CHALMERS

Variations in protein and fatty acid content in the kelp *Saccharina latissima*

Effect of season and harvest location

Degree project

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Gothenburg, Sweden, 2013

Abstract

The world's population is growing at a fast rate and the demand for food grows with it. We are already exploiting our lands at almost full capacity with plantations, livestock and more. It is important for us to be able to have multiple sources of some of our most basic nutritional needs, such as proteins and fatty acids, so we do not put too much pressure on and lose some of our sources used today.

The aim of this project was to determine if there is any seasonal variation in protein or fatty acid content in the kelp *Saccharina latissima* from three different locations around Tjärnö (Ulvillarna, Ursholmen and Yttre Vattenholmen), Sweden. The seasonal variation is interesting in order to determine the optimal time of harvest of the kelp, if it were to be cultivated. Five different methods were tested for the extraction of protein in order to see which one gave the highest yield of protein. The five methods included: 1: water extraction followed by an alkaline extraction. 2: only alkaline extraction. 3: extraction by boiling in a high concentration solution of NaOH. 4: SDS extraction. 5: trichlorolacetic acid extraction. For proteins there was a significant difference in content between harvest locations and harvest dates. The month of June was the most optimal month for harvest of the kelp, June gave an average of 0.7 % (dry weight) whilst August gave 0.4 % and October just under 0.7 % protein content in the kelp. The protein extraction method yielding the highest amount of protein was the SDS method giving about 2 % protein whilst method 1 (the main extraction method) only gave about 0.7 % protein. Only method 1 was tested on samples from all harvest dates and locations.

For fatty acid extraction a newly developed method was used involving the fatty acids being extracted during alkaline conditions and followed by methylation of fatty acids. October was the optimal month with a fatty acid content of 0.4 % compared to June and August which had a content of approximately 0.25 %. The harvest location for maximal protein and fatty acid content was Yttre Vattenholmen, the protein content in June was 0.7 %, whilst both at Ulvillarna and Ursholmen there was about 0.5 % protein in June.

Sammanfattning

Variation i protein- och fettsyrahalt i brunalgen *Saccharina latissima*

Effekt av säsong och skördeplats

Världens befolkning växer i snabb takt och efterfrågan på livsmedel växer med den. Vi utnyttjar vår mark vid nästintill full kapacitet med planteringar, för boskap med mera. Det är viktigt för oss att kunna ha flera källor för några av våra mest grundläggande näringsämnen såsom proteiner och fettsyror, så att vi inte sätter för mycket press på våra källor som används idag och förlorar en del av dem.

Syftet med detta projekt är att avgöra om det finns någon säsongsvariation i protein- eller fettsyrainnehåll i brunalgen *Saccharina latissima* från tre olika platser runt Tjärnö (Ulvillarna, Ursholmen och Yttre Vattenholmen), här i Sverige. Den säsongsmässiga variationen är intressant för att bestämma den optimala tidpunkten för skörd av kelp, om den skulle odlas. Fem olika extraktionsmetoder testades för utvinning av protein, för att se vilken av dem som ger högst proteininnehåll. De olika metoderna var: 1: vatten extraktion följt av en alkalisk extraktion 2: endast alkalisk extraktion 3: extraktion genom kokning i en hög koncentrerad lösning av NaOH 4: SDS extraktion 5: triklorättiksyra extraktion. För protein fanns det en signifikant skillnad mellan skördeplats och skördedatum. Juni är den mest optimala månaden för skörd med avseende på proteininnehåll för brunalgen. Juni gav ett genomsnitt på 0,7 % (torrvikt) medan augusti gav 0,4 % och oktober knappt 0,7 % protein innehåll i brunalgen. Proteinextraktionsmetoden som resulterade i den högsta mängden protein var SDS-metoden (metod 4), som gav ca 2 % protein medan metod 1 (den huvudsakliga utvinningsmetoden) endast gav ca 0,7 % protein. Endast metod 1 testades på prover från alla skördedatum och platser.

För fettsyraextraktion användes en nyligen utvecklad metod som innebär att syrorna först extraheras under alkaliska förhållanden för att sedan metyleras. För fettsyror är oktober den mest optimala månaden med ett cell innehåll på 0,4 % jämfört med juni och augusti som uppvisade ett cellinnehåll på ungefär 0,25 %. Skördeplats för maximal protein- och fettsyrainnehåll var Yttre Vattenholmen, proteinhalten i juni var 0,7 % medan för både Ulvillarna och Ursholmen återfanns ca 0,5 % protein i juni.

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1. Introduction

As Earth´s population is growing, its land where we can grow crops and breed livestock does not expand with it. We have a limited amount of land to use therefore we must look for alternatives or options for our land-based food production. One of those vital options can be seaweed. Macroalgae (seaweed) can contain proteins and lipids suitable for consumption and there are several ongoing and completed initiatives aiming to determine if seaweeds can potentially fill the nutritional needs of humans or other animals [Galland-Irmouli et al. 1999, Kuntze et al. 2004, Wong et al. 2001].

The commercial market for seaweed is growing fast. Seaweed has many different industrial applications, but the largest are the food and the pharmaceutical industries where it is being used as a thickening agent [Fitton et al. 2008]. Seaweed is also being used for fodder but only in small scale and in coastal regions [Yuan et al. 2008, Kumar et al. 2007]. By examining nutritional values of different algal species, the potential for using algae as a nutrient-rich and cheap fodder can be assessed.

Humans have consumed algae for centuries around the world, especially in Japan were it is considered being a delicacy. In the western world, there is an increase in sushi consumption and the view of certain macroalgae as health foods; we are possibly ready to change our consumption pattern. Marine algae could constitute an important source of nutrients, such as protein and lipids. The nutritional content of algae varies with the environmental conditions, so it is important to investigate when and where to harvest to achieve the optimal nutritional value.

In this report, the protein and fatty acid contents of the seaweed *Saccharina latissima* will be described, as well as the variation in content depending on time-point and location of harvest. The goal of the project is to obtain knowledge on variations in content since such knowledge is needed in order to determine the optimal time of harvest for the kelp. The algae for this project were collected at Ulvillarna, Ursholmen and Yttre Vattenholmen, which are all located close to Tjärnö outside of Strömstad, Sweden. The harvest dates were in June, August and October. All samples were examined to determine the optimal harvest date and location to obtain the greatest amount of protein and lipids.

2. Background

2.1 Seaweeds

Marine algae represent over 90 % of all marine primary producers (macro- and microalgae included). There are over a thousand different species of macroalgae (seaweeds) and they are classified into three major groups: green (*Chlorophyta*), red (*Rhodophyta*) and brown (*Phaeophyta*) [Fitton et al. 2008]. This report will only focus on the brown species *Saccharina latissima*, which is one of the most common species of macroalgae found in the seas around Scandinavia.

Figure 1: Picture of *Saccharina latissima* taken by Cwmhiraeth on 18 September 2011 (available at http://en.wikipedia.org/wiki/File:Saccharina_latissima_2.jpg)

Saccharina latissima can be found in most parts of the North Sea. The algae stalk is called stipe and its blades lamina, when new tissue develops on algae it starts to grow near the base of the lamina close to the stipe. Because of this the old tissue is coming further away from the stipe and can continuously erode away to give place for new younger tissue. This also makes the stipe constant and the lamina annual. Kelp can store photosynthesis products and therefore grow as usual even if there is a shortage of sunlight during its growth season. This type of growth strategy ensures that the kelps annual growth is about the same each year. Algae reproduce using spores [Wegeberg 2010].

2.2 Historical applications of seaweed

Archaeological findings in Chile indicate that humans were using seaweeds approximately 14000 years ago. According to early historical written texts, seaweeds were consumed in Japan approximately 1500 years ago [Buchholz et al. 2012]. Historical records show that seaweed was not only used in foods but also as a medicine for treating snakebites, lung diseases, gout and more [Fitton et al. 20008]. When the historical findings were found in

Chile, four different species of seaweed were found: *Sargassum*, *Gracilaria*, *Porphyra* and *Durvillea*. It is believed these four types of seaweed were the most common species used as medicine at that time.

Seaweed has been considered a “health food” throughout history around, it is said to prevent iodine deficiency, alleviate indigestion or heartburn and to be used as a laxative, an antiseptic poultice for a deep cut or swelling, and a broken collarbone [Fitton et al. 2008]. In Asian countries seaweed have had a dominant use in food, dating back to the Asukaand Nara Era in Japan approximated 1500 years ago [Buchholz et al. 2012].

2.3 Uses of seaweed today for food and fodder

Seaweeds have a variety of applications today; it is commonly used in foods around the world as seasonings and condiments (“Arame”, “Wakame” and “Hijiki” in Japan). Another common use is however to extract the polysaccharides agar, alginate, and carrageenan, which constitute large parts of certain seaweeds and can be used as thickening agents in foods and pharmaceuticals. Seaweed has many applications in foods and it is used in different kinds of cuisines around the world such as: “Hai dai”, a soup from China, Laver bread in Wales and even in a chewing gum “Karengo” in New Zealand [Yuan et al. 2008]. Seaweed today is also sold as a “health food” in health stores around the world where it is said to reduce body weight, be anticancer, blood-purifying and more [Food matters 2013]. But, most of these stores sell all kind of seaweed without the proper knowledge of which species of seaweed is the best at example weight-loss [Food matters 2013].

During the last 50 years the global food production of seaweed has rapidly grown, although it has been a tradition in Asia for centuries. In 2010, 16.8 million tons of seaweed and other marine plants were harvested [FAO 2010] and 99.95 % of that were farmed in Asian countries [Buchholz et al. 2012].The reason the seaweed demand has grown rapidly the last 50 years (outside of Asia) is because it can be used for a commercial value, it can be used in the food and pharmaceutical industry as said before, because it is a rich source for polysaccharides.

Seaweed has also been used as fodder for livestock, especially in coastal regions in Scandinavia and some European countries. It was used because it provides additional vitamins and minerals. Brown kelp and *Rhodophyceae* (red algae) species were mostly used in diets for poultry, swine, cattle, and sheep [Yuan et al. 2008, Kumar et al. 2007].

2.4 Uses of seaweed in medical applications

Studies have shown that a diet with daily intake of seaweed can reduce the risk of various diet-related chronic diseases, such as for example atherosclerosis and hyperlipoproteinemia related to cardiovascular disease (CVD) as well as breast and colon cancer [Yuan et al. 2008]. It was shown that the mortality rate in CVDs was much lower in Japan (approximately 50 % lower than in Europe and North America) [Yusuf et al. 2001], where almost 100 % of the population consume seaweed on a daily basis. These results indicate that seaweeds could have

anticancer and lipid lowering effects, which partly are explained by that they are a good source of antioxidants [Yuan et al. 2008].

Scientists have found that some species of brown kelp can have anti-HIV activity [Thomas et al. 2011, Ahn et al. 2004]. Research has also shown that phlorotannins from kelp (that also are antioxidants) have the same or even better inhibitory activity than some available commercial anti-HIV drugs; the inhibitory activity slows down the spread of HIV in the body and prevents it to develop into AIDS. But research is still at an early stage, it is too soon to say if algae can prevent the spread of HIV any further [Thomas et al. 2011]. Experiments also indicates that some species of algae can have anti-cancer effect, but this research is also at an early stage as the anti-HIV research and only red algae types have been tested [Harada et al. 1997, Thomas et al. 2011].

2.5 Seaweed and energy

Seaweeds contain up to 50 % carbohydrates, which make them an attractive raw material for bioenergy production. There is ongoing research on the feasibility to produce biogas from seaweed. It is possible to produce biogas from seaweed and it even gives a higher methane yield than other sources used today. But, due to its high feedstock cost, seaweed is not yet an option for biogas production from an economical point of view [Jung et al. 2012]. The high amount of carbohydrates also makes seaweed a potential candidate in bioethanol production. A microbial platform for the conversion of macroalgal carbohydrates into ethanol was recently developed [Wargacki et al. 2012], but there is still a need for development of both biomass production and processing before seaweed can be considered a sustainable raw material for bioethanol production. Combining the bioethanol production with extraction of more valuable compounds, in a biorefinery concept, could however increase the possibilities for economic sustainability. Such compounds could be the above mentioned antioxidants, as well as nutritionally valuable lipids and proteins.

2.6 Protein and lipid in macroalgae

Proteins and lipids are essential for human survival. Therefore it is important to have a sufficient amount of sources. Proteins are large biological molecules which consist of one or more chains of amino acids; they perform essential functions in our bodies such as catalyzing metabolic reactions, replicating DNA and transporting molecules around our bodies. Lipids consist of different types of molecules, of which fatty acids are the dominant ones (this project will only focus on fatty acids). The main biological functions of lipids are energy storage and structural composition of cell membranes. In short, proteins and lipids are vital for the survival of humans and animals.

Tests have shown that the essential amino acids histidine, leucine, cysteine, phenylalanine, tyrosine and threonine and the nonessential amino acids glutamic acid, serine and alanine are found in the red algae *Palmaria palmate*, which can be found in waters around Europe [Yuan et al. 2008]. Some essential fatty acids have been found in algae, such as C20 PUFA chains.

These long-chain fatty acids are often associated with fish oils [Yuan et al. 2008, Sánchez-Machado et al. 2004]

2.7 Growth locations and harvest dates

Already during the 1940s there was a scientific interest in in the seasonal variations in algal composition. Studies from that particular time show the difference in weight and content of compounds such as mannitol, cellulose and fat. These tests were performed on the most common seaweeds around the British Isles, such as *Laminaria digitata* and *Laminaria saccharina* [Black 1950].

Most studies on marine algae have been done on red algae (*Rhodophyta*) due to the fact that they have a long history of consumption in Asia, America and some European countries. Many tests have been made to see if there is any difference in nutrient values (proteins and lipids in this case) from different geographical locations and harvesting dates. In one study, red algae were collected from the French Brittany coast, Ireland, Spain and Norway. In Algae from these locations the protein value varied throughout the year, with the highest values during winter/spring (approximately 25 % protein content) and the lowest during the summer/fall (approximate 12 % protein content). Lipids content varied similarly as proteins, when it came to location and harvest date, but the amount of lipid was approximately the same throughout the year (1-5%) [Yuan et al. 2008].

The reason that protein contents differ over the year is because there are more nutrients in the water during the winter and spring than during the summer and fall. This difference in nutrient availability gives that the algae can accumulate more nutrients during the times with high levels and produce more amino acids that constitute the proteins. When it comes to geographical location, the locations with the most available nitrogenous nutrients had the highest yield of protein content. The “best” location from the study mentioned above was Northern Ireland with a protein yield of about 25 % [Yuan et al. 2008]. The salinity and wave action also play a large role in the growth of alga. A relative high salinity level in the sea is usually followed by a high level of nutrients, which often is favorable for the algae. An adequate level of wave action can prevent fouling of the seaweed and will also replenish the nutrient supply in the water. As for all other photosynthetic organisms, sunlight is crucial for the survival and growth of algae. Therefore, they must grow on a depth where the sunlight can reach it. This depth usually does not extend 10 meters below the surface [Wegeberg 2010].

The aim was to examine the differences in protein and fatty acid content in relation to time and location of harvest. Since the chemical composition of seaweed varies over the year [Black 1950, Yuan et al. 2008], it is important to know when to harvest to obtain the optimal amount of wanted components.

3. Materials and methods

3.1 Sampling of kelp

Six specimens of the kelp *Saccharina latissima* were collected at three occasions, June (5/6-2012), August (8/8-2012) and October (23/10-2012), and at three sites in the sea outside Tjärnö, Strömstad, Sweden, Ursholmen (UR), Yttre Vattenholmen (VH,) and Ulvillarna (UL), by the scientists at the Marine Biology station, Tjärnö. Because of storms in October the kelp was absent at two of the sites, and thus in total 42 samples were used in this study. After collection, the whole blade were put in the freezer within 8 hours and stored at -20°C. After collection of all samples the stipes were removed and the blades were crushed and homogenized with a mortal in liquid nitrogen. Portions of the homogenized samples were stored at -20 and -80 °C, respectively. The kelp samples were freeze-dried and grinded by a mortal to a fine powder and subsequently stored at -20 and -80 °C, respectively, until use.

3.2 Determination of moisture content in samples

All homogenized samples (before freeze drying) were each weighed and put in individual metal containers and placed in an oven at 105°C for 19 hours. Samples were cooled to room temperature in vacuum and weighed again to acquire the dry mass content.

3.3 Extraction of total proteins from kelp biomass

The measurement of total proteins in biomass was done according to Harnedy et al [2013], in which the proteins were solubilized by water followed by creation of alkaline conditions and detected using the Lowry principle. Biomass (approx. 25 mg of -20 °C stored material) in 500 µl of mQ-water was homogenized by glass beads (approx. 0.2 g) in a Fastprep-24 (MP) at speed 6.5 M/S for 40 s and repeated 8 times. To extract water soluble proteins the tubes with sample were shaken at 4 °C for 16 hours. The supernatants were recovered by centrifugation (20 min at 4°C, 12000 xg) and stored at -20 °C. The pellet was extracted with alkaline reagent (0.12 M NaOH, 0.5 % β-mercaptoethanol) twice. The samples were shaken at room temperature for 1 hour. The supernatants were recovered by centrifugation (20 min at 4°C, 12000 xg) and stored at -20 °C and all three extracts were pooled.

Alternative methods for the extraction were also evaluated. In the first method, all above steps were made except the water extraction (alkaline reagent was used during the Fastprep step). In the second method, 500 µl of 1 M NaOH with 5 % β-mercaptoethanol was used during the Fastprep step and the sample was boiled for 15 min. In the third method, 500 µl of denaturation solution (2 % SDS, 5 % β-mercaptoethanol) was used during the Fastprep step and boiled for 15 min afterwards. The final method was done based on Slocombe et al. [2013], in which 500 µl of 24 % trichloroacetic acid (TCA) was used during the Fastprep step and the sample was subsequently incubated at 95°C for 15 min. To the sample 600 µl of mQ-

water was added and subsequently it was centrifuged at 12000 xg for 20 min at 4°C. The supernatant were removed and discarded, 500 µl of 0.12 M NaOH was added to the pellet and samples were incubated at 55°C for 3 hours. To recover the final supernatant with solubilized proteins, samples from all different methods were centrifuged at 12000 xg for 20 min at 4°C (except the final method, which was at room temperature) and supernatants were frozen at -20°C until analysis.

3.4 Measurement of solubilized proteins

Solubilized proteins were measured with the biorad Dc kit (BIO-RAD), which is based on the Lowry method, with Bovine Serum Albumin as standard in the range 0-1.35 µg. Before measurement, proteins were precipitated by TCA, 500 µl of sample (in the alternative methods 100 µl of sample), 50 µl of 1.5 g/l deoxycholic acid and 50 µl of 70 % TCA were added sequentially. Precipitated proteins were pelleted by centrifugation (15000 xg for 10 min) and the supernatant was discarded. The pellet was dissolved in 250 µl kit reagent A and 1000 µl kit reagent B were added. Samples were incubated in room temperature for 30 min and 200 µl of sample was transferred to a 96 well microtiter plate (Sarstedt). Absorbance was measured at 750 nm using a plate reader (FLUOstar Omega, BMG LABTECH).

3.5 Measurement of fatty acids in kelp

The extraction and measurement of fatty acids in kelp biomass was done according to an unpublished method by Carlsson and Cavonius, 2013, in which the lipids first were extracted using an alkaline reagent, followed by methylation of fatty acids and detection using gas chromatography. Biomass (approx. 400 mg of - 80°C stored material), 50 µl internal standard (1.02 g/l C17:0 in toluene), 4 ml of solvent (ethanol:methanol 3:2 and 0.5 % pyrogallol) and 1 g of KOH tablets were incubated for 2 hours at 70°C. 5 ml of toluene and 2 ml of 6 M HCl were added before centrifugation (2000 g for 6 min), the organic phase was recovered and the remaining aqueous phase was washed with 2 ml of toluene and centrifuged once more. The organic phases were pooled together and evaporated under N_2-gas. To the residues, 1 ml of toluene and 1 ml of methylation solution (10 % acetyl chloride in methanol) were added before incubation at 70°C for 2 hours. After incubation, 0.2 ml of milliQ-water and 5 ml of ether solvent (petroleum ether / diethyl ether 80:20) were added and the organic phase was recovered by centrifugation (2000 xg for 6 min), 1 ml of sample was evaporated under N_2 atmosphere and 0.5 ml of iso-octane (2,2,4-trimethylpentane) were added. 200 µl of sample were transferred to a GC-vial before being analyzed by GC (HP, 5890). GC was run with a polar column (Agilent J&W DB®-WAX), the oven temp at 100-250°C, injector at 250°C, He & H_2 (50/50) as driving-gas, and flame ionization (FID) was used as detector at 275°C.

4. Results

In this study, the contents of proteins and lipids in the kelp *Saccharina latissima* were evaluated regarding variations depending on time of the year and growth locality.

4.2 Water content in kelp *S. latissima*

The water content of the initially homogenized kelp samples was determined and it was found that the samples could retain substantial amounts of water, 60-80%, see figure 2. The content of water varied depending on harvest location and date. In order to facilitate for a more fine mixing all samples were freeze-dried before any analyses were done on them.

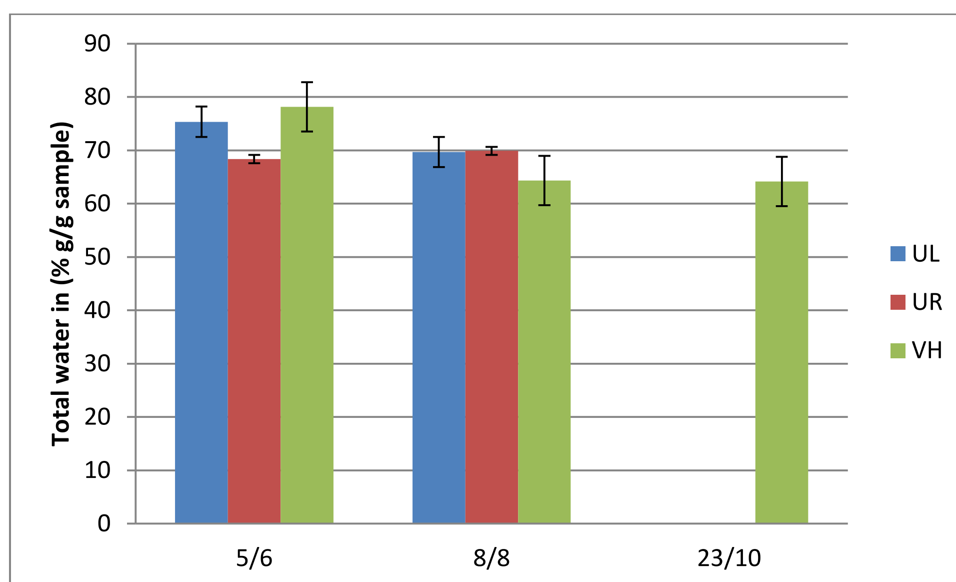


Figure 2: Content of total water (% g/g sample) in *S. latissima.* Data shown are average amount of water in six kelp blades for each harvest date with standard deviation as error bars. Locations are Ulvillarna (UL), Ursholmen (UR) and Yttre Vattenholmen (VH).

4.2 Content of protein in the kelp *S. latissima*

The protein content in kelp was measured for all 42 samples by the first method described [Harnedy et al, 2013], in which proteins were extracted first by water and then by alkaline conditions. The data were both related to the dry and wet weight of kelp biomass (for all data see table 1, Appendix A) and as seen in figure 3 and 4 there is a difference between trends between the two figures. In figure 3 (dry weight) the month of June gave the greatest protein content, while in figure 4 (wet weight) August gave the greatest protein content. Unfortunately there were only samples in October from Yttre Vattenholmen due to storms when the samples were harvested. According to both figure 3 and 4 the optimal harvest location of *S. latissima* is Yttre Vattenholmen (VH).

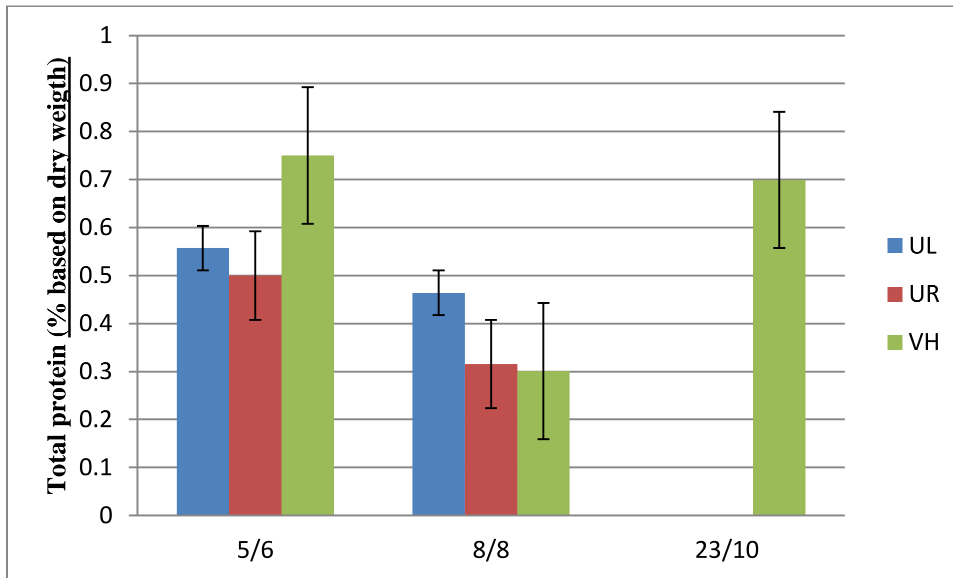


Figure 3: Content of total proteins (% based on dry weight) in S. *latissima*. Data shown are average amount of protein in six kelp blades for each harvest date with standard deviation as error bars. Locations are Ulvillarna (UL), Ursholmen (UR) and Yttre Vattenholmen (VH).

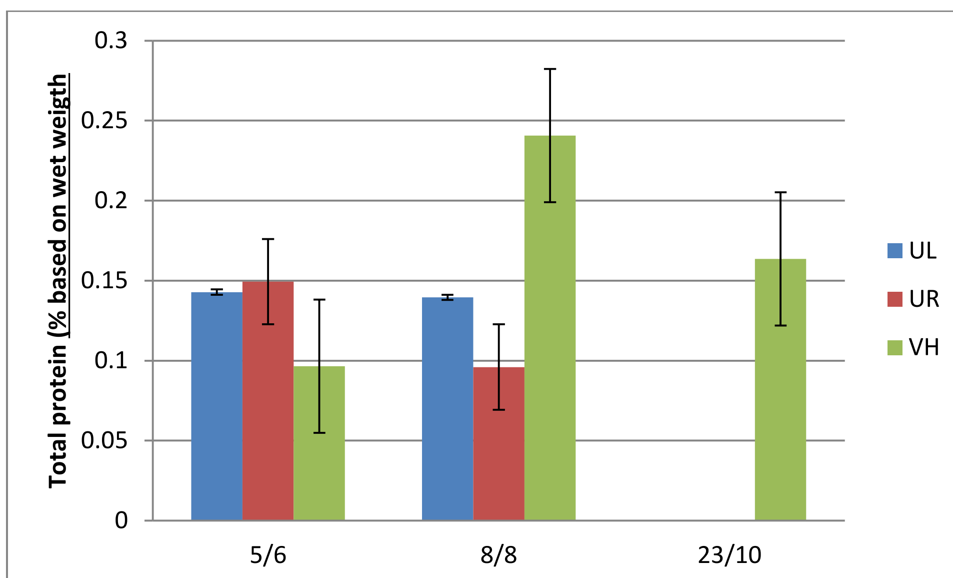


Figure 4: Content of total proteins (% based on wet weight) in S. *latissima*. Data shown are average amount of protein in six kelp blades for each harvest date with standard deviation as error bars. Locations are Ulvillarna (UL), Ursholmen (UR) and Yttre Vattenholmen (VH).

4.3 Evaluation of efficiency for various protein extraction methods for the kelp *S. latissima*

Overall the protein values reported in figure 3 and 4 were very low and far below literature data. This could be a result of poor extraction of proteins from biomass using the selected method. Thus, alternate methods for extraction need to be evaluated to identify a more efficient method for the protein analysis. To identify a protein extraction method that more efficiently solubilize proteins from kelp biomass, four alternative extraction methods for protein were evaluated. The methods selected were compared with the initial method (method 1) and were as follows; 2: extraction by alkaline conditions without water extraction, 3: extraction by boiling in strong NaOH, 4: SDS extraction and 5: TCA extraction. These tests were only made on one single algae sample (Ulvillarna 5/6-5) and duplicate tests were made for all alternative methods. The data showed that the initially used method resulted in the lowest values (compared with all protein samples). The best method was method 4 (SDS extraction), which gave the highest amount of protein in the sample with up to 3 % per dry weight of biomass, seen in figure 5.

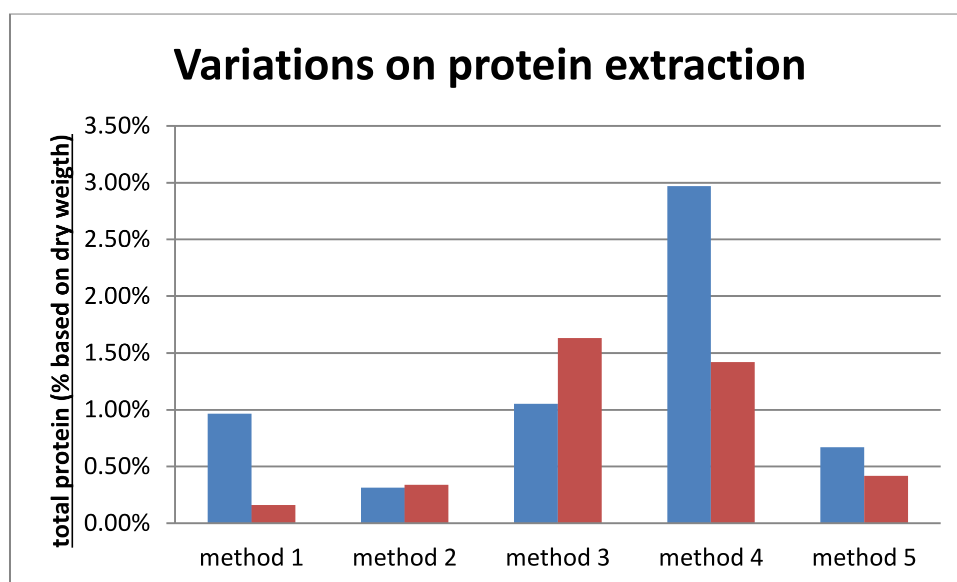


Figure 5: The protein content (% g/g dry weight) in Ulvillarna (UL) 5/6-5 determined by the alternative protein extraction methods. The blue and red bars represent duplicate tests using each method

4.4 Content of fatty acids in the kelp S. *latissima*

The fatty acid extraction test was only done on 21 of the samples, due to time constrains. The fatty acids were extracted using alkaline conditions, followed by methylation of fatty acids. The data were both related to the dry and wet weight of kelp biomass (for all data see table 1, Appendix A). As seen in figure 6 and 7, the same trends were seen irrespectively if related to dry or wet weight. The fatty acid content is more or less constant throughout the summer (June-August). However, higher levels of fatty acids were found in October as seen in figure 6 and 7. There were slight differences in the lipid content between samples from different

growth localities, where Yttre Vattenholmen had a slightly lower average than Ulvillarna (UL) and Ursholmen (UR) (October month excluded). In general, the fatty acid content in *S. latissima* was low, 0.2-0.4%, which is in accordance with reported data from other kelp species [Dawczynski et al 2007].

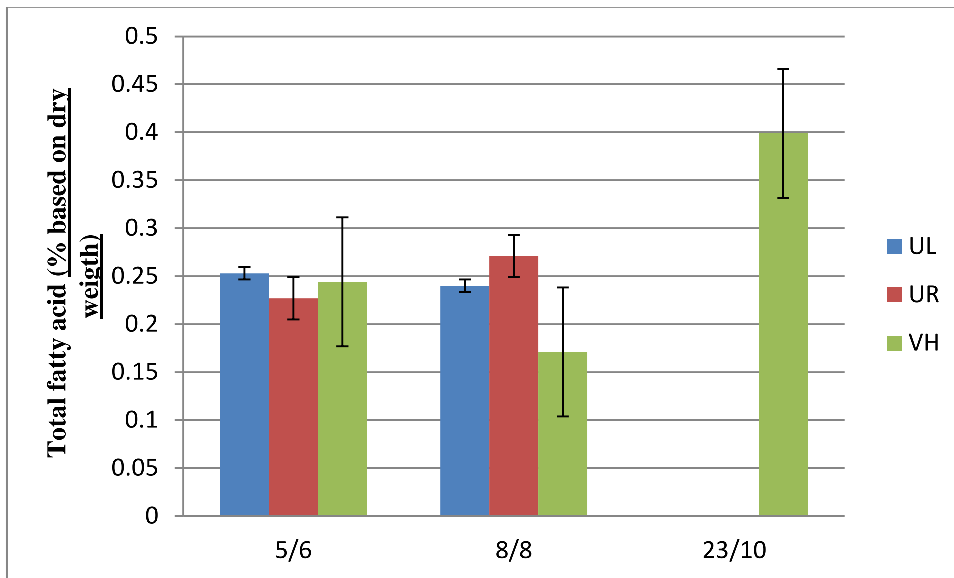


Figure 6: Content of total fatty acids (% based on dry weight) in *S. latissima*. Data shown are average amount of fatty acids in three kelp blades for each harvest date with standard deviation as error bars. Locations are Ulvillarna (UL), Ursholmen (UR) and Yttre Vattenholmen (VH).

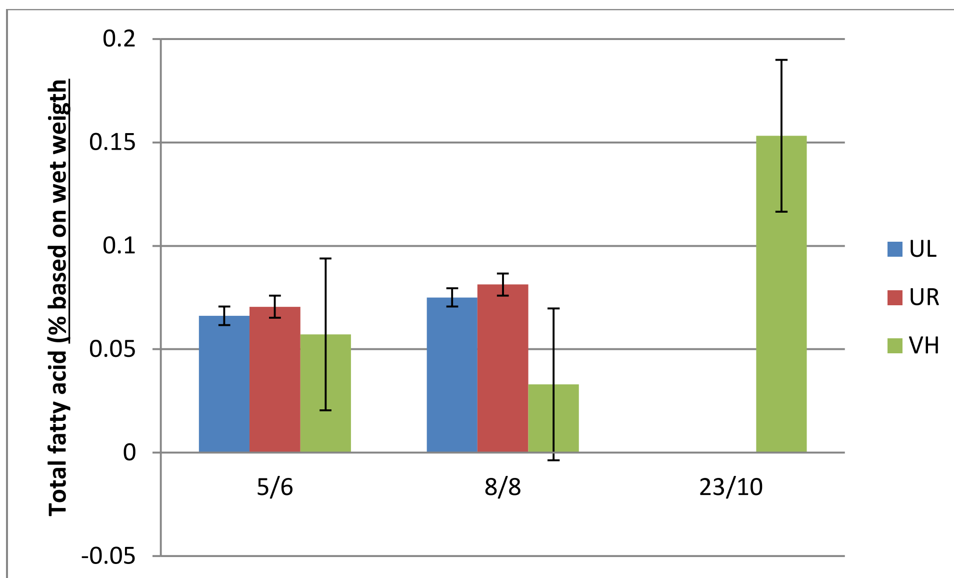


Figure 7: Content of total fatty acids (% based on wet weight) in *S. latissima*. Data shown are average amount of fatty acids in three kelp blades for each harvest date with standard deviation as error bars. Locations are Ulvillarna (UL), Ursholmen (UR) and Yttre Vattenholmen (VH).

4.5 Types of fatty acids in the kelp S. *latissima*

The different peaks of the GC chromatogram (as exemplified in figure 8) each represent a fatty acid (see Appendix table 2 for all samples). The fatty acids found in highest amounts in the samples were identified to be the following: methyl tetradecanoic acid (C14:0), palmitic acid (C16:0), oleic acid (C18:1), arachidonic acid (C20:4n6), eicosapentaenoic acid (C20:5n3, EPA) and docosahexaenoic acid (C22:6, DHA). These fatty acids were found in all samples except DHA which was only found in 6 samples, it is however included because it is one of the important omega-3 fatty acids (worth to notice is that several peaks were higher than the added internal standard (50 µg)).

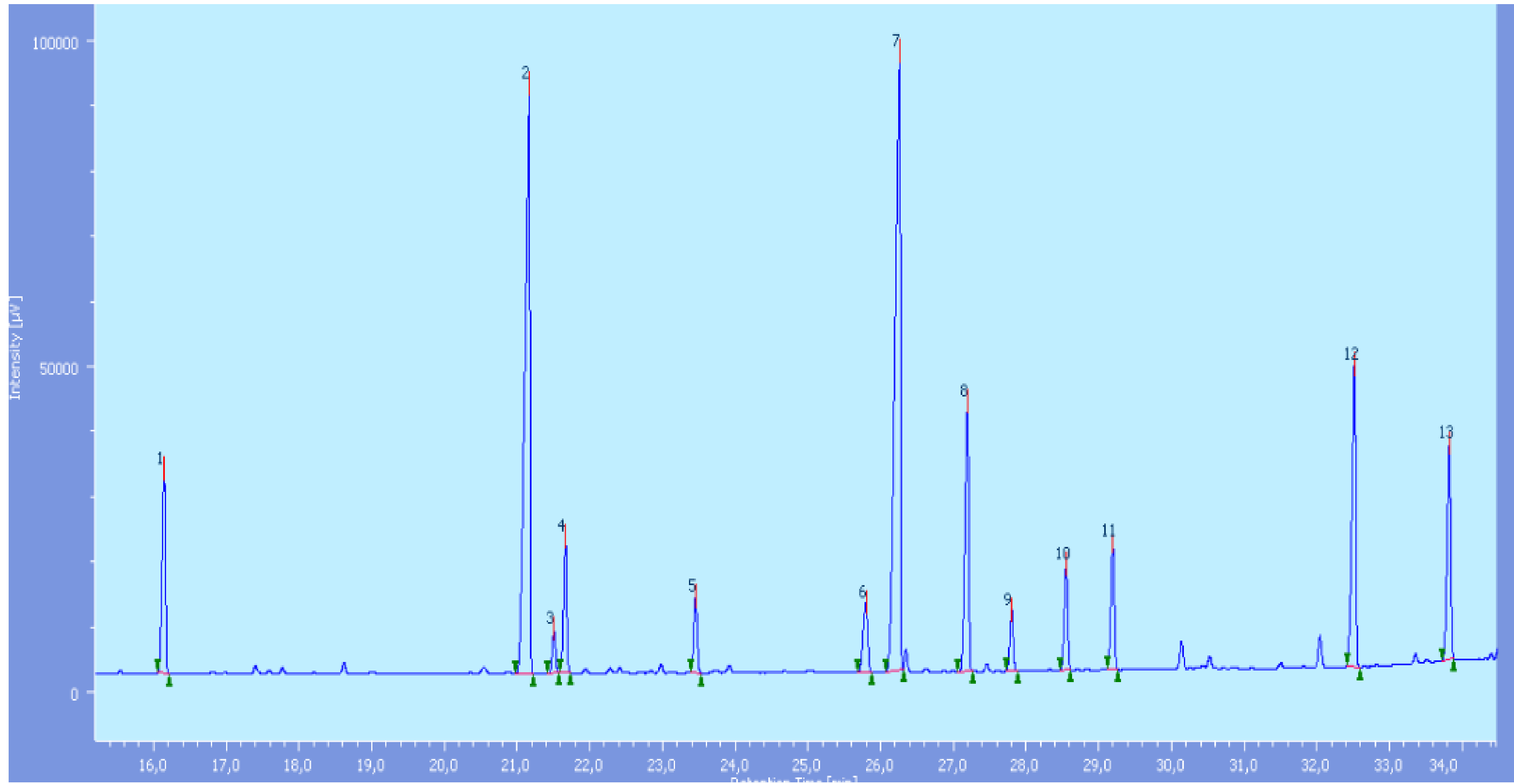


Figure 8: Chromatogram from GC analysis of VH 23/10-6 (VH =Yttre Vattenholmen). Used peaks represent 1. methyl tetradecanoic acid (C14:0) (0.033 % g/g dry weight, 2. palmitic acid (C16:0) (0.140 % g/g dry weight), 5.internal standard C17:0 (50 µg), 7. oleic acid (C18:1) (0.161 % g/g dry weight), 12. arachidonic acid (C20:4n6) (0.056 % g/g dry weight), and 13. eicosapentaenoic acid (C20:5n3, EPA) (0.039 % g/g dry weight).

Different patterns regarding time point and growth location were found for the average amount of different fatty acids. Figure 9 through 14 show the averaged amount of each fatty acid (C14:0, C16:0, C18:1n-9, C20:4n6, EPA C20:5n3 and DHA C22:6n3) as % based on dry weight in all 21 samples. The samples from Ulvillarna (UL) and Ursholmen (UR) had almost the same amount for each fatty acid throughout the year. Yttre Vattenholmen (VH) had a more varied amount in between the different fatty acids, as exemplified in figure 11 the amount of C18:1n-9 were about 0.04 % in August and October and almost double (0.08 %) in June. As noted in figure 14 there are only bars for Ulvillarna (UL) and Ursholmen (UR) in August since the fatty acid DHA was only found in those samples.

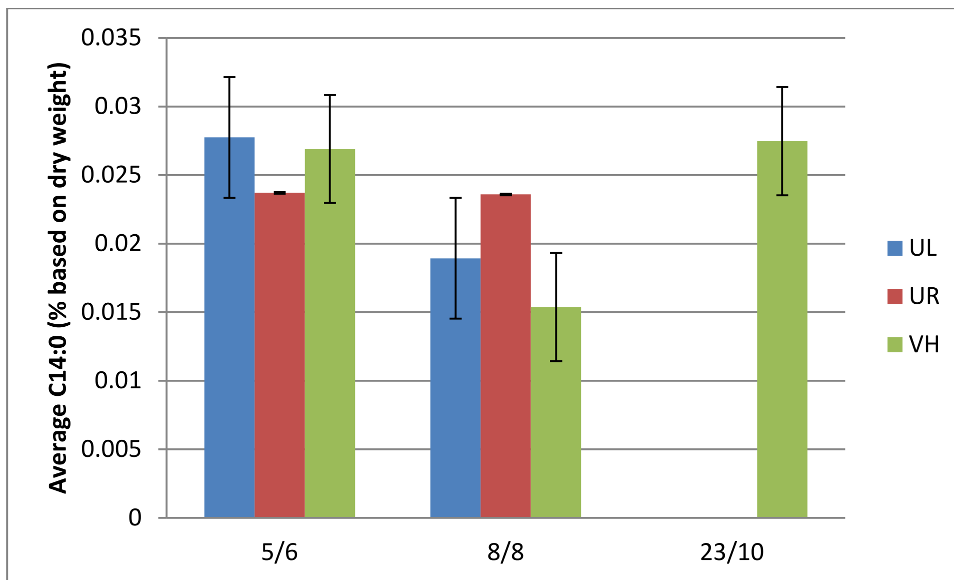


Figure 9: Content of average amount fatty acid C14:0 (% based on dry weight) in *S. latissima*. Data shown are average amount of C14:0 in three kelp blades for each harvest date with standard deviation as error bars. Locations are Ulvillarna (UL), Ursholmen (UR) and Yttre Vattenholmen (VH).

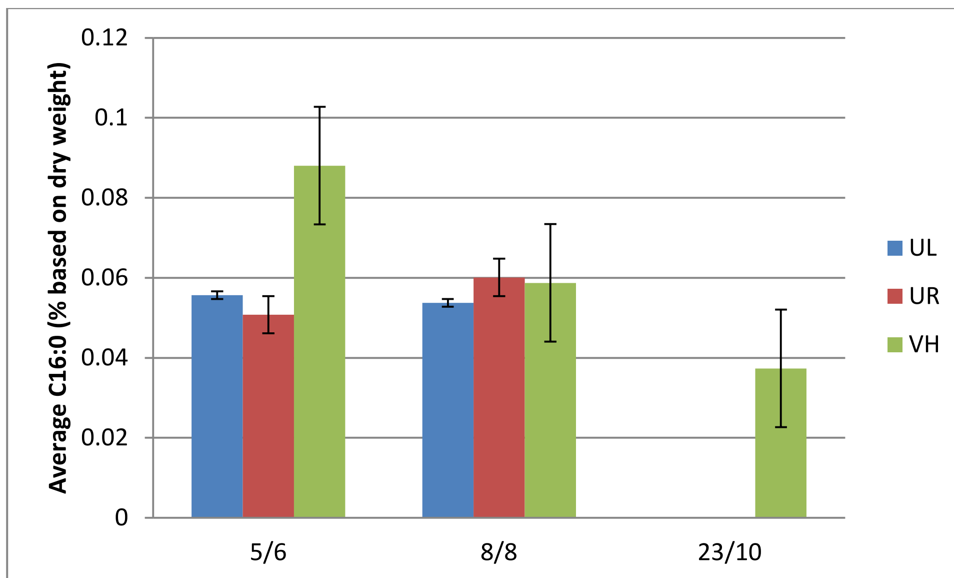


Figure 10: Content of average amount fatty acid C16:0 (% based on dry weight) in *S. latissima*. Data shown are average amount of C16:0 in three kelp blades for each harvest date with standard deviation as error bars. Locations are Ulvillarna (UL), Ursholmen (UR) and Yttre Vattenholmen (VH).

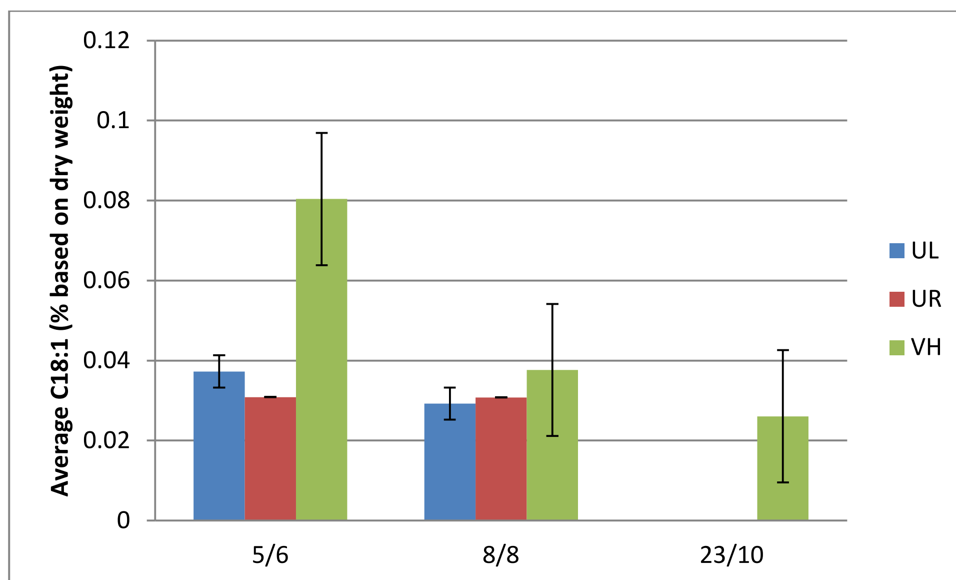


Figure 11: Content of average amount fatty acid C18:1 (% based on dry weight) in *S. latissima*. Data shown are average amount of C18:1 in three kelp blades for each harvest date with standard deviation as error bars. Locations are Ulvillarna (UL), Ursholmen (UR) and Yttre Vattenholmen (VH).

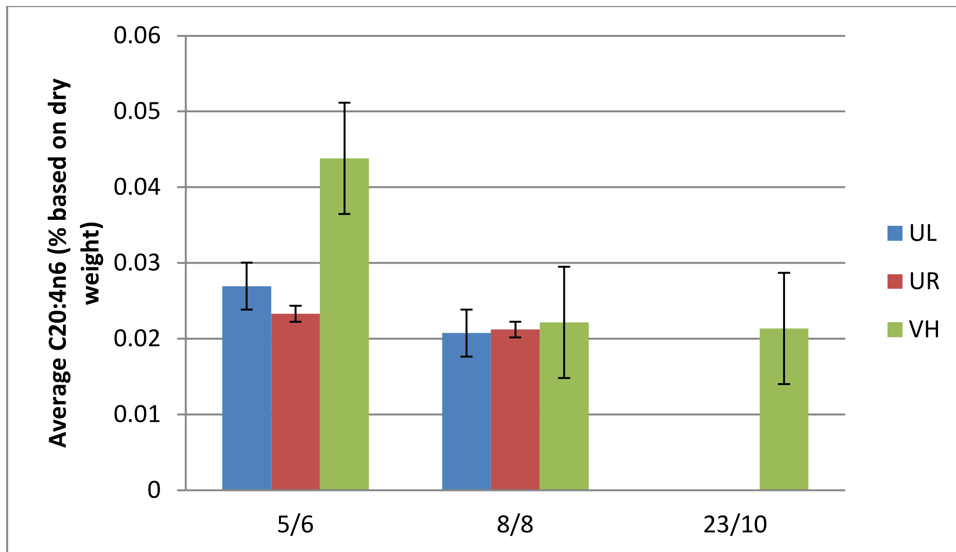
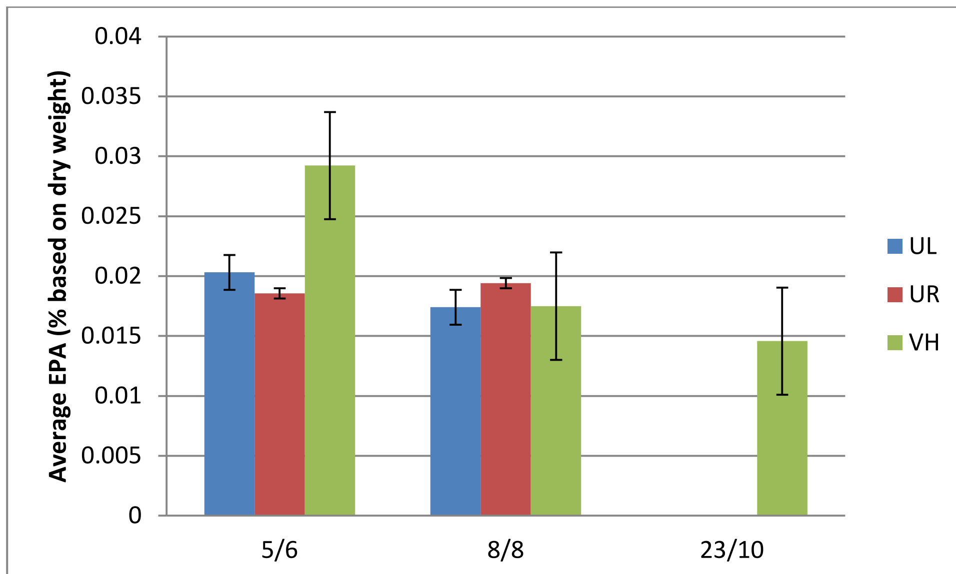


Figure 12: Content of average amount of fatty acid C20:4n6 (% based on dry weight) in *S. latissima*. Data shown are average amount of C20:4n6 in three kelp blades for each harvest date with standard deviation as error bars. Locations are Ulvillarna (UL), Ursholmen (UR) and Yttre Vattenholmen (VH).



Figur 13: Content of average amount of fatty acid EPA (% based on dry weight) in *S. latissima*. Data shown are average amount of EPA in three kelp blades for each harvest date with standard deviation as error bars. Locations are Ulvillarna (UL), Ursholmen (UR) and Yttre Vattenholmen (VH).

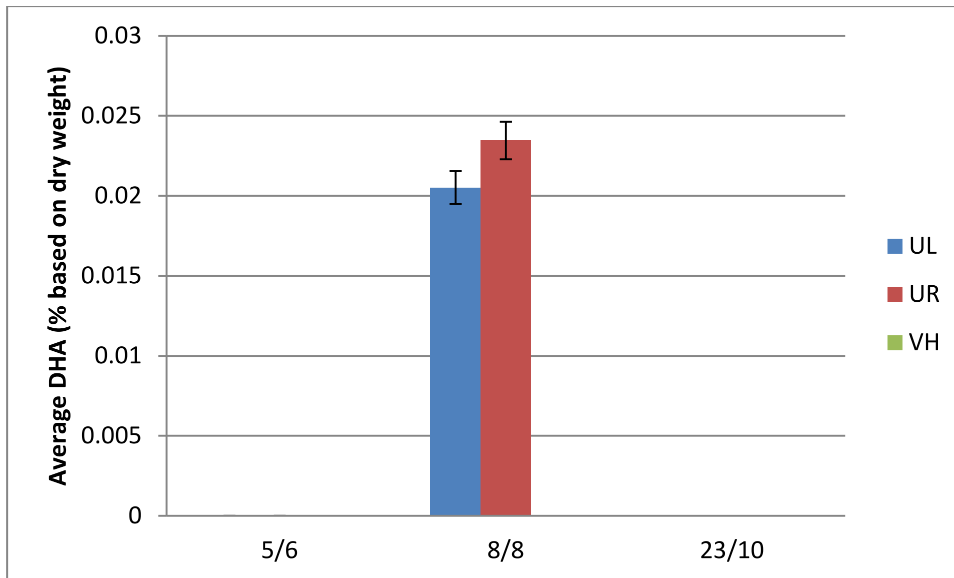


Figure 14: Content of average amount of fatty acid DHA (% based on dry weight) in *S. latissima*. Data shown are average amount of DHA in three kelp blades for each harvest date with standard deviation as error bars. Locations are Ulvillarna (UL), Ursholmen (UR) and Yttre Vattenholmen (VH).

5. Discussion

The aim of this project was to determine seasonal variations in the amounts of protein and fatty acids in the kelp *Saccharina latissima* collected from waters around Sweden.

The results obtained from the protein analysis were lower than expected. Data reported in the literature are found in the range 8-15 % dry weight basis for *Laminaria digitata* [Indergaard, M et al, 1991], which are about 20 times higher than the results in this project. Obtaining such low values could be because of many factors. One of the main factors is that the extraction method used had a poor efficiency for solubilizing proteins from the biomass. That this was the case for our work was supported by the evaluation of alternative methods for the extraction using samples from the same algae (UL 5/6-5). The SDS extraction method gave a higher percentage of protein than both the initial method used and the others tested. However, the duplicates done with this method gave rather different results, which may be because of errors. Those can be of human nature or some of the chemical reactions failed along the way or the extraction method used was not the most optimal one. In order to confirm which method that gave the highest protein yield, the different protein extraction methods should be performed on some additional samples with distinctly different protein content. Also more than two replicates should be made. Another factor explaining the low protein values is that the water the algae are coming from can have low concentrations of nitrogenous nutrients whereas a high level can help the growth of the proteins [Kuntze et al. 2004]. Also, a low level of salinity (which the Swedish sea water has) can prevent the biomass of the algae to grow and the level of wave action could have been too low to prevent fouling of the seaweed [Wegeberg 2010].

The results on the average seasonal variation on protein content as shown in figure 2 and 3 almost show the same trend as the ones in literature [Fleurence, 1999], that algae contain the most protein during the winter/spring (according to literature) than the rest of the year. The protein samples from this experiment however show a slight higher protein yield in June then the other months (based on dry weight). It is unfortunate that there were samples only from one location (VH) in October; otherwise our findings should have been more solid. It has been found that seaweeds have their highest amount of protein during the winter/spring [Fleurence, 1999]. This means that a later harvest than October may result in an even higher content of protein. However *Saccharina latissima* cannot be consider a “good” source of protein.

A final factor contributing to low protein results compared to those reported in literature can be that the algae samples from Swedish waters simply contain such a low amount of protein in general. Content of proteins in Swedish seaweed is very little studied. The measurements done in this study on *Saccharina latissima* are among the first ones made. To distinguish an effect of Swedish growth localities on protein content require further experiments are needed to be done on the kelp *Saccharina latissima* from Swedish waters.

The values for the amount of protein in *Saccharina latissima* were much lower than those of other algae species (especially red algae as mentioned in the text before). This does not necessarily mean that we in this project have measured the total amount of protein in the algae correctly, perhaps there is better extraction method for protein that gives a greater yield than the ones used in this project. Method 3 gave a higher yield than method 1 so if there would have been more time perhaps it would be good to extract the protein from all 42 samples using method 3 to see if there is much difference between all the samples or not.

The fatty acid analysis was not done on all 42 samples, because of lack of time, but on 21 samples. Only three kelp blades were used from each harvest date and location instead of all six as in the protein analysis. The fatty acids mentioned in the result section were identified based on their retention times and compared with other tests using the same GC. They have the highest possibility of being just those fatty acids (the human error has a large effect on the selection of which fatty acids representing which peaks in the chromatogram). The results show that the amounts of lipid in *S. latissima* are almost constant throughout the sampling period (about 0.15-0.40 % of dry biomass, see figure 5). These levels are similar compared to literature values [Sánchez-Machado et al. 2004]. There are some samples that have a lower and higher value than the rest and this can be due to experimental error. The lipid extraction method used is newly developed and used for the first time on macroalgae biomass. Since it contains quite some experimental steps there is a risk for handling errors during the procedure. The fatty acid values however are more consistent with the literature value around 1-5 % and in some brown kelp even as low as 0.3 % (found around Hawaii) [Yuan et al. 2008]. The lipid content obtained from these results is average about 0.25 % (not striving far from the kelp found around Hawaii with 0.3 % fatty acid content) from all tests, conforming that also *Saccharina latissima* has a low amount of fatty acids. With this said in that 0.25 % there is some high value fatty acid chains as DHA and others but due to the low total fatty acid content *Saccharina latissima* cannot be consider being a “good” source for fatty acids.

The amount of water in the sample cannot be trusted completely due to that there was some frost on the algae sample (and in some cases a lot of frost). This was due to that during collection and the initial sample preparation, water was not removed in a standardized way. Different amounts of frost were present on the algae samples why the test results may not be 100 % accurate.

6. Conclusion

According to the results obtained during this project the best month for harvest of *Saccharina latissima* is June for optimal protein content and October for fatty acid content. But the protein results obtained are low (0.5 % based on dry weight averaged on all 42 samples). The optimal harvest location for greatest amount of protein and lipid is Yttre Vattenholmen. The best method for extracting protein from *S. latissima* was the SDS extraction method which gave a much higher protein content than the method used on all 42 samples (the SDS extraction method gave a protein content of 3 % and the other method gave a content under 1 % based on dry weight). The essential fatty acids EPA and DHA were both found in *S. latissima* in samples from Ulvillarna and Ursholmen harvested in August but at a low rate of 0.025 % g/g dry weight, therefore cannot *Saccharina latissima* be considered a good source for either protein or lipids.

7. Acknowledgment

I would like to thank my supervisors Eva Albers, Jenny Vilg and Ingrid Undeland for great support and guidance. I would also like to thank Nils-Gunnar Carlsson and Lillie Cavonius for helping me with the fatty acid extractions and the interpretation of the GC chromatograms. Finally, I would like to thank Göran Nylund and Henrik Pavia at the University of Gothenburg for collecting all the algae samples used in the experiments.

8. References

Ahn, M.J., Yoon, K.D., Min, S.Y., Lee, J.S., Kim, J.H., Kim, T.G., Kim, S.H., Kim, S.E., Kim, N.G., Huh, H., Kim, J. (2004).Inhibition of HIV-1 Reverse Transcriptase and Protease by Phlorotannins from the Brown Alga *Ecklonia cava. Handbook of Pharmaceutical Biotechnology,* 27, 544-547.

Barbarino, E., Lorunco, S.O. (2005). An evaluation of methods for extraction and quantification of protein from marine macro- and microalgae. *Journal of Applied Phycology*, 14, 447-460.

Black, W.A.P. (1950). The seasonal variation in weight and chemical composition of the common British Laminariaceae. Scottish Seaweed Research Association, Musselburgh.

Buchholz, C.M, Krause, G., Buck, B.H. Seaweed and Man. (2012). *Seaweed Biology. 22,* 471-493.

Dawczynski, C., Schubert, R., Jahreis, G. (2007). Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chemistry*, 103, 819-899.

FAO Yearbook of Fishery and Aquaculture Statistics. (2010). *Food And Agriculture Organizations Of The United Nations*.

Fitton, J.H, Irhimeh, M.R, Teas, J. (2008). Marine algae and polysaccharides with therapeutic applications. *Marine Nutraceuticals and Functional Foods*. 345-365.

Fleurence, J. (1999).Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends in Food Science & Technology*, 10, 25-25

FOOD MATTERS. (2013). *Seaweed superfoods.* Available at: http://foodmatters.tv/Health_Resources/Seaweed_Superfoods. [Accessed 28.06.2013].

Galland-Irmouli, A.V., Fleurence, J., Lamghari, R., Luçon, M., Rouxel, C., Barbaroux, O., Bronowicki, J.P., Villaume, C. and Guéant, J.L. (1999). Nutritional value of proteins from edible seaweed *Palmaria palmata* (Dulse). *The Journal of Nutritional Biochemistry*, 10, 353–359.

Harada, H., Kamei, Y. (1997). Selective cytotoxicity of marine algae extracts to several human leukemic cell lines. *Cytotechnology,* 25, 213-219.

Indergaard, M., Minsaas, J. (1991). Animal and human nutrition. *Seaweed Resources in Europe: Uses and Potential*, John Wiley & Sons, chichester, 21-64.

Jung, K.A, Lim, S., Kim, Y., Park, J.M. (2012). Potential of macroalgae as feedstocks for biorefinery. *Biosource Technology,*135, 182-190.

Kumar, V., Kaladharan, P. (2007). Amino acids in the seaweeds as an alternate source of protein for animal feed. *Journal of the Marine Biological Association of India,*49, 35-40.

Kuntze, R., Ruth, S. H., Vårum, K.M., Larsen, B.A., Myklestad, S.M.(2004). Seasonal and geographical variation in the chemical composition of the red alga *Palmaria palmata* (L.). *Botanica Marina,* 47, 125-133.

Puja Kumari, C.R.K. Reddy, Bhavanath Jha (2011). Comparative evaluation and selection of a method for lipid and fatty acid extraction from macroalgae. *Analytical Biochemistry*, 415, 134-144.

Sánchez-Machado, D.I., López-Cervantes, J., López-Hernández, J., Paserio-Losada (2003). Fatty acids, total protein and ash contents of processed edible seaweeds. *Food Chemistry,* 85, 439-444.

Slocombe, S.P, Ross, M.,Thomas, N., McNeill, S., Stanley, M.S. (2013). A Rapid and general method for measurement of protein in micro-algal biomass. *Bioresource Technology*, 129, 51-57.

Thomas, N.V., Se-Kwon, K. (2011). Potential pharmacological applications of polyphenolic derivatives from marine brown algae. *Environmental Toxicology And Pharmacology,* 32, 325-335.

Wargacki, A.J., Leonard, E., Win, M.N., Regitsky, D.D., Santos, C.N.S., Kim, P.B., Cooper, S.R., Raisner, R.M., Herman, A., Sivitz, A.B., Lakshmanaswamy, A., Kashiyama, Y., Baker, D., Yoshikuni, Y. (2012). An Engineered Microbial Platform for Direct Biofuel Production from Brown Macroalgae. *Science,* 335, 308-313.

Wegeberg, S. (2010). Cultivation of kelp species in the Limfjord, Denmark. Department of Biology, SCIENCE, Copenhagen University.

Wong, K.H., Cheung, P.C.K. (2001). Nutritional evaluation of some subtropical red and green seaweeds Part II. In vitro protein digestibility and amino acid profiles of protein concentrates. *Food Chemistry*, 72, 11–17.

Yuan, Y.V. (2008). Marine algal constituents. *Marine Nutraceuticals and Functional Foods*, 259-296.

Yusuf, S., Reddy, S., Ôunpuu, S., Anand, S. (2001). Global Burden of Cardiovascular Diseases. *Circulation,* 104, 2746-2753.

9. Appendix A

Table 1: shows all the results from the protein, fatty acid and water content measurements in each algae sample. The blank spaces are because those samples were excluded from the fatty acid tests. **UL = Ulvillarna, UR = Ursholmen and VH = Ytrre Vattenholmen.**

Sample name	Fatty acid dry weight in %	Fatty acid wet weight in %	Protein dry weight in %	Protein wet weight in %	Water dry weight in %
UL-5/6-1	0.228	0.052	0.643	0.147	77.1
UL-5/6-2			0.333	0.079	76.3
UL-5/6-3	0.235	0.058	0.427	0.107	74.9
UL-5/6-4			0.171	0.037	78.6
UL-5/6-5			0.855	0.217	74.6
UL-5/6-6	0.297	0.087	0.915	0.270	70.5
Average±SD	**0.253±0.04**	**0.061±0.022**	**0.557±0.297**	**0.140±0.091**	**75.3±2.8**
UL-8/8-1	0.253	0.069	0.324	0.089	72.5
UL-8/8-2			0.545	0.169	68.9
UL-8/8-3	0.181	0.056	0.439	0.138	68.5
UL-8/8-4			0.401	0.122	69.7
UL-8/8-5			0.700	0.189	72.9
UL-8/8-6	0.286	0.098	0.374	0.129	65.4
Average±SD	**0.24±0.05**	**0.072±0.023**	**0.464±0.138**	**0.143±0.041**	**69.6±2.8**
UR-5/6-1	0.225	0.054	0.664	0.160	75.9
UR-5/6-2			0.235	0.087	63.0
UR-5/6-3	0.263	0.092	0.299	0.105	64.8
UR-5/6-4			0.429	0.153	64.3
UR-5/6-5			0.731	0.175	76.1
UR-5/6-6	0.192	0.064	0.638	0.215	66.2
Average±SD	**0.227±0.04**	**0.071±0.020**	**0.500±0.207**	**0.151±0.052**	**68.3±6.0**
UR-8/8-1	0.265	0.078	0.377	0.111	70.5
UR-8/8-2			0.329	0.095	71.2
UR-8/8-3	0.296	0.092	0.524	0.164	68.8
UR-8/8-4			0.402	0.118	70.6
UR-8/8-5			0.329	0.107	67.5
UR-8/8-6	0.251	0,073	0.065	-0.019	70.8
Average±SD	**0.271±0.02**	**0.082±0.013**	**0.316±0.200**	**0.011±0.060**	**69.9±1.4**
VH 23/10-1	0.295	0.119	0.312	0.126	59.6
VH-23/10-2			1.237	0.375	69.7
VH-23/10-3	0.285	0.121	0.588	0.251	57.3
VH-23/10-4			0.469	0.163	65.2
VH-23/10-5			0.791	0.249	68.5
VH-23/10-6	0.618	0.218	0.792	0.280	64.6
Average±SD	**0.399±0.19**	**0.150±0.064**	**0.699±0.323**	**0.242±0.093**	**64.2±4.9**
VH-5/6-1	0.270	0.051	0.758	0.145	80.8
VH-5/6-2			0.522	0.096	81.7
VH-5/6-3	0.260	0.047	0.723	0.131	81.9
VH-5/6-4			0.604	0.134	77.8
VH-5/6-5			0.632	0.198	68.6
VH-5/6-6	0.201	0.004	1.263	0.276	78.1
Average±SD	**0.244±0.04**	**0.033±0.031**	**0.750±0.265**	**0.159±0.064**	**78.2±5.0**
VH-8/8-1	0.194	0.070	0.526	0.191	63.8
VH-8/8-2			0.019	0.009	51.8
VH-8/8-3	0.117	0.021	0.345	0.065	81.2
VH-8/8-4			0.162	0.067	58.7
VH-8/8-5			0.542	0.164	69.7
VH-8/8-6	0.203	0.079	0.214	0.084	60.9
Average±SD	**0.171±0.05**	**0.062±0.029**	**0.301±0.208**	**0.101±0.072**	**64.4±10.1**

Table 2: Shows the different fatty acids found in the samples as percentage to the sample dry weight. UL = Ulvillarna, UR = Ursholmen and VH = Ytrre Vattenholmen.

Sample name	methyl tetradecanonate (C14:0) in %	Methyl hexadecanoate (C16:0) in %	Methyl elaidate (C18:1n-9) in %	Arachidonic acid methyl ester (C20:4n6) in %	eicosapentaenoic acid (C20:5n3, EPA) in %	Docosahexaenoic acid (C22:6, DHA) in %
UL 5/6-1	0.028	0.056	0.033	0.023	0.017	0.000
UL 5/6-3	0.027	0.053	0.033	0.025	0.020	0.000
UL 5/6-6	0.029	0.058	0.046	0.033	0.023	0.000
average	**0.027±0.001**	**0.055±0.003**	**0.037±0.007**	**0.027±0.005**	**0.020±0.003**	**0±0**
UL 8/8-1	0.014	0.052	0.028	0.022	0.020	0.028
UL 8/8-3	0.016	0.042	0.024	0.018	0.014	0.009
UL 8/8-6	0.026	0.068	0.036	0.022	0.018	0.024
average	**0.019±0.006**	**0.054±0.013**	**0.029±0.006**	**0.021±0.002**	**0.017±0.003**	**0.021±0.009**
UR 5/6-1	0.025	0.050	0.029	0.023	0.017	0.000
UR 5/6-3	0.029	0.058	0.035	0.026	0.022	0.000
UR 5/6-6	0.017	0.045	0.028	0.021	0.017	0.000
average	**0.024±0.006**	**0.051±0.006**	**0.031±0.004**	**0.023±0.003**	**0.018±0.003**	**0±0**
UR 8/8-1	0.024	0.057	0.030	0.021	0.019	0.024
UR 8/8-3	0.024	0.066	0.031	0.022	0.022	0.023
UR 8/8-6	0.021	0.057	0.032	0.021	0.017	0.023
average	**0.023±0.002**	**0.060±0.005**	**0.031±0.001**	**0.021±0.001**	**0.019±0.002**	**0.023±0.0004**
VH 23/10-1	0.024	0.060	0.043	0.036	0.025	0.000
VH 23/10-3	0.026	0.064	0.037	0.039	0.024	0.000
VH 23/10-6	0.033	0.141	0.161	0.056	0.039	0.000
average	**0.027±0.005**	**0.088±0.046**	**0.080±0.069**	**0.044±0.011**	**0.029±0.008**	**0±0**
VH 5/6-1	0.030	0.067	0.040	0.024	0.017	0.000
VH 5/6-3	0.030	0.064	0.044	0.023	0.020	0.000
VH 5/6-6	0.020	0.045	0.029	0.019	0.015	0.000
average	**0.027±0.006**	**0.059±0.012**	**0.037±0.007**	**0.022±0.003**	**0.017±0.002**	**0±0**
VH 8/8-1	0.018	0.042	0.027	0.021	0.018	0.000
VH 8/8-3	0.010	0.028	0.021	0.015	0.008	0.000
VH 8/8-6	0.018	0.041	0.030	0.028	0.017	0.000
average	**0.015±0.004**	**0.037±0.008**	**0.026±0.005**	**0.021±0.006**	**0.014±0.005**	**0±0**