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The intensive land-based production of the green seaweeds *Derbesia tenuissima* and *Ulva ohnoi:* biomass and bioproducts

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Abstract The green seaweeds *Derbesia tenuissima* and *Ulva ohnoi* were assessed comparatively for yields of biomass and bioproducts (fatty acids, soluble fibres and amino acids) under controlled land-based culture over 6 months. The intensive cultivation of these seaweeds yielded an average biomass productivity of 15 g dry weight (dw) m^{-2} day^{-1} (56 t dw ha^{-1} $year^{-1}$) for *D. tenuissima* and 38 g dw m^{-2} day^{-1} (138 t dw ha^{-1} $year^{-1}$) for *U. ohnoi*. The production of *D. tenuissima* was comparatively consistent, ranging between 8 and 20 g dw m^{-2} day^{-1}, while that of *U. ohnoi* was highly variable and stochastic, ranging between 16 and 77 g dw m^{-2} day^{-1}. The major bioproducts were lipids (13 % dw) and fatty acids (5 % dw) for *D. tenuissima* and soluble fibres (ulvan, 12 % dw) for *U. ohnoi*. These concentrations were consistent over time, irrespective of the variation in environmental conditions and biomass productivity. In addition, *D. tenuissima* and *U. ohnoi* are potential bioresources for the extraction of proteins (amino acids). The amino acid content of *D. tenuissima* (24 % dw) was higher than that of *U. ohnoi* (13 % dw). However, the annual amino acid productivity of *U. ohnoi* (18 t ha^{-1} $year^{-1}$) was higher than that of *D. tenuissima* (14 t ha^{-1} $year^{-1}$) due to the higher annual productivity of biomass. Notably, both species offer niche opportunities to deliver multiple products through a biorefinery process.

Keywords Seaweed · Chlorophyta · Land-based cultivation · Bioproducts · Fatty acid · Amino acids · Ulvans · Biorefinery

Introduction

Land-based cultivation systems provide the opportunity to produce novel species of seaweed with high value, many with morphologies or sizes not suitable for traditional ocean-based production methods (Mata et al. 2010; Magnusson et al. 2014). Research on the land-based cultivation of seaweeds was initially driven by the use of seaweed as a nutrient scrubber of the waste waters from the intensive aquaculture of animals (Ryther et al. 1975; Lapointe et al. 1976). However, it is the high biomass productivity per unit surface area that has maintained commercial interest in land-based cultivation systems with biomass productivities above 20 g dry weight (dw) m^{-2} day^{-1}, and up to 100 g dw m^{-2} day^{-1}, being achieved in pilot-scale cultivation systems (Mata et al. 2010; Bruhn et al. 2011). Notably, these values exceed those of highly productive kelp forests (Kelly and Dworjanyn 2008) or terrestrial crops (McKendry 2002) and approach the upper theoretical limits of photosynthetic productivity (Grobbelaar 2009). These systems are highly suited to cultivating seaweeds with vegetative or clonal propagation, avoiding the need to control complex life cycles. Furthermore, they provide a higher level of control over the quantity and quality of biomass, traceability and security compared to traditional cultivation methods (Hafting et al. 2012; Pereira et al. 2013). However, despite all of the advantages offered by land-based cultivation, it is most challenging because of its capital-intensive nature with high infrastructure, maintenance and energy requirements (Huguenin 1976; Bidwell et al. 1985). These demands have restricted the production of seaweeds in land-based systems to those with high value as food or as a feedstock for bioproducts

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such as nutraceuticals and cosmaceuticals (Gellenbeck 2012; Hafting et al. 2012).

One of the key steps in the development of the production of land-based biomass at a commercial scale is the demonstration that high productivities of algal biomass, and subsequently derived bioproducts, can be attained consistently and at scales and timeframes relevant to commercial production (Gellenbeck 2012; Hafting et al. 2012). Few studies to date have reported the continuous production of biomass at a scale greater than thousands of litres and timeframes of more than 6 months (but see Bidwell et al. 1985; Capo et al. 1999; Bolton et al. 2009; Buschmann et al. 1994). Furthermore, there are even fewer studies that quantify the biochemical composition of the produced biomass over this timeframe. This is important as the value derived from species targeted for intensive land-based culture will be, to a large part, dependent on their biochemical composition, which can vary markedly across the year in both wild harvested and cultivated biomass (Buschmann et al. 1994; Adams et al. 2011; Schmid et al. 2014; Shuuluka et al. 2013).

In this study, we cultivated the green seaweeds *Derbesia tenuissima* and *Ulva ohnoi* in an intensive land-based cultivation system (10,000-L tank units) over 6 months and assessed the comparative production of algal biomass and bioproducts. The filamentous seaweed *D. tenuissima* was selected as it has high lipid content (up to 12 % dw) and total fatty acid (TFA) content of 5 % dw of which 30 % are nutritionally important omega-3 polyunsaturated fatty acids (PUFAn-3) (Gosch et al. 2012). *D. tenuissima* has undergone initial assessment in intensive land-based cultivation with high biomass productivity and the highest productivity of lipids and fatty acids from cultivated macroalgae (Magnusson et al. 2014). Consequently, *D. tenuissima* is a novel target for production in land-based cultivation systems. In contrast, *U. ohnoi* is a species of the genus *Ulva* which has been a benchmark for land-based cultivation. *Ulva* is cultivated in tanks to remediate waste waters from the commercial production of abalone, and the produced biomass is used as feeds for the cultured animals (Bolton et al. 2009). However, *Ulva* biomass also has potential as a resource for high-value bioproducts as it contains unique soluble fibres—sulphated polysaccharides termed ulvans—which are functional biopolymers with uses in biomedical tissue engineering, regenerative medicine and drug delivery (Lahaye and Robic 2007; Chiellini and Morelli 2011). Therefore, *U. ohnoi* is both a suitable benchmark for *D. tenuissima* and a target for the production of ulvans in tropical regions. Furthermore, both *D. tenuissima* and *U. ohnoi* are a potential resource for protein for human and animal nutrition (Boland et al. 2013; Angell et al. 2014; Neveux et al. 2014). Finally, the extraction of multiple co-products (the biorefinery concept) including lipids (fatty acids), proteins (amino acids) and carbohydrates provides an opportunity to enhance the value of the biomass (Neveux et al. 2014).

Therefore, the specific objectives of this study were to (1) quantify the seasonal productivity of biomass of both seaweeds by assessing weekly variation in growth, (2) quantify the lipid, fatty acid and PUFAn-3 content in *D. tenuissima* and soluble fibres (ulvans) in *U. ohnoi* by assessing temporal variation and finally (3) quantify the lipid, fatty acid, soluble fibre and amino acid content in the biomass produced within 6 months to calculate the productivity of bioproducts from *D. tenuissima* and *U. ohnoi*.

Materials and methods

The seaweed *D. tenuissima* (Moris & De Notaris) P.L. Crouan & H.M. Crouan was collected from a shallow subtidal rock platform at Rowes Bay, Townsville, Australia (latitude: 19.14 S, longitude 146.48 E) in August 2010. The seaweed *U. ohnoi* Hiraoka and Shimada was collected from the bioremediation ponds of an aquaculture facility in Guthalungra, Queensland, Australia (19° 55′ 27″ S, 147° 50′ 37″ E) in December 2010. This species was genetically identified using DNA barcoding with the markers ITS and tufAas *U. ohnoi* (Lawton et al. 2013). Both seaweeds were maintained at the Marine & Aquaculture Research Facility Unit (MARFU) at James Cook University (JCU), Townsville, Australia (latitude 19.33 S, longitude 146.76 E) in 60-L cylindrical tanks and scaled up to 2000-L cylindrical tanks, where they were held for almost 3 years prior to being transferred to the large-scale cultivation trial using 10,000-L parabolic tanks in another recirculation cultivation system at MARFU.

Cultivation system

The large-scale recirculation cultivation system consisted of six white fibre glass parabolic tanks (7.2 m long by 2.2 m wide and 0.89 m deep in the centre; 13 m^3) with opaque walls, exposed to full natural sunlight (Online Resource 1). To circulate the algae within the tanks, an air blower (Dargang, model DG-400) provided aeration and agitation at a rate of 2.8 m^3 min^{-1} through perforated airlines (25 mm in diameter with 2-mm holes every 15 cm) installed longitudinally along the bottom centreline of the parabolic tanks. The seawater level was set to 10 cm below the rim of the tank (total volume approx. 10,000 L of water) by fitting a 50-mm-diameter PVC stand pipe in the bottom outflow of the tanks. To retain the algae in the tanks, a 300-mm-diameter stand pipe with open windows covered by a 450-μm mesh screen was placed around the internal 50-mm stand pipe.

The water from the six tanks flowed by gravity to a 5000 L water reservoir (sump; Fig. 1). This water was then pumped from the sump and redirected in two ways: (1) recirculated back to the sump passing through a heater/chilling unit (Evoheat, DHP603, water volume of 9 m^3 h^{-1}) and a protein

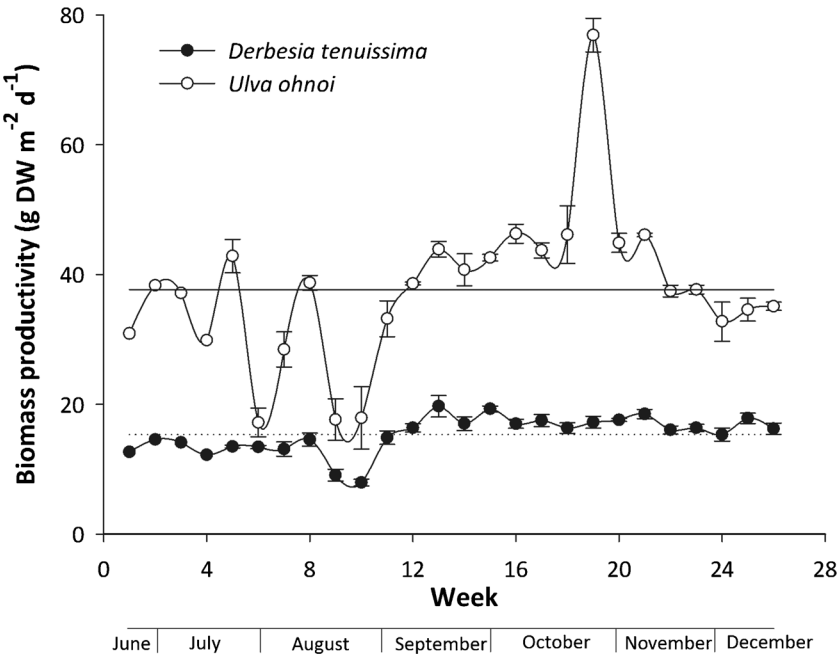

Fig. 1 Biomass productivity (g dw m^{-2} day^{-1}) of *D. tenuissima* and *U. ohnoi* with a biomass stocking density of 1 g fresh weight L^{-1} during 6 months (26 weeks). Mean±SE, n=3, except the first four weeks (n=1)

skimmer and (2) through a water filtration system which consists of a sand filter (Olga, Panter series ii P33), two C75 Trimline bag filters (100 μm; 0.27 m^2) and a UV filter (Smart HO UV Sterilizer 150 Watt 230 VAC), followed by a speece cone to dissolve CO_2 in the water before entering the six tanks and flowing back to the sump. The heater/chiller unit was set to operate when the water temperature in the sump was outside the range of 24–28 °C. A pH solenoid connected to a pH probe submerged in the water in the sump triggered the injection of CO_2 through the speece cone when the pH was above 8. To ensure that all six tanks had similar nutrient and carbon availability, the valves of the inflow water were manually adjusted to supply a water flow of approximately five tank volumes per day (vol day^{-1}). The volume of water entering the tanks was measured before and after the sand filter backwash (twice a day) and adjusted when necessary. The measured water flowing to the tanks ranged between 27 and 45 L min^{-1} throughout the experimental period, equivalent to water renewal rates between 4 and 6.5 vol day^{-1}.

The seaweed cultivation system (total volume of 65,000 L) received nutrient-rich waste water from a fish (barramundi) broodstock facility at MARFU. The waste water (approximately 600 L) from the backwash of sand-filters in the fish system was drained to the sump of the seaweed system daily, adding inorganic nutrients and compensating for water evaporation losses from the seaweed system. The levels of nitrate and phosphate in the seaweed system were analysed three times a week (Hach, DR800 portable colorimeter), and N ($NaNO_3^-$) and P (NaH_2PO_4) were added to maintain levels at approximately 10 and 2 mg L^{-1}, respectively. To supplement the culture with trace elements, a comprehensive algae culture medium (MAF, Manutech Pty Ltd) was added fortnightly to the water in the system at 0.1 g L^{-1} (6 kg).

Abiotic data collection

The incident irradiance (photosynthetic active radiation; PAR) on the culture system was logged at 5-min intervals using a Li-190SA Quantum Sensor connected to a Li-1400 Data Logger (Li-CorUSA). The temperature of the water entering the tanks was recorded every 15 min using a temperature logger (Onset, HOBO UA-001-08). The total PAR was summed for each week of production (mol photons m^{-2} $week^{-1}$). Salinity and pH (inflow and outflow water of each tank) were recorded daily (2 pm) using a TPS WP-81 probe, and salinity was adjusted when necessary with dechlorinated water or sea salt to maintain values between 32 and 35 ‰.

Biomass productivity

The biomass productivity of *D. tenuissima* and *U. ohnoi* was quantified weekly from June to December 2013 (6 months). Tanks were initially stocked at three densities for each species (n=1) to determine the best density to optimise weekly productivity (data not shown). After 4 weeks, all the tanks (n=3) were stocked at the optimum density of 1 g fresh weight (fw) L^{-1} (0.63 kg fw m^{-2}; 10 kg $tank^{-1}$) for each species for the remainder of the experiment. The seaweeds were manually harvested from each tank every 7 days using large nets and then placed inside cloth bags. The bags were spun in a domestic washing machine (1000 rpm) to remove excess water, and then 0.30 kg of spun biomass was combined from all of the tanks and subsequently divided equally between the three

tanks, at an initial stocking density of 10 kg tank^{-1}, to ensure homogeneous cultures. Five sub-samples of freshly spun algae were immediately weighed (10 g fw of *U. ohnoi* and 30 g fw of *D. tenuissima*) at every harvest and dried overnight at 60 °C to determine the fresh weight to dry weight ratios (fw/dw). Initial and final fw/dw ratios were used to calculate the dried biomass productivity per surface area using the equation:

$$P = \left[(\mathrm{fw}/(\mathrm{fw}:\mathrm{dw}))_{\mathrm{f}} - (\mathrm{fw}/(\mathrm{fw}:\mathrm{dw}))_{\mathrm{i}}\right]/A/t$$

where *fw* and *dw* are, respectively, the spun fresh and dry weights at the end ($_f$) and beginning ($_i$) of the production weeks, A is the culture surface area of the tanks (m^2) and t is the number of days in culture.

Biomass samples for biochemical analysis were also collected from each of the six tanks at every harvest, rinsed in running freshwater for 1 min to remove the excess salt, spun and subsequently dried in a food dehydrator at 60 °C for at least 24 h. The dried biomass (approximately 30 g dw) was homogenised using a domestic food processor and stored inside a zip lock bag at −20 °C prior to biochemical analysis.

Bioproducts

Temporal variation in key bioproducts The quantity of key bioproducts was analysed for each species every 4 weeks (beginning at week 6) to assess any potential variation over the 6-month period. For *D. tenuissima*, this is the oil bioproducts of total lipids, TFA and PUFAn-3, and for *U. ohnoi* the soluble fibres, which are mainly comprised of the high-value polysaccharide ulvan (Lahaye and Robic 2007). Carbon (C), nitrogen (N) and ash (dry inorganic) content were also analysed for both species to assess the variation in the ultimate composition over time and to complement the analysis of lipids and fatty acid in *D. tenuissima* and of soluble fibre in *U. ohnoi*.

Overall quantity and productivity of bioproducts A bulk sample (100 g dw) was generated for each species at the end of the 6-month trial period to represent the overall biomass produced during this time. To provide representative biomass for the 6 months of production, sub-samples of the stored dried biomass from each tank were added in proportion to their contribution to overall production. The bulk sample of each species was subsequently milled, homogenised and analysed in duplicate for total lipids, fatty acids, fibres, amino acids, ash and moisture content. The theoretical protein content was calculated from the sum of all amino acids. Total carbohydrates were determined by difference, by subtracting ash, moisture, total lipid and protein contents from 100 %. These values used to calculate the projected annual productivities of each bioproduct for each species were calculated by multiplying the content of individual bioproducts in the bulk sample by the annual biomass productivity estimation for each species using the equation:

$$P_{\mathrm{bioproduct}} = (P_{\mathrm{biomass}} * C)/100$$

where $P_{\mathrm{bioproduct}}$ is the productivity of a bioproduct (t ha^{-1} year^{-1}), P_{biomass} is the productivity of the biomass (t ha^{-1} year^{-1}) and C is the concentration of the bioproduct as percentage of algal dw. This is important because the higher biomass productivity of a species can offset the lower content for a specific bioproduct.

Biochemical analyses

A sub-sample of 200 mg was used for carbon (C) and nitrogen (N) analysis at the OEA Laboratory Ltd., UK (http://www.oealabs.com). Ash content (dry inorganic) was determined by combusting a sub-sample (~500 mg dw) of the dried material in a muffle furnace at 550 °C for 6 h. Moisture content was determined by drying a sample (~1.5 g fw) to constant weight at 110 ° C in a moisture balance (MS70, A&D Company Ltd., Japan). Total lipid was quantified on a 200±0.1 mg sub-sample using solvent extraction (Folch et al. 1957) as described in detail in Gosch et al. (2012). TFAs were extracted and trans-esterified from a separate sub-sample of biomass (30±0.1 mg) following a one-step extraction/trans-esterification method (methanol/acetylchloride, 95:5 *v*/*v*) as described in detail in Gosch et al. (2012). Fatty acid methyl esters (FAMEs) were separated and quantified by gas chromatography–mass spectrophotometry (GC/MS) on an Agilent 7890 GC/5975C EIMS system equipped with a DB-23 capillary column (cyanopropyl stationary phase [60 m×0.25 mm id×0.15 μm], Agilent Technologies, Australia; see Gosch et al. (2012) for further details). The quantity of fatty acids was determined by comparison of peak areas of external standards (SigmaAldrich, Australia) and was corrected for recovery of internal standard (nonadecanoic acid, C19:0). The total, soluble and insoluble fibre content was analysed on a 10-g sample, following standard methods (AOAC Official Method 985.29 total dietary fibre in Foods and AOAC Official Method 993.19 soluble dietary fibre in food and food products) by Grain Growers Ltd (www.graingrowers.com.au). Amino acids were quantified using the Water AccQTag method at the Australian Proteome Analysis Facility (Sydney, Australia). The content of essential amino acids was calculated from the sum of histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine.

Data analysis

The monthly variation in lipid, TFA and PUFAn-3 content in *D. tenuissima* and soluble fibres content in *U. ohnoi* was

analysed by one-way ANOVA and Tukey's honest significant difference (HSD) post hoc tests using one sample from each of the three tanks (one-way ANOVA, Tukey's HSD) using Sigma plot version 12.5 (Systat Software Inc., USA). Relationships between biomass productivity and temperature or light levels were analysed with curves of best fit, and the strength of the relationship is presented as *r* for each fit and a p-value for any linear correlations using Sigma Plot version 12.5.

Results

Biomass productivity

The average biomass productivity of *D. tenuissima* over the 6-month period was 15.3±0.6 g dw m^{-2} day^{-1} (Fig. 1). These values were 2.5 times lower than the average biomass productivity of *U. ohnoi* of 37.7±2.2 g dw m^{-2} day^{-1} over the same period. However, the distinguishing feature of the production of *D. tenuissima* was the consistency of productivity throughout the cultivation period, with weekly productivity within a narrow range of 12.2 and 19.7±1.6 g dw m^{-2} day^{-1}, with the exception of a 2-week period when biomass productivity dropped below 10 g dw m^{-2} day^{-1} (Fig. 1). In contrast, the production of *U. ohnoi* was more stochastic with weekly productivity ranging between 17.2±2.2 and 46.3±1.5 g dw m^{-2} day^{-1}, with an outlier of very high productivity of 76.9 ±2.6 g dw m^{-2} day^{-1} (Fig. 1).

There was a positive relationship between light and biomass productivity with maximum productivities as day length (light) increased into the austral wet season (summer). However, this trend was not as strong for *U. ohnoi* with a number of clear outliers from the fit (p=0.04, r=0.4), compared to *D. tenuissima* (p=0.001, r=0.6) (Fig. 2a). There was a similar positive relationship between temperature and biomass productivity where the productivity of *U. ohnoi* increased up to a daily average water temperature of 28 °C but then declined beyond this optimal temperature over the summer (polynomial fit, r=0.5) (Fig. 2b). The productivity of *D. tenuissima* increased with water temperature to a daily average of almost 30 °C, but in a similar manner to *U. ohnoi*, the maximum productivity values were achieved at 28 °C (r=0.6) (Fig. 2b). Both species tolerated minimum and maximum water temperatures of 18 and 34.5 °C, respectively, over the study period (Online Resource 2).

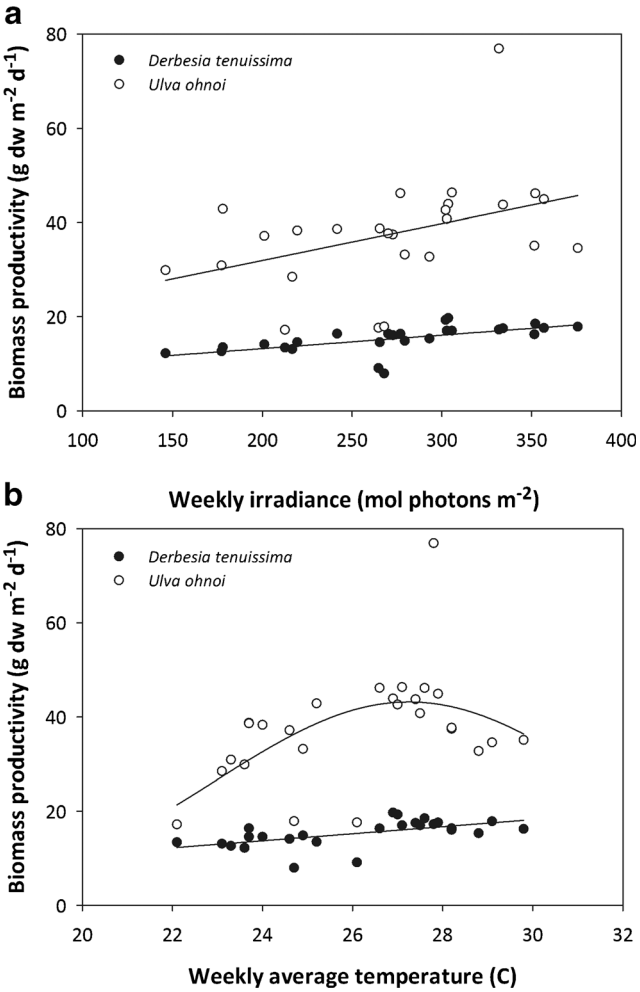

Fig. 2 Correlation of biomass productivity(g dw m^{-2} day^{-1}) of *D. tenuissima* and *U. ohnoi* with weekly irradiance (mol photons m^{-2} $week^{-1}$) (**a**) and mean weekly water temperature (°C) (**b**)

Bioproducts

Temporal variation in key bioproducts The content of total lipids in *D. tenuissima* was consistent over the experimental period at 13 % dw, with the exception of week 6 where it reached a maximum of at 15.8±0.5 % dw (ANOVA: $F_{5,17}$= 9.434, p<0.05, Tukey's HSD) (Fig. 3). In a similar manner, the total fatty acid content was relatively consistent with a minimum of 4.4±0.3 % dw and a maximum of 5.9±0.2 % dw, with different values only between week 6 and week 10 (ANOVA: $F_{5,17}$=21.626, p<0.05, Tukey's HSD) (Fig. 3). However, the quantity of the total PUFAn-3 was more variable over time with values above 2 % in week 6 (2.6±0.1 % dw) and week 22 (2.1±0.1 % dw), with more consistent values between 1.5 % dw and 1.7 % dw for the remaining weeks (ANOVA: $F_{5,17}$=23.623, p<0.05, Tukey's HSD) (Fig. 3). Although variations in biomass productivity of *D. tenuissima* were partially explained by changes in light and water temperature, there were no significant trends between these environmental parameters and the content of lipids or fatty acids (p>0.1 for all correlations; data not shown). The content of soluble fibres in *U. ohnoi* was also consistent throughout the cultivation period, varying between 11.3±2.7 and 12.6±1.6 % dw (Fig. 4). The mean nitrogen content in *D. tenuissima* was 6 % dw and was higher and more consistent than the nitrogen content in *U. ohnoi* (Table 1). Both species had approximately

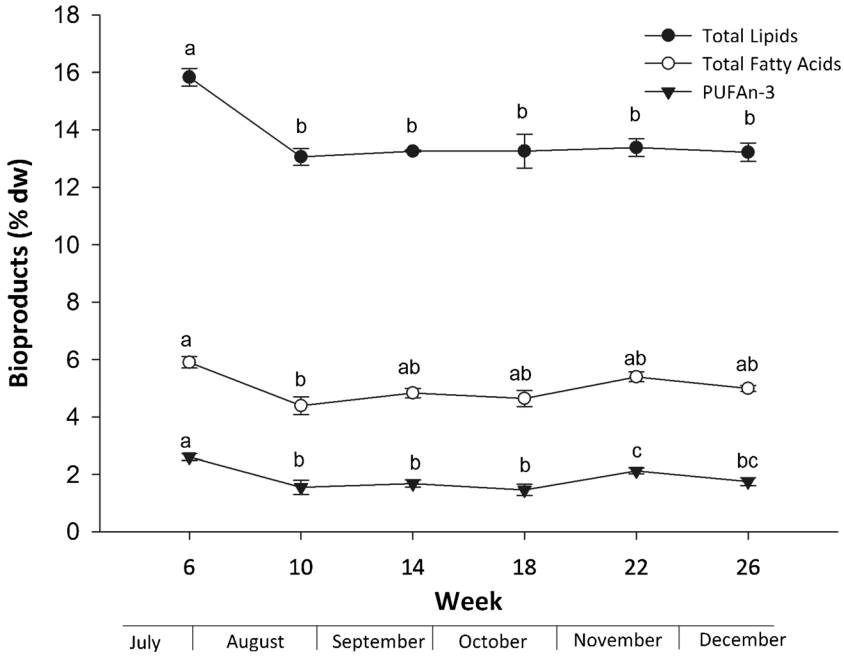

Fig. 3 Total lipids, total fatty acids and omega-3 polyunsaturated fatty acids content(% dw) in *Derbesiatenuissima* measured every fourth week (starting in week 6). Mean±SE, n=3

25 to 27 % of ash in dry matter. However, the ash content in *U. ohnoi* peaked up to 40 % dw (Table 1), coincident with peaks in biomass productivity.

Overall quantity and productivity of bioproducts Overall, the two species had distinct bioproduct profiles (Table 2) with *D. tenuissima* having seven times more lipids (15.2 % dw) and almost twice the protein (24.4 % dw) of *U. ohnoi* (2.3 % dw lipids and 13.3 % dw protein). Consequently, *U. ohnoi* had almost twice the carbohydrate (43.3 % dw) of *D. tenuissima* (27.1 % dw). Ash was the second largest component of the biomass after carbohydrate for *U. ohnoi* and after protein for *D. tenuissima*, with 33 and 28 % dw, respectively (Table 2).

D. tenuissima was richer than *U. ohnoi* in all fatty acids, except for stearidonic acid (SDA, 18:4 n-3) with 0.07±0.0 % dw (3.3 % of the TFA), five times lower than in *U. ohnoi* with0.34 ±0.01 % dw (17 % of the TFA) (Table 3). The most abundant PUFAn-3 in *D. tenuissima* was α-linolenic acid (ALA, 18:3) (1.14±0.02 % dw and 22.6 % of the TFA), which was an order of magnitude lower in *U. ohnoi* biomass (0.31±0.01 % dw and 15 % of the TFA). The proportion of saturated and unsaturated fatty acids (expressed as a relative amount of TFA) in

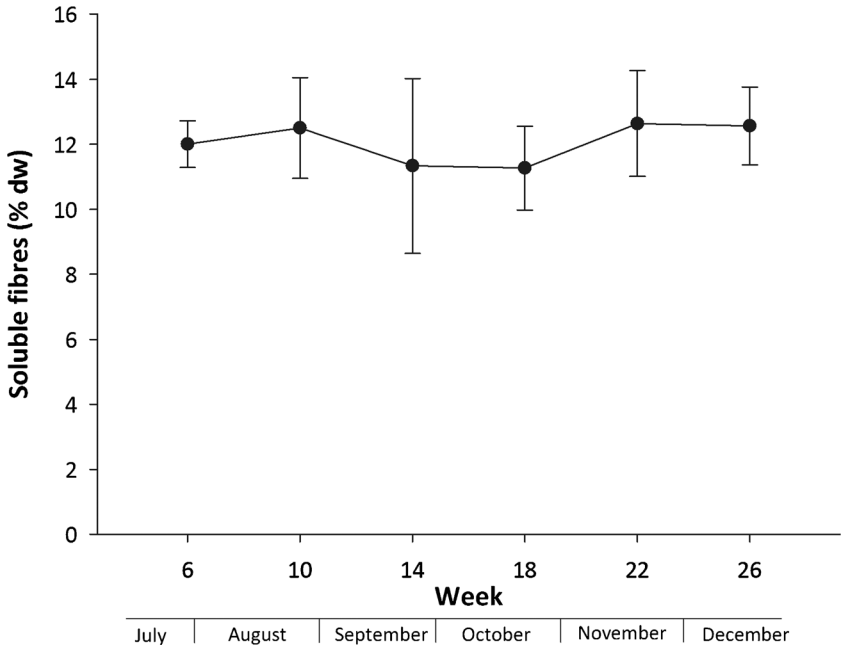

Fig. 4 Soluble fibres (% dw) in *Ulva ohnoi* measured every fourth week (starting in week 6). Mean± SE, n=3

Table 1 *Derbesia tenuissima* and *Ulva ohnoi* composition in terms of carbon (C), nitrogen (N) and ash in the three culture tanks every 4 weeks (mean % dw±SE, n=3)

	D. tenuissima			*U. ohnoi*		
Week	C	N	Ash	C	N	Ash
6	36.7±0.7	6±0.1	26.5±0.6	22.9±0.4	3.3±0.1	33.1±0.9
10	35.1±0.3	5.2±0.1	26.6±0.4	27.4±0.1	4.0±0.0	24.5±0.5
14	34.7±0.4	6.1±0.2	28.7±0.6	29.7±0.1	4.7±0.1	25.9±0.5
18	36.4±0.3	6.1±0.2	27.3±0.8	24.9±0.8	3.9±0.2	39.5±2.3
22	36.7±0.7	6.2±0.1	27.7±0.5	28.5±0.1	4.2±0.0	26.6±0.9
26	36.0±0.6	6.0±0.1	27.3±0.3	29.9±0.1	4.6±0.0	26.2±0.6

both species was similar, with unsaturated fatty acids representing approximately 60 % of the TFA (Table 3). PUFAn-3 made up 38 % of the TFA content in *D. tenuissima* and 42 % in *U. ohnoi*, resulting in nutritionally beneficial low PUFAn-6/n-3 ratios of 0.32 and 0.06, respectively (Table 3). *D. tenuissima* had a much higher content of protein and all amino acids than *U. ohnoi*, but the quality of the protein (expressed as a relative amount of essential amino acids to total amino acids) was similar between the two species (Table 3). The overall essential amino acids are well represented (41 % of the total amino acids) in the protein of both species. Furthermore, the protein of *D. tenuissima* and of *U. ohnoi* contained 4.9±0.4 % dw (1.6 % of the TAA) and 2.0±0.1 % dw (1.2 % of the TAA) of methionine and 15.2±0.1 % dw (5.2 % of the TAA) and 8.1±0.1 % dw (4.9 % of the TAA) of lysine, respectively (Table 3).

Conversely, *U. ohnoi* carbohydrates were richer in fibres, particularly of soluble fibres, than *D. tenuissima* (Table 2).

Table 2 Proximate and biochemical analysis of bulk sample of *D. tenuissima* and *U. ohnoi*. Data show values of lipids (total fatty acids and polyunsaturated omega-3), proteins (essential and non-essential amino acids), carbohydrates (soluble and insoluble fibres), ash and moisture contents of a combined sample containing all the biomass produced during the entire cultivation period (mean % dw±SE, n=2). Protein equals the sum of amino acids. Carbohydrate content was determined by difference

Composition (% dw)	*D.tenuissima*	*U. ohnoi*
Lipids	15.2±1.5	2.3±0.3
TFA	5.1±0.7	2.0±0.0
PUFAn-3	1.9±0.1	0.9±0.0
Proteins	24.4±0.1	13.3±0.3
Essential amino acids	10.0±0.0	5.5±0.1
Non-essential amino acids	14.3±0.1	7.9±0.2
Carbohydrates	27.1±0.4	43.3±0.3
Soluble fibres	0.9±0.8	11.9±0.3
Insoluble fibres	14.8±0.3	19.1±0.1
Ash	27.6±1.9	32.5±4.2
Moisture	5±0.9	8.6±1.7

U. ohnoi had 11.9 % dw of soluble fibres with *D. tenuissima* less than 1 % dw.

Extrapolating the data in this study to estimate the productivities of these bioproducts, the land-based cultivation of *D. tenuissima* has the potential to produce 8.5 t lipids ha^{-1} $year^{-1}$, more than twice that of *U. ohnoi* at 3.2 t ha^{-1} $year^{-1}$ (Table 4). However, due to the higher ratio of TFA/lipids in *U. ohnoi* than in *D. tenuissima*, the productivity of TFA between the two species was similar with 2.8 t ha^{-1} $year^{-1}$. Furthermore, the productivity of PUFAn-3 was also similar, with approximately 1.2 t ha^{-1} $year^{-1}$, due to the lower ratio of PUFAn-6/n-3 in *U. ohnoi* than in *D. tenuissima*. Similarly, despite the protein content in *D. tenuissima* being almost double that of *U. ohnoi* (Table 2), this was offset by the higher annual productivity of *U. ohnoi* with a higher protein productivity of 18.4 t ha^{-1} $year^{-1}$ for *U. ohnoi* compared to 13.7 t ha^{-1} $year^{-1}$ for *D. tenuissima* (Table 4). And finally, *U. ohnoi* has the potential to produce 42.8 t ha^{-1} $year^{-1}$ of carbohydrates, almost three times that of *D. tenuissima* at 15.2 t ha^{-1} $year^{-1}$. Furthermore, *U. ohnoi* can produce 16 t ha^{-1} $year^{-1}$ of soluble fibre (including ulvan) with *D. tenuissima* producing negligible amounts of this bioproduct.

Discussion

This study has demonstrated that intensive land-based seaweed cultivation systems at scale are highly productive with the advantage of producing biomass with a consistent biochemical profile for the extraction of multiple bioproducts. *D. tenuissima* has been established as a novel species in large-scale land-based cultivation with consistent productivities of biomass and bioproducts, specifically fatty acids and the nutritionally important PUFAn-3. In contrast, the biomass production of the benchmark species *U. ohnoi* was more stochastic with a lower content of lipid and protein than *D. tenuissima*. However, the productivity of ulvans in *U. ohnoi* was consistent regardless of fluctuations in productivity, and the lower content of lipid and protein was compensated for in terms of annual productivity by higher biomass productivity.

Biomass Overall, the biomass productivity of *D. tenuissima* was 2.5 times lower than the biomass productivity of *Ulva ohnoi*; however, it was more consistent throughout the cultivation period with the productivity of *U. ohnoi* varying stochastically by up to 100 % between weeks. Consistency in biomass productivity has been highlighted as an imperative feature for the successful production of seaweeds as it simplifies the management routines of biomass harvest and processing and assures a continuous and stable supply of the same raw material into the market (Gellenbeck 2012). Interestingly, highly variable rates of productivity are a common rather than exceptional feature in the land-based production of species of

Table 3 Fatty acids and amino acids profile of *Derbesia tenuissima* and *Ulva ohnoi*. Data show the values of fatty acids (mg g^{-1} dw) and values of α-amino acids (mg g^{-1} dw, tryptophan and cysteine not included) of a combined sample containing proportional amounts of all the biomass produced during the entire cultivation period (mean % dw±SE, n=2)

Elements	Seaweed species		Elements	Seaweed species	
Fatty acid concentration	*Derbesia*	*Ulva*	Amino acid concentration	*Derbesia*	*Ulva*
C14:0	0.55±0.01	0.14±0.00	Histidine	4.8±0.0	2.6±0.0
C15:0	0.86±0.01	0.27±0.01	Serine	12.1±0.0	6.7±0.1
C16:0	14.59±0.18	7.06±0.15	Arginine	13.8±0.0	8.7±0.3
C16:1 (n-7)	0.63±0.01		Glycine	14.1±0.0	8.5±0.2
C16:1 (n-9)	2.46±0.07	1.21±0.03	Aspartic acid/asparagine	24.0±0.3	16.4±0.5
C16:2 (n-6)	0.59±0.01	0.04±0.00			
C16:3 (n-3)	5.49±0.10	0.08±0.00	Glutamic acid/glutamine	41.8±0.2	16.7±0.5
C16:4 (n-3)	0.54±0.01	1.92±0.08			
C18:0	0.74±0.01	0.09±0.01	Threonine	12.5±0.0	6.1±0.1
C18:1 (n-9)	2.07±0.04	1.43±0.02	Alanine	17.8±0.1	11.3±0.2
C18:2 (n-6)	2.33±0.02	0.52±0.02	Proline	11.1±0.1	7.1±0.1
C18:3 (n-6)	1.04±0.03		Lysine	15.2±0.1	8.1±0.1
C18:3 (n-3)	11.44±0.22	3.05±0.06	Tyrosine	9.2±0.2	3.3±0.2
C18:4 (n-3)	0.68±0.01	3.38±0.10	Methionine	4.9±0.4	2.0±0.1
C20:3 (n-6)	0.46±0.01		Valine	16.2±0.0	9.5±0.1
C20:4 (n-6)	1.76±0.05		Isoleucine	11.4±0.0	6.6±0.1
C20:5 (n-3)	1.11±0.04	0.18±0.01	Leucine	20.6±0.0	11.6±0.1
C22:0	0.86±0.02	0.33±0.00	Phenylalanine	14.6±0.0	8.5±0.1
C20:5 (n-3)	1.11±0.04	0.18±0.01	Total AA	244.0±0.2	133.5±2.0
C22:0	0.86±0.02	0.33±0.00	Essential (%TAA)	41.2	41.1
C22:2 (n-6)	0.55±0.04		Methionine (%TAA)	1.6	1.2
C24:0	1.92±0.08	0.70±0.07	Lysine (%TAA)	5.2	4.9
Total FAs	50.7±1.17	20.4±0.3	Non-essential (%TAA)	58.8	58.9
SFA (%TFA)	38.3±0.38	42.13±0.60			
MUFA (%TFA)	10.12±0.16	12.91±0.10			
PUFA (%TFA)	51.58±0.37	44.96±0.69			
PUFAn-3 (%TFA)	38.0±0.48	42.22±0.64			
PUFAn-6 (%TFA)	12.4±0.19	2.74±0.08			
n-6/n-3	0.33±0.01	0.06±0.00			

FAs fatty acids, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *AA* amino acids

Table 4 Annual productivities (t dw ha^{-1} $year^{-1}$) of bioproducts from *D. tenuissima* and *U. ohnoi* produced over 6 months

Productivity (t dw ha^{-1} $year^{-1}$)	*D.tenuissima*	*U.ohnoi*
Lipids	8.5	3.2
Total fatty acids	2.8	2.8
PUFAn-3	1.1	1.2
Proteins	13.7	18.4
Essential amino acids	5.6	7.6
Non-essential amino acids	8.0	10.9
Carbohydrates	15.2	37.4
Soluble fibres	0.50	16.4
Insoluble fibres	8.3	26.4

PUFAn-3 polyunsaturated omega3 fatty acids

Ulva (Ryther et al, 1984; Del Campo et al. 1998; Robertson-Andersson et al. 2008; Bolton et al. 2009; Bruhn et al. 2011). Several causative factors have been proposed for these variable productivities, ranging from epiphytic and endophytic infestations (Robertson-Andersson et al. 2008; Bolton et al. 2009), perforations of the thalli from unknown causes (Ryther et al, 1984; Del Campo et al. 1998) or reproductive events (Bruhn et al. 2011). There is, however, commonality in symptoms associated with these events with the end result being a systemic degradation of the thallus, with a significant decrease in productivity and, in extreme cases, the complete loss of biomass. Notably, neither *D. tenuissima* nor *U. ohnoi* biomass had to be replenished with new inoculum throughout the 6-month culture period, even though *U. ohnoi* went through changes in size, morphology and thickness of the thallus (Online Resource 3).

In intensive seaweed cultivation, where nutrients are not limited, fluctuations in the production of biomass are commonly related to seasonal changes in abiotic factors such as light (irradiance) and temperature (Friedlander et al. 1990). We found that there were predictable effects of both light and water temperature on biomass productivity of the two species, noting the two seasons in the austral tropics (dry April–September and wet October–March). The productivity of both species peaked at 28 °C, and both tolerated the minimum and the maximum water temperatures of 18 and 34.5 °C, respectively, over the study. This is in accordance with laboratory experiments on the response of specific growth rate of *U. ohnoi* to temperature, where this strain had maximum growth at 28.5 °C, the upper monthly average sea surface temperature at Townsville (Lawton et al. 2013). In contrast to *U. ohnoi*, there are no data on physiological tolerances and responses of *D. tenuissima* to environmental variables. A broad tolerance to temperature is a desirable characteristic for target species in large-scale land-based cultivation systems, where the control over abiotic factors is limited.

The extrapolated annual biomass productivities were high for both species at 56 t dw ha^{-1} $year^{-1}$ for *D. tenuissima* and 138 t dw ha^{-1} $year^{-1}$ for *U. ohnoi*. This study ran for 6 months across two seasons in the austral tropics, and therefore the extrapolated annual productivities are defensible. These annual productivities are within the range of values reported for the long-term production of seaweeds at scales relevant to commercial production (thousands of litres) (Bidwell et al. 1985; Capo et al. 1999; Bolton et al. 2009). Annual productivities (t dw ha^{-1} $year^{-1}$) in long-term studies range from 84 t dw ha^{-1} $year^{-1}$ [*Ulva lactuca* over two consecutive years (Bolton et al. 2009)] through to 99 t dw ha^{-1} $year^{-1}$ [*Chondrus crispus* over 4 years (Bidwell et al. 1985)] and 146 t dw ha^{-1} $year^{-1}$ [*Gracilaria ferox* over 4 years (Capo et al. 1999)]. The annual productivity of *D. tenuissima* is the lowest of these, but notably it is still more than twice the annual productivity of highly productive terrestrial crops such as wheat (McKendry 2002) and at least an order of magnitude higher than traditional terrestrial oil seeds (Jaeger and Siegel 2008) and soybean (Ainsworth et al. 2012). The high productivities of land-based systems for the cultivation of seaweeds are a consequence of the high photosynthetic rates achieved by the algae in culture conditions with vertical mixing (Grobbelaar 2009, 2010).

Bioproducts This study highlights that intensive land-based cultivation systems, even at large-scale where the control over abiotic factors is limited, can assure a continuous and stable supply of the same raw material quality into the market. The content of key bioproducts, such as total lipids and TFA, in *D. tenuissima* was consistent over the experimental period. In contrast, seaweeds in the wild generally show steep fluctuations in the content and quality of lipids and fatty acids across seasons (Gosch et al. 2015; Schmid et al. 2014). The content of soluble fibres in *U. ohnoi*, which are mainly comprised of the high-value sulphated polysaccharide ulvan (Lahaye and Robic 2007),was also consistent throughout the cultivation period. Despite some inferences in the literature to seasonal variation in the concentration of ulvans, no studies have yet demonstrated links to environmental conditions (Robic et al. 2009). Castelar et al. (2014) measured similar concentrations of ulvan in *Ulva flexuosa* cultivated in tanks and at sea despite the contrasting environments. In our study, the concentrations of soluble fibre of *U. ohnoi* are lower than those in other species of *Ulva*, either collected from the wild or cultivated in tanks, which range from 15 to 21 % dw (Lahaye and Jegou 1993; Lahaye et al. 1995). The differences in concentration may be associated with taxonomy, post-harvest treatment of the biomass and/or extraction protocols (Lahaye and Robic 2007). However, the relatively low concentration of soluble fibres in *U. ohnoi* may also be explained by a high protein content (up to 13.3 % dw of amino acids; Table 2) characteristic of biomass grown in non-nitrogen-limiting conditions (Lahaye et al. 1995).

Both the content of nitrogen and of ash in the dry biomass of *D. tenuissima* were consistent over the cultivation period, in contrast to that of *U. ohnoi*. The peaks of ash content in *U. ohnoi* were coincident with peaks in biomass productivity. Interestingly, the concentration of soluble fibres in *U. ohnoi* was not affected by the drastic changes in the ash content over the study period, and correspondingly the productivity of this bioproduct can be calculated directly from biomass productivity values.

Overall, the largest difference in the biochemical composition of species was between the content and quality of the carbohydrates. The total level of carbohydrates was substantially lower in *D. tenuissima* (27 % dw) than in *U. ohnoi* (43 % dw). Generally, the contents of carbohydrates in seaweed species are greater than 35 % dw and can reach up to 74 % dw (Ito and Hori 1989). However, green seaweeds of the order Bryopsidales, including *D. tenuissima*, are "giant single-cell" algae, which lack or have few cellular cross walls. Because carbohydrates are mainly involved in the formation of the cell wall (Lobban and Harrison 1996), these species have a low content of carbohydrates. Furthermore, the constituents of the cell walls of *D. tenuissima* and *U. ohnoi* are distinct. The cell wall of *U. ohnoi* is rich in uronic acids with polysaccharides containing rhamnose (ulvans), whereas the cell walls of *D. tenuissima* are rich in insoluble fibres (Domozych et al. 2012).

D. tenuissima was much richer in lipids than *U. ohnoi*. However, the proportion of saturated and unsaturated fatty acids (expressed as a relative amount of TFA) in both species was similar, with unsaturated fatty acids representing approximately 60 % of the TFA. This is of particular importance because the quantity of specific fatty acids determines the

potential application and the value of the biomass. Of the unsaturated fatty acids, the PUFAn-3 are the most critical with roles in human health and of particular interest for the nutraceutical market (Saravanan et al. 2010). Both species present nutritionally beneficial low PUFAn-6/n-3 ratios similar to those of other species of seaweeds collected from the sea (Gosch et al. 2012; Schmid et al. 2014) but considerably lower than most traditional terrestrial oil crops, such as soybean and palm oil, which have ratios between 7 and 30 (Dubois et al. 2007). A typical western diet with a high n-6/n-3 ratio has been linked to health problems including cancer and cardiovascular diseases (Russo 2009). The most abundant PUFAn-3 was α-linolenic acid (ALA, 18:3) in *D. tenuissima* and stearidonic acid (SDA, 18:4 n-3) in *U. ohnoi*. These two PUFAn-3 are important in cardiovascular health (Guil-Guerrero 2007) and are associated with decreased blood pressure, improved heart and liver function and the reduction of body fat in animal trials (Poudyal et al. 2012, 2013). Both fatty acids are precursors for the nutritionally essential eicosapentaenoic acid (EPA C20:5n-3), but SDA has a higher conversion efficiency (Guil-Guerrero 2007). EPA is present in fish oils and algal oils but is absent in traditional terrestrial oil crops, such as flaxseed or canola, which have the precursor in the form of ALA but lack the most efficient precursor, SDA (Dubois et al. 2007). These seaweeds can therefore provide specific nutritionally beneficial fatty acids that are notably distinct to the fatty acids from traditional terrestrial oil crops.

D. tenuissima had a much higher content of all amino acids, but the quality of the protein (expressed as a relative amount of total amino acids) was similar between the two species. Among the essential amino acids, methionine and lysine are often limiting in terms of the nutritional value of plant-based diets for animal production (Boland et al. 2013). The protein of *D. tenuissima* and of *U. ohnoi* contained 1.6 and 1.2 % of methionine and 5.2 and 4.9 % of lysine, respectively, making them suitable to complement terrestrial plant protein (soybean) meal in food and animal feed industries. Soybean meal (defatted) with a protein content above 45 % contains 1.3 % of methionine and 6.2 % of lysine as a comparison (Li et al. 2011).

Despite the higher amounts of fatty acids and amino acids in *D. tenuissima* than in*U. ohnoi*, the amount of the bioproducts produced per unit area was similar or even higher for *U. ohnoi* due to its higher annual biomass productivity. These scenarios are relevant where a biorefinery approach is taken for the biomass, with multiple co-products being extracted from a feedstock to maximise the value of the biomass (Neveux et al. 2014). As a comparison, the average annual productivity of soybean is ~3 t ha^{-1} $year^{-1}$ (Ainsworth et al. 2012). Soybean has an average lipid concentration of 18 % and crude protein concentration of 40 % (Grieshop and Fahey 2001), resulting in productivities of 0.5 and 1.2 t ha^{-1} $year^{-1}$, respectively. The land-based cultivation of *U. ohnoi*can produced 2.8 t ha^{-1} $year^{-1}$ of lipids, 18.4 t ha^{-1} $year^{-1}$of protein and has the further advantage of producing 16.4 t ha^{-1} annually of soluble fibre, including the high-value sulphated polysaccharide ulvan, which offers a potential niche opportunity for a biorefinery process.

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References

Adams JM, Ross AB, Anastasakis K, Hodgson EM, Gallagher JA, Jones JM, Donnison IS (2011) Seasonal variation in the chemical composition of the bioenergy feedstock *Laminaria digitata* for thermochemical conversion. Bioresour Technol 102:226–234

Ainsworth EA, Yendrek CR, Skoneczka JA, Long SP (2012) Accelerating yield potential in soybean: potential targets for biotechnological improvement. Plant Cell Environ 35:38–52

Angell AR, Mata L, de Nys R, Paul NA (2014) Variation in amino acid content and its relationship to nitrogen content and growth rate in *Ulva ohnoi* (Chlorophyta). J Phycol 50:216–226

Bidwell RGS, Mclachlan J, Lloyd NDH (1985) Tank cultivation of Irish moss, *Chondrus crispus* Stackh. Bot Mar 28:87–97

Boland MJ, Rae AN, Vereijken JM, Meuwissen MPM, Fischer ARH, van Boekel MAJS, Rutherfurd SM, Gruppen H, Moughan PJ, Hendriks WH (2013) The future supply of animal-derived protein for human consumption. Trends Food Sci Technol 29:62–73

Bolton JJ, Robertson-Andersson DV, Shuuluka D, Kandjengo L (2009) Growing *Ulva* (Chlorophyta) in integrated systems as a commercial crop for abalone feed in South Africa: a SWOT analysis. J Appl Phycol 21:575–583

Bruhn A, Dahl J, Nielsen HB, Nikolaisen L, Rasmussen MB, Markager S, Olesen B, Arias C, Jensen PD (2011) Bioenergy potential of *Ulva lactuca*: biomass yield, methane production and combustion. Bioresour Technol 102:2595–2604

Buschmann AH, Mora OA, Gómez P, Böttger M, Buitano S, Retamales C, Vergara PA, Gutierrez A (1994) *Gracilaria chilensis* outdoor tank cultivation in Chile: use of land-based salmon culture effluents. Aquacult Eng 13:283–300

Capo TR, Jaramillo JC, Boyd AE, Lapointe BE, Serafy JE (1999) Sustained high yields of *Gracilaria* (Rhodophyta) grown in intensive large-scale culture. J Appl Phycol 11:143–147

Castelar B, Reis R, Santos Calheiros A (2014) *Ulva lactuca*and *U. flexuosa* (Chlorophyta, Ulvophyceae) cultivation in Brazilian tropical waters: recruitment, growth, and ulvan yield. J Appl Phyco l26:1989–1999

Chiellini F, Morelli A (2011) Ulvan: a versatile platform of biomaterials from renewable resources. In: Pignatello R (ed) Biomaterials—physics and chemistry. InTech, Rijieka, pp 75–98

Del Campo E, García-Reina G, Correa JA (1998) Degradative disease in *Ulva rigida* (Chlorophyceae) associated with *Acrochaete geniculata* (Chlorophyceae). J Phycol 34:160

Domozych D, Ciancia M, Fangel JU, Mikkelsen MD, Ulvskov P, Willats WGT (2012) The cell walls of green algae: a journey through evolution and diversity. Front Plant Sci 3. doi:10.3389/fpls.2012.00082

Dubois V, Breton S, Linder M, Fanni J, Parmentier M (2007) Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. Eur J Lipid Sci Technol 109:710–732

Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226: 497–509

Friedlander M, Galai N, Farbstein H (1990) A model of seaweed growth in an outdoor culture in Israel. Hydrobiologia 204(205):367–373

Gellenbeck KW (2012) Utilization of algal materials for nutraceutical and cosmeceutical applications—what do manufacturers need to know? J Appl Phycol 24:309–313

Gosch B, Paul N, de Nys R, Magnusson M (2015) Seasonal and within-plant variation in fatty acid content and composition in the brown seaweed *Spatoglossum macrodontum* (Dictyotales, Phaeophyceae). J Appl Phycol 27:387–398

Gosch BJ, Magnusson M, Paul NA, de Nys R (2012) Total lipid and fatty acid composition of seaweeds for the selection of species for oil-based biofuel and bioproducts. GCB Bioenergy 4:919–930

Grieshop CM, Fahey GC (2001) Comparison of quality characteristics of soybeans from Brazil, China, and the United States. J Agric Food Chem 49:2669–2673

Grobbelaar JU (2009) Upper limits of photosynthetic productivity and problems of scaling. J Appl Phycol 21:519–522

Grobbelaar JU (2010) Microalgal biomass production: challenges and realities. Photosynth Res 106:135–144

Guil-Guerrero JL (2007) Stearidonic acid (18:4n-3): metabolism, nutritional importance, medical uses and natural sources. Eur J Lipid Sci Technol 109:1226–1236

Hafting J, Critchley A, Cornish ML, Hubley S, Archibald A (2012) On-land cultivation of functional seaweed products for human usage. J Appl Phycol 24:385–392

Huguenin JE (1976) An examination of problems and potentials for future large-scale intensive seaweed culture systems. Aquaculture 9: 313–342

Ito K, Hori K (1989) Seaweed: chemical composition and potential food uses. Food Rev Int 5:101–144

Jaeger W, Siegel R (2008) Economics of oilseed crops and their biodiesel potential in Oregon's Willamette Valley. Oregon State University, Department of Agricultural and Resource Economics

Kelly ME, Dworjanyn S (2008) The potential of marine biomass for anaerobic biogas production: a feasibility study with recommendations for further research. Crown Estate 103

Lahaye M, Gomez-Pinchetti J-L, del Rio MJ, Garcia-Reina G (1995) Natural decoloration, composition and increase in dietary fibre content of an edible marine algae, *Ulva rigida* (Chlorophyta), grown under different nitrogen conditions. J Sci Food Agric 68:99–104

Lahaye M, Jegou D (1993) Chemical and physical–chemical characteristics of dietary fibres from *Ulva lactuca* (L.) Thuret and *Enteromorpha compressa* (L.) Grev. J Appl Phycol 5:195–200

Lahaye M, Robic A (2007) Structure and functional properties of ulvan, a polysaccharide from green seaweeds. Biomacromolecules 8:1765–1774

Lapointe BE, Williams LD, Goldman JC, Ryther JH (1976) The mass outdoor culture of macroscopic marine algae. Aquaculture 8:9–21

Lawton RJ, Mata L, de Nys R, Paul NA (2013) Algal bioremediation of waste waters from land-based aquaculture using *Ulva*: selecting target species and strains. PLoS One 8(10):e77344

Li X, Rezaei R, Li P, Wu G (2011) Composition of amino acids in feed ingredients for animal diets. Amino Acids 40:1159–1168

Lobban CS, Harrison PJ (1996) Light and photosynthesis. In: Seaweed ecology and physiology. Cambridge University Press, Cambridge, pp 146–150

Magnusson M, Mata L, Nys R, Paul N (2014) Biomass, lipid and fatty acid production in large-scale cultures of the marine macroalga *Derbesia tenuissima* (Chlorophyta). Mar Biotech 16:456–464

Mata L, Schuenhoff A, Santos R (2010) A direct comparison of the performance of the seaweed biofilters, *Asparagopsis armata* and *Ulva rigida*. J Appl Phycol 22:639–644

McKendry P (2002) Energy production from biomass (part 1): overview of biomass. Bioresour Technol 83:37–46

Neveux N, Magnusson M, Maschmeyer T, de Nys R, Paul NA (2014) Comparing the potential production and value of high-energy liquid fuels and protein from marine and freshwater macroalgae. GCB Bioenergy:1–17. doi:10.1111/gcbb.12171

Pereira R, Yarish C, Critchley A (2013) Seaweed aquaculture for human foods in land-based and IMTA systems. In: Christou P, Savin R, Costa-Pierce B, Misztal I, Whitelaw CB (eds) Sustainable food production. Springer, New York, pp 1405–1424

Poudyal H, Panchal SK, Waanders J, Ward L, Brown L (2012) Lipid redistribution by α-linolenic acid-rich chia seed inhibits stearoyl-CoA desaturase-1 and induces cardiac and hepatic protection in diet-induced obese rats. J Nutr Biochem 23:153–162

Poudyal H, Panchal SK, Ward LC, Brown L (2013) Effects of ALA, EPA and DHA in high-carbohydrate, high-fat diet-induced metabolic syndrome in rats. J Nutr Biochem 24:1041–1052

Robertson-Andersson DV, Potgieter M, Hansen J, Bolton JJ, Troell M, Anderson RJ, Halling C, Probyn T (2008) Integrated seaweed cultivation on an abalone farm in South Africa. J Appl Phycol 20:579–595

Robic A, Sassi J-F, Dion P, Lerat Y, Lahaye M (2009) Seasonal variability of physicochemical and rheological properties of ulvan in two *Ulva* species (Chlorophyta) from the Brittany coast. J Phycol 45:962–973

Russo GL (2009) Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. Biochem Pharmacol 77:937–946

Ryther JH, Goldman JC, Gifford CE, Huguenin JE, Wing AS, Clarner JP, Williams LD, Lapointe BE (1975) Physical models of integrated waste recycling-marine polyculture systems. Aquaculture 5:163–177

Ryther JH, Debusk TA, Blakeslee M (1984) Cultivation and conversion of marine macroalgae (*Gracilaria* and *Ulva*). SERI/STR-231-2360, pp. 1–88

Saravanan P, Davidson NC, Schmidt EB, Calder PC (2010) Cardiovascular effects of marine omega-3 fatty acids. Lancet 376: 540–550

Schmid M, Guihéneuf F, Stengel D (2014) Fatty acid contents and profiles of 16 macroalgae collected from the Irish Coast at two seasons. J Appl Phycol 26:451–463

Shuuluka D, Bolton J, Anderson R (2013) Protein content, amino acid composition and nitrogen-to-protein conversion factors of *Ulva rigida* and *Ulva capensis* from natural populations and *Ulva lactuca* from an aquaculture system, in South Africa. J Appl Phycol 25:677–685