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Seasonal variations in the amino acid profile and protein nutritional value of *Saccharina latissima* cultivated in a commercial IMTA system

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Abstract Seaweeds have potential for the provision of biomass for food and feed supplements. The demand is increasing especially for proteins as ingredients; however, the amino acid profile is essential for evaluation of the nutritional value of proteins. The year-round protein concentration and amino acid profiles of *Saccharina latissima* were determined, and the harvest time and nutritional potential were evaluated. Bi-monthly samples were analyzed from *S. latissima* (including epiphytes, when present) cultivated commercially at an integrated multi-trophic aquaculture (IMTA) site and a reference site in Denmark in 2013–2014. Overall, there was no significant difference for the tested parameters between the two sampling sites; however, seasonal variations were found. The protein concentration varied markedly reaching a maximum of 10.8 % dry weight (DW) in November and a minimum of 1.3 % DW in May 2013. Aspartic and glutamic acids dominated the amino acid profile, accounting for up to 49 % of the total. Greatest seasonal differences in amino acid composition occurred in July, with leucine contributing most (22.7–26.7 %) of the observed differences. A maximal essential amino acid (EAA) score of 68.9 % (based on WHO/FAO/UNU requirements) was achieved in November 2013. The presence of epiphytes in July to November changed neither the amino acid content nor the EAA score. *S. latissima* is comparable with wheat as a protein ingredient for fish feed and appears to be a suitable protein/amino acid source for human consumption. This study proposes that there may be a mismatch between harvest time and nutritional value. The preferable harvest time for *S. latissima* is November, due to high protein content and EAA score. However, higher yield and cleaner biomass for human consumption would be found in May.

Keywords Integrated multi-trophic aquaculture · Epiphytes · Biomass composition · Marine proteins · Essential amino acid score · Fish feed supplement · Seasonal changes

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Introduction

Increasing human population has driven the search for unconventional food sources (Rosegrant and Cline 2003). Seaweed was recently promoted in the cuisine of several American and European countries as a healthy food (McHugh 2003). Nevertheless, the utilization of seaweed as a high nutritional value feedstock is still barely explored in western countries.

Protein nutritional value is mainly defined by its amino acid composition and digestibility. Seaweed protein generally contains most amino acids especially glycine, alanine, arginine, proline, and glutamic and aspartic acids (Černá 2011). Threonine, lysine, tryptophan, sulfur amino acids (cysteine and methionine), and histidine are usually the limiting amino acids, even though their levels are generally higher than those found in vegetables and cereals (Holdt and Kraan 2011). In some seaweed species, essential amino acids (EAAs) can account for almost half of the total amino acids (Černá 2011) and the amino acid score of edible seaweeds such us *Saccharina latissima* (82), *Porphyra* complex (Amanori; 91), and *Undaria pinnatifida* (Wakame; 100) is comparable to that of beef (100; Murata and Nakazoe 2001). EAAs in human nutrition include leucine, isoleucine, valine, lysine, threonine, tryptophan, methionine, phenylalanine, and histidine (Friedman 1996; WHO/FAO/UNU 2007), while arginine is also an EAA in diets for aquaculture species such as fish and abalone (Metailler et al. 1973; Allen and Kilgore 1975; Hardy 2002).

Fishmeal has long been used as the main source of protein in aquafeeds; however, this resource is already showing signs of overexploitation (FAO 2012). In this context, the replacement of fishmeal by vegetable protein sources, such as soybean, corn gluten, cotton seed, and canola, has been extensively studied (see review by El-Sayed and Tacon 1997). Dietary deficiencies or imbalanced amino acid profiles have been highlighted as one of the most important factors leading to poor fish growth performance when fish meal is completely or partially replaced for alternative vegetable protein sources (Dias et al. 2005; Goda et al. 2007; Silva et al. 2010).

The chemical composition of seaweeds, including protein content, varies markedly according to the species, season, geographic distribution, and even population (e.g., Ito and Hori 1989; Fleurence 1999). A study showed that the protein content of *S. latissima* was higher in February–March (13–14 % dry weight), while the lowest values were found during July–September (5–8 % dry weight; Black 1950). Although the amino acid composition of *S. latissima* has been reported before (Mai et al. 1994; Murata and Nakazoe 2001), no information is available on the seasonal changes in the amino acid composition of this species.

In this study, seasonal variations of dry weight, ash, protein content, and amino acid profile were determined in *S. latissima* cultivated at an integrated multi-trophic aquaculture (IMTA) and a reference site. The nutritional value of the protein of *S. latissima* (including epiphytes) was evaluated based on the EAA composition and compared with the reference pattern from WHO/FAO/UNU (2007). Moreover, the EAA composition was compared with reference fish feed ingredients and dietary requirements for optimal growth of fish. Harvest time was evaluated based on seasonal variation in the protein quality and seasonal presence of epiphytes on *S. latissima.*

Materials and methods

Sampling site *S. latissima* samples were collected from two commercial cultivation areas by Hjarnø Havbrug A/S outside, but in the vicinity of, Horsens Fjord, in the inner Danish waters. The IMTA site was located in the area As Vig (55° 47.529′ N, 10° 03.027′ E), approximately 100 m from a blue mussel SmartFarm™ (35 tubes incl. nets) and 500 m from rainbow trout (*Oncorhynchus mykiss*) farm cages (175 t per year). The reference (REF) site was established in a 100-ha seaweed cultivation area (55° 49.045′ N 10° 06.824′ E) located at approximately 2000 m from the same fish farm cages. The rainbow trout grow-out season in the sea is from April–May to October–December. This area is expected to be out of range of the nutrient load from the mussel and fish farms. Both locations commercially produce *S. latissima*, where 7 and 90 km of *S. latissima* cultivation lines were deployed at the IMTA and REF sites, respectively, during 2011–2013 (Marinho et al. 2015).

Of the 90 km commercially cultivated seaweed lines at the REF site, a longline with 200 droppers, with *S. latissima* seedlings from the same hatchery batch, deployed in 15 January 2013, was selected, and tagged for further monitoring. On 21 May 2013 (i.e., just prior to the start of the experiment), 64 droppers from this selected longline were sampled haphazardly and moved from the REF site to a new longline at the IMTA site shortly after the fish had been stocked in the farm cages.

Seaweed sampling *S. latissima* biomass was sampled bimonthly from May 2013 to May 2014 at both cultivation sites, to determine seasonal variations on the protein content and amino acid composition. In each sampling, three droppers were selected at random (haphazard sampling; triplicates) and the biomass growing in the first meter (approximately 1–2-m depth) was harvested. At least ten individuals were haphazardly sampled from three droppers (n=3) from both cultivation sites and their fresh weights (FWs) recorded. All collected samples were frozen until chemical analysis.

Chemical analyses Seaweed samples were freeze-dried and ground to fine powder before chemical analyses, using a Siebtechnik Screening disc mill TS 250. Chemical analyses were performed according to AOAC procedures (AOAC 2006), and samples were run in duplicates. Dry matter was determined after drying the samples in an oven at 105 °C until constant weight; ash content was determined by incineration in a muffle furnace at 550 °C for 6 h. Protein concentration was calculated by summing up the amino acid masses retrieved after acid hydrolysis, minus the water mass (18 g H_2O mol^{-1} of amino acid) incorporated into each amino acid after the disruption of the peptide bonds according to Lourenço et al. (2002). Samples were hydrolyzed for 1 h with 6 M HCl in a microwave (Microwave 3000 SOLV, Anton Paar), followed by derivatization using a Phenomenex EZ:faast amino acid analysis kit according to the user's manual (Phenomenex). Amino acid composition was determined by liquid chromatography using mass spectrometry (Agilent 1100 Series, LC/MSD Trap) with an EZ:faast 4u AAA-MS new column (250× 3.0 mm, Phenomenex).

The EAA ratio was calculated by the total EAA divided by the total amino acids. To evaluate protein quality, the EAA score was determined as follows according to FAO/WHO (1991) based on the EAA requirement pattern from WHO/FAO/UNU (2007).

$$\text{EAA score}(\%) = \frac{\text{g of first limiting EAA in 100 g of test protein}}{\text{g of limiting EAA in 100 g of WHO/FAO/UNU reference pattern}} \times 100$$

Statistical analysis Data are expressed as mean ± standard deviation. Data were tested for normality using Kolmogorov-Smirnov and homogeneity of variance using Levene's test. A two-way ANOVA was used to analyze the influence of the cultivation site and season on the dry weight and ash content. When F values showed significance, individual means were compared using Tukey's post hoc test. Means were considered different at a significance level of $P<0.05$. ANOVA was performed using OriginPro 9.0 (OriginLab Corporation).

A permutational ANOVA (using Euclidian distances) was used to test the effect of time (random) and site (REF and IMTA; fixed) on various response parameters: total amino acids, EAA content, EAA score, and EAA ratio and concentration of amino acids: methionine, lysine, and arginine (PERMANOVA package in PRIMER+; Anderson et al. 2008; type III sum of squares and unrestricted permutation (9999) on raw data (α=0.05)). This was followed by a SIMPER analysis (based also on Euclidian distances) to identify those amino acid species that contributed most to the observed differences (between pairs of time) among time and/or site. Prior to this multivariate analysis of variance (MANOVA), all amino acid values were standardized and thereby expressed relative to the highest value in each dataset. MANOVA analyses therefore only changes in composition, not in total amount of amino acids. The results were also visualized in the multidimensional scaling (MDS) plot, one point for each sample, where points that were close were more similar in composition (standardized samples by maximum resemblance, D1 Euclidian distance; 2-D stress, 0.09). May 2013 was excluded from the analysis due to less data compared to all other sampling months.

Results

Epiphyte cover and general biochemical composition of *S. latissima*

Dry matter content changed seasonally with the highest values found in July–September (21.7–22.5 %) and the lowest values found in January (11.3 %), with no significant difference between cultivation sites (Fig. 1a). Fluctuation in the ash content was similar in both cultivation sites ($P>0.05$), with the lowest values observed in July (15.3–19.9 % dry weight; DW) and the highest values found in January (38.2–40.2 % DW; Fig. 1b).

Growth of epiphytes was observed in the samples collected from July to November in both cultivation sites (Fig. 2). Epiphytic organisms mainly included bryozoans, but also barnacles, blue mussel juveniles, and filamentous seaweeds. Samples of *S. latissima* including epiphytes from July and November showed a higher ash content than those cleaned of epiphytes, being significantly different from the November samples ($P<0.05$).

Protein content also varied markedly seasonally with the lowest values recorded in May–July 2013 (1.3–1.7 % DW) and the highest values recorded in November (9.7–10.8 % DW), in both sites (Fig. 3a). Presence of epiphytes did not significantly alter the protein content in samples collected from July to November.

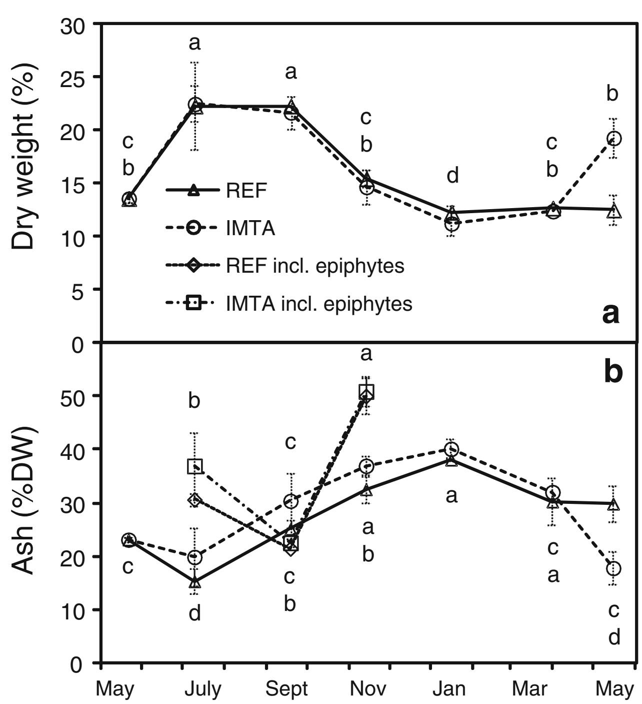

Fig. 1 Year-round variation in the **a** dry weight (%) and **b** ash (%DW) of *Saccharina latissima* cultivated at both reference (REF) and at an integrated multi-trophic aquaculture (IMTA) and including epiphytes when present from July to November. The standard deviations are presented as *bars* (n=3), and *different letters* represent significant difference ($P<$-0.05) between sampling months

Fig. 2 *Saccharina latissima* biomass harvested in **a** May representing clean seaweed and **b** September representing seaweed covered by epiphytes of, e.g., bryozoans, barnacles, other filamentous seaweed, and young blue mussels

Composition of total amino acids and EAA content

Data on the bimonthly amino acid composition of *S. latissima* are presented in Table 1. All of the analyzed amino acids were found in most of the samples, except tryptophan, which is destroyed during acid hydrolysis.

While significant differences were observed in the composition of amino acids seasonally (PERMANOVA; $F=3.6841$, $P<0.001$), site type (REF and IMTA) did not show any significant effects ($F=0.54631$, $P=0.693$). The pairwise test revealed significant differences in the composition of amino acids between July and all the other months ($P=0.003$–0.034) and in September compared to November and May 2014 ($P=0.005$–0.034). This observation is further corroborated by the data presented in the MDS plot, suggesting that July samples appear to be "isolated" (Fig. 4; samples cluster to the right, except one sample) and furthermore that September was different from November 2013 and May 2014. The SIMPER analysis showed that in July, the main observed dissimilarities (average squared distance between samples) were mainly caused by changes in the relative abundance of leucine (22.7–26.7 %), glutamic acid (10.4–16.4 %), and phenylalanine (11.5–14.3 %). Aspartic acid (21.4–32.0 %), leucine (16.3–16.9 %), glycine (11.2–14.4 %), alanine (10.4–20.6 %), and arginine (6.2–7.6 %) mainly caused the changes in relative abundance in September compared to November 2013 and May 2014.

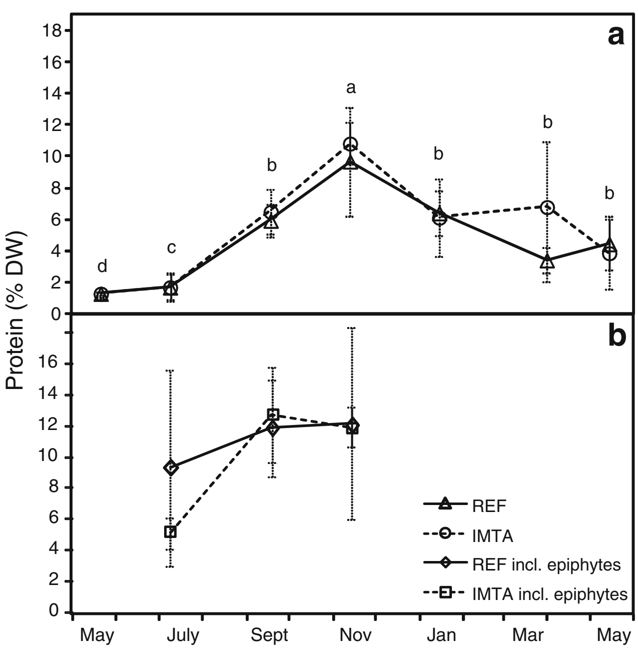

Fig. 3 Year-round variation in the protein content (%DW) of *Saccharina latissima* cultivated at **a** reference (REF) and at an integrated multi-trophic aquaculture (IMTA) sites and **b** including epiphyte concentrations when present from July to November. The standard deviations are presented as *bars* ($n=3$), and different letters represent significant difference ($P<0.05$) between sampling months

The highest total amino acid content was found in November in both cultivation sites (11.3–12.7 % DW; $P<0.05$), while the lowest value was found in May–July 2013 (1.5–2.0 % DW; Table 1). Aspartic and glutamic acids together accounted for up to 42–49 % of total amino acids in March, with lowest values found in July (19–26 %) in both sites. No significant differences were found between sites.

The EAA content varied markedly seasonally with lower values found in March in both sites (248 mg g^{-1} protein) and significantly higher values found in July (481–494 mg g^{-1} protein).

Table 1 Year-round variation in the amino acid composition (mg amino acid g^{-1} protein), total amino acid content (∑ AA; % DW), total essential amino acids (∑ EAA), essential amino acid ratio (EAA/AA), and EAA score of *S. latissima* cultivated at both reference (REF) and IMTA sites

Amino acid	May	July		September		November		January		March		May		Pattern[c]	FM[d]	SM[d]	WM[d]
	Initial	REF	IMTA	REF	IMTA	REF	IMTA	REF	IMTA	REF	IMTA	REF	IMTA				
LYS[a]	53.1±0.9	61.8±8.2	58.7±16.1	56.7±8.3	64.6±14.9	65.1±7.3	62.8±1.4	58.4±11.1	53.5±2.4	47.4±8.1	45.9±16.3	52.3±8.9	52.7±14.9	52.0	113.15	139.56	21.68
ALA	155.2±17.5	121.2±31.2	95.7±27.7	141.8±6.9	135.9±8.8	112.9±30.9	120.8±12.3	109.9±31.0	105.2±28.0	197.8±56.9	138.4±17.0	107.0±58.9	90.6±45.1				
ARG[b]	58.6±2.4	69.4±6.1	66.3±6.1	40.9±17.1	49.9±20.8	80.6±32.3	50.3±18.5	44.0±13.0	32.5±7.2	68.2±16.9	59.5±5.6	71.1±25.9	69.1±36.9		83.45	154.22	31.47
CYS	4.1±0.6	1.0±0.6	1.1±0.3	2.9±0.7	2.9±2.4	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d				
MET[a]	29.8±5.1	60.4±55.9	39.6±17.5	26.3±9.0	26.7±5.5	24.3±9.2	23.2±0.7	22.9±16.2	26.2±15.7	31.4±13.2	24.3±15.0	44.2±6.1	43.0±19.6	26.0[e]	41.02	30.67	13.99
LEU[a]	102.5±1.2	128.7±35.4	128.1±43.3	82.3±15.0	107.3±36.8	64.2±8.7	66.1±10.4	79.2±6.4	73.3±10.4	53.0±17.7	53.1±37.2	69.1±11.4	59.4±11.8	63.0	108.91	167.56	65.73
TYR	28.9±1.8	23.6±1.7	22.9±0.5	19.9±1.4	22.4±4.5	20.2±0.2	17.6±2.0	19.7±4.3	21.7±9.0	15.1±3.6	17.2±8.3	25.3±5.8	21.6±2.8	46.0[f]	16.97	26.22	8.39
PHE[a]	112.6±7.3	83.1±11.8	97.3±31.0	54.7±7.4	63.8±17.5	38.3±9.1	38.8±1.3	49.2±3.0	42.0±6.3	33.9±6.7	36.4±14.9	47.2±10.7	44.7±7.3		59.41	111.78	44.76
PRO	51.6±3.5	61.3±4.4	51.5±4.6	46.7±2.6	50.7±4.5	47.4±5.0	45.8±10.9	59.1±9.3	65.5±10.2	47.1±10.8	43.7±23.7	42.8±3.9	37.4±6.5				
THR[a]	26.5±2.3	33.7±2.5	38.4±7.7	35.5±3.9	32.9±7.6	33.7±15.8	37.8±2.4	39.3±3.2	25.8±3.6	23.9±4.7	27.0±8.5	41.4±9.7	43.0±13.8	27.0	62.23	109.33	24.48
ASP	157.5±10.9	121.6±23.4	117.3±19.5	112.0±19.1	117.4±15.9	193.4±36.8	181.5±35.8	182.5±32.1	190.7±49.7	211.2±85.3	270.1±114.4	205.3±12.4	174.7±17.4				
SER	75.2±2.3	79.5±25.6	68.4±6.3	55.5±8.1	43.6±17.7	54.3±25.6	52.9±19.4	69.7±34.2	125.1±50.6	n.d	n.d	73.3±7.1	70.1±8.1				
HYP	4.1±0.4	19.1±13.3	8.4±3.5	31.7±11.5	28.3±10.7	18.4±16.5	17.9±8.6	12.0±2.8	8.7±3.7	10.3±2.8	5.8±1.4	n.d	n.d				
GLU	160.2±36.6	146.9±0.6	190.1±62.9	266.5±33.3	204.2±40.6	233.6±17.2	260.9±93.2	236.1±72.8	213.5±32.3	281.9±64.0	304.4±142.0	225.3±8.3	299.9±29.5				
VAL[a]	25.1±4.6	57.0±12.7	50.0±4.2	53.6±6.0	57.0±7.7	45.0±19.6	64.2±14.3	52.1±5.4	44.0±4.3	21.7±4.8	22.8±23.7	37.5±8.6	39.5±10.9	42.0	76.38	104.89	37.06
HIS[a]	10.5±0.9	9.4±1.3	6.7±2.4	3.0±1.0	8.9±2.9	8.7±5.1	12.4±6.2	8.1±1.0	12.2±7.3	n.d	n.d	7.1±3.3	6.2±2.6	18.0	35.36	58.67	18.88
TRP[a]	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d				
ILE[a]	12.0±0.1	35.9±2.4	38.1±3.3	28.1±4.6	43.2±9.1	30.8±14.7	33.6±2.4	39.5±5.0	28.7±3.1	17.4±5.1	16.6±9.9	28.1±13.2	31.4±10.9	31.0	66.48	111.33	34.97
GLY	109.4±8.1	119.9±9.3	91.8±7.4	121.1±21.3	118.5±15.7	101.6±36.1	86.1±12.9	91.7±41.6	112.8±44.9	87.5±21.7	77.8±39.4	87.5±37.1	77.4±32.6				
∑ AA (%DW)	1.5±0.2 d	2.0±0.9	2.0±1.1 c	7.0±1.2	7.6±1.7 b	11.3±4.0	12.7±1.6 a	7.5±1.6	7.2±2.9 b	4.0±1.0	8.0±4.9 b	5.2±1.9	4.5±2.7 b				
∑ EAA	405.0±20.4 b	494.2±40.8	480.7±65.3 a	368.3±13.1	428.6±76.0 b	330.4±73.8	356.5±35.1 bc	368.5±18.2	327.5±36.9 bc	247.5±54.7	248.2±141.6 c	352.1±58.0	341.5±48.4 bc	305.0			
EAA/AA	0.34±0.02 b	0.42±0.03	0.41±0.06 a	0.31±0.01	0.36±0.07 b	0.28±0.06	0.30±0.03 bc	0.31±0.01	0.28±0.03 bc	0.21±0.05	0.21±0.12 c	0.30±0.05	0.29±0.04 bc				
EAA score (%)	38.7±0.4	52.0±7.3	37.1±13.3	16.7±5.7	49.5±16.2	48.4±28.4	68.9±34.4	45.0±5.5	67.7±40.8	n.d	n.d	39.3±18.6	34.2±14.4				

Data expressed as mean±standard deviation (n=3). Different letters in the same row indicate significant differences (P<0.05) for total amino acids, total EAA, EAA ratio, and EAA score between sampling months

n.d. not detected

[a] Essential amino acids in human and fish nutrition

[b] Essential amino acid in fish nutrition

[c] EAA requirement pattern (WHO/FAO/UNU 2007)

[d] Essential amino acid composition of fish meal (FM) soybean meal (SM) and wheat meal (WM) reported by Hertrampf and Piedad-Pascual (2000)

[e] Methionine+cysteine

[f] Phenylalanine+tyrosine

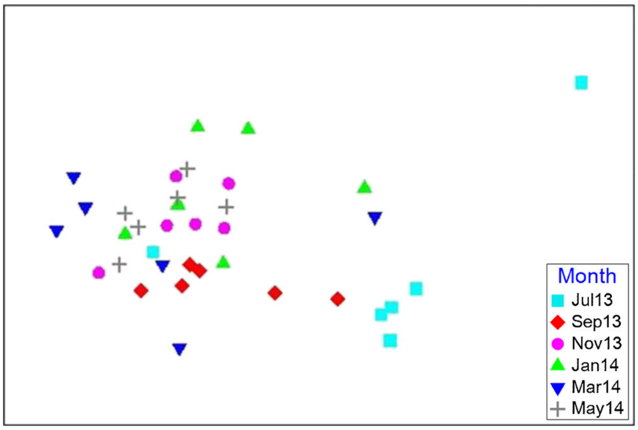

Fig. 4 Multidimensional scaling (MDS) plot, one point for each sample (standardized samples by maximum resemblance, D1 Euclidian distance; 2-D stress, 0.09) considering the similarities of amino acid composition of *Saccharina latissima* (*n*=6)

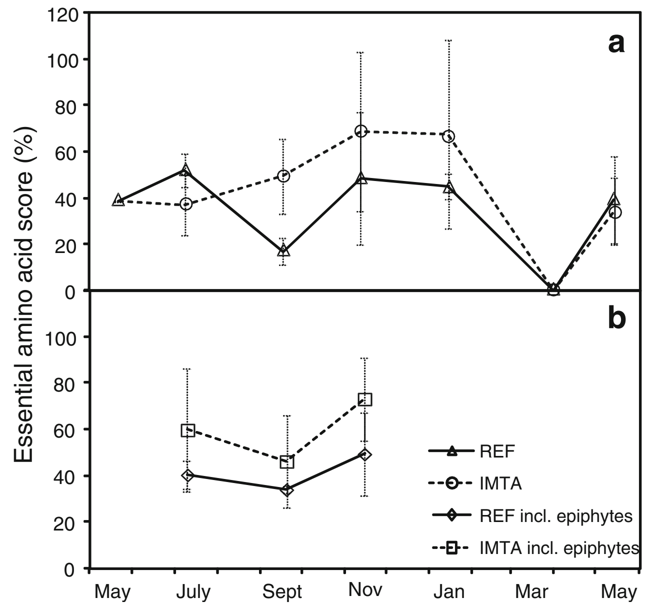

Fig. 5 Year-round variations of essential amino acids score (%; based on the amino acid requirements by WHO/FAO/UNU (2007)) of *Saccharina latissima* cultivated at **a** reference (REF) and at an integrated multi-trophic aquaculture (IMTA) sites and **b** including epiphyte concentrations when present from July to November. The standard deviations are presented as *bars* (*n*=3)

EAA ratios and scores

The lowest EAA ratio was found in March in both cultivation sites (0.21; 0.26–0.27 including arginine), but not significantly lower compared to November 2013 and January and May 2014, while the highest values were found in July (0.41–0.42; 0.47–0.48 including arginine; $P<0.05$).

Histidine was not detected in the samples collected at both sites in March and was the first limiting amino acid seasonally, with the exception of May 2013 samples, where isoleucine was the first limiting EAA with a score of 38.7 %. The highest EAA scores were found in July and November at the REF site (48.4–52.0 %) and November and January at the IMTA site (67.7–68.9 %; Fig. 5), however, without significant difference in none of the sites compared to the other months. Lysine and methionine concentrations varied from a minimum of 0.68 and 0.38 mg g^{-1} DW in May 2013 to a maximum of 6.5–7.2 and 2.1–2.4 mg g^{-1} DW in November, respectively, in both sites (Figs. 6 and 7). However, differences in the content of both amino acids for all sampling periods as well as between sites were not significant ($P>0.05$). The same pattern was found for arginine content that increased significantly from 0.75 mg g^{-1} DW in May 2013 to 6.1–9.8 mg g^{-1} DW in November in both sites. Cysteine was not detected from November to May 2014 while it peaked in September in both sites (0.15–0.19 mg g^{-1} DW).

Impact of epiphytes on EAAs

Amino acid composition, total amino acid and EAA content, EAA ratio, and EAA score of *S. latissima* samples collected from July to November including epiphytes are presented in Table 2. Analyses showed that only the EAA content and the EAA ratio were significantly lower in the samples including epiphytes compared to cleaned seaweed biomass (July and September samples; $P<0.05$; Tables 1 and 2). EAA content and the EAA ratio of the samples including epiphytes were significantly higher in July compared to those in September (Table 2). Lysine content was significantly higher in the samples including epiphytes compared with cleaned samples collected in July and September ($P<0.05$; Fig. 6). Similarly, the

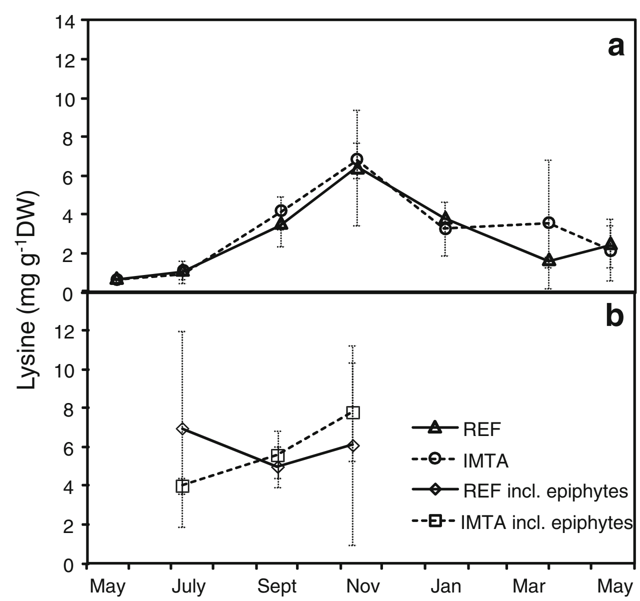

Fig. 6 Year-round concentration of the amino acid lysine (mg g^{-1} DW) of *Saccharina latissima* cultivated at **a** reference (REF) and at an integrated multi-trophic aquaculture (IMTA) sites and **b** including epiphyte concentrations when present from July to November. The standard deviations are presented as *bars* (*n*=3)

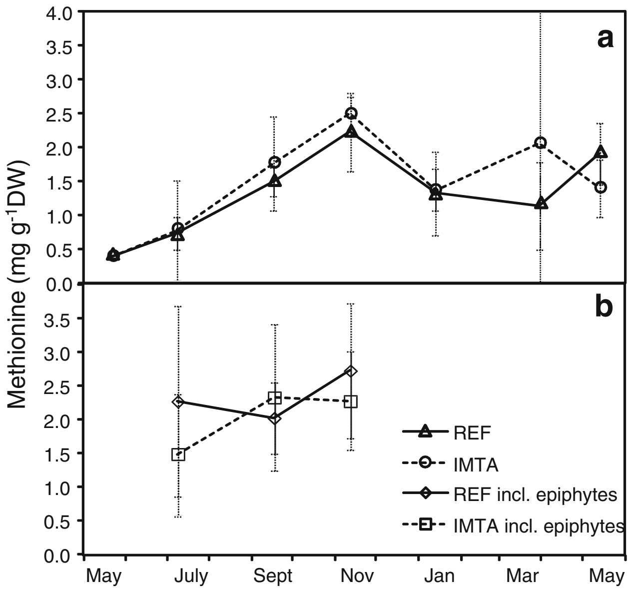

Fig. 7 Year-round concentration of the amino acid methionine (mg g^{-1} DW) of *Saccharina latissima* cultivated at **a** reference (REF) and at an integrated multi-trophic aquaculture (IMTA) sites and **b** including epiphyte concentrations when present from July to November. The standard deviations are presented as *bars* (*n*=3)

presence of epiphytes significantly increased arginine content of the samples compared to the cleaned seaweed samples ($P<0.05$). However, the methionine content did not show any significant differences between epiphytized and cleaned samples. No significant differences were found seasonally for lysine (Fig. 6), methionine (Fig. 7), and arginine contents between samples including epiphytes.

Discussion

Overall, season had an effect on the chemical composition of *S. latissima*; however, no differences were found between cultivation sites. The seasonal variation in the dry matter content of *S. latissima* matches the pattern reported by Black (1950) and Haug and Jensen (1954), indicating higher values in July–September and lower values in January–March. In contrast with these studies, the maximum and minimum values for ash content were found slightly earlier in the year in the present study.

Protein content varied markedly year-round from 1.3 to 10.8 % DW which is within the lower range of concentrations previously reported for brown seaweeds (3–21 % DW; Fleurence 1999; Holdt and Kraan 2011). The highest concentrations of protein found in November in both sites (9.7–10.8 % DW) are somewhat comparable to the highest annual concentrations reported for natural populations (13–14 % DW February-March; Black 1950). However, the lowest concentrations found in May 2013–July (1.3–1.7 % DW) are lower than the minimum annual values (5–8 % DW July–September) reported by Black (1950). Even though brown seaweeds have been observed to have generally lower protein content than green and red seaweeds, the highest protein content found in this study (9.7–10.8 % DW) is within range of some of the concentrations reported in green seaweeds, e.g., *Ulva lactuca* and *U. pertusa* (10–26 %) and red seaweeds, e.g., *Chondrus crispus* (6–29 %), *Gracilaria* spp. (5–23 %), *Porphyra* spp. (7–44 %), or *Palmaria palmata* (8–35 %; Fleurence 1999; Holdt and Kraan 2011). It is worth noting that in the present study, the protein content of the seaweed was determined by summing up the amino acid masses retrieved after acid hydrolysis, minus the water mass incorporated into each amino acid after the disruption of the peptide bonds (Lourenço et al. 2002). This protein content estimation method is more accurate than the commonly used nitrogen-to-protein conversion factors, as these may change with species (Lourenço et al. 2002) and season (author's own observations).

To our knowledge, our study is the first evaluating the seasonal variation on the amino acid composition of brown seaweed. With few exceptions (cysteine and histidine in some sampling periods), all samples contained ten EAAs (except tryptophan lost during acid hydrolyses), confirming seaweed as a source of most EAA (Černá 2011). Aspartic and glutamic acids were the dominant amino acids in May 2013 and from November 2013 to May 2014, regardless of site or epiphytic coverage. These results are in agreement with those reported for other brown seaweeds where these two amino acids accounted for 20–44 % of total amino acids (Fleurence 1999; Wong and Cheung 2000). On the other hand, our results showed seasonal variation in the amino acid dominance with glutamic acid present in highest concentration together with, respectively, leucine and alanine in July and September.

Changes in amino acid composition were driven by time/season while site did not show significant effect. Generally, the late autumn (November), winter (January), and spring (March and May 2014) were rather similar in composition, and the large changes occurred in summer (July and partly September). This can most likely be explained by partial or complete nitrogen limitation of *S. latissima* during this season, as shown by Marinho et al. (2015). Leucine and aspartic acid were the main amino acids contributing to the observed differences in composition in July and September, respectively.

EAA ratio varied significantly from 0.41 to 0.42 (0.47–0.48 including arginine) in July to 0.21 (0.26–0.27 including arginine) in March in both REF and IMTA sites. The values obtained in July were within the range of values reported for other edible brown seaweeds such as *Himanthalia elongata* (0.47) and *U. pinnatifida* (0.48; Cofrades et al. 2010) but also within range for green and red seaweeds (42–48 %; Wong and Cheung 2000). The first limiting amino acid in *S. latissima* is lysine according to Murata and Nakazoe (2001), with an amino acid score of 82, based on the amino acid requirements by FAO/WHO (1973). However, histidine was not acknowledged as

Table 2 Amino acid composition (mg amino acid g^{-1} protein), total amino acid content (∑ AA; % DW), total essential amino acids (∑ EAA), essential amino acid ratio (EAA/AA), and EAA score of *S. latissima* cultivated at both reference (REF) and IMTA sites, including epiphytes (July–November)

Amino acid	July		September		November	
	REF	IMTA	REF	IMTA	REF	IMTA
LYS[a]	72.92±7.76	83.74±11.37	43.17±2.64	44.83±1.25	48.14±19.49	65.31±15.41
ALA	89.68±19.70	83.85±18.37	132.32±39.85	122.54±16.86	103.39±9.41	111.75±22.68
ARG[b]	84.02±38.83	96.85±39.07	63.93±2.70	51.41±17.32	83.91±37.21	73.52±28.19
CYS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MET[a]	27.48±5.99	29.53±13.19	17.35±0.10	17.98±4.01	24.42±6.61	19.16±4.71
LEU[a]	66.13±13.00	63.86±11.90	54.26±7.41	48.62±2.13	63.18±7.49	60.06±10.96
TYR	19.98±1.67	22.38±4.19	14.01±2.23	13.69±2.44	26.97±9.07	18.46±3.13
PHE[a]	39.45±9.19	39.24±14.47	29.15±5.73	29.14±6.01	37.51±8.30	36.15±11.52
PRO	55.56±2.18	45.70±3.97	41.66±2.40	37.48±2.05	51.28±3.74	54.22±14.03
THR[a]	34.08±14.47	33.25±19.82	27.92±6.18	29.10±6.38	35.06±9.42	31.63±3.55
ASP	189.94±43.89	180.37±24.82	198.74±15.46	169.16±25.24	202.22±40.44	200.72±35.71
SER	49.14±9.46	63.12±17.12	44.54±7.01	36.47±23.01	69.97±20.19	37.62±6.71
HYP	20.33±10.77	10.83±2.00	17.21±4.78	31.19±30.33	30.20±16.82	28.90±6.04
GLU	212.42±41.24	211.16±27.15	342.86±0.10	366.65±24.59	184.87±34.70	220.93±39.29
VAL[a]	41.37±9.98	42.47±17.60	40.70±7.43	47.76±11.35	37.82±4.45	48.83±15.47
HIS[a]	7.36±1.10	10.86±4.80	6.22±0.26	8.41±3.56	8.98±3.19	12.19±5.59
ILE[a]	37.60±10.64	30.67±7.36	27.21±6.60	25.56±2.45	33.70±8.27	35.95±15.92
GLY	126.38±22.73	124.81±15.09	68.10±6.62	91.18±38.52	135.64±31.11	119.48±14.43
∑ AA (% DW)	10.69±7.41	5.72±1.16	13.65±3.74	14.69±3.66	14.08±7.24	13.78±1.46
∑ EAA	346.37±53.6	355.99±53.6 a	259.98±38.1	265.09±1.8 b	315.79±12.1	327.74±63.5 ab
EAA/AA	0.30±0.05	0.30±0.05 a	0.22±0.03	0.23±0.00 b	0.27±0.01	0.28±0.05 ab
EAA score (%)	40.89±6.09	60.32±26.68	34.53±1.42	46.73±19.75	49.91±17.74	73.69±18.10

Data expressed as mean±standard deviation (n=3). Different letters in the same row indicate significant differences (P<0.05) for total amino acids, total EAA, EAA ratio, and EAA score between sampling months

n.d. not detected

[a] Essential amino acids in human and fish nutrition

[b] Essential amino acid in fish nutrition

being required, and the suggested requirement pattern in that report was recently considered underestimated (WHO/FAO/UNU 2007). In this study, histidine was the first limiting amino acid year-round with exception of May 2013. The EAA score peaked in July and November at the REF site and November and January at the IMTA site, while the histidine was not detected in the samples collected in March. The highest EAA score found in this study is within the range of values reported for edible seaweeds in Japan (60–100 %; Murata and Nakazoe 2001) and is higher than the scores for oats, rice, soybeans, wheat, or peanuts (43–57 %; Brody 1999). Furthermore, EAA scores found in this study year-round and in both cultivation sites are within the range of values reported for other seaweed species (20–67 %; Černá 2011). In addition, the EAA content found in this study in all sampling periods and locations was above the requirement pattern (305 mg g^{-1} protein) by WHO/FAO/UNU (2007), with exception of the samples collected in March in both sites.

Dietary protein requirements for optimal growth of fish vary from 28 to 50 % DW (Wahbeh 1997), which is above the maximum values found for *S. latissima* in this study. Regarding amino acid, maximum concentrations of lysine (6.4–6.8 mg g^{-1} DW), methionine (2.2–2.5 mg g^{-1} DW), and arginine (5.4–7.8 mg g^{-1} DW) were achieved in November in both cultivation sites, while highest cysteine concentration (0.15–0.19 mg g^{-1} DW) was found in September. Data suggest that *S. latissima* is not able to provide adequate amounts of these amino acids, considering that EAA requirement of fish (% diet) ranges from 1.2 to 2.1 % for lysine, 0.56 to 1.35 % for methionine, 1.59 to 2.4 % for arginine, and 0.54 to 0.74 % for cysteine (Alliot et al. 1974; Harding et al. 1977; Jackson and Capper 1982; Thebault et al. 1985; Tibaldi and Lanari 1991). However, *S. latissima* seems to be able to contribute with protein levels comparable to that of wheat meal (14.3 %; Hertrampf and Piedad-Pascual 2000) and its protein presents higher lysine, methionine, and arginine content than the protein of wheat meal, regardless the harvest time. On the other hand, compared with fish meal and soy meal composition reported by Hertrampf and Piedad-Pascual (2000), *S. latissima* has a lower protein content and poorer amino acid profile. These data suggest that *S. latissima* may have potential to partially replace terrestrial vegetable protein sources such as wheat meal in fish diets without impairing dietary protein content or amino acid composition. Moreover, reported benefits of seaweed supplementation in fish diets (0.5–5 %) must be considered including enhanced growth performance, feed utilization, body composition, and immune response of fish (see review by Mustafa and Nakagawa 1995; Ergün et al. 2008).

While the maximum EAA score was achieved in November, cultivated *S. latissima* yielded maximum biomass production in both cultivation sites in August–September followed by loss of biomass (Marinho et al. 2015). Thus, there is an apparent mismatch between maximum biomass production, highest protein content, and most likely the EAA score. At this time (November), a large part of the thallus is free of epiphytes and suitable for food. However, the apical and oldest part of the frond is still coved by epiphytes, making it unsuitable for human consumption and thus must be removed. That rejected biomass might be used as an ingredient for fish feed, as the concentration of lysine and methionine is similar, and the concentration of arginine is even higher, compared to that of the cleaned seaweed. If maximum biomass yield is prioritized (e.g., nutrient bioremediation), then harvest time should be established in September resulting in higher epiphyte coverage. This biomass has a similar amino acid composition as biomass harvested in November including epiphytes and thus utilization as ingredient or additive for fish feed would be a potential commercial application. On the other hand, harvest time must be carried out in May, if clean biomass is desired. However, according to our results, the protein content is very low while the EAA score is most likely also lower than that found in November, and thus, the biomass would have lower protein nutritional value.

Nutritional value of protein is mainly defined based on amino acid composition and digestibility (Černá 2011). In order to fully evaluate the biological value of *S. latissima* protein either for human consumption or fish feed, further in vivo protein digestibility trials must be carried out, so the protein digestibility-corrected amino acid score can be determined (PDCAAS; WHO/FAO/UNU 2007).

In conclusion, this study draws attention to the seasonal variations of protein and amino acid profiles for the nutritional evaluation of *S. latissima*, which has implications for utilizing seaweed for, e.g., human consumption and fish feed. While the biomass appears to be suitable as a protein/EAA ingredient, the entire seaweed for food or feed inclusion will also have other beneficial effects. Protein content varied considerably depending on time of harvest, which needs to be considered for optimal utilization. The natural biomass including epiphytes changed neither the amino acid content nor the EAA score. However, EAA content and the EAA ratio were significantly reduced compared to clean seaweed biomass. When epiphytes are present, they need to be accounted for and evaluated within the potential use of the biomass. Harvest time is therefore important to consider and may mismatch with utilization, yield, and best nutritional value.

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