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ORIGINAL ARTICLE

Biomass, Lipid and Fatty Acid Production in Large-Scale Cultures of the Marine Macroalga *Derbesia tenuissima* (Chlorophyta)

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Abstract Biomass productivity was quantified for the marine macroalga *Derbesia tenuissima* cultivated outdoors at seven stocking densities from 0.25 to 8 g L^{-1} for 5 weeks. Total lipids and fatty acid quantity and quality was measured from samples that were freeze-dried, dried by oven (75 °C), food dehydrator (60 °C), or outdoor in the sun (40 °C) or shade (38 °C). Stocking densities of 0.25 to 2 g L^{-1} yielded the highest biomass productivities (>20 g dry weight m^{-2} day^{-1}) with no effect on total lipid quantity (11 %), or fatty acid quantity (5.3 %) or quality at any density tested. However, there was an interactive effect of stocking density and drying technique, with a decrease of up to 40 % in polyunsaturated fatty acids in sun-dried compared to freeze-dried biomass. Notably, while fatty acid and biomass productivity may be inseparable in macroalgae, cultivation conditions have a significant carryover effect in the post-harvest delivery of high-quality bio-oils.

Keywords Algal biomass productivity · Lipid productivity · Post-harvest processing · Drying · Lipid oxidation · Bio-oils

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Introduction

The majority of research on algal oils (lipids) has concentrated on microalgae, mainly due to the high lipid content of up to 30 % of dry weight (DW) reached in some species (Huerlimann et al. 2010). In contrast, macroalgae have typically been perceived as low in oil (Kumari et al. 2013) and therefore unsuitable as feedstock for production of bio-oils. This traditional view is questionable with several studies reporting macroalgal lipid-contents above 10 % of DW (McDermid and Stuercke 2003; Gosch et al. 2012) while highlighting the relative advantages of the lower costs of harvest and drying (Gosch et al. 2012). The tropical marine macroalga *Derbesia tenuissima* (Bryopsidales) is a key example with a total lipid content of 12 % and a fatty acid (FA) content of 4 %, of which 30 % are nutritionally important omega-3 polyunsaturated fatty acids (PUFA *n*−3) (Gosch et al. 2012). However, for *Derbesia* or any alga to be considered as a viable bio-oil resource it is critical that high total lipid and fatty acid productivities can be achieved (on a per unit area basis).

Total lipid and fatty acid productivities are a function of lipid or fatty acid content (grams per gram) and biomass productivities (grams DW per square meter per day). These parameters cannot be considered in isolation and are best evaluated simultaneously in outdoor cultures to estimate productivities for scale-up cultivation. Both biomass production (Mata et al. 2006) and fatty acid quantity and quality (Khotimchenko and Yakovleva 2004) can potentially be manipulated by varying cultivation conditions, with the expectation that there are trade-offs between growth rate and fatty acid quantity and quality driven by light and nutrient availability (Li et al. 2008; Pal et al. 2011). For example, high-biomass productivities may be

attained through low stocking densities with high growth rates or through high stocking densities with low growth rates, and consequently the biochemical composition of individuals within each culture may be very different (Bruhn et al. 2011). These differences may be more pronounced at either extreme of stocking density, as very low densities may limit biomass productivity if light exceeds the demands of photosynthesis (Osmond 1994; Mata et al. 2006), while at very high densities severe light limitation may lead to decreased biomass productivity (Schuenhoff et al. 2006; Bruhn et al. 2011). These same factors may also affect the quantity of fatty acids, by increasing synthesis and desaturation under low light (Klyachko-Gurvich et al. 1999), and the quality of fatty acids, by stimulating particular fatty acid pathways (Khotimchenko and Yakovleva 2004) but see Dawes et al. (1993). Given the recent interest in algal oils for nutraceutical applications, primarily PUFA (Gosch et al. 2012; Kumari et al. 2013; Poudyal et al. 2013a, b), it is notable that the links between total lipid quantity, fatty acid quantity and quality, and the scalable, high-productivity culture of macroalgae are not well established.

The links between biomass cultivation and the practical post-harvest processing of biomass, specifically the efficacy of modes of drying from freeze-dried through to air dried, have also not been quantified. Drying is an additional and critical step in oil production that has typically been considered in isolation from biomass production, either through processing of wild collections (Wong and Cheung 2001; Sánchez-Machado et al. 2004) or laboratory-cultured material (Esquivel et al. 1993; Ryckebosch et al. 2011). One emphasis has been on methods to retain and stabilise bio-active compounds, in particular polyunsaturated fatty acids (PUFA), that are susceptible to oxidative degradation (Shahidi and Zhong 2010) in the initial drying (dewatering) steps (Ratti 2001). The method of drying influences product quality (Esquivel et al. 1993; Chen et al. 2007), and freeze-dried products typically have the highest retention of bioactivity (antioxidants, vitamins, etc.) (Ratti 2001; Sablani 2006; Le Lann et al. 2008). However, the method is costly and air drying remains the most accessible and widespread method for dehydration of food products (Ratti 2001). The effects of these drying methods on the lipids in algae has yielded conflicting results, with only a negligible decrease in % PUFA in sun-dried compared to freeze-dried *Sargassum hemiphyllum* (Chen et al. 2007), but a 60 % loss of lipids in two species of diatoms after freeze-drying compared to extraction of fresh biomass (Esquivel et al. 1993). Notably, enhanced yields in the quantity and quality of total lipids and fatty acids may be lost through post-harvest processing through drying prior to the extraction and delivery of bio-oils.

Therefore, the first objective of this study was to quantify biomass productivities in the green macroalga *D. tenuissima* across a range of stocking densities. The second objective was to quantify total lipids and fatty acids in this biomass, and consequently determine total lipid and fatty acid productivities across stocking densities in 2,000-L outdoor cultures of *Derbesia*. Finally, the third objective was to determine the effects of drying techniques on the quantity and quality of fatty acids in biomass produced over a range of stocking densities, examining for the first time, the interactive effects of culture and post-harvest processing on fatty acid quantity and quality in macroalgae.

Materials and Methods

Study Organism

D. tenuissima (Chlorophyta, Bryopsidales) was selected as it has a high total lipid content (>12 % DW) rich in PUFA (Gosch et al. 2012) and has a filamentous growth habit suitable for tumble culture in tanks (Online Resource 1a), and with siphonous filaments approximately 25 μm in diameter (Online Resource 1b). Biomass samples were collected from a shallow sub-tidal rock platform at Rowes Bay, Townsville, Australia (latitude, 19.14°S; longitude, 146.48°E) and brought to the Marine & Aquaculture Research Facilities Unit (MARFU), at James Cook University (JCU), Townsville (latitude, 19.33°S; longitude, 146.76°E). Stock cultures of the biomass were scaled up to 35 kg fresh weight (FW) required to begin the stocking density experiments.

Effect of Stocking Density on Biomass, Lipid and Fatty Acid Productivities

A 5-week cultivation experiment was conducted using seven stocking densities (SD) (0.25, 0.5, 1, 2, 4, 6 and 8 g FW L^{-1}) relevant to large-scale, high-biomass productivity conditions. This experimental component focussed on lipid and fatty acid contents determined from freeze-dried biomass.

Biomass Productivity and Growth Rates

The experiment was conducted in seven circular polyethylene tanks (3.33 m^2, 80 cm height) filled with 2,000 L of saltwater (65 cm deep) with a ring of vigorous aeration in the bottom to maintain the macroalgae biomass in suspension. One week prior to the start of the experiment, 5 kg FW of *D. tenuissima* was stocked in each tank (2.5 g FW L^{-1}) with water turnover rates of 6.5 volumes per day to acclimate biomass to experimental conditions. Biomass was then combined and redistributed at the seven stocking densities and the effects on biomass productivity and growth rates were quantified on a weekly basis for 5 weeks.

Every week, the biomass was harvested using mesh bags (0.1 mm mesh), drained in a standard domestic centrifuge (MW512; Fisher & Paykel) at 1,000 rpm for 5 min to constant

fresh weight (FW) and weighed to quantify biomass productivity. The biomass was then immediately re-stocked at the same density but in a different tank that was randomly selected in the array to minimise any tank effects. Biomass productivity and specific growth rate (SGR) were calculated using the equations:

$$\text{Productivity}_{\text{Biomass}}\left(\text{g DW m}^{-2}\ \text{day}^{-1}\right) = \frac{(W_f - W_i)}{(\text{FW/DW}) \times A \times t} \quad (1)$$

$$\text{SGR}\left(\%\,\text{day}^{-1}\right) = \frac{100 \times \ln\left(\frac{W_f}{W_i}\right)}{t} \quad (2)$$

where W_f and W_i are the final and initial algal fresh weights, FW/DW is the fresh to dry weight ratio, A is the culture surface area of the tanks (square meters) and t is the number of days in culture. Each week, the fresh weight to dry weight ratio was determined for each density by drying five samples of freshly spun algae from each treatment overnight at 60 °C. Cultivation density dependent total lipid and fatty acid productivity was determined for freeze-dried biomass as described in the section "Total Lipid and Fatty Acid Quantity and Quality".

The general culture conditions were as follows: saltwater was recirculated continuously within a 25,000 L system, passing through two serially connected micron fibreglass sand filters (S800, Waterco, Australia) and a chilling unit to keep the water temperature between 25–34 °C. The water turnover in each tank was monitored daily and re-adjusted when necessary to 6.5 volumes per day. Salinity varied between 30 and 35 ppt and temperature between 25 °C (minimum night time) and 34 °C (maximum day time), with a 2° average temperature increase over the experimental period. The pH (carbon supply) in the water was maintained at a daytime average of 8.5 by controlled injection of CO_2 into the inlet water. Nitrate concentration was kept between 1 and 5 mg L^{-1} at all times by measuring its concentration in the water twice a week and adding *f*/2 medium to the water when necessary. The photosynthetically active radiation (PAR) was monitored continuously using a Li-190SA underwater Quantum Sensor connected to a Li-1000 Data Logger (Li- Cor, Lincoln, NE, USA). The light available to the algae inside the seven tanks was determined the day after restocking the biomass in the tanks. Noontime PAR was measured just below the water surface (10 cm depth) and close to the base of the tanks (60 cm).

Total Lipid and Fatty Acid Quantity and Quality

Total lipid and fatty acid quantity and quality were measured in biomass sampled each week of the culture experiment. Biomass (60 g FW) from each stocking density was freeze-dried for 24 h (Virtis benchtop 2K, VWR, Australia) and maintained in the dark during processing. The dried biomass was homogenised using a standard domestic food processor and immediately processed for internal moisture and ash content and weighed out for total lipid and fatty acid analyses. Moisture content was measured on a minimum of 1.0 g biomass from each drying treatment and stocking density at 105 °C to constant weight (less than 0.01 % change in weight per minute) (MS-70 moisture analyser, A&D Company Ltd.). The same biomass sample was then weighed to 0.001 g precision followed by combustion in air (550 °C, 6 h) (SEM muffle furnace, LabTek, Australia) to determine ash content per gram dry weight. Biomass weights for total lipid and fatty acid quantification were corrected for sample specific internal moisture content and the results are reported on a true dry weight basis. Weighed samples were stored at −20 °C in airtight containers and analysed for total lipid and fatty acids within 5 days.

Total lipid was quantified on a 200±0.1 mg sample from each stocking density using traditional organic solvent extraction (Folch et al. 1957) as described in detail in Gosch et al. (2012). Fatty acids were extracted and transesterified from separate dried biomass (30±0.1 mg) from each stocking density following a one-step extraction/transesterification method (methanol/acetylchloride; 95:5 v/v) adapted from Cohen et al. (1987) and Rodriguez-Ruiz et al. (1998) 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 EI-MS 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 (Sigma Aldrich, Australia), and was corrected for recovery of internal standard (nonadecanoic acid, C19:0).

Total lipid and total fatty acid quantity (milligrams per gram DW), the latter being the sum of all FAMEs, were used to calculate respective productivity as:

$$\text{Productivity}_{\text{Lipid/Fatty Acid}}\left(\text{g m}^{-2}\text{day}^{-1}\right) = \text{Productivity}_{\text{Biomass}} \times C_{\text{L/FA}} \quad (3)$$

where $C_{\text{L/FA}}$ is the concentration of lipids/fatty acids as grams per gram DW algae. Similarly, the concentration of the PUFA was used to calculate PUFA productivity.

A sub-sample of each stocking density (week five) was analysed for carbon (C) and nitrogen (N) content (OEA labs, UK (http://www.oealabs.com/)). These values were used to derive the C/N ratio, which is an indicator of the nutrient status of the biomass (Atkinson and Smith 1983).

Post-Harvest Drying Techniques

At the same time as measuring the total lipid and fatty acid productivity using freeze-dried material, the relationship between stocking density and post-harvest quality after drying was also evaluated by treating biomass in a range of drying methods. Each week following harvest, biomass subsamples (240 g FW) from each stocking density were divided into four equal portions (n=28) and dried in an oven (75 °C) (Thermoline D367), food dehydrator (60 °C) (Ezidri Ultra FD1000, Harvey Norman, Australia) or outdoors in ambient air conditions (sun-dried or shade-dried) for 24 h before total lipid and fatty acid extraction as described in the section above for freeze-dried biomass. Air dried (sun and shade) biomass was placed on perforated plastic trays (XL6 Aquatray, Tooltech Pty Ltd) that were raised 140 mm off the ground to allow airflow. For the shade treatment, a 280-mm high protective cover was constructed using a PVC frame (1,280× 2,320 mm) covered with two shade cloth layers (extra heavy [84–90 %] UV block, Gale Pacific, Ltd.) to prevent direct exposure to sunlight while allowing airflow between the drying algae and the frame itself. The biomass for each drying treatment was sub-divided into two 30 g FW portions which were positioned within each treatment to correct for possible edge effects, and spread to a 1–2-mm-thin layer over a similar area to ensure an even drying profile and surface exposure. The average maximum temperatures were 51±6 °C for sun-dried biomass and 37±3 °C for shade-dried biomass, while minimum (night time) average was 21±2 °C for both treatments.

Data Analysis

Biomass, total lipid and fatty acid productivities relative to stocking densities were analysed separately using permutational analysis of variance (PERMANOVA, Primer 6) (Anderson et al. 2008). Because these large-scale cultures were run back-to-back for 5 weeks, a two-factor mixed-model PERMANOVA with stocking density as the fixed factor was used, treating week as an unreplicated blocking (random) factor as each week had only a single replicate of stocking density. The fatty acid quantity related to drying treatments was analysed in a similar manner using a three-factor mixed-model PERMANOVA, with drying treatment as an additional fixed factor.

Non-metric multidimensional scaling (nMDS, Primer 6) (Clarke 1993) was used to evaluate patterns in fatty acid quality (% of total FAME rather than quantity) relative to stocking density and drying treatment. MDS is a useful tool to visualize similarities in multivariate data (18 fatty acids), with increasing distances between points in the ordination plot representing decreasing similarity between those points. The Bray-Curtis similarity coefficient was used as a distance measure, derived from individual fatty acid proportions averaged over the 5 weeks for each drying treatment and stocking density. Shaded portions on the ordination plot are three arbitrary clusters of data points with similar fatty acid profiles. Pearson product correlations for the relative proportions of the individual FAs (>0.7, 10 in total) are shown in the vector loading plot, where the length and direction of the vectors indicate the strength of the correlation and direction of change between the two axes, i.e. the relative differences in specific fatty acids between the demarcated groups.

Results and Discussion

Biomass Productivity

Stocking densities of 0.25 to 2 g L^{-1} yielded the highest biomass productivities, exceeding 20 g DW m^{-2} day^{-1} (Fig. 1a) averaged over the five-week period. This effectively resulted in a doubling of the biomass every three days. Higher stocking densities (≥4 g L^{-1}) had a significant 40 % to 80 % reduction in productivity, while growth in some weeks was zero or negative in 8 g L^{-1} cultures (Fig. 1a, PERMANOVA, $F_{6,24}$=15.93, p<0.01, Online Resource 2). There was no overall effect of week on productivity at any stocking density (PERMANOVA, $F_{6,24}$=15.93, p<0.01, Online Resource 2). The high productivities for lower stocking densities lead to an estimated annual biomass production of over 70 t DW ha^{-1} $year^{-1}$ for *D. tenuissima* cultured under these conditions. These productivity values for *Derbesia* (>20 g DW m^{-2} day^{-1}, 70 t DW ha^{-1} $year^{-1}$) are among the highest estimated annual biomass productivities reported for land-based cultivation of macroalgae (Bruhn et al. 2011, and references therein) but see Schuenhoff et al. (2006). Despite the similar productivities in stocking densities from 0.25 g L^{-1} to 2 g L^{-1} the biomass had very different specific growth rates (SGR), ranging from 29.8 % day^{-1} at 0.25 g L^{-1} to 9.4 % day^{-1} at 2 g L^{-1} (Fig. 1b). In a comparative context, while specific growth rates for *Derbesia* were much higher than those of the green macroalga *Ulva lactuca* (Bruhn et al. 2011), a similar inverse relationship between stocking density and SGR also occurs for *U. lactuca* cultivated in 600 L tanks, with the highest average SGR (10.5 % day^{-1}) being recorded at the lowest stocking densities (1.7 g L^{-1}) (Bruhn et al. 2011). A high SGR is regularly cited as a criterion for the selection of species for large-scale algal production (Griffiths and Harrison 2009). In the current study, the fastest growth rates did not result in the highest biomass productivities. Rather, specific growth rates were inversely related to stocking densities and stocking densities from 0.25 to 2 g L^{-1} resulted in similar biomass productivities despite large differences (67 %) in SGR. This is in accordance with recent studies for both marine (Bruhn et al. 2011) and freshwater macroalgae (Lawton et al. 2013), where

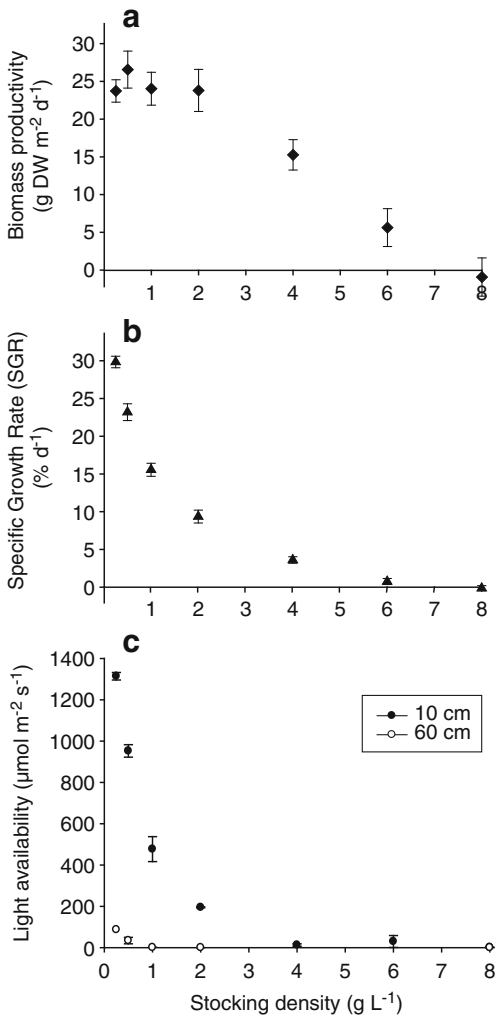

Fig. 1 Growth and culture characteristics of *D. tenuissima* related to stocking density **a** biomass productivity [grams DW per square meter per day], **b** specific growth rate [percent per day], and **c** light availability in tanks [micromoles per square meter per second], taken at 10 cm depth and at the base of the tanks (60 cm)

SGR alone also provide inaccurate estimates for biomass productivities. The algal biomass received very different light regimes depending on stocking density, and SGR (Fig. 1b) was closely related to the availability of light (Fig. 1c). The most limiting factor for growth, and therefore biomass productivity, in high stocking density treatments (>2 g L^{-1}) is light availability and there was effectively no light available to biomass at or below 10 cm depth (i.e., >80 % of the biomass) in tanks at >4 g L^{-1} (Fig. 1c). Nutrients (N, P) and carbon were available in excess in all cultures (NO_3^->1 mg L^{-1}, PO_4^{3-}>1 mg L^{-1}; 7.5<pH<9.3).

These results clearly identify how the simple manipulations of cultivation conditions through the application of low initial stocking density can dramatically increase productivity, assuming excess nutrients and carbon. They also provided the platform to evaluate changes in fatty acid quantity or quality in relation to the fundamental changes in growth rate and productivity of the biomass between 0.25–8 g L^{-1} stocking densities.

Lipid and Fatty Acid Productivities

The average maximum total lipid productivities (3.6 g m^{-2} day^{-1}; Fig. 2) reported here are the highest recorded for outdoor tank systems (≥2,000 L), and well above previous estimates for marine macroalgae of 2 g m^{-2} day^{-1} (Gosch et al. 2012). This is also approaching the 5-g lipid m^{-2} day^{-1} benchmark productivity proposed for the economic feasibility of microalgal oil production (Stephens et al. 2010). While average maximum total lipid (3.6 g lipid m^{-2} day^{-1}) and fatty acid (1.4 g FA m^{-2} day^{-1}) (Fig. 2) productivities over the 5 weeks were reached in lower stocking densities (≤2 g L^{-1}), neither stocking density nor variability in culture conditions (described in section "Biomass Productivity and Growth Rates": two-degree mean temperature increase, N-concentrations between 1–5 mg L^{-1}, salinity between 30 and 35 ppt) had any effect on the quantity of total lipids, or the quantity or quality of fatty acids in the freeze-dried biomass (see Figs. 3, 4 and also Online Resource 3 and 4). The mean fatty acid concentration of 5.3 g 100 g^{-1} DW was dominated by PUFAs (53–55 % of total fatty acids) (Table 1) in all cultures, irrespective of stocking density or growth rates. This means that total lipid, total fatty acid, and PUFA productivities are all driven solely by variation in biomass productivity. The cultivation of macroalgal biomass with a consistent quality for nutraceutical applications, coupled with the relative advantages of technically simpler harvesting and dewatering (drying) compared to microalgae, is highly attractive, with an estimated biomass production of over 70 t DW ha^{-1} $year^{-1}$ corresponding to 13 t total lipid ha^{-1} $year^{-1}$ for *D. tenuissima*.

Some influence of stocking density on the fatty acid quantity and/or quality might have been expected either as an effect of differences in growth rate or light availability in the cultures. For example, an increased degree of unsaturation (as %

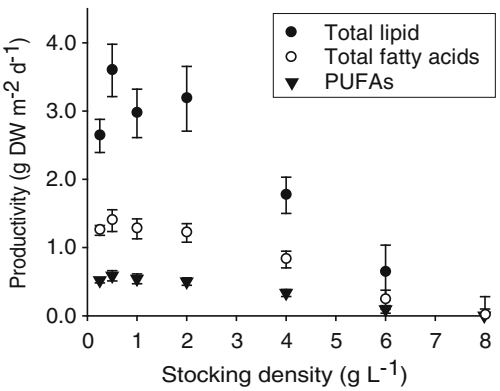

Fig. 2 Productivity of total lipid, total fatty acid, and sum of polyunsaturated fatty acids [grams DW per square meter per day] of *D. tenuissima* related to stocking density

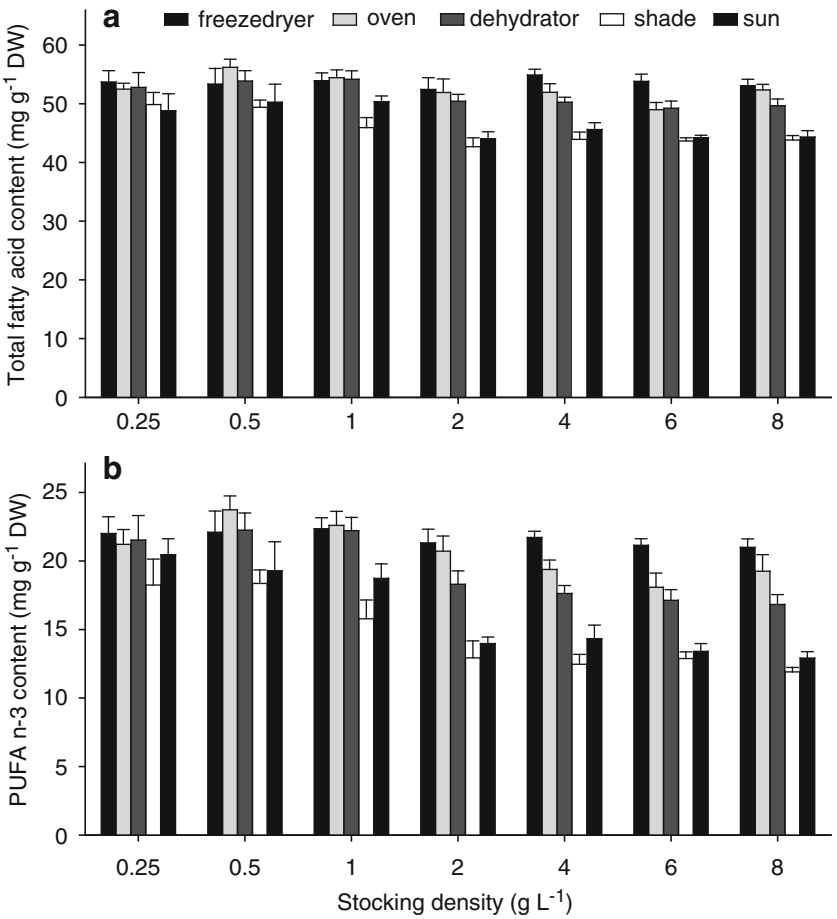

Fig. 3 Fatty acid quantity of *D. tenuissima* in different stocking densities and drying treatments **a** total fatty acids and **b** PUFA *n*−3 content [milligrams per gram DW]

C20:5) was recorded in faster growing *Porphyridium cruentum* (Rhodophyta) in small laboratory scale systems (Cohen et al. 1988). Increased accumulation of storage lipid is also commonly assumed to be connected with both lower growth rates and nutrient deprivation in microalgae (Griffiths and Harrison 2009; Pal et al. 2011). However, a recent review challenged this assumption for microalgae and showed there is no clear correlation between lipid content and growth rate in nutrient replete cultures (Griffiths and Harrison 2009). The very low light availability (i.e. <10 μmol m^{-2} s^{-1} below 10 cm depth in the tanks) for at least 85 % of the biomass at high densities, at and above 4 g L^{-1}, likely only provided enough solar energy for cellular maintenance. Given that the siphonous Bryopsidophyceae macroalgae may store starch rather than lipid as their energy reserve, it is unclear whether any effect of excess fixed carbon through photosynthesis would be stored as lipids in these species.

In addition to growth, the pronounced differences in light intensity experienced at the different stocking densities, from 70 % of surface irradiance at 10 cm depth in 0.25 g L^{-1} cultures to 10 % in 2 g L^{-1} and below 1 % of surface irradiance in denser cultures (Fig. 1c), were also expected to affect fatty acid quality. One mechanism for changes in the quality of fatty acids is that a higher degree of unsaturation in membrane lipids leads to increased fluidity and thus an increased velocity of electron flow as a response to low light to improve photosynthesis rates (Mock and Kroon 2002). Lower light intensity has also led to relatively higher PUFA content in microalgae (Klyachko-Gurvich et al. 1999) and the macroalga *Ulva fenestrata* (Khotimchenko and Yakovleva 2004), however, these changes are mainly detected at the level of lipid class with no concurrent change in the total fatty acid pool. Conversely, neither growth nor light affect any aspect of the fatty profile of *Derbesia* with a remarkable consistency in total lipids and the quantity and quality of fatty acids. In a similar context, the C/N ratios also remained constant at approximately 6:1 in *Derbesia* biomass at all stocking densities (Table 1). This is a comparatively low C/N ratio for benthic marine macroalgae corresponding to high nutrient conditions (Atkinson and Smith 1983) corroborating no limitation of resources, as carbon, nitrogen and phosphorous in the culture medium.

Post-Harvest Drying

While there were no effects on the intrinsic fatty acid composition of *D. tenuissima* with growth rates associated with different stocking densities (light availability), there was a

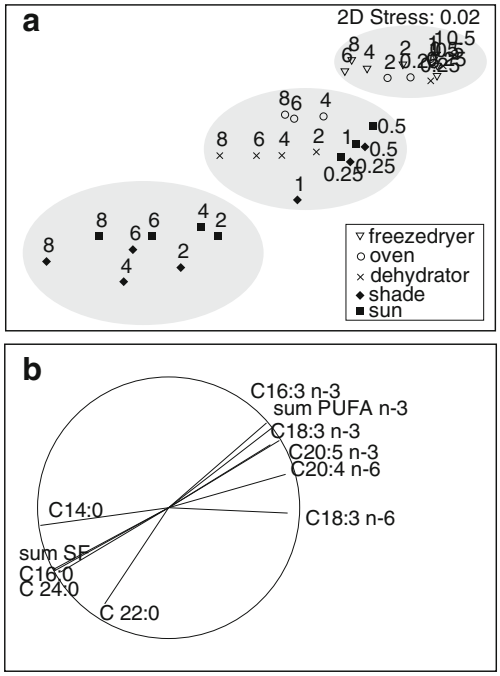

Fig. 4 Fatty acid quality of *D. tenuissima* in different stocking densities and drying treatments. **a** Multidimensional scaling (MDS) ordinations showing clusters of samples based on the similarity of their fatty acid profiles [% of total FAME] and **b** vector loadings of the specific fatty acids relating to the clusters of samples in **a** above (correlations of $R>0.7$). The direction and size of each vector indicates the relative abundance of that variable in samples in the corresponding section of the ordination plot (vector loadings are normally overlaid on the ordination but have been separated for ease of viewing)

clear link between biomass culture conditions and the influence of drying method on both fatty acid quantity and quality. There was a strong interactive effect between stocking density and drying technique on fatty acid quantity (PERMANOVA, $F_{24,96}=1.83$, $p<0.05$) (Fig. 3a, Online Resource 2) and quality (PERMANOVA, $F_{24,96}=3.31$, $p<0.05$) (Fig. 3b, Online Resource 2). Freeze-dried material typically had the highest quantity of fatty acids (5.3 % of DW) (Fig. 3) and also the highest degree of unsaturation of all drying treatments, with over 60 % of fatty acids present as monounsaturated fatty acids (MUFA) and PUFA across all stocking densities (Fig. 3, see also Online Resource 3 and 4). Air (sun and shade)-dried biomass had a decrease in PUFA *n*−3 (mainly C16:3 and C18:3) of up to 40 % compared to freeze-dried material, while the effect was smaller in food dehydrator (20 % decrease) and oven (15 % decrease) dried biomass, primarily in stocking densities >4 g L^{-1}. However, this interactive effect of stocking density and drying method manifested in biomass from 2–8 g L^{-1} cultures, showing incremental decreases in both fatty acid quantity and quality compared to freeze-dried material (Fig. 3). MDS analysis highlighted three distinct groupings (Fig. 4a) separated mainly by the abundance of PUFAs vs. saturated fatty acids (SF) (stress 0.02), as indicated by the vector loading of PUFA to the top right of the plot opposite in direction to the SF loading to the bottom left (Fig. 4b). Sun- and shade-dried material from 2–8 g L^{-1} cultures grouped based on fatty acid profiles rich in the saturated fatty acids C14:0, C16:0, C22:0 and C24:0 with the sum of SFA>50 % of total FA. Biomass from the same drying treatments but at lower stocking densities (0.25–1 g L^{-1}) grouped with food dehydrator and oven-dried biomass from 2–8 g L^{-1}, with slightly more unsaturated fatty acids. Food dehydrator and oven-dried biomass from lower stocking densities formed a separate group together with freeze-dried biomass from all stocking densities, characterised by higher amounts of nutritionally important C16:3, C18:3, C20:4 and C20:5 (Fig. 4).

Lipids are inherently susceptible to oxidation through multiple pathways, with their stability being dependent on the specific fatty acids present, environmental conditions, or the sample matrix. For example, the oxidation rate of individual fatty acids increases exponentially with degree of unsaturation (Wagner et al. 1994) so that C18:3 has a 2,500-fold faster oxidation rate than the saturated C18:0 (Shahidi and Zhong 2010). Elevated temperatures can accelerate the auto-oxidation process and there are also enzymes that specifically

Table 1 Biomass composition (freeze-dried material) from all stocking densities over five cultivation weeks. Average±SE, $n=5$, except for %C and C/N which was analysed at week 5 only in duplicate from each stocking density

Stocking density [g FW L^{-1}]	% of DW		% of total FA			n6:n3	Ash [%]	C [%]	C/N
	Total lipid	Total FA	SF	MUFA	PUFA				
0.25	11.1±0.4	5.3±0.2	36.9±0.7	8.1±0.3	55.0±0.9	0.33±0.01	21.9±1.4	37.0±0.2	5.9±0.00
0.5	12.0±1.3	5.3±0.3	36.9±0.7	8.0±0.3	55.1±0.8	0.32±0.01	22.9±1.6	36.2±0.0	6.0±0.01
1.0	12.5±1.4	5.3±0.1	36.9±0.3	7.8±0.6	55.3±0.8	0.31±0.01	23.1±0.2	36.1±0.3	6.1±0.03
2.0	13.4±0.8	5.2±0.2	37.6±0.6	8.2±0.1	54.2±0.5	0.31±0.01	22.1±0.9	37.3±0.0	6.3±0.02
4.0	11.6±0.3	5.4±0.1	39.0±0.6	7.6±0.3	53.4±0.4	0.33±0.01	22.1±1	37.4±0.6	6.5±0.02
6.0	13.3±0.7	5.3±0.1	40.0±0.3	7.2±0.1	52.7±0.2	0.32±0.01	21.5±0.6	37.9±0.1	6.5±0.00
8.0	12.7±0.8	5.2±0.1	39.7±0.8	6.9±0.4	53.3±0.6	0.32±0.01	20.7±0.7	41.0±0.0	6.5±0.05

FA fatty acids, *SF* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *n6* omega-6 PUFA, *n3* omega-3 PUFA

catalyze the oxidation of PUFA in vivo (Shahidi and Zhong 2010). For these reasons, freeze-drying was expected to yield the highest quality lipid product (the highest levels of PUFA) of any drying method as the low temperature and oxygen-free atmosphere limit oxidation processes. In contrast, drying in sunlight was expected to result in lipid deterioration; particularly at the high temperatures recorded during the experiment described here (average maximum 51±6 °C). Despite these expected effects on lipid oxidation state, the choice of drying technique has previously only shown a marginal influence on fatty acid quality in macroalgae. Following extended sun drying (4 days), oven-drying (60 °C, 15 h), or freeze-drying (5 days), the PUFA content of wild collected *S. hemiphyllum* was only slightly lower in sun-dried (55.3 % of total FA) compared to freeze-dried material (58.5 % of total FA) (Chan et al. 1997). Brown macroalgae contain high levels of antioxidants as polyphenols and vitamin C (Chan et al. 1997; Le Lann et al. 2008) that may have protected the fatty acids from oxidation reactions. Although green macroalgae typically contain comparatively lower amounts of polyphenols (Løvstad-Holdt and Kraan 2011), vitamins C and E, and other compounds such as pigments, peptides and small molecular weight polysaccharides, have antioxidant activity (Heo et al. 2005). The results presented here indicate that *Derbesia* produced at lower stocking densities has an inversely proportional, and incrementally higher, level of such antioxidants that play an inherent role in preventing the oxidation of PUFA. The interaction between stocking density and the prevention of oxidation of high value fatty acids provides an innovative opportunity to manipulate macroalgal culture conditions to improve and optimise biomass quality for downstream processing and consumption.

Conclusions

D. tenuissima is a high-productivity feedstock for biotechnology applications, yielding (1) biomass with a remarkable consistency in quality across a range of cultivation conditions, and (2) the highest reported productivities of lipids for macroalgae cultivated in intensive outdoors systems. Importantly, a strong interactive effect was demonstrated between cultivation conditions and drying methods on the amount of PUFA in the dried biomass, manifesting as a 40 % loss of PUFA in sun-dried compared to freeze-dried biomass from high stocking densities, while low stocking densities delivered a high-quality product regardless of drying treatment.

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