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The effects of cultivation deployment- and harvest-timing, location and depth on growth and composition of *Saccharina latissima* at the Swedish west coast

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ABSTRACT

A study has been conducted to shed light on the effect of cultivation parameters on growth and chemical composition of *Saccharina latissima*. Longline cultivation took place at two separate locations in the Koster archipelago on the Swedish west coast, centred around three experiments that explored duration of pre-deployment hatchery processes (Exp 1), deployment and harvest time-frames (Exp 2), and cultivation at 2 m vs. 4 m depth (Exp 3). For all experiments the effects of these parameters were evaluated in terms of size/weight of specimens, and for experiments Exp 2 and Exp 3, concentrations of moisture, ash, carbohydrates, proteins, fatty acids, phlorotannins and common heavy metals were determined.

The specific parameters used in this study are likely to vary from site to site, nevertheless trends were observed that resonate in the literature, and these lead to some general recommendations. The weight of harvested kelp blades was higher for the later harvest, earlier deployment and when grown at a shallower depth. Carbohydrates concentration increased with later harvests and at shallower depth. Later harvests also increased the concentration of phlorotannin, while ash and total fatty acids decreased. Protein and fucoidan content was higher for the deeper lines. Growth and chemical composition of the seaweeds showed only minor differences between the two study sites, mostly relating to heavy metal content.

1. Introduction

The cultivation of seaweeds in Europe is developing at a fast pace following calls for a more bio-based economy (European Commission, 2012b) and enhanced blue growth opportunities (European Commission, 2012). Pilot cultivations and commercial sites are springing up all along the Atlantic coast, developing technologies and skills while adapting to European markets, regulations and local conditions. Cultivated seaweeds have a lot of advantages compared to terrestrial crops: they require no arable land, irrigation, fertilisers or other agricultural products. Kelps or brown seaweeds in particular show promise as a multi-product biorefinery raw material to support a more bio-based European economy, seeing as they are relatively easy to cultivate and can be used to produce both lower value/high volume products as biofuels, fertilisers and animal feed, as well as higher value/low volume products for human consumption such as biobased materials, food ingredients and medically active compounds (McHugh, 2003). Indeed, direct use of kelp in foods, without advanced pre-processing is also an important use that is currently growing in the Western world. A recent Swedish investigation showed that consumers have a positive attitude toward seaweed containing food products (Wendin and Undeland, 2020). Research has also suggested that kelp cultivations have few detectable local environmental impacts (Visch et al., 2020a) while providing a range of ecosystem services (Hasselström et al., 2018). Life Cycle Assessment studies also suggest that cultivated kelp production systems have potential to produce a low impact (low carbon and

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eutrophication mitigating) biomass for subsequent use (Seghetta et al., 2017; Thomas et al., 2021).

There are some significant challenges with cultivating kelps in the North Atlantic region. It is fairly expensive at current operational scales and is not yet competitive with the harvesting of wild stocks (Hasselström et al., 2020). There are also technical challenges to be overcome relating to working at sea, notably with regards to mechanisation, cultivation systems, identifying suitable cultivation locations, obtaining licenses and scaling up supply chain operations while remaining profitable (Bak et al., 2020). Furthermore, just as is the case for any terrestrial crop, kelp and other seaweeds generally show important seasonal variation in their chemical composition, thus making it a difficult raw material to work with in industry.

Generations of marine biologists have documented the seasonal variation in chemical and structural composition of seaweeds but most notably kelps sampled from natural populations, dating back to the early and mid-19th Century (Lapicque, 1919; Ricard, 1931; Black, 1950). Black (1950) presents a comparison of the seasonal variation in chemical composition in a range of species sampled from wild stocks in Scotland. Vilg et al. (2015) explored seasonal and spatial variations in the macromolecular, fatty acid and sugar content of one kelp species, *Saccharina latissima*, sampled wild from three locations in the Koster Archipelago in Sweden and discussed these results in terms of biorefinery applications. A series of studies from Denmark have explored seasonal variation of target compounds linked to specific industrial uses: Marinho et al. (2015a) explored seasonal variation in amino acid profiles and protein content; Marinho et al. (2015b) provided perspective on the seasonality of bioremediation in integrated multi-trophic aquaculture; Nielsen et al. (2016) shed light on the challenge of the salinity gradients in Danish waters on the chemical composition of *Saccharina latissima* and *Laminaria digitata*, and their corresponding potential suitability for use to produce biofuels and feed or as a bioremediation tool; and Bruhn et al. (2017) explored seasonal yield fluctuations in fucoidan, a bioactive compound exhibiting properties desirable in the medical and pharmaceutical industries, also in wild sampled *Saccharina latissima* and L. *digitata*. In view of optimising biofuel production, Anastasakis et al. (2011) and Adams et al. (2011) assessed how thermochemical and biochemical conversion pathways, respectively, to biofuels are affected by seasonal variation in the composition of wild stocks of L. *digitata*. Similar studies aiming to optimise biofuel yield according to harvest season have also been conducted by Tabassum et al. (2016) on wild stocks of *Ascophyllum nodosum* in Ireland and by Ometto et al. (2018) on wild stocks of Fucales and Laminariales kelps in Norway.

In all these studies, seasonal variations in chemical composition were identified and led to recommendations of best practice, notably for optimised harvest times in relation to specific target products. However, these studies predominantly sampled wild stocks or natural populations and thus suffer from some inherent problems: there is no practical way to control specimen age, nor to control key variables that affect kelps such as breeding, juvenile development conditions and spatial variability. Furthermore, kelp cultivation has to take place at certain times of the year: regardless of location in the North Atlantic, deployment typically happens in the fall and harvests take place in the end of spring or early summer. It is therefore most relevant for practitioners to focus on compositional variations occurring during these short harvest windows, among cultivated biomass and resulting from variations of key cultivation parameters.

With these aims in mind, variations in the chemical composition of cultivated kelp within practicable cultivation and harvest windows have only been subject of a handful of studies. Handå et al. (2013) shed light on the effects of depth and proximity to a fish farm while also assessing seasonal variation to carbohydrates, amino acids and minerals in cultivated *Saccharina latissima*. Sharma et al. (2018) explored seasonal aspects of cultivated *Saccharina latissima* with a particular focus on the effects of harvest time and cultivation depth on the composition of carbohydrates, amino acids, minerals and phenolics. Most recently, Forbord et al. (2020a) shed light on the effects of seasonality and cultivation depth on the chemical composition of *Saccharina latissima* across a latitudinal gradient stretching along most of the Norwegian coast. In the Faroe Islands, studies have explored how growth, protein content and yield of *Saccharina latissima* are affected when cultivated in varying degrees of wave and current exposure (Mols-Mortensen et al., 2017), and how the protein and amino acid profiles cary across the seasons (Bak et al., 2019). Adding to this body of literature, the present study contributes by assessing how the chemical composition of the kelp *Saccharina latissima* cultivated on longlines varied (i) at two different locations and depths on the Swedish west coast, (ii) with different key cultivation parameters: cultivation depth, time of deployment and time of harvest. As a whole, this study aims to contribute knowledge toward the development of best practices for both kelp farmers and associated prospective industries in Sweden and across the North-Atlantic region.

2. Materials and methods

2.1. Parental plants and induction of sporogenesis

Parental plants (20 individuals) were collected by snorkelling from a natural population in the Koster Archipelago on the Swedish west coast (N 58°50.1′, E 10°59.4) four times during 2014 (11 Jun, 5 Jul, 6 Aug and 10 Sep). To induce sporogenic tissue formation, kelp blades were cut 20 cm from the stipe-meristem intersection and incubated under artificial light in 100 L tanks with flow through water for minimum 5 weeks (Lüning and Pang, 2003). The light intensity was 115 μmol m^{-2} s^{-1} at the water surface with 8 h light per day and the water temperature was maintained at 10 °C. The kelp were tumbled from the bottom of the tanks to the water surface by water currents generated from compressed air released from the bottom of the tanks.

2.2. Hatchery

After induction of sporogenesis, sorus portions of the blades (6 blades with well-developed sori) were used for release of zoospores and subsequent seeding of 2 mm Kuralon twine wrapped around spools (diam. 90 mm, PVC) for cultivation of juvenile sporelings. The zoospore density for seeding was 2000 spores per ml. The spools with seeded twine were incubated for 2, 4 and 6 weeks (Exp 1) and 4 weeks (Exp 2 and Exp 3), in 40 L seawater tanks enriched with half strength Provasoli's medium (PES) and germanium dioxide (final concentration 0.5 mg/L) at 10 °C and 12 h light. The culture medium was renewed every week and a central air pump provided aeration and water mixing within the tanks. The irradiance at the water surface was 35 μmol m^{-2} s^{-1} for the first two weeks where after it was increased to 70 μmol m^{-2} s^{-1} (Redmond et al., 2014). The densities of the juvenile sporophytes after 2, 4 or 6 weeks of incubation were not specifically measured at the time of deployment. However, since we used a high seeding density (2000 spores per ml) and visual inspection of the spools showed an even distribution of juveniles the density can be considered high (Redmond et al., 2014). The mean length of the sporophytes was around 0.2 mm after 2 weeks of incubation, 1 mm at 4 weeks and 15 mm at 6 weeks.

2.3. Cultivation and sampling

Three separate experiments were performed to explore cultivation timing aspects: duration of juvenile sporophyte maturation in the hatchery (Exp 1), deployment and sampling times (Exp 2), and cultivation depths (Exp 3) on biochemical composition of *Saccharina latissima*. The cultivation experiments were conducted within previously established experimental seaweed farms at two localities (Vedskär: N58°51.55, E11°04.10, Lådderskär: N58°51.61, E11°00.67) in the Koster archipelago on the Swedish west coast. The seaweed farms consisted of parallel 100 m long horizontal lines (long line culture ropes), spaced 4 m apart. Strings with juvenile sporophytes were rolled around

the horizontal culture ropes at each deployment. Exp 1 was deployed at 3 Oct, 17 Oct and 31 Oct, Exp 2 at 9 Sep, 21 Oct and 21 Nov, and Exp 3 at 3 Sep. The experimental strings were randomly distributed within and among three different 100 m longlines (two at 2 m and one at 4 m), at each locality. Each treatment covered approximately 10 m of a longline. The cultivation depth for Exp 1 and Exp 2 was 2 m, while for Exp 3 half of the samples were deployed at 2 m and half at 4 m. Light penetration at these depths was not specifically measured, however for reference, the average of Secci depths in the Kosterfjorden over 10 years is 8.4 m (SMHI, 2021). The sampling of the three experiment was carried out on 9 Mar, 22 Apr and 27 May, when a number (n) of individuals were collected from the cultivation lines (n for Exp 1, 2 and 3 being 20, 10 and 20 respectively). The sampling was randomized by throwing a grappling hook consisting of two parallel hooks distanced 25 cm apart at the stretch of the culture rope with a specific treatment (10 m long) and haphazardly choosing a blade between the hooks. The sampled individuals (holdfasts and stipes removed) were measured (length and width), removed from excess water by manually spinning them in a meshed bag, weighed in this wet state (wet weight) and frozen at −20 °C. The frozen samples were then freeze-dried and re-weighed to determine the water content, before being ground into a fine powder and stored at −60 °C until chemical analysis. Chemical analysis was therefore conducted on whole individuals (excluding the stipes and holdfasts), not on separate parts of the blade. Whereas size and weight measurements were made on all samples, chemical analysis for Exp 2 and the two later harvests of Exp 3 were only performed on 5 out of 10 samples (randomly selected), except for ash, protein and metal analysis which were performed on 3 out of 10 samples (randomly selected).

2.4. Analysis of ash

Ash content was determined gravimetrically from combustion of freeze-dried samples at 550 °C, applying the same method employed by Olsson et al. (2020).

2.5. Analysis of proteins

Proteins were extracted from approx. 20 mg dry biomass by 1 mL of 2% (*w/v*) SDS, 1 mM DTT at 100 °C for 5 min repeatedly three times with bead beating in between. Appropriate amount (to fit the standard curve) of solubilised proteins in the supernatant after centrifugation (14,000 x*g*, 20 min, 4 °C) were precipitated by 125 mg/L deoxycholate and 58 g/L trichloroacetic acid (Vilg and Undeland, 2017). Precipitated proteins were then quantified by BioRad DC™ kit based on the Lowry assay which was started with solubilizing the precipitated proteins in Reagent A of the kit. Absorbance at 750 nm was measured in a microtiter plate reader (FLUOstar Omega, BMG LABTECH, Ortenberg, Germany) and Bovine Serum Albumin was used as standard in the range 0.062–0.31 g/L.

2.6. Analysis of carbohydrates

Freeze dried and milled seaweed samples were hydrolyzed in order to perform carbohydrate analysis. Deviations were made from the standard method (SCAN-CM 71:09, 2022) to handle lower sample volumes and more individual samples simultaneously (Sterner and Edlund, 2016; Rugiu et al., 2021). Volumes were decreased in order to use vials of 15 mL, glass fiber filters were replaced by syringe filters and hydrolysis temperature was decreased to the expense of longer hydrolysis times. As a first step in the hydrolysis process, 25 mg of freeze-dried sample was soaked in 0.80 mL 72% (*w*/w) sulfuric acid for 1.5 h in room temperature and in capped vials. The final 0.5 h of soaking time the samples were shaken in standing position 200 rpm with shake radius of 1 cm. As a second step in the procedure 10 mL of deionized water was added to the sample-vials, so that the sulfuric acid concentration reached approximately 8% (w/w). As a third step the vials were shaken at 80 °C for 72 h in standing position 100 rpm with shake radius of 1.5 cm. To measure eventual non-hydrolyzed material the samples were filtered through 0.2 μm nylon filters, which had been dried in a desiccator and weighted. The filters were cleaned by pressing through 60% (w/w) ethanol solution, dried in a 60 °C oven for a day, put in the same desiccator as previously and weighted again the day after. The carbohydrate compositions of the hydrolyzed samples were determined using a high-performance anion exchange chromatograph (Dionex, Sunnyvale, CA, USA) equipped with a pulsed amperometric detector (HPAEC-PAD, Dionex ICS-3000) and CarboPac PA1 column (4 × 250 mm), using Milli-Q water and solutions of sodium hydroxide and sodium acetate. The eluent was pumped at 1.5 mL/min with a program starting with 0.10 M sodium hydroxide and increasing to 0.16 M sodium hydroxide with 0.16 M sodium acetate during the run. The data were processed with Chromeleon 7.1 software. The carbohydrate standards used for calibration were mannitol, fucose, glucose and commercial alginate with a determined mannuronic/guluronic acid composition.

2.7. Analysis of fatty acids

Fatty acid extraction and analysis methods were adapted from (Cavonius et al., 2014) and (Khoomrung et al., 2012). For direct methylation 1.0 mL of toluene containing 100 μg of heptadecanoic acid was added to 50 mg of freeze-dried kelp material, followed by addition of 1 mL freshly prepared 10% (*v*/v) acetyl chloride in methanol. Tubes were capped tightly, incubated for 120 min at 70 °C, and allowed to cool to room temperature before the reaction was terminated by adding 1.0 mL ultra-pure water. Thereafter, 1.0 mL hexane was added followed by 1 min vortexing and 6 min centrifugation at 100 x*g*. The upper phase was transferred to new vials, diluted 10 times with hexane and stored at −20 °C for maximum 5 days before analysed. Analysis of fatty acids was performed with a Thermo Scientific ISQ series single quadrupole GC–MS system equipped with a Phenomenex ZB-Wax column (30 m length, 0.25 mm inner diameter, 0.25 μm film thickness). The inlet temperature was maintained at 240 °C and the column held at 50 °C for 1.5 min, ramped at 25.0 °C/min to 220 °C followed by 1.5 °C/min up to 240 °C and 10.0 °C/min up to 255 °C where after the temperature was held for 3 min. Helium was used as carrier gas at a constant flow rate of 0.6 mL/min and interfaced to the mass spectroscopy detector in electronic ionization mode. The injection volume was 1 μL in splitless mode. Identification of the fatty acids was based on GLC 502 standard fatty acid methyl esters obtained from Nu-Check Prep (Elysian, USA).

2.8. Analysis of phenolics

Total phlorotannin levels were quantified colorimetrically using the Folin-Ciocalteu method (van Alstyne 1995). Approximately 12 mg of powdered freeze-dried kelp was extracted in 1.5 mL aqueous acetone (60% by volume) on a vortex in the dark for 18 h. The extracts were centrifuged and 1 mL of the supernatant was transferred to new tubes and subsequently evaporated in a speed-vac to remove the acetone. The samples were diluted to 1 mL with MQ (Milli-Q®), centrifuged, and 500 μL of the supernatant was diluted with MQ water to a final volume of 8 mL in 15 mL falcon tubes. Thereafter, 0.5 mL Folin-Ciocalteu's reagent (Merck, Art. 109,001) and subsequently 1.5 mL sodium carbonate were added to the samples. After 2 h incubation in the dark the absorbance at 740 nm was measured using a spectrophotometer. Phloroglucinol (1,3,5-Trihydroxybenzene, Sigma, Art. 6099-90-7) was used as standard.

2.9. Analysis of heavy metals

Analysis of heavy metals was conducted by Eurofins Environmental Testing Sweden AB using ICP-MS (method NMKL No 1611998).

2.10. Statistical analysis

For single variable analysis, type III factorial ANOVA-tests were performed followed by pairwise comparison of means by Student–Newman–Keuls method, and (Tukey for chemical component composition of Exp 2). In the cases when the data showed signs of heteroscedasticity a log transformation was performed before conducting the ANOVA-test.

Multivariable analysis was used in the case of chemical composition analysis of *Saccharina latissima*. The chemical composition of *Saccharina latissima* was divided into four groups: (1) major chemical compound groups consisting of ash, water content and total levels of carbohydrates, fatty acids, proteins and phenolics; composition of (2) fatty acids, (3) sugars and (4) heavy metals. Variations in these compound groups due to deployment and harvest time, depth and locality were analysed using permutational multivariate analysis of variance (PERMANOVA; Primer-E v7) (Anderson, 2001). Two separate PERMANOVA tests were performed, one 2-factorial analysis with "Cultivation depth" and "Locality" as fixed factors (Exp 3) and one 3-factorial analysis with "Deployment time", "Harvest time" and "Locality" as fixed factors (Exp 2). Significant factors were further analysed using PERMANOVA pairwise comparisons. Monte Carlo (MC) tests were included to obtain more reliable *p*-values in cases with few possible permutations (Anderson et al., 2008). Tests were carried out using 9999 unrestricted permutations. Similarities among samples and importance of individual chemical components were visualized and evaluated with canonical analysis of principal coordinates (CAP) (Anderson and Willis, 2003), using the results from PERMANOVA to determine which factors to include in the analysis. Prior to CAP, the distribution of the dependent variables was examined with draftsman plots and the quality of the CAP models was evaluated by cross validation (Anderson et al., 2008). PERMANOVA and CAP analyses were based on Euclidian distance measures, using unit variance scaled data.

3. Results

The results of this study are presented in 6 parts: first growth data is presented, second an overview of chemical compositional changes, and thereafter, specific sections bring emphasis to the results of the multivariate analysis of major component groups as a whole (ash, water content and total levels of carbohydrates, fatty acids, proteins and phenolics), then specifically for fatty acid composition, sugar composition, and finally, the heavy metal composition.

3.1. Growth data of Saccharina latissima

Exp 1. (Fig. 1a) shows growth data after varying the duration of juvenile sporophyte maturation before deployment to sea. For all three harvests, and for all three dependent variables (length, width and weight), there was a significant effect for juvenile maturation time (ANOVA, $p < 0.05$) with significant interaction between either locality or locality and population. A multiple comparison test (SNK, $p < 0.05$) could not significantly distinguish harvested specimen based on their juvenile maturation time. (Data in supplementary table S1). No significant trend regarding the effect of juvenile maturation time on length, width or weight at harvests could be concluded.

Exp 2. (Fig. 1b) shows growth data of algae with different deployment times to sea. For blade length no significant effect was seen at any harvest. (Data in supplementary table S1). For blade width, deployment time had a significant effect for harvests on 9 Mar and 27 May and a significant interaction with locality on 22 Apr (ANOVA, $p < 0.05$). Samples harvested on 9 Mar, 22 Apr at Vedskär and 27 May had significantly higher width when deployed in Sep compared to Oct [+41%, +24% (for Vedskär), +38%] and compared to Nov [+82%, +54% (for Vedskär), +38%] (SNK, $p < 0.05$). (Data in supplementary table S1). For blade weight, deployment time had a significant effect for harvests on 9 Mar and 27 May and a significant interaction with locality on 9 Mar and 22 Apr (ANOVA, $p < 0.05$). Samples harvested on 9 Mar, 22 Apr at Vedskär and 27 May had significantly higher weight when deployed in Sep compared to Oct [68%, 70% (for Vedskär), 124%] and compared to Nov [156%, 161% (for Vedskär), 140%] (SNK, $p < 0.05$). (Data in supplementary table S1).

Exp 3. (Fig. 1c) show growth data of algae deployed at different depth, 2 m and 4 m. For all three harvests and for the dependent variables length, width and weight there was a significant effect of cultivation depth, with a significant interaction between weight and locality at harvest 22 Apr and 27 May (ANOVA, $p < 0.05$). Samples deployed at 2 m depth, compared to 4 m depth, had a higher length, width and weight, for samples harvested on 9 Mar [112%, 95%, 552%], 22 Apr [39%, 45%, 199%] and 27 May [39%, 44%, 251%] (SNK, $p < 0.05$). (Data in supplementary table S1).

Information from type III factorial ANOVA-tests for Exp 1–3 and numeric data of averages and standard deviation for each of weight, length and width is found in supplementary table S1 and S2.

3.2. Chemical composition of Saccharina latissima

The chemical composition of *Saccharina latissima* was studied for all harvests of Exp 2 which was designed to explore the effects of deployment time and locality and the two later harvests of Exp 3, designed to explore the effects of depth and locality. The composition of the seaweed was analysed for its content of water, ash, carbohydrates, fatty acids, proteins, phenolics and heavy metals. The change in concentration during the harvest window for both Exp 2 and Exp 3 are presented in Figs. 2–4 and found in numerical form in tables S2-S5 in the supplementary material with averages and standard deviations. In Fig. 2a these are expressed in milligrams per gram (mg/g) of dry matter and in Fig. 2b per gram of wet weight (which give the concentration of compounds in the fresh biomass). These results are presented below, initially for Exp 2 (i.e. Fig. 2 a_i, b_i) and then for Exp 3 (i.e. Fig. 2 a_{ii}, b_{ii}). For Exp 2 a statistical analysis was done with parametric ANOVA type III for the main component groups (Supplementary Table S12), and multivariate PERMANOVA for the main component groups and the components within each group in Section 3.3. As a method for multiple comparison of means, Tukey was used for ANOVA on chemical composition data of Exp 2, in contrast to SNK for all other data, since Tukey is more conservative and the treatment numbers were high with small sample sizes for chemical composition data of Exp 2.

In Fig. 2a_i, the dry weight concentration of some chemicals or chemical groups can be seen for algae harvested on 9 Mar, 22 Apr and 27 Maj. Exp 2. (Fig. 2a_i, 2b_i) shows growth data of algae deployed at different times to sea. Exp 3. (Fig. 2a_{ii}, 2b_{ii}) shows growth data of algae deployed at different depths, at 2 m and 4 m.

For water content (Exp 2. Fig. 2b_i), there was a significant effect of harvest time, with a significant interaction between harvest time, deployment time and location (ANOVA, $p < 0.05$). A multiple comparison test (Tukey, $p < 0.05$) could in most cases not significantly distinguish harvested specimen based on the harvest time, except for samples deployed Oct 21 at Vedskär when samples at 27 May harvest contained significantly more water than 9 Mar, and samples deployed Sep 11 at Lådderskär when samples at 27 May harvest contained significantly more water than 9 Mar and 22 Apr. For water content (Exp 3. Fig. 2b_{ii}) there was a small but significant increase in water content for samples harvested on 27 May compared to samples harvested on 22 Apr, with no significant interactions. There was no significant difference in water content between samples deployed at different depth. (Data in supplementary table S12).

For ash content (Exp 2. Fig. 2a_i), there was a significant effect of harvest time, with no significant interactions (ANOVA, $p < 0.05$).

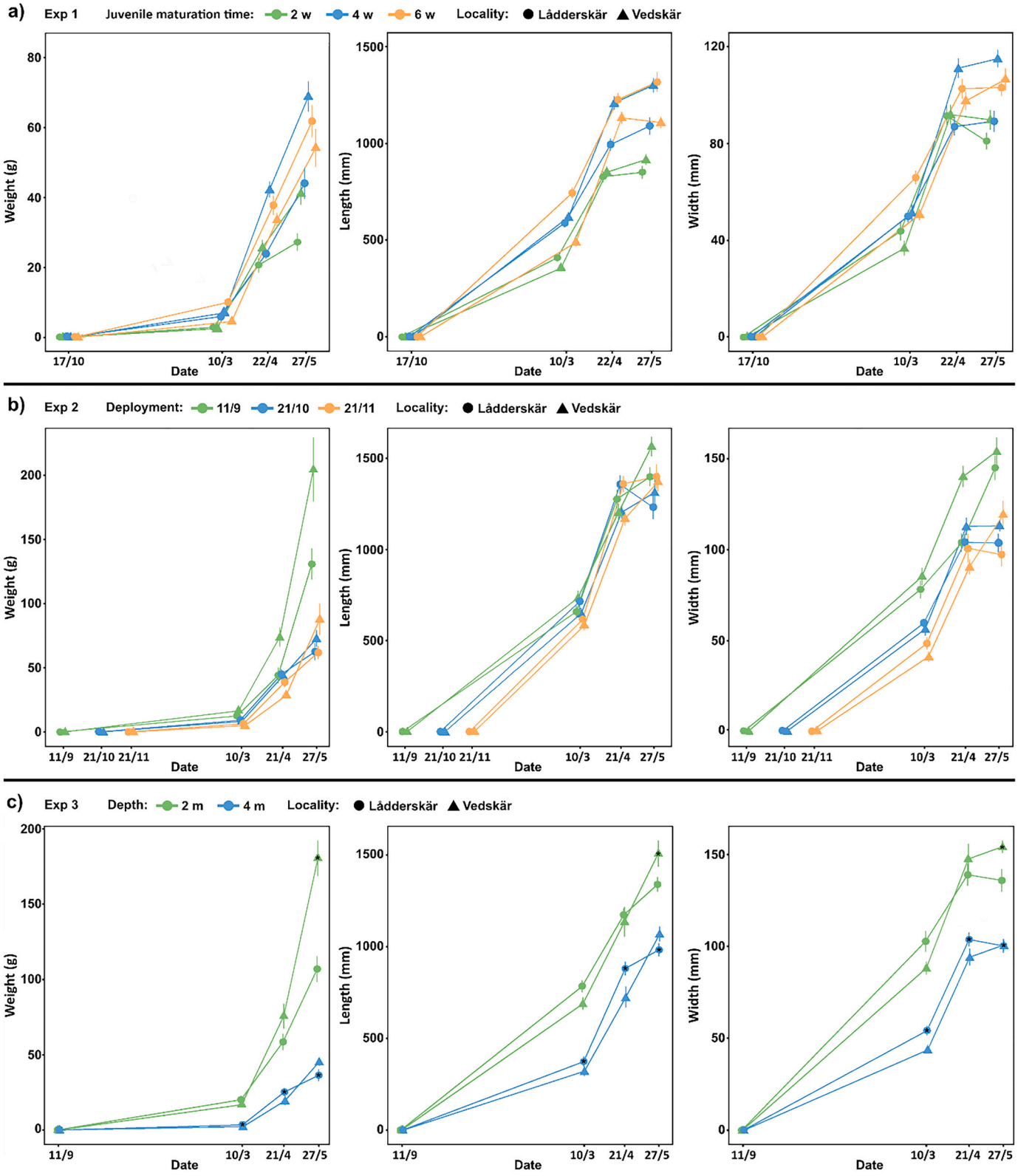

Fig. 1. Changes in blade weight, length and width over time. For Exp 1, (a) each datapoint represents 10 × 2 specimens of *Saccharina latissima,* for Exp 2, (b) 10 × 1 specimens, and Exp 3 (c) 10 × 2 specimens. The exception for Exp 3 (c) was when one longline was lost due to storms and only 10 × 1 specimens could be used, marked with a black star on the data point. The long-lines were positioned at Lådderskär (circles) and Vedskär (triangles). Bars represent the standard error. The colours in each figure (a, b and c) represent different variables, as specified in each figure. Plotted data is found in supplementary table S2.

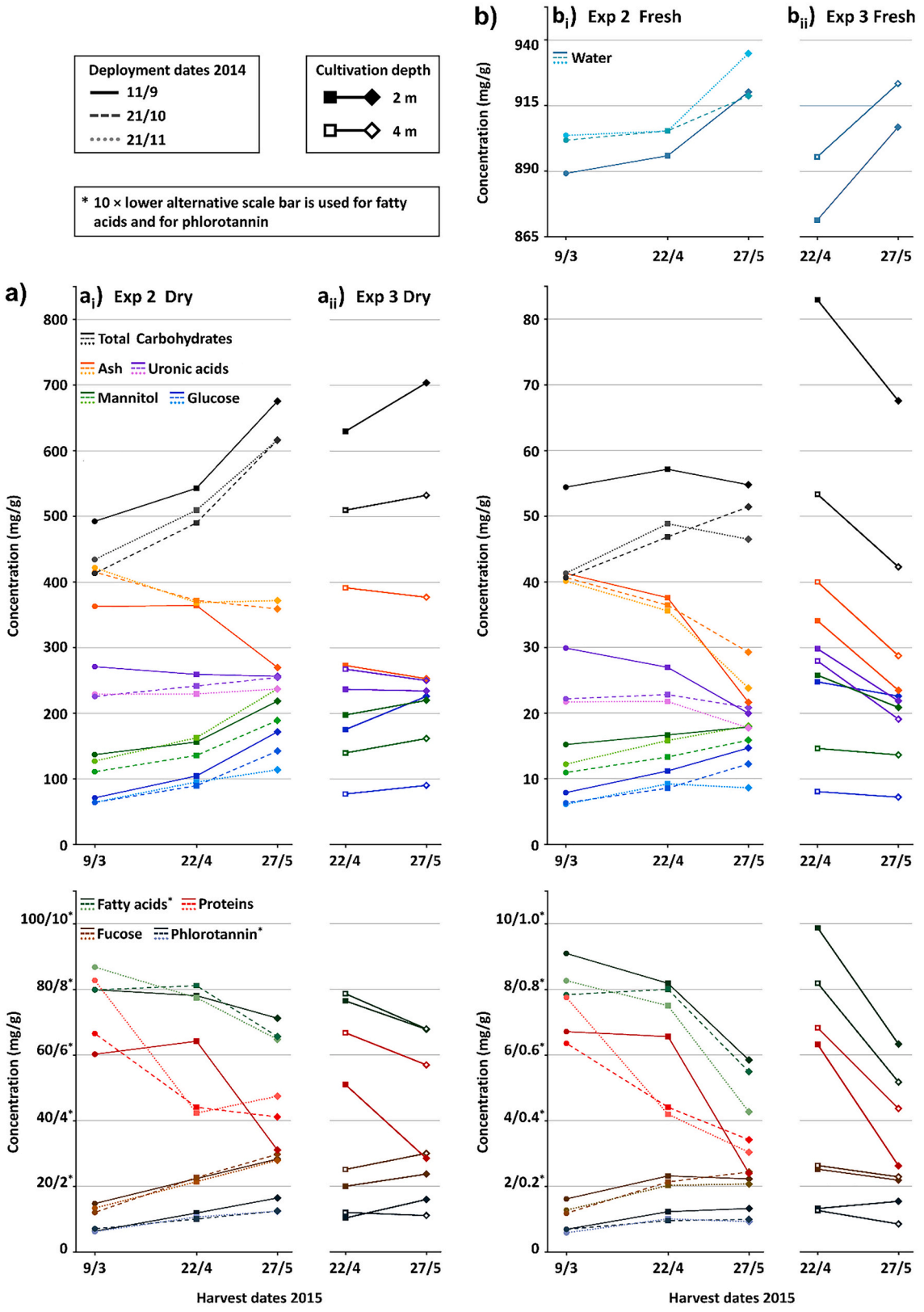

Fig. 2. Chemical composition in dry (a) and fresh (b) seaweed for Exp 2 (a_i, $b_{i)}$ and Exp 3 (a_{ii}, b_{ii}). Straight, striped and dotted line are used for seaweed deployed 11 Sep, 21 Oct and 21 Nov 2014. Circle, square and diamond marker for harvest at 9 Mar, 22 Apr and 27 May 2015. Filled and hollow markers for deployment at 2 and 4 m depth. Data point are averages of combined samples from both cultivation location, giving 10 samples, except fatty acids, ash and protein which are averages of 6 samples. Numeric data is available in tables S2 and S3 in supplementary materials.

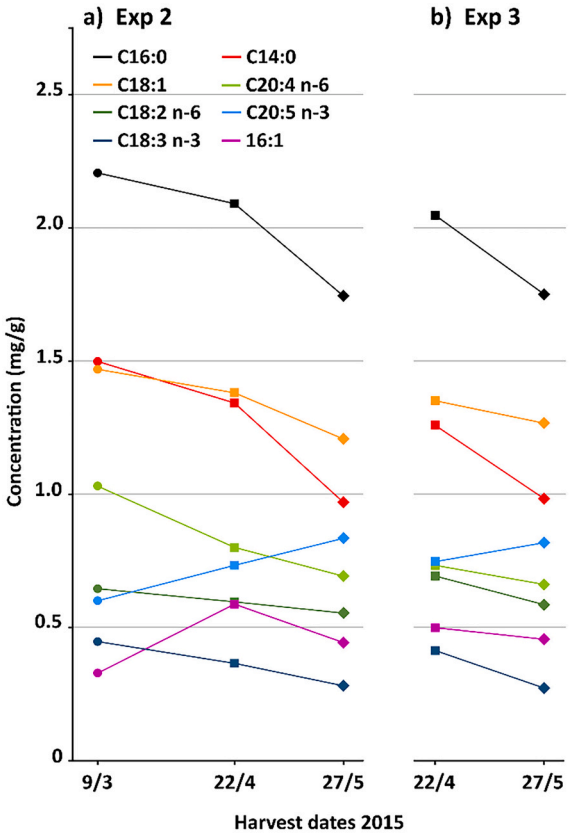

Fig. 3. Fatty acid content in dry biomass of Exp 2 (a) and Exp 3 (b). Circle, square and diamond marker represent harvests on the 9 Mar, 22 Apr and 27 May 2015. The average composition of all specimens at each harvest is presented, based on 6 specimens from each of the 3 deployment times (11 Sep, 21 Oct and 21 Nov 2014) for Exp 2, and 6 specimens from each of two cultivation depths for Exp 3. Numeric data is available in table S4 in supplementary materials. Name of acids: Palmitic (C16:0), myristic (C14:0), vaccenic (C18:1), arachidonic (C20:4 n-6), linoleic (C18:2 n-6), eicosapentaenoic (C20:5 n-3), α-linolenic (C18:3 n-3) and palmitoleic acid (C16:1).

Samples harvested on May 27 contained significantly less ash than samples harvested on 9 Mar and 22 Apr (Tukey, $p < 0.05$). For ash content (Exp 3. Fig. 2a$_{ii}$), there was no significant main effect but a just barely significant, ($p = 0.0497$), interaction between harvest time, locality and depth. A multiple comparison test (SNK, $p < 0.05$) revealed that the only condition that was significantly different from the rest was samples harvested at Vedskär at 2 m depth on 27 May, so no general difference could be found. (Data in supplementary table S12).

For carbohydrate content (Exp 2. Fig. 2a$_i$), there was a significant effect of harvest time, with no significant interactions (ANOVA, $p < 0.05$). Samples harvested on May 27 contained significantly more carbohydrates than samples harvested on 9 Mar (Tukey, $p < 0.05$). For carbohydrate content (Exp 3. Fig. 2a$_{ii}$) there was a significant effect of harvest time, locality and depth with no significant interaction (even though close to significant between harvest time, depth and locality, $p = 0.0504$). A deployment at 2 m compared to 4 m, a harvest on 27 May compared to 22 Apr, and cultivation at Vedskär compared to Lådderskär all led to significantly higher carbohydrate content. (Data in supplementary table S12).

For fatty acid content (Exp 2. Fig. 2a$_i$), there was a significant effect of harvest time, with significant interaction between harvest time and deployment time (ANOVA, $p < 0.05$). Samples harvested on May 27 contained significantly less fatty acids than samples harvested on 9 Mar (Tukey, $p < 0.05$). For fatty acid content (Exp 3. Fig. 2a$_{ii}$) there was a significant effect of harvest time and locality with no significant

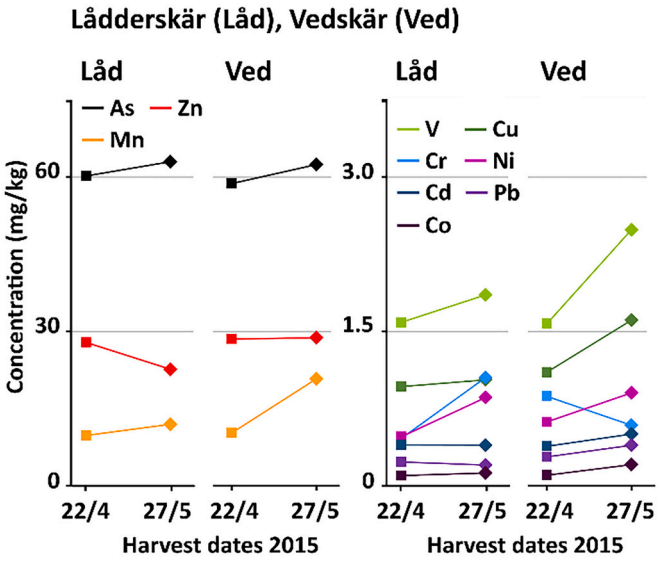

Fig. 4. Heavy metal composition in dry biomass for Exp 2. Square and diamond markers represent samples harvested on 22 Apr and 27 May 2015. For each location Vedskär (Ved) and Lådderskär (Låd), the average composition of all specimens harvested is presented, based on 3 specimens from each of the 3 deployment times (11 Sep, 21 Oct and 21 Nov 2014). Numeric data is available in table S5 in supplementary materials. Hg was also analysed for but was in all cases found below detection limit (0.02 mg kg^{-1}).

interactions. There was significantly more fatty acids in samples harvested on 22 Apr compared to 27 May, and significantly more in samples harvested at Vedskär compared to Lådderskär. (Data in supplementary table S12).

For phlorotannin content (Exp 2. Fig. 2a$_i$), there was a significant effect of harvest time, with no significant interactions (ANOVA, $p < 0.05$). Samples harvested on May 27 contained significantly more phlorotannins than samples harvested on 9 Mar (Tukey, $p < 0.05$). For phlorotannin content (Exp 3. Fig. 2a$_{ii}$) there was no significant main effect but a significant interaction between harvest time and depth. A multiple comparison test (SNK, $p < 0.05$) however revealed that no group was significantly different from each other. No statistically significant conclusion could be drawn regarding phlorotannin in Exp 3. (Data in supplementary table S12).

For protein content (Exp 2. Fig. 2a$_i$), there was a significant effect of harvest time, deployment time and locality with significant interaction between harvest time and deployment time, harvest time and locality, and harvest time, deployment time and locality (ANOVA, $p < 0.05$). Multiple comparison showed no easily understood or described trend for protein content which seemingly varied with deployment time, harvest time and locality (Tukey, $p < 0.05$). For protein content (Exp 3. Fig. 2a$_{ii}$) there was a significant effect of harvest time, locality and depth with no significant interaction. A deployment at 4 m compared to 2 m, a harvest on 22 Apr compared to 27 May, and cultivation at Lådderskär compared to Vedskär all led to significantly higher protein content. (Data in supplementary table S12).

Fig. 2b$_{i,ii}$ presents the same data as in Fig. 2a$_{i,ii}$, but expressed in milligrams per gram wet weight. As previously stated the water content in the harvested biomass increased significantly in harvested biomass between 9 Mar - 27 May (Exp 2) and 22 Apr – 27 May (Exp 3). As can be seen from Fig. 2b$_i$, this increase in water content gradually diluted all components. The average of total carbohydrates, for instance, increased per gram dry matter but remained approximately the same per gram wet weight. The average ash concentration in both dry and wet biomass decreased with later harvests (significant only for Exp 2). However, among specimen from the same harvest period, ash concentration was found to be closely tied with water content, with higher water content

giving more minerals which contribute to ash when dried. Numeric average values and standard deviations are available in supplementary table S2.

It should be noted that the results of Exp 2 and 3 should not be compared directly to one another as these originate in different experimental setups and are produced from different parent spore groups. In Exp 3, all individuals were deployed at the same time and relatively early (on 3 Sep 2014), with the focus of Exp 3 being to explore the effect of cultivation depth at 2 m vs 4 m. On the whole, the average values for each of carbohydrate, ash and water content of individuals deployed at 4 m depth showed similar average compositions as the late deployed individuals in Exp 2, while those deployed at 2 m depth showed similar average composition to early deployments.

Individuals grown in the same conditions in Exp 2 and Exp 3 (i.e. deployed at approximately the same time in early September 2014, cultivated at a depth of 2 m and sampled at similar harvest dates) also displayed important differences. This indicates that further to the variables of depth, deployment and harvest times, other control variables beyond the scope of this study seem to have an important influence on composition. There are several probable reasons for the observed differences relating to experimental set-up, but the most likely is thought to be due to Exp 2 and Exp 3 being conducted with individuals originating from different batches of spores (from different parent specimen), and thus perhaps being influenced by different genetic traits.

Heavy metals were measured in the biomass for Exp 2 and were seemingly more affected by cultivation site than deployment date. The heavy metal concentration for each location and harvest time is given in Fig. 4. Of all tested heavy metals, the amount of arsenic (As) was detected in the highest concentration and in rather similar amounts at both Lådderskär and Vedskär, while most other heavy metals varied between sites. To determine if there was any significant difference between the heavy metal composition between the two sites, multivariate analysis was used, results of which are presented in next section (Fig. 5d).

3.3. Multivariate analysis

PERMANOVA (Exp 2) followed by pairwise comparisons showed a significant difference in the content of major chemical compound groups between all harvest times (supplementary table S7). The effect of deployment time was dependent on the harvest time with significant differences between all deployment times for the March harvest, only between September and November deployment for the May harvest, and no differences for the April harvest. For the March harvest the localities were also significantly different but not for the other harvest times (supplementary table S7). A biplot of CAP for the data on first, second and third canonical axes is found in Fig. 5 a_i.

Consistent with PERMANOVA, CAP showed a separation between harvest times (Fig. 5a_i, supplementary table S8) with higher levels of carbohydrates, water and phenolics and lower levels of fatty acids, proteins and ash in May compared to March. CAP also showed a difference between localities in March with higher protein levels at Vedskär, but no effect of deployment time was detected (Fig. 5a_i, supplementary table S8). The largest effect of harvest time was observed for phenolics and carbohydrates, with means increasing approximately 2 and 1.5 times, respectively, from March to May (Fig. 2).

PERMANOVA (Exp 3) showed a significant interaction between cultivation depth and locality, and pairwise comparisons showed that cultivation depth affected the levels of major chemical compound groups at a significant level at one locality (Vedskär) and that localities had a significant effect at 4 m but not at 2 m (supplementary table S7). A biplot of CAP for the data on first and second canonical axes is found in Fig. 5a_{ii}. CAP also showed an effect of cultivation depth at Vedskär with higher content of carbohydrates and phenolics for kelp cultivated at 2 m while kelp cultivated at 4 m had higher levels of ash, proteins and water (Fig. 5a_{ii}, supplementary table S8). The total fatty acid concentration was not affected by cultivation depth.

3.4. Fatty acid composition of Saccharina latissima

The distribution of fatty acids is presented in Fig. 4 and numeric values are available in supplementary table S5. PERMANOVA (Exp 2) followed by pairwise comparisons showed a significant difference in composition of fatty acids between all harvest times (supplementary table S9). Kelp deployed in November also had a significantly different composition of fatty acids compared to kelp deployed in September and October, although this effect was only observed for kelp harvested in March and April (supplementary table S9). Consistent with PERMANOVA, CAP showed clear differences between harvest times, but not between deployment times or localities (Fig. 5b_i, supplementary table S8). The general pattern was a decrease in levels of myristic acid (C14:0), palmitic acid (C16:0), vaccenic acid (C18:1), α-linolenic acid (C18:3 n-3) and arachidonic acid (C20:4 n-6) with later harvest time, while kelp harvested in April and May had highest concentrations of palmitoleic acid (C16:1) and EPA (C20:5 n-3), respectively (Fig. 5b_i, Fig. 3).

PERMANOVA (Exp 3) showed a significant interaction between cultivation depth and locality (supplementary table S9). Pairwise comparisons showed that cultivation depth affected the fatty acid composition at one locality (Vedskär), and that kelp cultivated at 2 m differed between the localities. CAP also showed a separation between cultivation depths at Vedskär as well as a difference between localities at 2 m (Fig. 5b_i, supplementary table S8). Samples at 2 m from Vedskär were characterised by higher content of linoleic acid (C18:2 n-6), α-linolenic acid (C18:3 n-3), arachidonic acid (C20:4 n-6) and EPA (C20:5 n-3), while samples at 4 m had higher content of myristic acid (C14:0) and palmitic acid (C16:0) (Fig. 5 b_i, supplementary table S8).

3.5. Sugar composition of Saccharina latissima

The carbohydrate composition is described in Fig. 2 and numeric values are found in supplementary table S4. PERMANOVA (Exp 2) showed significant effects of both deployment time and harvest time on the carbohydrate composition (supplementary table S10). Subsequent pairwise comparisons showed a significant difference between all harvest times and between September deployment and deployment in October and November. Consistent with PERMANOVA, CAP showed a separation between all harvest times with largest difference for samples harvested in March (Fig. 5c_i, supplementary table S8). In general, the amount of fucose and glucose increased with harvest time with strongest effect for fucose (Fig. 5c_i). CAP also indicated an effect of deployment time for samples harvested in May with higher concentration of glucose for kelp deployed in September compared to November (Fig. 5c_i).

PERMANOVA (Exp 3) showed a significant effect of cultivation depth (supplementary table S10). CAP also indicated an effect of cultivation depth, with clearer effect for Vedskär (Fig. 5c_{ii}, supplementary table S8). Samples at 2 m tended to have higher content of glucose and lower content of guluronic acid and fucose compared to 4 m, although there was high variability between samples. (Fig. 5c_{ii}, Fig. 2).

3.6. Heavy metal composition of Saccharina latissima

The composition of heavy metals is presented in Fig. 4 and numeric values are found in supplementary table S6. PERMANOVA (Exp 2) showed a significant effect of locality on the composition of heavy metals, while cultivation depth had no effect (supplementary table S11). However, CAP did not discriminate between the localities (supplementary table S8). PERMANOVA (Exp 2) showed a significant effect of deployment time and a significant interaction between harvest time and locality (supplementary table S11). Subsequent pairwise comparisons showed that kelp deployed in September had a significant different composition of heavy metals compared to kelp deployed in November,

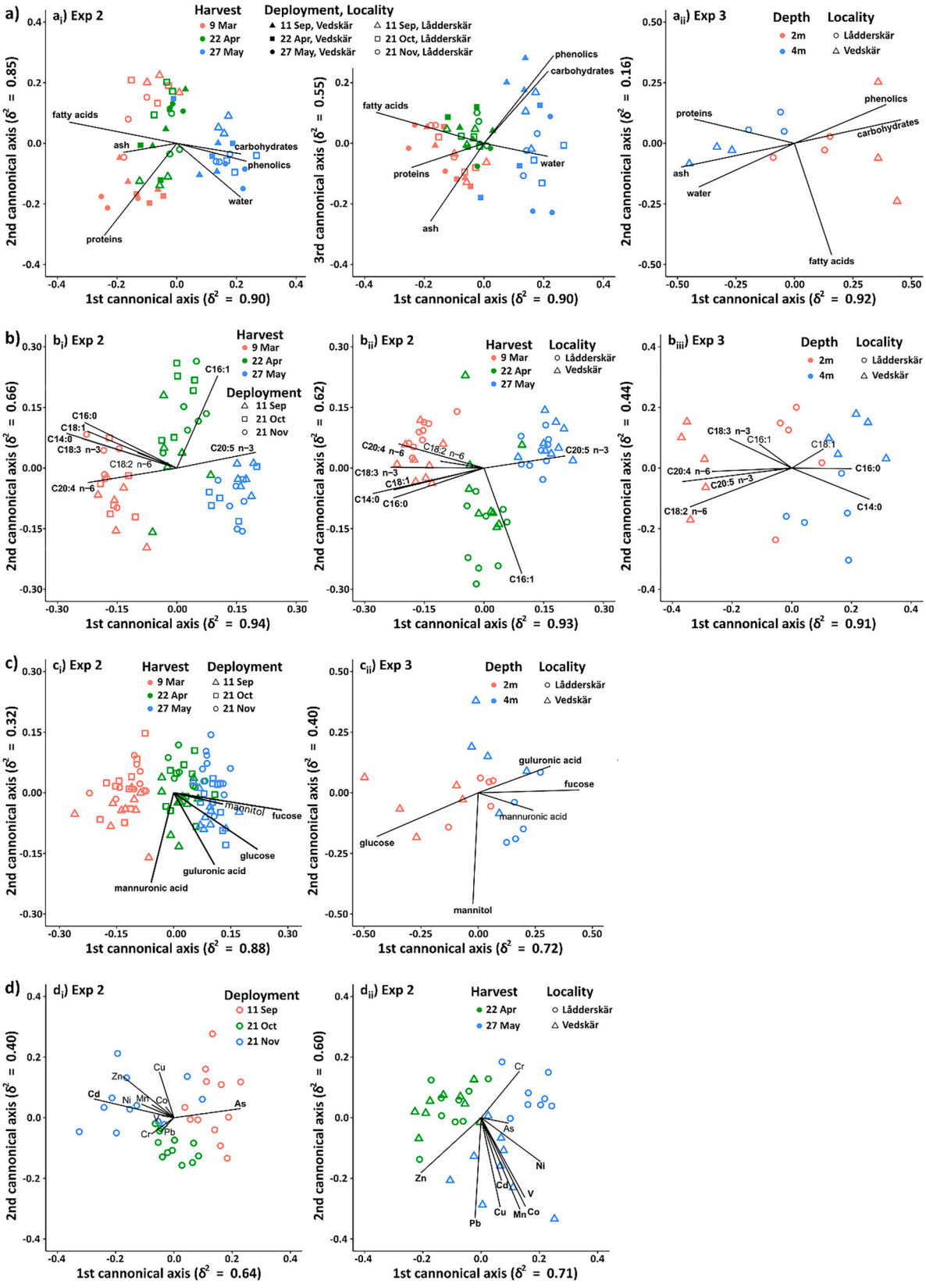

Fig. 5. Biplots of canonical analysis of principal coordinates (CAP) for data of chemical component concentration in cultivated *Saccharina latissima* specimens from Exp 2 and Exp 3. Results are organised as follows: (a) major component groups, (b) fatty acids, (c) carbohydrates and (d) heavy metals (only for Exp 2). Vectors represent the Pearson correlations, (values between −1 and 1), of each chemical component to the two canonical axes but scaled to fit the plot, (example −0.4 to 0.4 for a_i). Vectors for which the unscaled Pearson correlation coefficient exceed 0.5 for any of the 2–3 displayed canonical axes are named in bold font. Symbols for locality, depth and harvest times are found in the figure captions.

and that both harvest time and locality affected the heavy metal composition. CAP indicated a difference between all deployment times with arsenic content increasing and cadmium content decreasing in kelp with earlier deployment times (Fig. 5d_i, supplementary table S8). Consistent with PERMANOVA, CAP showed an effect of harvest time and locality although the locality effect was only observed for kelp harvested in May. In general, kelp tended to have higher content of heavy metals in May compared to April with largest differences for kelp cultivated at Vedskär that were characterised with higher content of cadmium, cobalt, copper, lead, manganese, nickel and vanadium (Fig. 5d_{ii}, supplementary table S8).

4. Discussion

Kelp mariculture on higher latitudes such the Swedish and Norwegian coasts are subject to a relatively short harvesting window, to benefit from rapid growth in spring due to more light and yet avoid fouling by epibionts in summer and associated loss of value (Forbord et al., 2020a; Visch et al., 2020b). In this study we did not measure yield, but focused on measures of blade weight, length and width.

During spring and early summer the size and weight of specimen varied greatly. This study covers three harvests on 9 Mar, 22 Apr and 27 May with 5 and 6 weeks between. For all experiments of this study, between first and second harvest the average weight of specimens increased by more than four times, and between second and third harvest it doubled, as seen in Fig. 1. The weight increases between second and third harvest can be explained by an increase in water content, so for the dry biomass there was less difference. The increase in water content shown in Fig. 2 supported by supplementary table S12 is also in line with literature on seasonal variation for wild *Saccharina latissima* specimens (Black, 1950).

Other than a late harvest, several additional factors were identified as having a positive effect on growth: earlier deployment on 11 Sep compared to 21 Oct or 21 Nov (significant for blade weight and width for the harvests on 9 Mar, 27 May and 22 Apr only at Vedskär), and cultivation at 2 m depth compared to 4 m, as shown in Fig. 1 supported by supplementary table S1. It is possible that an earlier deployment, shallower cultivation or later harvest time than in this study could increase growth even more. However, the time/depth-span of this study sections out a window that was relatively free from fouling, which might not be the case if deviating much for instance to later harvests. Early autumn deployment of cultivated *Saccharina latissima* has been found to result in increased size and yield in other studies (Peteiro and Freire, 2009; Edwards and Watson, 2011; Handå et al., 2013; Bruhn et al., 2016). Our study came to no significant conclusion regarding which juvenile sporophyte maturation duration was best suited for growth after deployment. A study with a similar method but further north in Norway and deployed in mid-February (63° 39′ N, 8° 39′ E) (Forbord et al., 2020b) came to the conclusion that 6 weeks was the optimal time for their conditions.

The chemical composition in the biomass varied considerably with harvest time, as seen in Figs. 2–4. In the dry biomass, the late harvest compared the early was characterised by significantly lower concentrations of ash (27 May vs 9 Mar), total fatty acids (27 May vs 22 Apr & 9 Mar) and protein (27 May vs 22 Apr for Exp 3 only) and higher concentrations of carbohydrates (Exp 2: 27 May vs 9 Mar, Exp 3: 27 May vs 22 Apr), phlorotannin (27 May vs 9 Mar). The main carbohydrates that increased were glucose, fucose, and mannitol and for fatty acids all decreased except EPA (C20:5 n-3) which increased. The water content also increased (Significant for 27 May vs 22 Apr for Exp 3, and deployment/location dependent for Exp 2), especially so in samples from the latest harvest. Increased water content had a dilution effect on the other components (as measured in g per kg fresh weight), and as such, most components either decreased or just barely increased when measured per unit of fresh biomass. That water content increased while ash in the dry biomass decreased, could be a sign that the seaweed regulated their salt balance to contain less salt. In a seasonal variation study of wild *Saccharina latissima* the increase in water content at a similar time was temporary, and once carbohydrate production had gained momentum, the water content got reduced to similar values as in autumn and spring, while ash concentration remained lower until late autumn/winter (Black, 1950). In our study the specimens with highest concentrations of carbohydrates (earlier deployment, later harvest and cultivated at 2 m), distinguished by a more than double glucose concentration compared to the average concentration at the harvest, showed a similar composition as samples in Blacks study when carbohydrate increase had occurred a reduced water content and yet lower ash content. The highly increased glucose concentration is a sign that the seaweed are producing laminarin, since cellulose concentration instead is known to decrease at the March–May interval (Black, 1950; Schiener et al., 2015), and if one could wait for more specimens to reach that composition stage it would be beneficial if one is interested in a high carbohydrate content with less ash and water, and also if one is interested specifically in laminarin. These patterns are in general agreement with the seasonal variations in recent literature (Handå et al., 2013; Marinho et al., 2015a; Sharma et al., 2018; Forbord et al., 2020a).

Other factors than changing the harvest time also affected the composition, as seen in Fig. 2. Early deployment (11 Sep) compared to late (21 Oct and 21 Nov) or with cultivation line at 2 m compared to 4 m depth, both affected the chemical composition in harvested seaweed in similar ways. Glucose and mannitol concentration became higher, protein and fucose concentration lower, and water content lower, which effectively increased the concentration of all components in the fresh biomass. The reduced water content also resulted in less ash in the dry biomass since the water had a significant salt content. The effect of depth was however more impacting on composition, than the effect of deployment time. While some samples deployed in the middle deployment 21 Oct for instance had produced as much glucose as samples from the first deployment 9 Sep, this was never the case for samples cultivated at 4 m depth compared to 2 m depth. For most practical uses of the biomass, an early deployment or less deep cultivation will likely be favourable given that the harvested specimen will be larger and contain more components other than water. The exception would be if the goal is to extract fucoidan (a polysaccharide containing fucose) or protein from dried biomass, for these compounds, a cultivation at 4 m depth or later deployment could give a higher starting concentration in the biomass. The depth parameter has also been studied in other investigations which found similar trends regarding growth and composition (Handå et al., 2013; Fossberg et al., 2018; Sharma et al., 2018; Forbord et al., 2020a).

Some variation was seen between cultivation sites for both growth data and composition but depth, deployment time and harvest time had a bigger impact on the composition. This is discernible in Fig. 5a–c from specimens from both localities that end up in mixed groups in the plot of multivariate analyses of principle components. The one exception from this trend is for heavy metals, where locality had a greater impact for many metals (Figs. 4, 5d), and it was found that in the late harvest all metals except for chrome and arsenic were detected in higher amount in Vedskär. An early deployment also seemed to decrease the amount of heavy metals, except for arsenic which showed the opposite trend. To show that timing and choice of locality may impact the metal composition is an important step in finding ways to cultivate with lower amounts of heavy metals. However, not enough information was collected about the two sites (e.g currents and exposure, bottom substrate, etc.) to determine the causality behind the differences between the two locations. Further research should continue to explore effects of different cultivation localities (e.g. Camus et al., 2018) and the causality of heavy metal absorption by kelp, building on the groundwork laid by Bruhn et al. (2016) and Ometto et al. (2018) which found differences in heavy metal composition between sites and time of harvest, notably regarding sediment types, sediment resuspension and the use of sediment heavy metal content as a proxy indicator for likely heavy metal absorption, thus potentially enhancing site selection.

5. Conclusion

The physical properties and chemical composition of *Saccharina latissima* varied significantly across the narrow harvesting window on the Swedish west coast which can stretch from March to May. Furthermore, cultivation parameters such as deployment date, cultivation depth and harvest date also had some significant impacts on both growth and composition. In the present study, some differences were also seen between two cultivation locations though the underlying reasons for these differences could not be determined based on available data.

Though the general trends identified in this study may hold true, the parameters used in these experiments are likely to vary spatially, due to differences in local environmental conditions. Nevertheless, some recommendations for kelp farming practitioners have emerged from this study, especially for those located at or near the Swedish west coast. To maximise growth and concentration of certain target components notably carbohydrates during the harvest window, practitioners should aim for an early deployment (e.g. September rather than later in October or November), at a shallower depth (e.g. 2 m rather than 4 m), and to harvest later in the harvest window (e.g. in late May rather than March or April). If such fast-growth promoting cultivation settings are selected, glucose was the component that increased the most especially in samples harvested later, which is also a sign of laminarin production. If instead the lines are deployed deeper (e.g. at 4 m depth instead of 2 m), growth is reduced but an increased concentration of protein and fucoidan (fucose) can be expected. Fatty acid concentrations decreased with later harvests, apart from eicosapentaenoic acid (EPA, C20:5 n-3), which increased. Since the carbohydrate's concentration increased rapidly during May and still had not reached its maximum, further delaying harvests could lead to a more carbohydrate rich biomass. However delayed harvests also increase the risk of epibiont fouling, which may or may not be acceptable depending on the intended use of the biomass.

Author statement

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Declarations

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Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

Declaration of Competing Interest

FG, HP and GN are co-founders of a kelp farming start-up, Nordic Seafarm AB. All other authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2022.738443.

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