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Variation in biochemical composition of *Saccharina latissima* and *Laminaria digitata* along an estuarine salinity gradient in inner Danish waters

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ABSTRACT

In European kelp cultivation, knowledge on the spatial variation in biomass productivity and quality needs to be established. The present study provides a detailed overview of the biochemical composition and biomass production potential of *Saccharina latissima* and *Laminaria digitata* along a salinity gradient (16–31 PSU) in inner Danish waters. We discuss the results in a cultivation perspective, and evaluate the potential use of Laminariales as an energy feedstock, a feed additive and a bioremediation tool for mitigating eutrophication. We found the highest biomass production potential, the highest protein content (7.5% of dry matter), and the highest capacity for bioremediation of nitrogen (1.88% N of dry matter) at high salinities, as opposed to the highest concentrations of fermentable sugars (90% of dry matter) and pigments at low salinities. Thus, areas suitable for high biomass production are not necessarily optimal for producing a specific biomass quality such as high carbohydrate concentration for bioenergy conversion, and this challenges the cultivation practice. Furthermore, concentrations of arsenic in the biomass were generally higher (up to 88 ppm) than allowed for animal diet (40 ppm) and could therefore impose challenges for utilizing *S. latissima* and *L. digitata* as animal feed additives.

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1. Introduction

The sublittoral zone forms the typical habitat for kelps and often represents a relatively stable environment. However, in estuaries and Arctic coastal waters, freshwater run-off and melt water may lead to stratification of the water column imposing major fluctuations of salinity in the sublittoral zone on different temporal scales. Inner Danish waters make part of the North Sea–Baltic Sea transition zone, known as the world's largest estuary, where high saline water (>30 PSU) from the North Sea meets low saline water (<10 PSU) from the south western part of the Baltic Proper [1]. As a consequence of estuarine mixing, the water column of inner Danish waters is stratified throughout most of the year, thereby limiting vertical nutrient transport and affecting the dynamics of primary production and nutrient cycling [2].

Kelps, belonging to the order Laminariales, are key organisms in marine ecosystems due to their high primary production and role in habitat structuring [3]. Three native species of Laminariales are found in Denmark; *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders, *Laminaria digitata* (Hudson) J.V. Lamouroux and *Laminaria hyperborea* (Gunnerus) Foslie. These are all known to tolerate major salinity changes [4] but only *S. latissima* and *L. digitata* are distributed far into the Kattegat [5]. In general, Laminariales are distributed in marine waters over a broad salinity range, but few studies have been conducted on their salinity tolerance [6]. Optimal growth of *S. latissima* is found between 27 and 33 PSU [7], and a relatively low length growth in Aarhus Bay, Denmark has been suggested to reflect the low salinity (23 PSU) [8]. Despite a direct impact of salinity on growth, only few publications have described the structural changes in seaweeds in response to salinity. Lu et al. [9] reported an effect of salinity on the antioxidant activity of *Ulva fasciata*, and increased salinity has been reported to increase the contents of fatty acids in *Ulva pertusa* and *Sargassum piluliferum* [10]. A recent study has further described the influence of salinity on the quality and quantity of amino acids of *Ulva ohnoi* [11]. However, studies on the impact of salinity on the biochemical composition of Laminariales are still scarce.

Knowledge on the biochemical composition of Laminariales and the controlling environmental parameters are of utmost importance in European macroalgae cultivation. Lately, the focus has been on the potential of mitigating negative impacts of open water aquaculture [e.g., 12–14] and on the development of biorefinery concepts to secure a cost-efficient utilization of the macroalgae through sequential

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applications of the biomass (e.g., for energy, protein feed, hydrocolloids, pharma- and nutraceuticals) [15]. Whatever the specific end-uses; knowledge on the spatial and temporal variation in the biomass composition is crucial for identifying optimal sites and seasons for cultivation and harvest.

Temporal variation in biomass quality of natural kelp populations has been described for a few locations in Britain, France, and Denmark [8,16–19], and has lead to the conclusion that Laminarian biomass for energy purposes should be harvested during late summer when contents of fermentable carbohydrates peak. However, the spatial variation in the biochemical composition of Laminariales is not investigated in spite of great importance in a cultivation perspective, since the biomass composition at any given time may vary considerably across environmental gradients. Spatial differences in composition will reflect phenotypic plasticity and/or genotypic adaptations to local conditions, and are thus relevant when 1) selecting a strain or ecotype for cultivation and breeding and 2) when selecting a site for cultivation.

In this study we explored the spatial variation in the size, as a proxy for biomass production, and the biochemical composition of Laminariales within inner Danish waters in relation to environmental conditions with a focus on salinity, depth, and inorganic nutrient concentrations. The sampling time was chosen to be within the predicted late summer peak of carbohydrate content [17,18,20]. The sampling area spanned a broad salinity range (16–31 PSU) with salinity at some sites being below the optimal range for growth. We hypothesized differences in frond size between sites, with the smallest fronds, reflecting the slowest growth, at the most brackish sites. Further, as salinity differences in inner Danish waters due to water column stratification are often coupled to variations of other physico-chemical parameters, such as temperature and nutrient conditions, we expected effects on the biochemical composition of the tissue as well. The findings are discussed with the perspective of evaluating the use of Laminariales as energy feedstock, feed additive for fish feed and as a bioremediation tool to mitigate eutrophication in estuarine areas.

2. Materials and methods

The study was based on whole-plant samples of *S. latissima* and *L. digitata* obtained during a one-week field campaign 14–20 August 2012, combined with data of physico-chemical variables retrieved from the database of the Danish National Aquatic Monitoring and Assessment Program (DNAMAP).

2.1. Sampling

Specimens of kelp were hand-collected by divers on a total of 15 stone reefs spanning inner Danish waters. *S. latissima* was collected at 10 reefs/sites (Fig. 1) covering a depth range of 7–16 m, and depending on the coverage, 4–16 specimens were collected from each site. A similar sampling of *L. digitata* was conducted, although less comprehensive: 1–5 specimens were collected at 13 reefs/sites covering a depth range of 4–15 m (Fig. 1). A total of 87 and 31 specimens of *S. latissima* and *L. digitata*, respectively, were included in the study.

For *S. latissima*, weight, length, and width (average of three measures) of the fronds were registered, whereas no such data were obtained for *L. digitata*.

2.2. Biochemical analyses

A tissue sample of a known area was retrieved from the meristem of each specimen using a cork borer. The samples were frozen, freeze-dried and finely ground prior to all subsequent biochemical analyses. A different approach was used for analyses of amino acid and carbohydrates (see below).

The dry matter (DM) content was calculated as the difference between the weight prior to and after freeze-drying and reported as the percentage of fresh weight (FW). Ash content was analyzed from a 0.2–0.4 g subsample heated at 550 °C for 2 h (only conducted for *S. latissima*). Carbon (C) and nitrogen (N) contents were analyzed using a Perkin Elmer 2400 Series II CHN Analyzer (PerkinElmer Inc. Waltham MA, USA).

Amino acids and protein: 50 mg dry tissue sample and 6 ml HCl (6 M) were flushed with N in closed vessels. Samples were then hydrolysed in a microwave oven (AntonPaar 3000 solv) at 500 W and 150 °C and subsequently frozen (−80 °C) and freeze-dried to eliminate the HCl before the amino acid content was determined with HPLC-FLD (Dionex UltiMate 3000, Thermo Scientific). The protein content was calculated as the sum of all individual amino acids subtracting the molecular weight of water. For *S. latissima*, biomass from three individuals per site was pooled and used for duplicate analysis. For *L. digitata*, three individuals were not available for all sites; hence biomass from 1–3 individuals per site was pooled for duplicate analysis.

Carbohydrates: Monomeric sugars, mannitol, and uronic acids were quantified by HPAEC-PAD after a 2-step sulphuric acid hydrolysis as described in detail by Manns et al. [21]. For *S. latissima*, biomass from the basal, mid and apical part of the frond from three individuals was pooled and used to perform triplicates of the carbohydrate composition. For *L. digitata* triplicate samples were analyzed for each individual also pooling tissue from the basal, mid and apical part of the frond.

Pigment analysis: Pigments were extracted from a 20–40 mg freeze-dried sample and analyzed by HPLC as described in Boderskov et al. [22]. Chlorophyll a, fucoxanthin, β-carotene and violaxanthin standards were obtained from DHI Laboratory Products (Hoersholm, Denmark).

Elemental analysis: Concentrations of As, Cd, Pb, Ni, Zn, Fe, Cu, Al, V, Cr, Mn and phosphorus (P) were determined by ICP-MS. In short, a 0.2 g dry subsample was digested in a closed vessel in a microwave oven using 5 ml of nitric acid (7 M) and 1 ml of hydrogen peroxide diluted to 50 ml with milliQ water, followed by ICP-MS determination using internal standards of Rh, Ir and Ge to correct for drift (for specifications see [23]). Certified reference material of macro-algae from IAEA-140 [24] was used for quality assurance.

2.3. Environmental variables

Data on salinity and nutrient concentrations was obtained from the Danish National Aquatic Monitoring and Assessment Program (DNAMAP) that has monitored these variables in Danish waters since 1989. Data from 7 monitoring stations with a maximum distance of 30 km from a given sampling site (Fig. 1) was used to estimate the environmental conditions at the different sites. No monitoring station was found within 30 km for sites 8, 10 and 15, and hence, no salinity and nutrient data were available for these sites.

Since the sampling frequency of nutrients was relatively low, nutrient conditions were described using concentrations of dissolved inorganic N (DIN) ($NO_3^- + NO_2^- + NH_4^+$) and ortho-phosphate (PO_4^{3-}) from late February/early March when concentrations are generally maximal [25]. As an indicator of the nitrogen concentrations experienced by the algae prior to sampling, the algae tissue N concentrations were used [26]. For salinity, an annual weighted average (August 2011–August 2012) was calculated based on monthly/bimonthly measurements with a vertical interval of 0.2 m. An average value within a depth interval of 5 m around the sampling depth was used for the salinity data, whereas an interval of 10 m around the sampling depth was used for nutrient data, due to coarser vertical sampling (every 5 m).

2.4. Data analysis and statistics

All statistical analyses were performed using SAS 9.3 (SAS Inc.). All data except data on amino acids, protein, and carbohydrates were log-transformed to achieve normal distribution and homogeneity of variance. Differences between sites were tested by ANOVA and the effects of salinity and depth were assessed with general linear modeling

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