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The yield and quality of multiple harvests of filamentous *Ulva tepida*

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Abstract Species of the genus *Ulva* are used for human consumption due to their nutritional qualities and we assess a new filamentous species, *Ulva tepida*. A critical step is to quantify the yield and quality of biomass over multiple harvests to ensure consistency throughout the production cycle. To do this, ropes were seeded with *U. tepida* and harvested fortnightly over 6 weeks of outdoor cultivation with biomass yield and quality quantified for each harvest. This cycle was repeated a further two times. The yield of biomass was not significantly different between harvests (13.6–23.0 g dry weight (dw) m^{-1} rope), however, the final harvest was highly variable. Consequently, we recommend a production cycle of two harvests. The quality of biomass, as determined by the key biochemical parameters for these two sequential harvests, was consistent. Carbohydrates were the major component (45 % dw) and were primarily dietary fibre (27 % dw) consisting of insoluble (18 % dw) and soluble (9 % dw, equates to ulvan) fibre, with consistent values between harvests. Protein, as the sum of amino acids (17 % dw), was also consistent between harvests. Similarly, the content of ash (31 % dw) and lipids (3 % dw), as well as the composition of minerals and fatty acids was consistent. These results quantify, for the first time, no negative effects of multiple harvests on the yield and quality of biomass and support this technique to optimise productivity and quality.

Keywords Macroalgae · Aquaculture · Nutritional composition · Ulvan · Aonori

Introduction

The green macroalgal genus *Ulv*a has compelling characteristics for biomass production and diverse applications (Holdt and Kraan 2011; Alves et al. 2013a; Carl et al. 2014a) of which food and functional food products represent high value markets (Hafting et al. 2012). Species of *Ulva* are generally rich in nutritional value and, therefore, widely used for human consumption (Mabeau and Fleurence 1993; Holdt and Kraan 2011). In Japan, a major market for *Ulva* is 'aonori' which refers specifically to species with a filamentous morphology which are dried for human consumption (McHugh 2003; Ohno 2006; Kawashima et al. 2013). A new filamentous species of *Ulva*, *Ulva tepida* (Masakiyo and Shimada 2014; Phillips et al. 2016), is a target species for cultivation as 'aonori' based on its robustness and consistently high growth rates (Shimada et al. 2008; Lawton et al. 2013; Carl et al. 2014a). The life cycle of *U. tepida* has been closed (Carl et al. 2014b) allowing for cultivation on substrates by artificial seeding with the high degree of control required for aquaculture production (Carl et al. 2014a). Dried biomass of *U. tepida* has a strong and pleasant flavour and, therefore, is suitable as a food product. However, the nutritional value of carbohydrates, dietary fibre content, mineral, protein, amino acid profiles, lipid, and fatty acid profiles of this new species is yet to be

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quantified. This knowledge is important for the development of this species as the biochemical composition will determine its biomass applications and value.

Besides food production, biomass of *U. tepida* has a potential as a resource of ulvans which are unique soluble dietary fibre. In general, dietary fibre of *Ulva* are cell wall polysaccharides differentiated as soluble and insoluble in water. Ulvans are complex sulphated polysaccharides representing a functional biopolymer with food, pharmaceutical, biomedical, agricultural and chemical applications based on their unique physiochemical properties (Lahaye and Robic 2007; Alves et al. 2012, 2013a; 2013b; Wang et al. 2014). However, the content of ulvan is highly variable between species ranging from 8 to 29 % dw (Lahaye and Robic 2007). To date, the ulvan content of *U. tepida* has not been quantified and, therefore, its suitability as a resource of this functional product remains unknown.

One fundamental requirement for the sustainable production of high quantities of algal biomass is effective cultivation at scale. The strategy of multiple harvests, also referred to as pruning, is widely applied in the seaweed industry to optimise cultivation, and filamentous species of *Ulva* are generally harvested two to three times during a production cycle of 2 to 3 months (Ohno 1993; Ohno 2006). However, information in the literature is sparse, and to our knowledge, only two studies have quantified the effects of multiple harvests on the biomass yield of filamentous species of *Ulva* with contrary results (Ohno et al. 1981; Pandey and Ohno 1985). While the yield decreased several-fold from the first to the second harvest for nets seeded with *Ulva flexuosa* and *Ulva compressa* (Ohno et al. 1981), and *Ulva intestinalis* (Pandey and Ohno 1985), the yield of *Ulva prolifera* increased from the first to the second harvest and approximately halved for the third harvest (Pandey and Ohno 1985). Notably, both studies were conducted under natural conditions. The effect of multiple harvests on the yield of biomass is a key factor in determining sustainable harvesting processes for *U. tepida*. Notably, multiple harvests can also affect the quality of the biomass (Adnan and Prose 1987) and this needs be quantified to deliver reproducible quality of biomass across harvests.

Therefore, the aim of this study was to optimise production of *U. tepida* and quantify biochemical composition to determine the suitability of the biomass as a food product for human consumption. As a first step, the effect of multiple harvests on the yield of the cultivated biomass was determined by harvesting the biomass fortnightly during 6 weeks of outdoor cultivation over three production cycles. Second, the quality of the harvested biomass was determined by quantifying the key biochemical parameters of ash, mineral content, elemental composition, carbohydrate, amino acid profile (protein), lipid, fatty acid profile and dietary fibre.

Materials and methods

Algal biomass and preparation of reproductive material

Biomass of *Ulva tepida* (Masakiyo and Shimada 2014; Phillips et al. 2016), previously referred to as *Ulva* sp. 3 (Shimada et al. 2008) (Genbank accession number KM406999), was collected at three times (22 April 2014, 7 July 2014 and 11 March 2015), hereafter referred to as batches, from James Cook University (JCU) in Townsville and an aquaculture facility at Ayr, Australia. To obtain seedlings for artificial seeding from each batch, the release of zoids was induced using a temperature shock at 4 °C for 10 min (Carl et al. 2014b), and the filaments were subsequently cut using a blender (Carl et al. 2014a). The release of zoids peaked after two days between 10:00 and 11:30 a.m. and the density of zoids was determined using a haemocytometer.

Cultivation and cultivation conditions

To determine the effect of multiple harvests on the yield and quality of the harvested biomass, the released swarmers were artificially seeded onto ropes at a density of 621,000 zoids m^{-1} rope ($n = 13$ for each batch). Each rope had a length of 580 mm and was maintained under nursery conditions for 5 days with a water change after 3 days (for details see Carl et al. 2014a). Subsequent to the time in the nursery, each seeded rope was individually placed into an aerated flow-through outdoor tank holding approximately 28 L of filtered seawater (1 μm and UV sterilised) under ambient light at the Marine and Aquaculture Research Facility at JCU. To immerse the ropes horizontally in the water at a depth of approximately 100 mm below the water surface, each seeded rope was individually attached to a weighted frame (380 × 500 mm) using cable ties, and each frame was placed on the bottom of a tank. To minimise temperature fluctuations, the tanks holding the ropes were placed in a circulating water bath. Holding tanks, frames and air lines were cleaned weekly.

Experiments were conducted from April 2014 to April 2015. The average water temperatures in the outdoor tanks ranged between 23.6 (±2.2 S.D.)°C and 26.3 (±1.7 S.D.)°C during the trial (Online Resource 1). The average salinity was 33.0 (±1.8 S.D.) ppt and the seeded ropes received an average photosynthetically active radiation ranging from 26.1 (±8.1 S.D.) to 35.0 (±4.9 S.D.) mol photons m^{-2} day^{-1}. The tanks had a flow rate of 0.5 L min^{-1} and were on a recirculating system. The nutrient concentration was measured three times per week, and the concentration of nitrogen as nitrate was maintained at 1–3 mgL^{-1} using MAF growth medium (Manutec Pty Ltd).

Biomass yield

After 14 days of outdoor cultivation, ropes were spun to remove excess water and the biomass was pruned (harvest 1) by cutting off *U. tepida* using scissors and leaving approximately 1 cm of biomass on each rope to ensure that the basal parts of the thallus were not damaged. The period of outdoor cultivation was based on previous work where the productivity declined for longer periods due to the reproductive maturation of biomass (Carl et al. 2014a). To ensure that this was the case, sporulation was monitored through the experiment and did not occur in pruned biomass in tanks. Ropes with the remaining biomass were then returned to the outdoor tanks under the previous conditions. Each replicate rope was weighed prior and post harvesting to determine the fresh weight (fw) and growth of the biomass. The algal fw for each replicate was calculated by subtracting the weight of the moist rope from the total weight of the seeded rope with the algal biomass. The specific growth rate (SGR) for each replicate was calculated using the equation SGR (% day^{-1}) = ln $(B_2/B_1)/(t_2-t_1) \times 100$, where B_1 and B_2 are the algal biomasses (g fw) at time t_1 and t_2 (days). Notably, algal fw was measured from 14 days of outdoor cultivation onwards and SGRs were calculated for the second and third cultivation cycle for the time period of 14 to 28 days and 28 to 42 days of outdoor cultivation, respectively. To determine the yield of the cultivated biomass for each harvest, the biomass from all replicate ropes of each batch was pooled, freeze-dried and weighed. The dry biomass yield was calculated as dry weight (dw) per linear metre of rope (g dw m^{-1} rope). Subsequently, the dried biomass was homogenised using a coffee grinder (Breville, CG2B) and Magic Bullet (MB1001). The ground biomass was then maintained in the dark at −20 °C in airtight containers until further processing (see *'Quality of harvested biomass'* below).

For the subsequent second (harvest 2) and third pruning (harvest 3), the biomass on the ropes was harvested as described above at day 28 and 42 of outdoor cultivation, respectively. This corresponds to a total of three cycles of culture and harvest at 14 days for each cultivation cycle. Experiments were conducted at different times with three batches of zoids from three independent reproductive events resulting in three independent production cycles as described above.

Quality of harvested biomass

There was a high variation in the biomass yield between batches for the final harvest supporting a production cycle of two harvests (see results). Consequently, the quality of biomass was determined for these two harvests. The quality of the biomass was defined by ash content and composition (23 elements), elemental composition (CHONS), carbohydrate, lipid, fatty acid profile, amino acid profile (protein) and dietary fibre. In addition, the colour of the dried biomass was quantified (see Online Resource 2).

The ash content was quantified by heating a 2 g homogenised subsample of dried biomass at 110 °C in a moisture balance until a constant dry weight was reached. The sample was then split into triplicates and subsequently combusted at 550 °C in a muffle furnace for 24 h until a constant weight was reached. In addition, a homogenised subsample was analysed for a total of 23 minerals (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, Se, Sr, V and Zn) following Roberts et al. (2013). Analyses were conducted by the Advanced Analytical Centre at JCU. Furthermore, a homogenised subsample of the dried biomass was analysed for the elemental composition of carbon (C), hydrogen (H), oxygen (O), nitrogen (N), sulphur (S) and iodine (I) content by ultimate analysis. The samples were analysed by the OEA Laboratories (Cornwall, UK). In addition, The total lipid content was analysed using traditional organic solvent extraction as described in detail in Gosch et al. (2012). Fatty acids were extracted and transesterified following a direct transesterification method described in detail in Gosch et al. (2012). Amino acid profiles were quantified by hydrolysis following analysis using the Waters AccQTag Ultra chemistry on a Waters ACQUITY UPLC at the Australian Proteome Analysis Facility (Sydney, Australia). The protein content was calculated as the sum of all proteomic amino acids. The content of essential amino acids was calculated as the sum of histidine, isoleucine, leucine, lysine, methiodine, phenylalanine, threonine and valine. The total carbohydrate content was determined by difference using the equation Carbohydrates (%) = 100 % − (ash + moisture + total lipids + protein content). The total insoluble and soluble fibre content was analysed using the enzymatic-gravimetric method and analyses were conducted by Grain Growers Ltd (North Ryde, Australia).

Statistical analysis

To formally test the effect of multiple harvests on the dry biomass yield, data were analysed by two-factor PERMANOVA using PRIMER 6 (v. 6.1.13) and PERMANOVA+ (v. 1.0.3) (Clarke and Gorley 2006) with partial harvest as a fixed factor and batch as a random factor. PERMANOVA is the equivalent of an ANOVA performed on similarity values and p values are obtained by permutation methods (Anderson et al. 2008). The method is non-parametric and distance-based pseudo-F statistics are calculated for each term. All PERMANOVA tests presented here used the Euclidean distance measure on normalised data and p values were calculated using permutation of residuals under a reduced model with 9999 random permutations (Anderson et al. 2008).

Differences in the quality parameters of ash, mineral content, elemental composition, amino acids, lipids, fatty acids,

carbohydrates and fibre between the first two harvests were assessed by paired *t* tests on log-transformed data using IBM SPSS Statistics (v. 20). All data are reported as mean ± 1 standard error (S.E.) unless stated otherwise.

Results

Biomass yield

The average yield of dry biomass was similar between harvests (Fig. 1a) with the lowest yield for the first harvest (13.6 ± 3.4 g dw m^{-1} rope) and higher yields for the second (23.0 ± 13.6 g dw m^{-1}) and third harvest (15.2 ± 13.7 g dw m^{-1}). Notably, the biomass yield was highly variable between batches and the variation increased with increasing number of harvests from two- to 60-fold from the first to the third harvest, respectively (Fig. 1b). Similarly, the variation of the average specific growth rate increased from the second (day 14–28; SGR 15.6 ± 4.2 % day^{-1}) to the third (day 28–42; SGR 10.4 ± 8.0 % day^{-1}) cultivation cycle and growth generally decreased over time. Although there was a significant difference between batches ($F_{(2108)} = 882.68$, $p < 0.001$), there was also a significant interaction between 'batch' and 'harvest' on biomass yield ($F_{(4108)} = 102.20$, $p < 0.001$) driven by opposing trends between batches. While the yield of batch 2 decreased with increasing number of harvests, the yield of batch 1 and 3 increased from the first to the second harvest followed by a decrease for the third harvest.

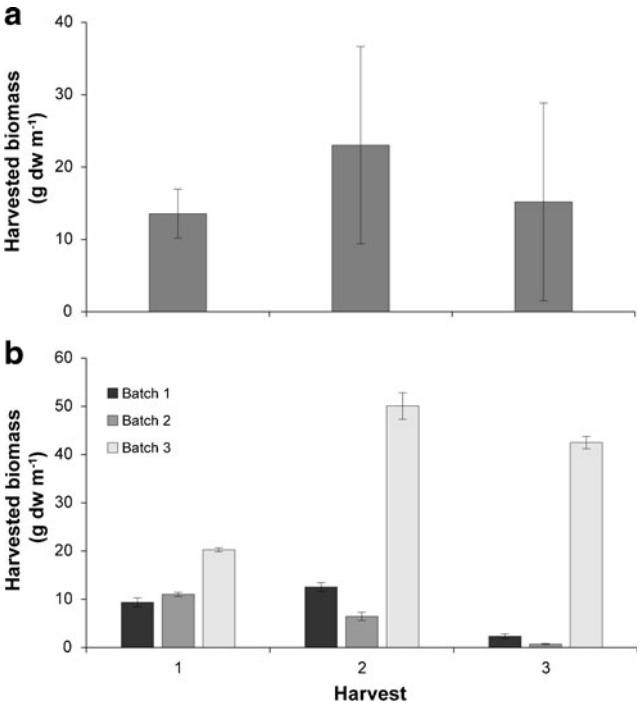

Fig. 1 Mean (±S.E.) weight (g dw m^{-1} rope) of harvested biomass for each harvest. **a** Mean harvested biomass of three batches over time ($n = 3$). **b** Mean harvested biomass ($n = 13$) of each batch for successive harvests. The ropes were independently seeded on 24 April 2014 (batch 1), 9 July 2014 (batch 2) and 13 March 2015 (batch 3)

Quality of harvested biomass

The quality parameters of average ash content, elemental composition, mineral, carbohydrate, lipid and fatty acid composition content were consistent between harvests. In contrast, total dietary fibre content increased from the first to the second harvest. Interestingly, most quality parameters were relatively consistent between batches for the first harvest, yet the biochemical features of the second harvest varied substantially between batches.

The content of ash and moisture was consistent between harvests with ash ranging from 31.2 ± 2.1 % dw for the first harvest to 30.2 ± 4.2 % dw for the second harvest (Table 1). Similarly, the average elemental composition of *U. tepida* was consistent between harvests. Carbon (30 % dw) and oxygen (26–28 % dw) were the major elements characterised by ultimate analysis with sulphur being the lowest (3 % dw). However, variation in the carbon content of the harvested biomass increased six-fold for the second harvest resulting in an order of a magnitude increase in variation of the C:N ratio compared to the first harvest (Online Resource 3).

The average content of the 24 minerals measured in the harvested biomass was relatively consistent between harvests (Table 2). Potassium (K; >3486 mg 100 g^{-1} dw) and sodium (Na; >3423 mg 100 g^{-1} dw) were the main minerals, followed by magnesium (Mg; >1292 mg 100 g^{-1} dw), calcium (Ca; >252 mg 100 g^{-1} dw) and phosphorous (P; > 186 mg 100 g^{-1} dw). There was more variation in the content of potassium in the second harvest ranging from 2670 to 4990 mg 100 g^{-1} dw compared to the first harvest from 4200 to 5190 mg 100 g^{-1} dw (Online Resource 4). Notably, the content

Table 1 Proximate and ultimate analysis of *U. tepida*. Data are mean (± S.E.; $n = 3$) values (% dw). No significant differences were found between harvests (paired *t*-test, $\alpha = 0.05$)

	Harvest 1	Harvest 2
Proximate (% dw)		
Ash	31.2 ± 2.1	30.2 ± 4.2
Moisture	5.4 ± 1.1	4.7 ± 0.8
Ultimate (% dw)		
C	29.7 ± 0.3	30.1 ± 1.7
H	5.1 ± 0.2	5.2 ± 0.3
O	26.4 ± 4.8	27.8 ± 3.9
N	4.8 ± 0.01	4.0 ± 0.7
S	3.2 ± 0.2	3.4 ± 0.3
C:N ratio	6.2 ± 0.1	8.0 ± 1.2

Table 2 Mean (±S.E.; $n = 3$) mineral content of *U. tepida* (mg 100 g^{-1} dw) for the first and second harvest. Means in a row with different superscript letters differ significantly (paired *t*-test, $\alpha = 0.05$)

Element	Harvest 1	Harvest 2
Al	1.48 ± 0.41	1.54 ± 0.11
As	0.07 ± 0.02 [a]	0.11 ± 0.02 [b]
B	15.49 ± 5.66	19.37 ± 3.36
Ba	0.10 ± 0.01	0.13 ± 0.02
Ca	252.00 ± 40.62	305.67 ± 40.13
Cd	0.08 ± 0.04	0.05 ± 0.03
Co	0.01 ± 0.004	0.02 ± 0.002
Cr	0.24 ± 0.03	0.29 ± 0.02
Cu	1.22 ± 0.19	1.69 ± 0.35
Fe	5.86 ± 1.78	5.69 ± 1.78
Hg	≤0.05	≤0.05
I	1.13 ± 0.47	3.78 ± 2.61
K	4823.33 ± 313.28	3486.67 ± 752.60
Mg	1295.00 ± 175.10	1292.00 ± 156.26
Mn	1.53 ± 0.16	1.47 ± 0.04
Mo	0.03 ± 0.01	0.03 ± 0.01
Na	3423.33 ± 694.39	3980.00 ± 536.94
Ni	0.29 ± 0.08	0.47 ± 0.05
P	208.00 ± 47.06	186.23 ± 68.25
Pb	0.01 ± 0.01	0.01 ± 0.002
Se	0.27 ± 0.01	0.32 ± 0.03
Sr	3.28 ± 0.04	3.71 ± 0.21
V	0.08 ± 0.01	0.16 ± 0.04
Zn	3.72 ± 1.62	2.96 ± 0.95

of remaining minerals was at least an order of a magnitude lower than phosphorous. The content of arsenic, representing less than 0.0001 % dw, increased significantly from the first to the second harvest. Similarly, although not statistically significant, the content of boron and calcium increased by 20 %, and that of iodine and vanadium doubled, while the content of potassium and zinc decreased by approximately 20 %.

Carbohydrates were the main biochemical component of *U. tepida* and making up 44.6 % dw of the biomass (Table 3). Dietary fibre was the main component comprising between 56 % (harvest 1) and 67 % of total carbohydrate (harvest 2). The total dietary fibre content increased significantly by more than 20 % from the first to the second harvest (*paired t-test:* $t = -10.60$, $df = 2$, $p = 0.009$) up to 29.7 ± 0.7 % dw of *U. tepida*. The majority of the total dietary fibre content was insoluble fibre, approximately double that of soluble fibre. The contents of both insoluble and soluble dietary fibre were more variable in the second harvest. For example, insoluble fibre (ulvans) ranged from 8.2 to 8.5 % dw for the first harvest, and from 7.0 to 14.9 % dw for the second harvest (Online Resource 5).

Table 3 Biochemical analysis of *U. tepida*. Data are mean (±S.E.; $n = 3$) values (% dw) for the first and second harvest. Carbohydrate content was determined by difference. Protein content equals total amino acid contents (sum of all analysed amino acids). Means in a row with different superscript letters differ significantly (paired *t*-test, $\alpha = 0.05$)

	Harvest 1	Harvest 2
Carbohydrate	43.6 ± 3.4	44.6 ± 1.9
Total dietary fibre	24.5 ± 0.4 [a]	29.7 ± 0.7 [b]
Insoluble	16.2 ± 0.3	19.5 ± 3.6
Soluble	8.3 ± 0.1	10.2 ± 2.9
Lipid	3.5 ± 0.9	2.6 ± 0.5
Protein	16.3 ± 0.5	17.9 ± 3.5

The average protein content (sum of all amino acids) was consistent between harvests with a similar proportion of essential and non-essential amino acids between harvests (Table 4). More than half of the total amino acid content (TAA) consisted of aspartic and glutamic acids, with each approximately 28 % TAA (Table 4). While the quantity of the essential amino acid methionine, as a proportion of total amino acids, was consistent between harvests, lysine decreased by more than 10 % TAA from the first to the second harvest (*paired t-test:* $t = 6.38$, $df = 2$, $p = 0.024$). Notably, the content of lysine was consistent between batches for the first harvest (9.1–9.2 % dw) and highly variable for the second harvest (5.5–10.6 % dw) (Online Resource 6). Similarly, the protein content was more variable in the second harvest. Protein content (TAA) ranged from 15.3 to 16.9 % dw for the first harvest, and from 11.0 to 22.4 % dw for the second harvest (Online Resource 5).

Total lipids were the smallest component with an average of less than 3.5 % dw (Table 3). The profile of fatty acids was consistent between harvests (Table 5). The most abundant fatty acids were palmitic acid (C16:0), linoleic acid (C18:2) and α-linolenic acid (C18:3), together making up 62.7 to 65.3 % of total fatty acids (TFA). Polyunsaturated fatty acids (PUFAs) were the major component of total fatty acids with *n*-3 PUFAs being more abundant than *n*-6 resulting in a low *n*-6/*n*-3 ratio. The variation within a harvest (between batches) was relatively consistent (Online Resource 7).

Discussion

This study demonstrates that *U. tepida* can be harvested multiple times during a production cycle to optimise cultivation, with similar yields between harvests. However, the variation of the biomass yield and biochemical composition increased markedly with increasing number of harvests. Therefore, two harvests are recommended for *U. tepida* as a conservative

Table 4 Amino acid profiles of *U. tepida*. Data are means (±S.E.; $n = 3$) of α-amino acids (mg g^{-1} dw, tryptophan and cysteine not included) for the first and second harvest. Properties of total amino acids are expressed as essential and non-essential amino acids, lysine and methionine contents as proportion of total amino acid content (%TAA). Data also include ratios of methionine:lysine and protein:N. Means in a row with different superscript letters differ significantly (paired *t*-test, $\alpha = 0.05$)

	Harvest 1	Harvest 2
Essential Amino acid (mg g^{-1})		
Histidine	3.1 ± 0.1	2.8 ± 0.4
Threonine	7.3 ± 0.4	10.5 ± 2.1
Lysine	9.1 ± 0.03	8.7 ± 1.6
Methionine	1.9 ± 0.1	2.1 ± 0.2
Valine	10.9 ± 0.6	12.0 ± 2.1
Isoleucine	7.1 ± 0.4	7.6 ± 1.3
Leucine	12.9 ± 0.9	12.8 ± 1.7
Phenylalanine	9.6 ± 0.6	11.1 ± 2.0
Non-essential amino acid (mg g^{-1})		
Serine	7.8 ± 0.5	10.3 ± 2.2
Arginine	11.8 ± 2.3	11.0 ± 2.6
Glycine	9.3 ± 0.2	10.3 ± 1.9
Aspartic acid/aspartate	19.1 ± 0.5	25.1 ± 4.6
Glutamic acid/glutamate	25.9 ± 4.4	25.5 ± 6.4
Alanine	13.7 ± 0.7	16.3 ± 3.5
Proline	8.8 ± 0.3	8.3 ± 1.6
Tyrosine	4.0 ± 0.4	4.5 ± 0.8
Total amino acids (TAA) (mg g^{-1})	162.5 ± 5.0	178.8 ± 34.9
Proportion of total amino acids (%TAA)		
Essential amino acid	38.3 ± 2.5	38.3 ± 1.4
Non-essential amino acid	61.7 ± 2.5	61.7 ± 1.4
Lysine	5.6 ± 0.2 [a]	4.9 ± 0.1 [b]
Methionine	1.2 ± 0.1	1.3 ± 0.2
Ratio		
Methionine:lysine	0.2 ± 0.02	0.3 ± 0.03
Protein:N	3.4 ± 0.03 [a]	4.4 ± 0.1 [b]

Table 5 Fatty acid (FA) composition of *U. tepida*. Mean (± S.E.; $n = 3$) content of fatty acids (% of total FA) for the first and second harvest. Data also include saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and PUFA *n*-6/*n*-3 ratio. No significant differences were found between harvests (paired *t*-test, $\alpha = 0.05$)

	Harvest 1	Harvest 2
Fatty acids (% of total FA)		
C14:0	0.57 ± 0.06	0.57 ± 0.04
C14:1	0.83 ± 0.07	0.85 ± 0.08
C16:0	31.88 ± 0.72	33.86 ± 1.40
C16:1	4.37 ± 0.21	5.15 ± 0.51
C16:2 (*n*-6)	1.72 ± 0.22	1.70 ± 0.19
C16:3 (*n*-3)	2.74 ± 0.85	1.35 ± 0.62
C16:4 (*n*-3)	8.11 ± 1.00	8.50 ± 0.84
C18:0	0.19 ± 0.04	0.14 ± 0.01
C18:1	11.84 ± 5.18	9.09 ± 1.16
C18:2 (*n*-6)	12.12 ± 2.62	14.47 ± 1.30
C18:3 (*n*-3)	18.66 ± 1.51	16.94 ± 1.95
C18:4 (*n*-3)	5.04 ± 0.68	5.53 ± 0.56
C20:0	0.08 ± 0.02	0.03 ± 0.01
C20:3	0.45 ± 0.15	0.43 ± 0.15
C20:4 (*n*-6)	0.82 ± 0.24	0.86 ± 0.33
C22:0	0.58 ± 0.04	0.53 ± 0.09
Total SFA	33.30 ± 0.70	35.13 ± 1.43
Total MUFA	17.05 ± 5.34	15.09 ± 1.35
Total PUFA	49.65 ± 5.16	49.78 ± 2.50
PUFA (*n*-3)	34.55 ± 2.04	32.32 ± 2.94
PUFA (*n*-6)	14.66 ± 2.98	17.04 ± 1.78
Ratio		
*n*6/*n*3	0.42 ± 0.07	0.54 ± 0.10

approach to double the total biomass yield compared to one harvest, while maintaining consistent quality.

Notably, while there was no difference between the average yields of biomass between harvests of *U. tepida*, the variation within a harvest (between batches) increased with each harvest. This overall outcome in terms of harvest is in accordance with previous studies (Ohno et al. 1981; Pandey and Ohno 1985) where highly variable biomass yields are common for filamentous species of *Ulva,* ranging from 60 to 485 g dw m^{-2} nets for *U. prolifera* (Ohno 1993) and 5 to 44 g dw m^{-1} rope for *U. tepida* in previous studies (Carl et al. 2014a; Carl et al. 2016) and from 9 to 20 g dw m^{-1} rope for the first harvest. However, the present study demonstrates the effect of replication using independent batches, at different times, with high variation in biomass yield between batches.

The causes of high variation in the yield of *U. tepida* are poorly understood. However, there are likely to be both generational components and environmental effects (Carl et al. 2016). The main environmental drivers for algal growth are light, temperature, salinity, water flow and nutrients (Lobban and Harrison 1997) yet these remained relatively constant during the present study. Notably, biomass had an internal nitrogen content close to 5 % supporting non-N limiting resources (Angell et al. 2014; Neveux et al. 2014). Furthermore, the tissue N:P ratio supports neither nitrogen nor phosphorous limitation (Björnsäter and Wheeler 1990). Consequently, an inadequate nutrient supply resulting in decreased growth can be ruled out (Navarro-Angulo and Robledo 1999; Sterner and Elser 2002). Our findings clearly demonstrate that environmental effects were not driving the variation in yield between batches for *U. tepida*.

The average biochemical characteristics of *U. tepida*, analysed here for the first time, demonstrates a profile high in fibre and essential minerals with applications as a food product. The main biochemical component was

carbohydrates, which made up approximately 50 % dw of the biomass. The carbohydrates of *U. tepida* were mostly dietary fibre (56 - 67 %) and within the range for this genus (Mata et al. 2016; Lahaye 1991; Bobin-Dubigeon and Barr 1997). Dietary fibre, regardless of whether soluble or insoluble in water, are not digested or absorbed in the human small intestine (Fuentes-Zaragoza et al. 2010). Importantly, the intake of dietary fibre has health promoting effects by increasing faecal bulk, reduction of postprandial blood glucose response and lowering pre-prandial cholesterol (Champ et al. 2003; Elleuch et al. 2011). The biomass of *U. tepida* represents a valuable source of fibre (25–30 % dw) which is double that of most cereals (e.g. rice <1 % dw, oats <10 % dw), fruits (e.g. apple < 14 % dw, peach < 14 % dw) and vegetables (e.g. sweet potato < 10 % dw) (Englyst and Hudson 1996; Elleuch et al. 2011). The supplementation of fibre to food low in dietary fibre can create healthier products lower in calories, cholesterol and fat, while improving water- and oil-holding capacity, texture and the shelf life of food products (Elleuch et al. 2011). For example, the incorporation of seaweed positively affects the dietary fibre content of meat with an increased tenderness and the inhibition of bacterial growth (Cox and Abu-Ghannam 2013).

Importantly, the water-soluble dietary fibre in the genus *Ulva* corresponds to ulvans (Bobin-Dubigeon et al. 1997) and varies between 8 and 29 % dw (Lahaye and Robic 2007). For *U. tepida*, the ulvan content is in the lower range of 7 to 15 % dw. The factors of growth rate, environmental conditions, post-harvest treatments and extraction methods can impact the ratio of soluble to insoluble fibre (Lahaye et al. 1995; Bobin-Dubigeon and Barr 1997; Yaich et al. 2015) and therefore the ulvan content (Lahaye and Jegou 1993; Hernández-Garibay et al. 2011; Yaich et al. 2013). The biological activity and health benefits of ulvans are influenced by its degree of sulfation affecting anti-oxidant, anti-viral, anti-peroxidative and anti-hyperlipidemic activities (Alves et al. 2013a and references therein).

The second largest component of *U. tepida* was ash which is similar to other filamentous species of *Ulva* ranging from 21 to 29 % dw (McDermid and Stuercke 2003; Zhuang et al. 2012). The ash content of seaweeds is primarily made up of minerals (Holdt and Kraan 2011) and the ash and mineral content of *U. tepida* is much higher than terrestrial crops (Ross et al. 2008) with the ash content of most vegetables being ≤2 % on dry weight basis (Hanif et al. 2006; de Souza Araújo et al. 2014). This in turn makes this species suitable to offset nutritional deficiencies. All the main minerals of *U. tepida* are essential macro-minerals for human (Suter et al. 2002) and animal health (Suttle 2010). Seaweeds are used in a wide range of foods and feed supplements as a source of macro-minerals and trace elements (Holdt and Kraan 2011) and *Ulva* has been incorporated into food products to supply bioavailable iron to combat iron deficiency and anaemia (García-Casal et al. 2009). Similarly, the consumption of *U. tepida,* or addition to foods, would increase the dietary content of magnesium and calcium which are underconsumed in the Western diet (U.S. Department of Agriculture and U.S. Department of Health and Human Services 2010). Although the iodine content of *U. tepida* was lower than many seaweeds, it is approximately one order of magnitude higher than for most terrestrial crops (Mahesh et al. 1992) and would supplement nutritional deficiencies in both human and animal diets.

Interestingly, *U. tepida* has also a low Na:K ratio and is, therefore, of interest for food applications to reduce sodium content (Gupta and Abu-Ghannam 2011). Modern diets contain an excess of sodium primarily sourced (90 %) from manufactured salt (NaCl), and consequently, the Na:K ratio of modern diets has increased (Cordain et al. 2005). Diets high in sodium and low in potassium have been linked with hypertension (Du et al. 2014). Seaweeds have successfully been added to meat products without compromising the water and fat-binding properties when the salt (NaCl) content was reduced (Cofrades et al. 2008).

Protein (as the sum of amino acids) was the third largest component of *U. tepida* with an average content of 17 % dw which is in the range for *Ulva* (7–29 % dw; Wong and Cheung 2000; Ortiz et al. 2006; Marsham et al. 2007; Shuuluka et al 2013; van der Wal et al. 2013; Neveux et al. 2015). As with many macroalgae the quantity of protein is low relative to terrestrial crops; however, the quality is high (Grieshop and Fahey 2001; Cole et al. 2014; Angell et al. 2014). The nutritional value of proteins depends on the ratio of essential to non-essential amino acids. For *U. tepida*, the ratio was approximately 60:40 which matches the common range for macroalgae (Neveux et al. 2014), and is within the optimal range for protein synthesis in humans (Melnik et al. 1995). Furthermore, the levels of the non-essential amino acids aspartic and glutamic acids are high in *U. tepida*. Both aspartate and glutamate are natural flavour enhancers with the latter being a main component in the taste sensation of 'umami' (MacArtain et al. 2007).

Although the lipid content of *U. tepida* was low (<3 % dw), the quality was high. More than 60 % of the fatty acids of *U. tepida* were unsaturated fatty acids which are of nutritional importance for human health. PUFA *n*-3 (omega-3) in particular are important as they cannot be synthesised by humans and are obtained only through diet. The most abundant PUFA *n*-3 was α-linolenic acid (ALA, 18:3) which is important in cardiovascular health (Pan et al. 2012). Furthermore, the biomass of *U. tepida* had a low *n*-6/*n*-3 fatty acid ratio and the inclusion into diets can make a beneficial contribution to improve this ratio which is linked to inflammatory processes in the body (Schmitz and Ecker 2008). Ratios in most Western diets are approximately 15; however, low ratios of the range of 2 are optimal (Simopoulos 2002).

In conclusion, the biomass yield of *U. tepida* was optimised by multiple harvests and no negative effects were found in the yield and quality of biomass. However, the variation of yield and quality increased with increasing harvests, and therefore, we recommend a production cycle of two harvests. The harvested *U. tepida* is high in nutritional value with a high mineral and fibre content. Carbohydrates were the major component of *U. tepida* and were primarily dietary fibre, which has health promoting effects.

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