, is a cell wall component. Considering their likely stability over seasons, alginate levels can be assumed relatively steady as well and thereby not affecting the conclusions.

The seasonal variations in total carbohydrate content showed no consistent trends between sites, and variations observed within sites were relatively small. A conclusion of the optimal harvesting season may therefore be drawn based on when the plants are of their utmost size. *Laminaria digitata* grow mainly during winter and spring (Schaffelke B 1994), promoting an early harvest, prior to a decline in growth. Consideration should also be taken to fouling that cover the surface of the kelp, reducing the commercial value of the biomass. These fouling colonies settle in mid-June and spread thereafter, harvest in early June should therefore be preferred (Førde H 2016).

6. Suggestions for future research

- To measure the seasonal variations without genetic expressions are likely affecting the results. Thus, collecting samples from one single plant throughout the year is a thought worthy option. Although, consideration would have to be taken to the plant aging, which may affect the composition, and the stress the plant would be exposed to by repeated removal of biomass for sampling as well as difficulties in obtaining enough sample.
- Further notice should be taken to the achieved results from plants collected at Ulvillarna in an attempt at establishing the reasons why this site differs from the other two.
- Drawing a conclusion of the optimal harvesting season requires knowledge of the total carbohydrate content and therefor the alginate content should be quantified. Levels are considered stable over seasons, although, in order to confirm this, the amount needs to be measured.
- Environmental factors might affect the composition in the seaweed. Tracking the changes in environmental factors between sampling occasions could help further explaining the variations in composition. Such measurements would be extremely time demanding and expensive, a realistic option is to grow the plants in tanks with controlled environmental conditions to see how these affect the composition.
- Collecting samples over a longer period of time would increase the certainty of the results. Samples collected the same month and date over a course of two year or more could provide additional data necessary to draw a correct conclusion of the optimal harvesting season.

7. Acknowledgments

I would like to thank my examiner Eva Albers and supervisor Joakim Olsson for providing me this opportunity and for all the guidance and support along the way. I would also like to thank Joshua Mayers for offering support despite not being obligated to. This project has taught me more than I would have imagined about laborative work, data analysis, writing scientific reports and the discipline.

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9. Appendix

Specific compositional data of IC and elemental analysis

Table 2: Average saccharide content from samples collected at each site and month

Saccharide	Collection site and month		Percentage of dry weight	Standard deviation
Mannitol	URJ		18.029	2.599
	URA		20.038	2.314
	URO		9.801	1.063
	VHJ		18.696	3.550
	VHA		13.884	1.229
	VHO		12.505	1.174
	ULJ		18.713	1.877
	ULA		20.635	1.496
	ULO		14.566	2.040
Fucose	URJ		4.107	0.921
	URA		3.878	0.435
	URO		4.382	0.383
	VHJ		4.462	0.279
	VHA		3.810	0.876
	VHO		3.710	0.394
	ULJ		4.952	0.879
	ULA		4.832	0.854
	ULO		4.769	1.168
Galactose	URJ		0.658	0.077
	URA		0.738	0.071
	URO		0.761	0.111
	VHJ		0.781	0.078
	VHA		0.718	0.120
	VHO		0.858	0.060
	ULJ		0.928	0.126
	ULA		0.877	0.155
	ULO		0.937	0.243
Glucose	URJ		28.252	8.736

	URA		15.760	5.775
	URO		35.977	6.999
	VHJ		22.639	7.118
	VHA		30.783	6.591
	VHO		24.803	5.116
	ULJ		11.850	11.169
	ULA		16.540	6.034
	ULO		17.068	7.809
Xylose	URJ		0.383	0.045
	URA		0.501	0.076
	URO		0.502	0.071
	VHJ		0.442	0.038
	VHA		0.469	0.129
	VHO		0.474	0.066
	ULJ		0.598	0.056
	ULA		0.616	0.101
	ULO		0.648	0.129
Laminarin	URJ		32.543	7.969
	URA		19.301	7.236
	URO		41.004	6.458
	VHJ		25.794	7.449
	VHA		36.809	6.800
	VHO		29.111	6.068
	ULJ		12.990	11.216
	ULA		16.383	7.309
	ULO		15.559	5.056

Table 3: Average content of elements, carbon, hydrogen, nitrogen and sulphur by site and season

Collection site and month	N %	Standard deviation, N	C %	Standard deviation, C	H %	standard deviation, H	S %	Standard deviation, S
URJ	1.085	0.151	38.272	1.012	5.519	0.197	1.245	0.232
URA	1.335	0.154	36.563	0.906	5.068	0.190	1.389	0.208
URO	1.343	0.160	38.488	0.858	5.484	0.239	1.325	0.090
VHJ	1.317	0.084	36.976	0.862	5.457	0.103	1.344	0.148
VHA	1.308	0.107	36.976	0.760	5.184	0.140	1.259	0.225
VHO	1.705	0.166	37.597	0.772	5.545	0.746	1.252	0.169
ULJ	1.602	0.290	35.040	2.068	4.906	0.355	1.596	0.197
ULA	1.592	0.102	36.685	1.850	5.548	0.688	1.563	0.300
ULO	2.355	0.215	34.525	0.766	4.898	0.566	1.438	0.177