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# Effects of geographical location on potentially valuable components in *Ulva intestinalis* sampled along the Swedish coast

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**ABSTRACT**

Macroalgal biomass has the potential to become an important source of chemicals and commodities in a future biorefinery. Currently, production of macroalgal biomass is expensive and the content of high-value compounds is often low. Therefore, in this study the biochemical composition of *Ulva intestinalis* along the Swedish west coast and the east coast up to Stockholm was assessed with the aim of determining how the content of potentially valuable compounds, such as rhamnose, iduronic acid and PUFAs, could be maximized by utilizing natural variation in the choice of marine cultivation site. Along the investigated coastline, the salinity dropped from 19.4‰ at high latitudes along the west coast to 5.4‰ at Stockholm. Nitrogen and phosphorus availability varied, while temperature was similar at all locations. The two major components of biomass, carbohydrates and ash, varied inversely with the highest content of ash in the west and carbohydrates in the east. In addition, total fatty acids were significantly higher in west coast samples at 3.2 g 100 $g^{-1}$ dw, with a higher proportion of mono- and polyunsaturated fatty acids. Some health-beneficial fatty acids were found, including EPA and DPA, at 10–50 mg 100 $g^{-1}$ dw, respectively. The metal content and elemental composition varied widely, probably due to the influence of specific local conditions. The P content was correlated with the phosphorus concentration in waters at the locations. In PCA analysis, the monosaccharides constituting the cell wall polysaccharide ulvan were found to vary by geographical location, with higher levels possibly associated with lower salinities. However, only glucuronic acid differed significantly between sites. These results show the considerable geographical variability in the composition of Swedish *U. intestinalis* and suggest that different salinities could be used to create a lipid- or carbohydrate-rich biomass.

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## Introduction

Renewable biomass sources for use in biorefineries are needed for a transition to a sustainable society. One promising alternative to terrestrial biomass is macroalgae. As macroalgae are not widely exploited as a food source in Europe, with large-scale cultivation limited to Asia, there is a significant potential for increased production in Europe that could be used in biorefining to produce a host of valuable products (Mac Monagail, Cornish, Morrison, Araújo, & Critchley, 2017). Macroalgae are generally rich in carbohydrates, but also contain lower, but highly nutritious, quantities of lipids and protein (Holdt & Kraan, 2011), and are well suited for further processing (Jones et al., 2020).

Production of seaweeds for novel applications struggles to be profitable, even in cases with fully developed down-stream processing technology (Konda, Singh, Simmons, & Klein-Marcuschamer, 2015; van den Burg, van Duijn, Bartelings, van Krimpen, & Poelman, 2016). It has been suggested that production of high-value products in a cascading biorefinery scheme could increase the profitability of seaweed production (van Hal, Huijgen, & Lopez-Contreras, 2014). A range of high-value products could potentially be extracted, including omega-3 fatty acids, essential amino acids, colourants, bioactive oligosaccharides and some rare monosaccharides which are difficult to acquire elsewhere (Holdt & Kraan, 2011; Lahaye & Robic, 2007). It has also been shown that the biomass composition can vary depending on local growth conditions (Nielsen et al., 2016; Vilg et al., 2015). As such, the value of the biomass will vary with the growth location, limiting the ability to create predictable product streams in a biorefinery context.

Along the Swedish coast, conditions vary substantially in terms of salinity and nutrient concentrations. The Baltic Sea is well known for being eutrophicated

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Supplemental data for this article can be accessed here.

and polluted (Gustafsson et al., 2012). However, the major differences are the salinity, which goes from almost 0‰ in the northern Gulf of Bothnia to 20–30 ‰ at the west coast (Feistel et al., 2010). The effect of the salinity gradient is demonstrated by the diversity of seaweed species dropping from 250 species at the Norwegian border to a mere 42 in the northern area of the Gulf of Bothnia (Gothenburg University, 2018; Naturvårdsverket, 2009). Very few species are present all along the entire coastline and very little is known about how these gradients influence seaweed biochemical composition.

A group of seaweeds that grows fast and is regularly found in seaweed blooms is the green *Ulva* genus, both tubular and blade morphologies forming particularly dense populations, which are also seen in Swedish waters on the west coast (Bermejo et al., 2019; Pihl, Svenson, Moksnes, & Wennhage, 1999). In this study, we focus on the species *Ulva intestinalis*, which has a tubular morphology and is one of the few species that grows around the entire Swedish coastline. Some of the economic potential of *Ulva* spp. lies in its content of the sulphated polysaccharide ulvan. This polysaccharide is similar in structure to heparin and has a plethora of biological activity, such as antioxidant, anticoagulative, immunostimulative, immunomodulative, cancer chemopreventative and cytotoxic properties (Abd-Ellatef et al., 2017; Castro et al., 2006; Hussein, Mahmoud, Farrag, & Bishayee, 2015; Kaeffer, Benard, Lahaye, Blottiere, & Cherbut, 1999; Kim, Cho, Karnjanapratum, Shin, & You, 2011; Leiro, Castro, Arranz, & Lamas, 2007; Mao, Zang, Li, & Zhang, 2006; Qi et al., 2005a, 2005b; Tabarsa, Han, Kim, & You, 2012). The polysaccharide component also contains the rare monosaccharides, rhamnose and iduronic acid (Kidgell, Magnusson, de Nys, & Glasson, 2019). In addition, the fatty acid composition also has potential value. Although it is only a minor fraction of the biomass (Holdt & Kraan, 2011), if the fatty acids were present in other recovered products (i.e., a PUFA-enriched protein fraction) this could substantially improve the value as a food ingredient. The long coastline of Sweden offers a variety of conditions for targeted cultivation, and there is potential for a cultivation business to emerge as, presently, aquaculture activities around the coast are negligible. In the present study, we collected biomass of *U. intestinalis* and report its biochemical composition at seven different locations from north of Göteborg in the west to Stockholm in the east. Using available environmental data as well as extraction of data from environmental models, we correlate local conditions to biochemical composition. By understanding the locations at which valuable components are at their highest, production systems could be developed for the conditions where the produced biomass provides the highest value.

## Materials and methods

### Location choice and sample collection and preparation

A number of locations along the coast were selected, with more sample points in the south, where the Baltic meets the Skagerrak and the salinity changes rapidly. This variation is caused by the Baltic Sea being isolated from the Atlantic by several narrow straits between Denmark and Sweden, causing the water to become brackish due to the low inflow of saline oceanic seawater (salinity ~35‰). As there is also an output of low-salinity surface waters from the straits and along the coasts of Baltic countries, the entire Swedish coastline becomes a long gradient of salinity.

The main aim was to observe local effects caused by the water conditions, so as many factors as possible were controlled for in the selection of locations. Hence, locations with water movement, no recreational harbours or water outlets close by, and hard seabeds were chosen where available. In total, seven locations were investigated (Fig 1) and are abbreviated through the work as Tjörn north of Göteborg (GBG), Helsingborg (HBG), Trelleborg (TBG), Åhus (ÅHS), Karlskrona (KKR), Västervik (VSV), and Stockholm (STH). At each location, three samples of biomass were collected about 50–7000 m apart (for most sites a few hundred metres) during late summer over 9 days; for coordinates of sites and times of sampling see Supplementary table S1.

Biomass samples (ca 10 cm in length) were taken from organisms/tissues as morphologically similar as possible and the collected material was sorted on site, keeping only clean blades with a bright green colour. However, due to issues with biomass availability or site suitability, not all criteria were followed everywhere. These divergences were as follows: in Karlskrona, samples were collected near harbours, in Stockholm, the selected specimens were smaller, and in Helsingborg, the sampling points were sandy beaches. Excess water in the biomass was removed by a salad spinner and the samples were frozen in a portable freezer shortly after collection. The samples were freeze-dried (Drywinner Heto, Allerød, Denmark) and homogenized by milling to a homogeneous powder (Tissue Lyzer II, Qiagen, Hilden, Germany, at 30 Hz for 1 to a few min). Milling was performed in stainless steel-grinding jars (Retsch material number 1.4112) with 15–50 ml volume with a 15 mm bead. The jars were frozen in liquid nitrogen for 1 min prior to milling to avoid heating of samples. The milling could have resulted in contamination by Fe and Cr but considering the regular use of the jars (limits surface oxidation) and limited time of milling, it cannot have caused significant differences between samples. The samples were then stored at −80° C prior to analysis.

### Species identification

The samples were initially identified morphologically and were assessed as a feedstock for hydrothermal liquefaction (Raikova et al., 2020). However, recent studies have identified strong morphological plasticity within *Ulva* spp., as well as the occurrence of cryptic species within the genus (Steinhagen, Karez, & Weinberger, 2019). Thus, the *Ulva* samples were molecularly identified in this study after the morphological pre-identification.

Per sample, total genomic DNA was isolated from 100 mg of the respective homogenized algal tissue. The powder was extracted using an Invisorb Spin Plant Mini Kit (Stratec, Birkenfeld, Germany) following the manufacturer's protocol. The barcode marker *tuf*A was amplified by polymerase chain reaction (PCR) following the procedure as described (Steinhagen et al., 2019). The obtained amplicons were subsequently sequenced by Eurofins Genomics (Konstanz, Germany). Sequence alignment as well as reciprocal editing was carried out using Sequencher (v. 4.1.4; Gene Codes Co., Ann Arbor, Michigan). Obtained and controlled sequences were uploaded to GenBank and are publicly available (accession numbers: MT028279-MT02296; Supplementary table S1). The taxonomic identity of the samples was determined by applying the BLAST function implemented in GenBank. As a control for the correctness of the databank searches, representative, peer-reviewed sequences of *Ulva* spp. were downloaded from GenBank and included in a phylogenetic analysis. The phylogenetic analyses were performed using the maximum likelihood approach within the software RAxML v. 8 (Stamatakis, 2014), data not shown. One sample from the GBG location was found not to be *U. intestinalis*, and diverged strongly in composition; thus, this sample was excluded from the study. For TBG and STH locations, the sequencing was unsuccessful for one sample at each site, but as the morphology of the collected samples and the resulting chemical composition was similar between all


**Figure 1.** Sampling locations of *Ulva intestinalis*: Göteborg (GBG), Helsingborg (HBG), Trelleborg (TBG), Åhus (ÅHS), Karlskrona (KKR), Västervik (VSV), and Stockholm (STH).

samples at these locations, the species identification was taken as *U. intestinalis*.

### Analyses of biomass components

Carbohydrate content and composition were determined after acid hydrolysis of 25 mg samples according to Bikker et al. (2016) without neutralization, and subsequent analysis by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Olsson et al., 2020b). The total carbohydrate content was calculated as the sum of monosaccharides and sugar acids corrected for the addition of water during hydrolysis of polysaccharides.

Total fatty acid analysis was performed by direct transesterification of a 25 mg sample and GC-FID separation and quantification (Mayers et al., 2018). Protein content was determined by analysing the nitrogen content on an elemental analyser (Carlo Erba Flash 2000 elemental analyser, at London Metropolitan University) and the nitrogen was converted to protein by a conversion factor of 4.73 (Biancarosa et al., 2017). Metals and some non-metal elements were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES) after digestion in *aqua regia* as described (Raikova et al., 2020). ICP-OES was carried out externally by Yara UK Ltd using an Agilent 700 series inductively coupled plasma optical emission spectrometer. Ash was determined by heating 500 mg of sample in a Carbolite muffle furnace at 550°C for 5 h and the mass remaining was considered as ash.

### Statistics

Principal component analysis (PCA) was used to generate an overview and to analyse for systematic differences in concentration of macromolecules, monosaccharides, fatty acids and metals between different locations along the Swedish coast. PCA was assisted by correlation plots showing the correlation between the original variables and the principal components. PCA was accompanied with univariate analysis of variance (ANOVA) with "Location" as fixed factor, using the false discovery rate to adjust for multiple testing (Benjamini & Hochberg, 1995). For individual compounds significantly affected by "Location", further analysis was performed with a Student-Newman-Keuls (SNK) posthoc test ($\alpha = 0.05$).

### Acquiring environmental data for sampling locations

Nutrient data were obtained from the SHARK database (Havs-och Vattenmyndigheten, 2019) using the closest similar sampling point to our sampling locations. The SHARK database contains environmental data from monitoring by governmental agencies as well as deposited data from research projects. Data for salinity and temperature were averaged over June–September and came from the NEMO-Nordic ocean model (Hordoir et al., 2019), using the coordinates of the locations (Supplementary table S1). STRÅNG model data (Swedish Meteorological and Hydrological Institute (SMHI), 2020) were used to find photosynthetically active radiation (PAR) values at the sites for June–September 2016.

## Results

### Environmental data for sampling locations

The SHARK database showed that the concentrations of nitrogen and phosphate-phosphorus, averaged over our sampling period, were 0.3–1.2 µM and 0.05–0.46 µM, respectively (Fig 2). These concentrations are much lower than the yearly averages for seawater (30 µM nitrogen and 2 µM phosphate (Hurd, Lobban, Bischof, & Harrison, 2014a), which is to be expected during the summer period when biological activity is high. A large standard deviation was noted in the nitrogen data over the course of summer, both on a local-specific and month-to-month basis. Anthropogenic input from agriculture and other human activities from the Southern coastal regions surrounding Trelleborg, Åhus and Karlskrona (TBG, ÅHS and KKR) has impacted the levels of phosphorus for many years (Rosenberg et al., 1990). Consequently, the phosphorus concentrations at these locations are high (0.31–0.46 µM) vs. ≤0.25 µM for the other sites.

Salinity gradually decreased from 19.4‰ at GBG to 5.4‰ at STH, whereas the temperature was similar at all locations (14.8–16.9°C) (Fig 2). Using STRÅNG model data, the accumulated PAR for June–September 2016 was estimated for each site. The highest accumulated PAR was at KKR, 4400 mol $m^{-2}$ $month^{-1}$, and the lowest at GBG, 3800 mol $m^{-2}$ $month^{-1}$ (Supplementary table S2). However, the other sites were relatively similar, with minor differences between them when comparing individual months.

### Biomass composition

In the *U. intestinalis* samples, the components directly analysed summed to 70–80% of the total dry weight of biomass (Fig 3). The ANOVA analyses revealed significant effects of locality on carbohydrates ($F_{6,13} = 3.19$, $p = 0.050$), fatty acids ($F_{6,13} = 5.94$, $p = 0.014$), and ash ($F_{6,13} = 4.66$, $p = 0.019$), but not on proteins ($F_{6,13} = 1.28$, $p = 0.33$). The largest biomass component was the carbohydrate fraction, comprising 29–41 g

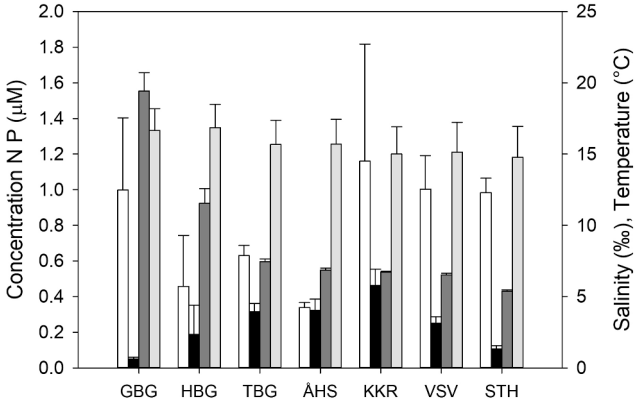


**Figure 2.** Environmental data for sampling locations along the Swedish coast (abbreviations, see Fig. 1) during summer months (June-September). Nitrogen (open bars) and phosphorus (black bars) concentration (µM), salinity (dark grey bars) of seawater (‰) and temperature (light grey bars) at locations (°C). Nitrogen is the sum of ammonium, nitrate, and nitrite concentrations and phosphorus is the phosphate concentration. Means ± standard deviation as error bars of data for the four months are shown (for TBG and VSV nutrient data were available only for three months). Data for nutrients are from the SHARK database. Data for salinity and temperature were modelled with the NEMO-Nordic model at each sampling site (2–3 sites per location, for coordinates see Supplementary table S1) and variation in data (standard deviation) between sites of a location were less than 1.8%.

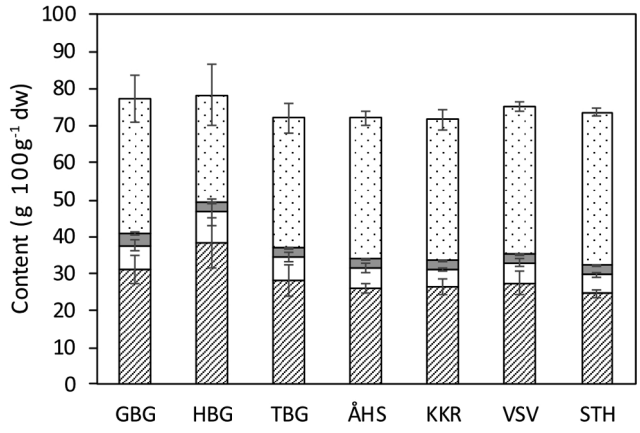


**Figure 3.** Biochemical components, ash (striped bars), proteins (open bars), total fatty acids (grey bars) and total carbohydrates (dotted bars) in *Ulva intestinalis* from sampling locations along the Swedish coast (for abbreviations, see Fig. 1). Averages of the three replicated samples (GBG duplicates) at each site with standard deviation and error bars are shown.

100 $g^{-1}$ dw. A significant difference in carbohydrate content (post hoc SNK test) was seen between the lowest and highest content found at Helsingborg and Stockholm, respectively, but all the east coast samples had high carbohydrate contents, ≥38 g 100 $g^{-1}$ dw (Table 1). The second largest component was ash, 24–38 g 100 $g^{-1}$ dw. The ash content was highest at Helsingborg, which was significantly different (SNK test, $p < 0.05$) from all other locations. This was presumably due to the HBG samples being contaminated by sand to different degrees, as they were collected at sandy beaches. The contamination only became obvious during sample preparation after drying. Despite the removal of all visible sand at collection, some sand was probably trapped inside the algal tubular thalli. Low ash content was seen in all east coast samples, with the lowest content of 24 g 100 $g^{-1}$ dw found at the Stockholm location, which could be expected as the salinity there is the lowest of all sample sites. Protein and total fatty acids (i.e., lipids) only constituted a small fraction of the biomass, 4.8–8.2 and 2.2–3.2 g 100 $g^{-1}$ dw, respectively. The lipid content was highest at the west coast location, GBG, which was significantly different compared to all the other locations (Table 2).

The monosaccharide profiling showed that the glucose and rhamnose were the main components, 10–17 g 100 $g^{-1}$ dw across all samples (Table 1). Xylose, galactose and glucuronic and iduronic acids were found at lower levels (Table 1). ANOVA was performed on ash-free dry weight data, as there was an influence of non-biomass components caused by the sand contamination

**Table 1.** Monosaccharide composition on dry weight (dw) basis of *Ulva intestinalis* from sampling locations along the Swedish coast (for abbreviations see Fig. 1). The mean and standard deviation of triplicated samples (GBG duplicated) at each location are given. Different letters indicate significant differences of means in post hoc SNK test.

| | GBG | | HBG | | TBG | | ÅHS | | KKR | | VSV | | STH | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | g 100 $g^{-1}$ dw | SD | g 100 $g^{-1}$ dw | SD | g 100 $g^{-1}$ dw | SD | g 100 $g^{-1}$ dw | SD | g 100 $g^{-1}$ dw | SD | g 100 $g^{-1}$ dw | SD | g 100 $g^{-1}$ dw | SD |
| Galactose | 0.81 | ± 0.14 | 0.56 | ± 0.03 | 1.01 | ± 0.09 | 1.06 | ± 0.16 | 1.10 | ± 0.05 | 1.04 | ± 0.17 | 0.88 | ± 0.19 |
| Glucose | 13.49 | ± 1.33 | 10.28 | ± 2.54 | 12.58 | ± 0.45 | 9.61 | ± 0.97 | 10.20 | ± 1.60 | 12.41 | ± 1.60 | 13.60 | ± 0.47 |
| Rhamnose | 14.21 | ± 0.93 | 11.74 | ± 3.97 | 14.11 | ± 2.77 | 17.22 | ± 2.10 | 17.39 | ± 1.12 | 16.50 | ± 0.26 | 16.88 | ± 1.02 |
| Xylose | 1.99 | ± 0.09 | 1.58 | ± 0.47 | 1.84 | ± 0.29 | 2.17 | ±0.56 | 1.91 | ± 0.40 | 1.93 | ± 0.20 | 2.96 | ± 0.43 |
| Iduronic acid | 2.71 | ± 0.42 | 1.77 | ± 0.29 | 2.30 | ± 0.35 | 2.99 | ± 0.12 | 3.10 | ± 0.15 | 3.51 | ± 0.28 | 3.26 | ± 0.49 |
| Glucuronic acid | 3.13 | ± 0.01 | 3.00 | ± 1.05 | 3.23 | ± 0.91 | 4.87 | ± 0.69 | 4.43 | ± 0.65 | 4.42 | ± 0.29 | 3.83 | ± 0.50 |
| Total | 36.33 | ± 6.41 ab | 28.94 | ± 8.30 b | 35.07 | ± 4.04 ab | 37.93 | ± 2.07 ab | 38.13 | ± 2.80 ab | 39.81 | ± 1.23 ab | 41.41 | ± 1.06 a |

**Table 2.** Fatty acid composition on dry weight (dw) basis of *Ulva intestinalis* from sampling locations along the Swedish coast (for abbreviations see Fig. 1) and lipid classes; saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The mean and standard deviation of triplicated samples (GBG duplicated) at each location are given. Results of ANOVA are shown by the letters in the total rows. For lipid classes SFA they were non-significant ($F_{6,13} = 0.57$, $p = 0.75$), MUFA ($F_{6,13} = 7.13$, $p = 0.004$) and for PUFA very significant ($F_{6,13} = 6.49$, $p = 0.004$).

| | GBG | | HBG | | TBG | | ÅHS | | KKR | | VSV | | STH | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mg 100 $g^{-1}$ dw | Stdev | mg 100 $g^{-1}$ dw | Stdev | mg 100 $g^{-1}$ dw | Stdev | mg 100 $g^{-1}$ dw | Stdev | mg 100 $g^{-1}$ dw | Stdev | mg 100 $g^{-1}$ dw | Stdev | mg 100 $g^{-1}$ dw | Stdev |
| C12:0 | 350 | ± 30 | 320 | ± 20 | 310 | ± 20 | 380 | ± 10 | 370 | ± 0 | 380 | ± 10 | 390 | ± 10 |
| C14:0 | 11 | ± 1 | 8 | ± 1 | 33 | ± 12 | 25 | ± 11 | 24 | ± 10 | 31 | ± 17 | 30 | ± 16 |
| C16:0 | 540 | ± 90 | 490 | ± 30 | 450 | ± 50 | 360 | ± 100 | 320 | ± 30 | 370 | ± 10 | 370 | ± 40 |
| C18:0 | 6.9 | ± 1.3 | 6.8 | ± 0.1 | 9.6 | ± 3.5 | 8.8 | ± 2.9 | 7.1 | ± 2.7 | 6.4 | ± 1.3 | 6.5 | ± 1.1 |
| C20:0 | 2.8 | ± 0.2 | 2.3 | ± 0.2 | 2.5 | ± 0.4 | 1.6 | ± 0.3 | 1.4 | ± 0.3 | 1.4 | ± 0.1 | 1.5 | ± 0.0 |
| C22:0 | 15 | ± 2 | 14 | ± 2 a | 11 | ± 1 | 11 | ± 1 | 10 | ± 1 | 11 | ± 1 | 11 | ± 1 |
| C24:0 | 0.7 | ± 0.0 | 0.6 | ± 0.1 | 1.9 | ± 0.8 | 1.9 | ± 0.9 | 1.2 | ± 0.3 | 1.4 | ± 0.6 | 1.8 | ± 0.9 |
| C16:1 | 150 | ± 60 | 160 | ± 70 | 110 | ± 20 | 70 | ± 20 | 60 | ± 20 | 80 | ± 20 | 80 | ± 10 |
| C18:1 | 1550 | ± 210 | 1140 | ± 130 | 1330 | ± 40 | 1210 | ± 50 | 1060 | ± 140 | 1170 | ± 20 | 1230 | ± 70 |
| C18:2 | 190 | ± 30 | 120 | ± 30 | 140 | ± 30 | 90 | ± 40 | 70 | ± 20 | 100 | ± 10 | 100 | ± 10 |
| C18:3 | 18 | ± 4 | 18 | ± 4 | 19 | ± 4 | 14 | ± 3 | 13 | ± 4 | 14 | ± 3 | 14 | ± 3 |
| C18:4 | 95 | ± 24 | 91 | ± 25 | 63 | ± 1 | 49 | ± 14 | 42 | ± 5 | 53 | ± 8 | 55 | ± 11 |
| C20:1 | 180 | ± 40 | 190 | ± 50 | 130 | ± 10 | 120 | ± 10 | 100 | ± 30 | 120 | ± 10 | 120 | ± 0 |
| C20:2 | 1.8 | ± 0.0 | 2.2 | ± 0.4 | 1.9 | ± 0.2 | 1.9 | ± 0.2 | 1.7 | ± 0.7 | 2.0 | ± 0.4 | 2.5 | ± 0.4 |
| C20:3 | 3.2 | ± 0.6 | 1.6 | ± 0.5 | 2.5 | ± 1.3 | 2.4 | ± 1.6 | 1.7 | ± 0.8 | 1.2 | ± 0.1 | 1.3 | ± 0.1 |
| C20:4 ARA | 6.5 | ± 0.8 | 4.3 | ± 0.9 | 4.4 | ± 0.8 | 3.5 | ± 1.0 | 2.8 | ± 0.5 | 3.7 | ± 1.5 | 4.0 | ± 1.5 |
| C20:5 EPA | 24 | ± 7 | 19 | ± 1 | 23 | ± 7 | 17 | ± 4 | 15 | ± 3 | 12 | ± 2 | 13 | ± 1 |
| C22:1 | 8.2 | ± 1.2 | 8.2 | ± 2.5 | 5.5 | ± 0.2 | 4.8 | ± 1.4 | 4.1 | ± 0.6 | 7.9 | ± 4.5 | 8.7 | ± 4.6 |
| C22:5 DPA | 29 | ± 1 | 29 | ± 4 | 53 | ± 18 | 48 | ± 14 | 42 | ± 6 | 44 | ± 18 | 47 | ± 16 |
| Total | 3220 | ± 520 a | 2640 | ± 110 b | 2720 | ± 150 b | 2440 | ± 250 b | 2170 | ± 190 b | 2430 | ± 80 b | 2510 | ± 40 b |
| SFA | 850 | ± 220 | 840 | ± 60 | 810 | ± 80 | 780 | ± 120 | 730 | ± 40 | 720 | ± 170 | 820 | ± 40 |
| MUFA | 1710 | ± 270 a | 1300 | ± 70 bc | 1440 | ± 40 b | 1290 | ± 60 bc | 1130 | ± 140 c | 1260 | ± 30 bc | 1310 | ± 60 bc |
| PUFA | 580 | ± 120 a | 500 | ± 60 ab | 460 | ± 50 abc | 370 | ± 80 bc | 310 | ± 50 c | 370 | ± 30 bc | 380 | ± 20 bc |

(Supplementary table S3). The analysis revealed that location resulted in significant variations in glucose, glucuronic acid and galactose contents. Most interesting is that the average glucuronic acid contents in samples from the east coast (STH, VSV, KKR) were significantly higher (SNK test) than those from the southern coast (HBG and TBG). Glucuronic acid is a constituent of the cell wall polysaccharide ulvan, and is also found in glucuronan (Redouan et al., 2009). Additionally, for other constituents of ulvan, rhamnose, iduronic acid and xylose (Wahlström et al., 2020), content increased in the east coast samples (Table 1). The ANOVA indicated effects on both iduronic acid and xylose when looking at the unadjusted p values, which were 0.05 and 0.07 for these components, respectively (Supplementary table S3).

The detailed fatty acid profile showed that the main fatty acids present at more than 150 mg 100 $g^{-1}$ dw were C12:0, C16:0, C16:1, C18:1, C18:2 and C20:1 (Table 2). In total, monounsaturated fatty acids were the most dominant class at 1.1–1.7 g 100 $g^{-1}$ dw. The ANOVA of lipid classes (dry weight basis) demonstrated that location had an effect on the MUFA and PUFA, which were both significantly higher in west coast samples compared to east coast samples. Levels of the health-beneficial polyunsaturated fatty acid EPA, i.e., C20:5, were found to be 12–24 mg 100 $g^{-1}$ dw, and DPA, i.e., 22:5, was 29–53 mg 100 $g^{-1}$ dw. The ANOVA for single fatty acids was also done on an ash-free dry weight basis (Supplementary table S4), and revealed effects of location on several fatty acids, C16:0, C20:0, C22:0, C18:1, C18:2, C18:4; C20:1 and EPA. For most of these, there were significant differences (SNK test) between the west coast and east coast locations, with higher content of these fatty acids in the west coast samples. Looking at the unadjusted p-value, location also had a significant effect on C16:1 and C20:4, ARA, fatty acids ($p \sim 0.04$).

Analysis of metal and non-metal elements showed that content of alkali and alkaline earth metals K, Ca and Mg was substantial, in the ranges 6900–10,600, 2400–5000, and 4600–7800 ppm, respectively (Table 3). The biomass also contained substantial amounts of P and S: 930–2980 and 47,400–79,800 ppm, respectively. In terms of potentially toxic metals and metalloids, As and Cr were found in the largest quantities, 33–51 and 16–25 ppm, respectively, and Pb and Tl were found in lower amounts, 4–13 ppm, while Hg and Cd were not detected (detection limits were 0.25 ppm and 0.05 ppm, respectively). For many elements the standard deviation was large, indicating large local differences among samples. The ANOVA revealed significant effects of location on Mg, Sr, Fe, P and S. For alkaline earth metals, Mg and Sr, the lowest content was found at the HBG location, which was significantly lower than all other locations. The Fe content in the GBG samples was much greater, up to five-fold higher, than at the other locations. The P content was significantly higher at the southern coast, at TBG, and lower at the west coast at GBG. S was also lowest at the southern coast, at HBG, and, although not statistically significant (SNK test), increasingly higher mean values of S were seen on the east coast, with the peak in STH.

PCA analysis showed a clear distinction between samples from the west and east coast, especially for the metals/other elements and fatty acids, as seen in the PCA analyses. The plot for monosaccharides indicated increased rhamnose, glucuronic and iduronic acids in the east coast samples, to the right and in the middle of the score plot, while some of the southern sampling locations (HBG and TBG) grouped mostly to the left together with the two GBG samples.

## Discussion

The biochemical composition of *U. intestinalis* collected along the coast of Sweden was determined and variations in some components were found to be related to geographic location. The fraction of biomass covered by the compositional analysis was only up to 80%, which is to be expected as some biomass components such as nucleic acids and phenolic compounds were not analysed. Total phenolics have previously been determined in *U. lactuca* and *U. intestinalis* to be in the range 2–5 mg $g^{-1}$ dw (Abd El-Baky, El-Baz, & El-Baroty, 2009; Farvin & Jacobsen, 2013), and it has been seen that this fraction responds to nutrient availability, with *U. rigida* having a higher content when grown at elevated nitrate concentrations (Cabello-Pasini, Macias-Carranza, Abdala, Korbee, & Figueroa, 2011). Thus, a higher phenolics content could explain, to some extent, the lower total mass recovery for the east coast samples (KKR, VSV and STH), due to the elevated nitrogen water concentrations at these locations relative to the other sites (Figs 2, 3). Pigments, such as chlorophyll *a* and carotenoids, make a small contribution towards the total mass balance in *Ulva* spp. (Grobe & Murphy, 1998). An additional contribution to a lower mass recovery is that the carbohydrate analysis potentially underestimates the content. Disaccharides of ulvan containing iduronic acid are known to be difficult to hydrolyse (De Ruiter, Schols, Voragen, & Rombouts, 1992), while some sugars are readily degraded, thus causing an underestimation of monosaccharides. However, none of these factors should affect the comparison between sites.

The metals and in particular some toxic metals detected in this study pose a potential barrier for

**Table 3.** Metal and non-metal elements on dry weight basis (dw) of *Ulva intestinalis* from sampling locations along the Swedish coast (for abbreviations see Fig 1). The elements Au, Be, Bi, Cd, Co, Hg, Li, Mo, Ni, Pd, Pt, Sb, Sc, Se, Sn, Te, Ti, V, and Zr were not detected. Because of disturbances Na was not measured. The mean and standard deviation of triplicated samples (GBG duplicated) at each location are given. Different letters indicate significant differences of means in post hoc SNK test.

| | | GBG | | HBG | | TBG | | ÅHS | | KKR | | VSV | | STH | | ANOVA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Elements | | ppm | Stdev | ppm | Stdev | ppm | Stdev | ppm | Stdev | ppm | Stdev | ppm | Stdev | ppm | Stdev | $F_{6,13}$ | p |
| Alkali metals | K | 10,600 | ± 1500 | 6900 | ± 1500 | 7500 | ± 1400 | 7700 | ± 500 | 7300 | ± 500 | 8500 | ± 1400 | 8900 | ± 1400 | 2.49 | 0.25 |
| | Rb | 35.6 | ± 0.5 | 30.5 | ± 4.8 | 33.4 | ± 2.9 | 32.2 | ± 3.7 | 32.0 | ± 0.6 | 34.4 | ± 2.1 | 37.1 | ± 2.5 | 1.71 | 0.33 |
| Alkaline earth metals | Ba | 9.9 | ± 2.9 | 11.9 | ± 0.1 | 12.0 | ± 0.2 | 12.1 | ± 0.1 | 12.0 | ± 0.2 | 11.9 | ± 0.1 | 11.9 | ± 0.1 | 1.84 | 0.31 |
| | Ca | 2430 | ± 70 | 2570 | ± 340 | 4150 | ± 1160 | 5000 | ± 990 | 3540 | ± 340 | 3640 | ± 680 | 4050 | ± 800 | 3.90 | 0.069 |
| | Mg | 5910 | ± 420 ab | 4580 | ± 1030 b | 6220 | ± 1420 ab | 7060 | ± 670 a | 7850 | ± 860 a | 7530 | ± 620 a | 6610 | ± 410 ab | 4.75 | **0.041** |
| | Sr | 25.7 | ± 2.4 ab | 19.9 | ± 4.0 b | 29.3 | ± 1.8 a | 34.9 | ± 4.4 a | 32.0 | ± 3.8 a | 31.8 | ± 3.7 a | 31.8 | ± 4.0 a | 5.61 | **0.034** |
| Other metals | Ag | 29.7 | ± 3.2 | 31.8 | ± 0.4 | 32.0 | ± 0.6 | 30.9 | ±2.5 | 32.0 | ± 0.6 | 31.8 | ± 0.4 | 30.5 | ± 2.2 | 0.76 | 0.64 |
| | Al | 288 | ± 68 | 289 | ± 17 | 213 | ± 19 | 212 | ± 28 | 187 | ± 32 | 196 | ± 14 | 229 | ± 95 | 2.15 | 0.30 |
| | B | 267 | ± 21 | 192 | ± 60 | 256 | ± 34 | 303 | ± 33 | 237 | ± 20 | 271 | ± 10 | 254 | ± 75 | 1.87 | 0.31 |
| | Cu | 11.8 | ± 5.4 | 11.9 | ± 0.1 | 10.7 | ± 4.9 | 8.1 | ± 0.1 | 10.7 | ± 4.6 | 7.9 | ± 0.1 | 7.9 | ± 0.1 | 1.02 | 0.59 |
| | Fe | 237 | ± 120 a | 138 | ± 33 b | 84 | ± 38 b | 82 | ± 32 b | 60 | ± 24 b | 45 | ± 6 b | 67 | ± 18 b | 5.58 | **0.034** |
| | La | 4.0 | ± 0.1 | 4.0 | ± 0.0 | 6.6 | ± 2.2 | 8.1 | ± 0.1 | 5.3 | ± 2.3 | 5.3 | ± 2.2 | 6.6 | ± 2.3 | 2.00 | 0.30 |
| | Mn | 10 | ± 9 | 20 | ± 11 | 57 | ± 22 | 187 | ± 198 | 129 | ± 84 | 28 | ± 8 | 79 | ± 55 | 1.56 | 0.35 |
| | W | 7.9 | ± 0.1 | 10.6 | ± 2.2 | 8.0 | ± 0.2 | 8.1 | ± 0.1 | 9.3 | ± 2.1 | 7.9 | ± 0.1 | 9.3 | ± 4.6 | 0.65 | 0.69 |
| | Zn | 13.8 | ± 2.6 | 14.6 | ± 2.2 | 14.6 | ± 2.1 | 14.8 | ± 2.4 | 12.0 | ± 0.2 | 11.9 | ± 0.1 | 31.2 | ± 2.1 | 1.26 | 0.47 |
| Potentially toxic metals | As | 41.6 | ± 2.2 | 37.0 | ± 12.3 | 33.4 | ± 2.9 | 48.2 | ± 17.1 | 50.7 | ± 3.2 | 39.7 | ± 14.0 | 39.6 | ± 13.8 | 0.85 | 0.64 |
| | Cr | 15.8 | ± 0.2 | 25.2 | ± 4.8 | 25.2 | ± 12.3 | 16.1 | ± 0.2 | 16.0 | ± 0.3 | 15.9 | ± 0.2 | 17.2 | ± 2.1 | 2.06 | 0.30 |
| | Pb | 4.0 | ± 0.1 | 4.0 | ± 0.0 | 5.4 | ± 2.4 | 5.4 | ± 2.3 | 4.0 | ± 0.1 | 4.0 | ± 0.0 | 4.0 | ± 0.0 | 0.78 | 0.64 |
| | Tl | 4.0 | ± 0.1 | 7.9 | ± 4.0 | 11.9 | ± 8.0 | 8.1 | ± 4.0 | 10.7 | ± 2.3 | 13.2 | ± 8.2 | 6.6 | ± 4.5 | 0.93 | 0.62 |
| Non-metal elements | P | 930 | ± 320 a | 1540 | ± 100 ab | 2980 | ± 1010 c | 2620 | ± 570 bc | 2300 | ± 570 abc | 1920 | ± 300 abc | 1440 | ± 250 ab | 4.71 | **0.041** |
| | S | 64,200 | ± 800 a | 47,400 | ± 10,200 b | 61,100 | ± 12,000 a | 72,700 | ± 3100 a | 74,400 | ± 4800 a | 75,100 | ± 4300 a | 79,800 | ± 3700 a | 7.62 | **0.025** |
| | Si | 396 | ± 6 | 357 | ± 42 | 394 | ± 84 | 404 | ± 39 | 376 | ± 99 | 377 | ± 40 | 493 | ± 35 | 1.63 | 0.34 |

utilization of these seaweeds, particularly in food and feed applications, for which European Union has various regulations and directives (European Commission, 2006, 2019). In addition, there are recommendations to monitor arsenic, cadmium, iodine, lead and mercury contents in seaweeds and seaweed products (European Commission, 2018). Seaweeds are well known for their accumulation of various elements from their surroundings and can be used as environmental indicators for anthropogenic inputs (Vasquez & Guerra, 1996); *Ulva* spp. in particular uptake metals strongly (Chakraborty, Bhattacharya, Singh, & Maity, 2014). Hence, the variations seen in the present study are probably related to the local environment of the sampling locations, both geochemistry and anthropogenic inputs from, for example, fertilizer run-off from land, which may have seasonal impacts. Without more data on the availability of elements in the local environment, it is difficult to elaborate further on other environmental differences and their effect on the metal content in seaweeds. For magnesium, the generally higher levels observed on the east coast could potentially have been due to increased chlorophyll *a* content, which has been seen in *Ulva pertusa* at low salinities (Choi, Kang, Kim, & Kim, 2010). The impact of metals, especially toxic metals, on a hypothetical seaweed industry will be strongly influenced not only by the actual content, but also in which process stream it accumulates. Such contaminated streams could potentially be treated by, for example, Hydrothermal Liquefaction (HTL) to enable removal of environmentally harmful materials (Raikova, Piccini, Surman, Allen, & Chuck, 2019).

For application of seaweeds in food and feed, a ranking of risks to human health implies that Cd, I, and the metalloid As provide major hazards, while Pb and Hg provide moderate hazards (Banach, Hoek-van den Hil, & van der Fels-klerx, 2020). However in our samples, a toxic metal at a problematic concentration is Pb at the level of 0.1 mg $kg^{-1}$ wet weight (ww), which is above the tolerated level for consumption (European commission, 2006) at all sites (calculation not shown). However, this level is not always exceeded by *Ulva* spp. in Swedish waters, as has been previously shown (Olsson, Toth, & Albers, 2020a), and factors influencing fluctuations of such metal content in biomass require further investigation. It has been previously shown that so-called "heavy metals" can influence the thickness of the cell wall in *U. intestinalis* (Zeroual et al., 2020). However, this cannot

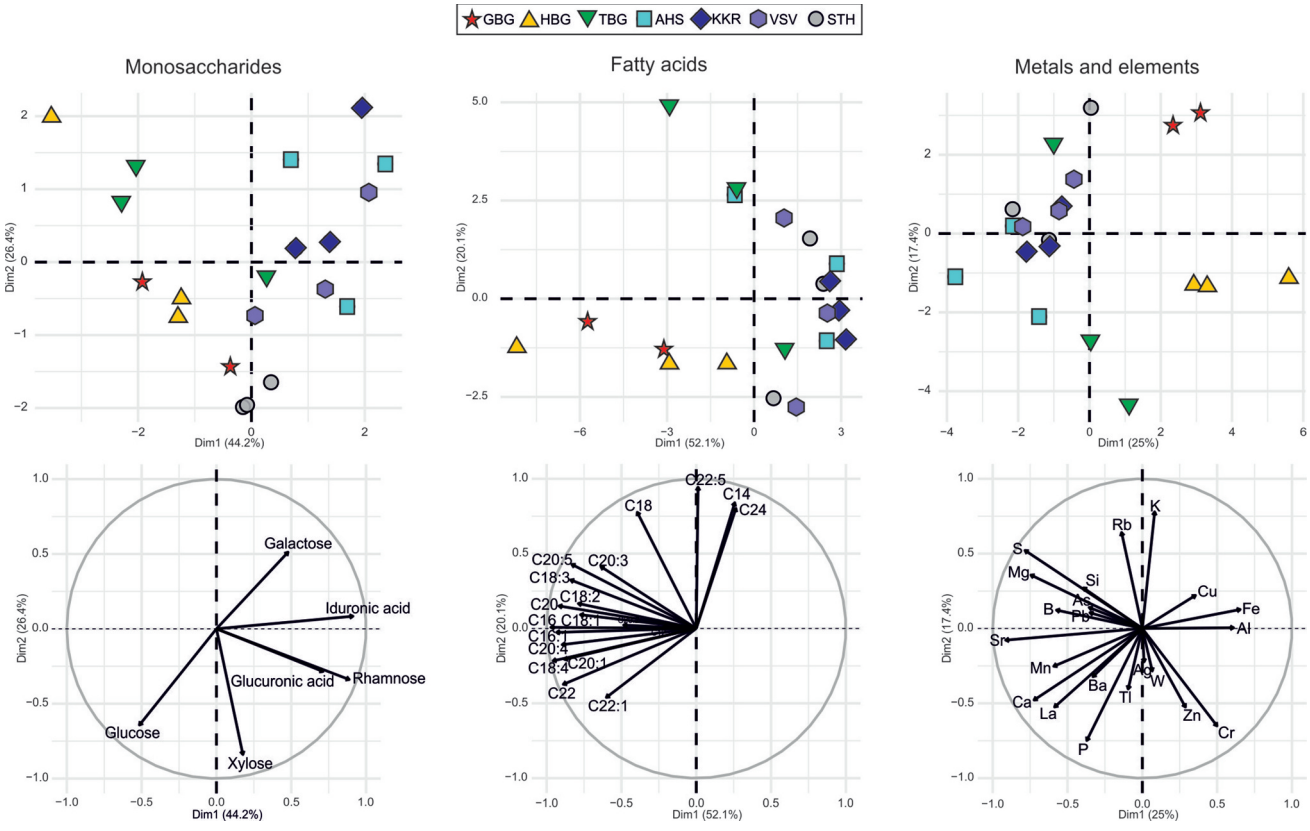


**Figure 4.** PCA analysis with score plots (upper row) and correlation plots (lower row) of monosaccharides (ash-free dry weight basis), fatty acids (ash-free dry weight basis), and metals and elements (dry weight basis) in *Ulva intestinalis* from sampling locations along the Swedish coast (for abbreviations, see Fig. 1).

be discerned in our study, as the potentially toxic metals vary differently at the different sites, as can be seen in the correlation plot (Fig. 4). Thus, with the available data a clear trend regarding the total metal content cannot be drawn and possible links to the effect of potentially toxic metals on these populations of *U. intestinalis* would need further investigation. It is clear, however, that the sites themselves mostly cluster close together. This points towards local geogenic (e.g., erosion) and anthropogenic (harbour, industrial, agricultural activities, etc.) inputs causing some level of change in content in the seaweed, though non-significant.

An assessment of the variation of *Ulva* species in Ireland also examined a range of metal content across different species (such as *Ulva compressa* and *U. intestinalis*) over various sites having differing Ecological Status (Wan et al., 2017). Over all the sites examined in the study, the potentially toxic metal Cr was demonstrated to be at a magnitude lower than found across all the Swedish sites presented here. Similarly, As was also seen in far smaller amounts in Ireland, with Pb being found at approximately the same values. Like the results presented here, no apparent link was observed between specific geographic areas, or Ecological Status categories and the levels of potentially toxic metals. However, the amount of Mn in the Irish *Ulva* species was related to the ecological quality index presented by the authors, whereas in the Swedish samples, in places of high fertilizer use, Mn was also observed in high concentrations. From both Ireland and the study presented here, no clear link between geographic location, anthropogenic input and metal content was found in the macroalgal species (Wan et al., 2017). Rather, it is the presence of elevated levels of macronutrients such as phosphorus in the water that enhance metal accumulation (Lee & Wang, 2001), as well as elevated runoff from the land that can lead to extensive deposition of metals in the sediments where the macroalgae are growing (Malea & Kevrekidis, 2014).

From the PCA, an indication of elevated ulvan content in *U. intestinalis* on the east coast can be discerned (Fig. 4) in the correlation vectors for rhamnose, xylose, iduronic acid and glucuronic acid, as is also seen for some of these sugars in the monosaccharide analysis. These data suggest that the seaweed responds to the growth conditions in the Baltic Sea by adapting its cell wall. *U. intestinalis* is well known for its resistance to varying salinities. It is a species with a large vertical range on the shore and can grow in salinities between 0 and 102‰ (Reed & Russell, 1979). There have been observations of thicker cell walls in *U. intestinalis* sampled from rock pools (Wærn, 1952), which could be an adaptation to dilute and/or concentrated seawater, but little is known about what cell wall components are influenced. However, the variability is thought to be caused by a need for increased cell wall flexibility as the cell swells at low-salinity conditions (Hurd, Lobban, Bischof, & Harrison, 2014b). Thus, it appears most likely that the increase in the ulvan components was due to the lower salinity on the east coast, although it cannot be ruled out that other conditions varying between the coasts and not measured in the present study could have caused this effect. However, the only ulvan component that was shown to change significantly was glucuronic acid. Since glucuronic acid also is the main component of the glucuronan polysaccharide in *U. intestinalis*, the link between ulvan and geographical location cannot be confirmed, although the numerous indications lend the argument some merit. In defined tank cultivation of *Ulva fenestrata*, it was found that low levels of nitrate increased the content of iduronic acid and low levels of phosphate increased rhamnose content in biomass, while increased irradiance affected both the iduronic acid and rhamnose contents (Olsson et al., 2020b). It is thus apparent that ulvan content and subsequently monosaccharide content is highly variable and allows *Ulva* species to acclimate to changes in variation in environmental conditions. The speed at which changes in these components occur has not been studied and may be interesting to use for even finer control of biomass composition in hydroponic/aquaculture cultivation systems.

For the fatty acid PCA, the majority of the increased types of fatty acids had loadings towards the west coast samples. The only exceptions were the very long C22:5 and C24:0 fatty acids, together with C14:0 that were instead greater in the east coast samples. This discrepancy could be partly linked to the difference in light intensity between the two coasts (Supplementary table S2), as higher irradiance has been shown to significantly increase the levels of C14:0 in *U. pertusa* (Floreto & Teshima, 1998). Fatty acid content has also been found to be affected by nutrient availability. McCauley, Winberg, Meyer, and Skropeta (2018) compared the lipid content in *Ulva* sp. grown under nitrogen starvation versus saturated nutrient uptake concentrations, with nutrient-deprived biomass having increased fatty acid content, whereas polyunsaturated fatty acids (PUFAs) were higher under saturated conditions as a percentage of total fatty acids. Phosphorus was found to not affect total fatty acid content, but has more subtle effects on some individual fatty acids, probably due to rearrangements of cellular membranes associated with

photosynthesis (Floreto, Teshima, & Ishikawa, 1996). Conversely, Gao, Clare, Chatzidimitriou, Rose, and Caldwell (2018) found that lipid (and protein) content increased at elevated nutrient levels. In line with these findings, *U. fenestrata* produced in defined tank cultivation increased the fatty acid and protein contents at high nitrate concentration and at low illumination (Toth et al., 2020). Reports also show positive effects of increased temperature (20–25°C) on lipid and protein contents, as well as lowered ash content for *Ulva* spp. (Gao et al., 2018; Liu & Zou, 2015). However, it seems unlikely that the small temperature differences found among our sampling locations, 1–1.5°C, could explain the compositional differences observed between localities. Consequently, the main influences are probably the variable nutrient levels and the salinity gradient.

In conclusion, we found some clear differences in content of *U. intestinalis* from different parts of the Swedish coast. The most interesting finding was the strong indication of a geographical impact on ulvan content, measured as iduronic, glucuronic acids and rhamnose, potentially as a response to lower salinity. Although these relationships would need to be verified in controlled growth experiments, these results suggest that it may be possible to increase the content of compounds of interest in seaweed, either by selecting a cultivation location to fit a particular profile, or by mimicking such a profile in tank cultivations. Ulvan is, to our knowledge, currently not utilized commercially, but the potential applications of its components are numerous in the synthesis of valuable compounds (Mohamed & Ferro, 2015; Muller et al., 2012). The fatty acids were also affected and changed both in content as well as profile. If optimized, the increase in valuable compounds would increase the value of *U. intestinalis* biomass as a raw material for high-value applications and provide better conditions for a financially sustainable seaweed industry.

## Disclosure statement

The authors have no conflicts of interests.

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