Algal Research 8 (2015) 153–160

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Manipulating antioxidant content in macroalgae in intensive land-based cultivation systems for functional food applications

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ARTICLE INFO

Article history:
Received 3 December 2014
Received in revised form 24 January 2015
Accepted 11 February 2015
Available online 18 February 2015

Keywords:
Biomass productivity
Antioxidant productivity
Bioproducts
Total phenol content
Nutraceutical
Seaweed

ABSTRACT

The recently domesticated marine macroalga *Derbesia tenuissima* is suitable for intensive land-based production and is a promising species for functional food applications and bioproducts as it is rich in bioactive components and has high biomass productivity. For the first time, we quantified the effects of inducing different degrees of light stress by managing culture conditions (as biomass density) in land-based 2000 L cultures, on the total phenolic content, antioxidant capacity (DPPH, FRAP, ABTS), and biomass and antioxidant productivity of *Derbesia*. We demonstrate that it is possible to manipulate the antioxidant content of *Derbesia* by managing culture conditions, with up to 88% higher antioxidant capacity in biomass stocked at 0.5 g L^{-1} than when stocked and maintained at 2 g L^{-1}, or stocked at 2 g L^{-1} and harvested weekly. Antioxidant productivity of tank-cultured *D. tenuissima* is high – up to 680 μmol gallic acid equivalents m^{-2} day^{-1} – and can be maximised by selecting low initial stocking densities without compromising productivity per unit land area.

1. Introduction

Marine macroalgae (seaweed) contain a wide variety of natural compounds with antioxidant activity, including polyphenols, ascorbic acid (vitamin C), peptides, free amino acids, carotenoids, and low molecular weight polysaccharides [1]. These functional ingredients are now seen as critical components in diets for preventing or reversing metabolic syndrome, which contributes to a number of chronic lifestyle disorders such as cardiovascular diseases, diabetes and various cancers [2]. The natural products of marine macroalgae in particular are considered one of drivers for the low incidences of metabolic syndrome in Asian populations where marine macroalgae constitute a regular and significant portion in the diets [3]. Research into the use of marine macroalgae for nutraceutical or functional food applications to address metabolic syndrome has previously focussed on the analysis of natural products from wild collected specimens [4,5]. However, if macroalgae are to be a reliable source of antioxidants for the functional food industry or other bioproducts, then the biomass will need to be sustainably sourced from culture and, more specifically, from intensive land-based facilities that enable traceability, control, and optimisation of the target compounds [6].

There is limited data on the antioxidant contents of cultured macroalgae [4,7], and most of the literature focuses on the antioxidant capacity of macroalgal extracts, without reporting extract yields or the antioxidant content of the whole biomass [5,8]. There is some evidence that culture conditions can be manipulated to influence the content of specific natural products. *Asparagopsis armata* cultured in fishpond effluents at higher nitrogen fluxes had an increased content of mycosporine-like amino acids (MAAs) which have a UV-protective and antioxidant function [9], and the reducing capacity of extracts from the red macroalga *Palmaria palmata* (dulse) harvested from a high UV-exposure environment was 86% higher than extracts from dulse harvested from a low UV-exposure environment [8]. In addition, there is substantial data on how the antioxidant content of marine macroalgae in natural habitats is influenced by abiotic stresses such as light, temperature, and desiccation stress [10,11]. On-land culture manipulations therefore provide the potential to exploit or enhance the antioxidant capacity of the biomass.

Light is perhaps the most common stress for shallow or intertidal macroalgae in nature. Light stress can be quantified directly in the biomass as photosynthetic capacity using pulse amplitude modulated (PAM) fluorometry as a decrease of the potential quantum yield (F_v/F_m) of photosystem II (PSII) [10,12]. Light is also the most variable input of intensive land-based algal culture as it is dynamic and affected by both external and internal factors. The external factors relate to the quantity of light in outdoor cultures and are influenced by photoperiod, season and day-to-day weather patterns. The internal factors relate to stocking density, which is also dynamic, increasing over time as the macroalgae grows if the biomass is harvested intermittently, for example, weekly. This creates a potential situation where the algae may be light stressed on certain days or at certain points in the culture cycle, particularly in low density cultures at noon time, and as self-shading increases light

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http://dx.doi.org/10.1016/j.algal.2015.02.007
2211-9264/

stress is reduced [13]. In addition, the 3-D nature of intensive cultures means that individuals in culture move in and out of light resulting in a light–dark cycle of seconds or minutes [14]. It is unclear how these culture conditions affect antioxidant content (either positively or negatively) of macroalgae in intensive production systems. It is also unclear whether the culture conditions can be manipulated to maximise the antioxidant potential of biomass while maintaining the high productivity of biomass that is characteristic of intensive land-based cultures.

Here, for the first time, we quantify the antioxidant capacity for the recently domesticated marine green macroalga (seaweed) *Derbesia tenuissima* in intensive land-based production under a range of culture conditions to assess the relationship between light availability and antioxidants in algal culture. The objectives of this research were to quantify the extent to which the total antioxidant content of *Derbesia* can be manipulated across three culture light conditions (by managing the biomass density), and to simultaneously quantify antioxidant and biomass productivity (yield per unit area of production) under these light conditions, examining the links between light availability, photosynthetic capacity of the biomass, and antioxidant response. In combination, these data were used to evaluate the potential of this macroalga in relation to recognised antioxidant crops focussing on two metrics — the antioxidant content and antioxidant yield per unit area of production.

2. Materials and methods

2.1. Chemicals and reagents

Gallic acid, Folin–Ciocalteu reagent, sodium carbonate, Trolox, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma Aldrich, Australia, while sodium acetate, potassium persulfate, and iron (III) chloride hexahydrate were purchased from Ajax Finechem Pty Ltd., Australia. All solvents were HPLC (methanol) or AR (acetone, acetic acid glacial, hydrochloric acid 32%) grade.

2.2. Study organism

The marine macroalga (seaweed) *D. tenuissima* (Chlorophyta, Bryopsidales) was originally collected from a shallow sub-tidal rock platform at Rowes Bay, Townsville, Australia (Latitude: 19.14°S; Longitude: 146.48°E) and stock cultures have been maintained for 5 years in outdoor culture tanks (2000–10,000 L) at the Marine & Aquaculture Research Facilities Unit (MARFU), James Cook University (JCU), Townsville (Latitude: 19.33°S; Longitude 146.76°E).

2.3. Biomass production and manipulation of antioxidant content

To quantify the extent to which the antioxidant capacity of *Derbesia* can be manipulated by changing the light available to the biomass, an outdoor culture experiment was conducted in 2000-L tanks over three consecutive production cycles of 7-days in November 2013. Light stress was induced in two ways, by altering the initial stocking densities (SD) and the harvest frequency. The culture conditions (defined here as the combination of stocking density and harvest frequency) were: high density-weekly treatment (2 g L^{-1} SD allowing a one-week growth period, these conditions yield a high and stable biomass productivity of 24 g dry weight (dw) m^{-2} day^{-1} [15]), high density-daily treatment (2 g L^{-1} SD continuously maintained at the initial density through daily harvest), and low density-weekly treatment (0.5 g L^{-1} SD allowing a one-week growth period). The degree of light stress response by the alga was measured using PAM-fluorometry, such that the high density-weekly treatment should represent the scenario of low initial light stress followed by decreasing light stress, the high density-daily treatment should represent the scenario of cumulative low initial light stress, and the low density-weekly treatment should represent the scenario of very high initial light stress followed by decreasing light stress (see Sections 2.7 and 3.3).

The experiment was conducted outdoors under ambient sun-light in circular polyethylene tanks (3.33 m^2, 80 cm height) filled with 2000 L of saltwater (65 cm deep) and connected to a 25,000 L recirculating system as described [15]. One week prior to the start of the experiment, 4 kg fresh weight (fw) of *Derbesia* was stocked in each tank (2.0 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 re-distributed at 2 g L^{-1} (n = 2 duplicate tanks for both high density-weekly and high density-daily treatments) and 0.5 g L^{-1} (n = 2 duplicate tanks, low density-weekly treatment). The effects on total phenol and antioxidant content (Section 2.5), and biomass and antioxidant productivity (Section 2.6) were then quantified on a weekly basis for 3 weeks, while photosynthetic capacity was quantified as dark-adapted photosynthetic yield on a daily basis for 3 weeks using PAM-fluorometry (Section 2.7). The biomass was harvested using 0.1 mm mesh bags, drained in a standard domestic centrifuge (MW512; Fisher & Paykel) at 1000 rpm for 5 min to constant fw and weighed to quantify biomass productivity. For the high density-daily treatment, on average 450 g fw biomass was harvested this way each day to maintain the standing stock at 2 g fw L^{-1}. After each 7-day cycle, biomass from the duplicate tanks of each treatment was combined and then re-stocked at the original density but in a different tank to account for any tank-effects. Each complete harvest (every 7 days) the fresh weight to dry weight (fw:dw) ratio was determined for each treatment by drying five samples of freshly spun algae from each treatment overnight at 60 °C.

Environmental conditions were as follows: salinity varied between 31.9 and 34.6 ppt and temperature was maintained between 23.7 °C (minimum night time) and 29.8 °C (maximum day time) by a water heater–chiller (Evoheat, DHP603, water volume of 9 m^3 h^{-1}); water pH (carbon supply) was maintained at a daytime average of 8.5 by controlled injection of CO_2 into the inlet water; nitrate concentration was maintained between 1 and 6 mg NO_3-N L^{-1} by measuring its concentration in the water twice a week and adding MAF nutrient concentrate (Manutec Pty Ltd., Australia) when necessary.

2.4. Biomass extraction

Freeze-dried and milled biomass (0.5 ± 0.0001 g dw) was extracted sequentially in 20 mL acidified methanol (50%, aq., pH 2, 1 h), followed by 20 mL acetone (70%, aq., 1 h) on a rotating table (100 rpm) at room temperature as modified from [16]. The samples were centrifuged at 3000 g for 15 min between extractions and the supernatants were collected and pooled, then filtered through 0.25 μm PTFE syringe filters prior to use in the antioxidant assays.

2.5. Antioxidant capacity

Four assays were chosen to quantify antioxidant content in the biomass; total phenol content using the Folin–Ciocalteu (FC) phenol reagent, DPPH radical scavenging ability, ABTS•+ radical scavenging ability, and ferric reducing antioxidant capacity (FRAP). Multiple antioxidant activity assays were used to ensure reliability of the results and to facilitate comparison of results with those in the literature. All assays were performed in triplicate wells per extract on 96-well microplates, and a new standard curve was prepared for each plate to account for any variability between plates. Total phenol content was analysed on a Spectramax Plus (Molecular Devices, Australia), while ABTS, DPPH, and FRAP were analysed on a Spectramax Plus M2 (Molecular Devices, Australia).

Total phenol was determined using the Folin–Ciocalteu (FC) phenol reagent method adapted for 96-well microplates as described in [16], with the modifications of diluting the extracts by 50% prior to the reaction, and incubating the plate with reactants at 40 °C for 30 min at 100 rpm (Innova 44R stackable incubator, John Morris Scientific,

Australia) prior to reading at 750 nm. Gallic acid was used as a reference standard (147 to 882 μM, $R^2 = 0.954$), and the results are expressed as μmol gallic acid equivalents (GAE) 100 g^{-1} dw.

DPPH radical scavenging ability was determined following [17] with some modifications. A 200 μM solution of DPPH• was prepared in 80% methanol, and then stored at −20 °C until needed. *Derbesia* extracts (40 μL) were allowed to react with 160 μL of the DPPH solution for 30 min in the dark before measuring absorbance at 517 nm. Gallic acid was used as a reference standard (10 to 100 μM gallic acid, $R^2 = 0.995$), and the results are expressed in μmol gallic acid equivalents (GAE) 100 g^{-1} dw.

ABTS•+ radical scavenging ability was determined following [18] with some modifications. The ABTS working solution was prepared by mixing 3.5 mL of 10 mM ABTS solution and 1.5 mL of 8.17 mM $K_2S_2O_8$ solution, and leaving the mixture overnight at room temperature in the dark to allow formation of the ABTS•+ radical. For the actual assays, 1 mL ABTS•+ working solution was mixed with 39 mL Milli-Q water to obtain an absorbance of 1.2 ± 0.01 units at 734 nm. 10 μL of sample or standard was added to the 96-well microplate, followed by addition of 190 μL ABTS•+ working solution. The plate was then incubated for 5 min at room temperature before reading at 734 nm. Trolox was used as a reference standard (65 to 1000 μM, $R^2 = 1.000$) and the results are expressed in μmol Trolox equivalents (TE) 100 g^{-1} dw.

Ferric reducing antioxidant power (FRAP) was determined following [19] with minor modifications in the use of Trolox as the standard (7 to 250 μM, $R^2 = 0.999$) rather than $FeSO_4$, and the results are expressed in μmol Trolox equivalents (TE) 100 g^{-1} dw.

2.6. Productivity

Biomass and antioxidant productivities were calculated using the equations:

$$\text{Productivity}_{\text{Biomass}}\left(\text{g dw m}^{-2}\ \text{day}^{-1}\right) = (W_t - W_i)/(\text{fw} : \text{dw})/A/t \quad (1)$$

$$\text{Productivity}_{\text{Antioxidant}}\left(\mu\text{mol m}^{-2}\ \text{day}^{-1}\right) = C_{\text{Antioxidant}} * \text{Productivity}_{\text{Biomass}} \quad (2)$$

where W_t is the total algal fresh weight after 7 days (including all the daily harvested biomass for high density-daily tanks), W_i is the initial algal fresh weight, fw:dw is the fresh to dry weight ratio, A is the culture surface area of the tanks (m^2), t is the number of days in culture and $C_{Antioxidant}$ is the concentration of antioxidant expressed as Trolox or gallic acid equivalents.

2.7. Light and photosynthesis measurements

Ambient photosynthetically active radiation (PAR, 400–700 nm) at the culture facility was monitored continuously using a Li-190SA underwater Quantum Sensor connected to a Li-1000 Data Logger (Li-Cor, Lincoln, NE, USA). The PAR available to the algae inside each of the six tanks was also measured daily at noon at the water surface, 5 cm below the surface and then every 10 cm to close to the bottom of the tanks (60 cm). Light stress for each treatment was measured in dark-adapted samples as differences in the potential quantum yield (F_v/F_m) of photosystem II (PSII) [12], using a portable pulse amplitude modulated fluorometer (Mini-PAM, Walz, Effeltrich, Germany). Replicate samples (n = 10 from each tank) of *Derbesia* were placed in the fluorometer leaf-clip holder for dark-adaptation (10 min) before a saturation pulse (approximately 4000 μmol photons m^{-2} s^{-1} for 0.4 s) was applied to determine F_v/F_m. Measurements of the stress response over the daily light cycle were taken at 8 am, 12 noon, and 4 pm, by which time the tanks were effectively in the shade. On the days of harvest, measurements were taken at least 1 h after the biomass had been re-stocked into the tanks to minimize any possible stress from the harvest procedure confounding the results.

2.8. Data analyses

The effect of culture conditions on total phenol and antioxidant content, biomass productivity, and antioxidant productivity, was analysed separately using two-factor mixed model permutational analysis of variance (PERMANOVA) [20], with culture condition (i.e. the combination of stocking density and harvest frequency) as a fixed factor and week as a blocked (random) factor. The daily potential quantum yield (F_v/F_m) measured at noon related to culture condition was analysed using a three factor mixed-model PERMANOVA, with culture condition as a fixed factor, week as a blocked factor and day (n = 7 treatments, 0–7 d) as the additional fixed factor. Analyses were conducted in Primer v6 (Primer-E Ltd., UK) using Bray–Curtis dissimilarities on fourth root transformed data and 999 unrestricted permutations of raw data. Tukey's multiple comparison was used to determine any differences between treatments. Pearson's correlations were taken between average F_v/F_m over the first three days of culture (where measurable differences were found between daily potential quantum yield using PERMANOVA) and antioxidant content in the harvested biomass (Statistica v12, StatSoft Inc.).

3. Results and discussion

3.1. Antioxidant content

The results show that it is possible to manipulate the antioxidant content of *Derbesia* by managing the culture conditions in intensive land-based culture-systems, with an overall higher antioxidant content in biomass stocked at 0.5 g L^{-1} and allowed to grow for one week, than when stocked and maintained at 2 g L^{-1}, or stocked at 2 g L^{-1} and allowed to grow for one week (Fig. 1; PERMAVOA, $F_{2,4} = 11.95$, P = 0.008). Specifically, culture conditions had a significant effect on total phenol content, DPPH, and FRAP, but not on ABTS (below, this section). While there was an effect of culture week, where the absolute content of antioxidants varied between weeks potentially in response to different environmental conditions, notably there was no interaction between culture condition and week ($F_{4,9} = 0.734$, P = 0.602). The absence of any interaction means that the pattern of antioxidant content was consistent for each culture week, with the low density-weekly treatment (0.5 g L^{-1} stocking density allowing a one-week growth period) > high density-daily treatment (2 g L^{-1} stocking density maintained at the initial density through daily harvest) > high density-weekly treatment (2 g L^{-1} stocking density allowing a one-week growth period). However, there were some differential effects in the relation to the magnitude of the different end

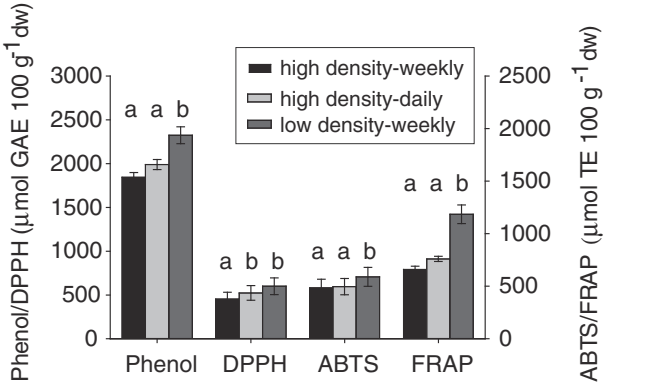

Fig. 1. Average antioxidant content as total phenol and DPPH (μmol GAE 100 g^{-1} dw), and ABTS and FRAP (μmol TE 100 g^{-1} dw), for *Derbesia tenuissima* related to culture conditions. High density-weekly = culture stocked at 2 g L^{-1}, high density-daily = continuously maintained at 2 g L^{-1}, low density-weekly = culture stocked at 0.5 g L^{-1}.

points with a stronger effect on the antioxidant content of low density-weekly treatment in TPC and FRAP, compared to DPPH and ABTS.

3.1.1. Total phenol content

Plant polyphenols are structurally diverse, abundant and highly effective antioxidants, and can therefore contribute substantially to the overall antioxidant capacity of a biomass sample [21]. Here, there were significant effects of culture conditions on the total phenol content over the 3-week production period (PERMANOVA $F_{2,4} = 4.88$, $P = 0.043$), for which total phenol content in biomass from the low density-weekly treatment (0.5 g L^{-1} stocking density, 2242 ± 228 SE μmol gallic acid equivalents [GAE] 100 g^{-1} dw) was 22% higher than in biomass from the high density-weekly treatment (1834 ± 46 SE μmol GAE 100 g^{-1} dw) and 14% higher than in biomass from the high density-daily treatment (Fig. 1). The slightly higher total phenol content in the high density-daily treatment compared to the high density-weekly treatment was not statistically significant (pair-wise test, $t = 1.92$, $P = 0.100$). The maximum total phenol content was detected in the low density-weekly treatment after 1 week of culture (2658 ± 137 SE μmol GAE 100 g^{-1} dw, duplicate tanks), and this was 40% higher than in the high density-weekly treatment and 27% higher than in the high density-daily treatment at the same sampling time. These levels of total phenolics (less than 0.5% of dw) are higher than other green (0.23–0.26%) and red (0.1–0.3%) marine macroalgae [4,7] (Table 1), but markedly lower than many brown (typically 3–14%) macroalgae [22], in which polyphenols make up structural components of the cell [23]. Total phenolics are also 70–80% lower than traditional high antioxidant foods such as fruits and berries (Table 1). It has been proposed that phenolic metabolites are produced in land plants as an alternative pathway for excess photochemical energy dissipation under stress, leading to increased antioxidant capacity of the biomass and also functioning as a carbon sink [24]. An 8-fold increase in total phenol content following a three-fold increase in light intensity in indoor batch cultures of *Spirulina platensis* suggests that this may also be true for cyanobacteria [25]. Our results support that the hypothesis that phenolic metabolites are produced to increase the photochemical energy dissipation of a plant under stress is also applicable to macroalgae.

3.1.2. DPPH radical scavenging ability

There was a significant effect of culture condition on DPPH radical scavenging ability (Fig. 1, PERMANOVA $F_{2,4} = 10.07$, $P = 0.039$), with a 33.6% higher content in biomass from the low density-weekly treatment (600 ± 96 SE μmol GAE 100 g^{-1} dw) compared to high density-weekly treatment (454 ± 31 SE μmol GAE 100 g^{-1} dw). Although there was a tendency for higher content in low density-weekly treatment biomass compared to high density-daily treatment, this was not statistically significant (Fig. 1, pair-wise test, $t = 2.02$, $P = 0.104$). In comparison, DPPH radical scavenging ability of wild collected *P. palmata* was an order of magnitude lower at 45 μmol Trolox equivalents 100 g^{-1} dw [26].

3.1.3. ABTS •+ radical scavenging ability

Culture conditions did not significantly affect the ABTS•+ radical scavenging ability (PERMANOVA, $F_{2,4} = 7.278$, $P = 0.051$). However, this was a marginal result and it is notable that the data was consistent with results from the other assays, with low density-weekly treatment (589 ± 48 SE μmol Trolox equivalents [TE] 100 g^{-1} dw) approximately 20% higher than high density-daily treatment and high density-weekly treatment (485 ± 30 SE μmol TE 100 g^{-1} dw) (Fig. 1). The radical scavenging ability of low density-weekly treatment biomass falls between the commonly consumed algae *Undaria pinnitafida* (181 μmol TE 100 g^{-1} dw) and *Himanthalia elongata* (1405 μmol TE 100 g^{-1} dw), however, it is much higher than in *Porphyra umbilicalis* (1 μmol TE 100 g^{-1} dw) [27].

3.1.4. Ferric reducing antioxidant potential

Ferric reducing antioxidant potential (FRAP) was strongly affected by culture conditions (Fig. 1, PERMANOVA, $F_{2,4} = 17.970$, $P = 0.009$), and followed the same trend as the other assays with low density-weekly treatment > high density-daily treatment > high density-weekly treatment. Maximum FRAP was recorded for low density-weekly treatment in week 3, at 1430 ± 74 SE μmol TE 100 g^{-1} dw, and averaged over the three weeks FRAP in biomass from low density-weekly treatment was 88% higher than high density-weekly treatment, and 55% higher than high density-daily treatment. This is similar to the reducing capacity of *P. umbilicalis* (1358 μmol TE 100 g^{-1} dw), and twice that of *Laminaria ochroleuca* (690 μmol TE 100 g^{-1} dw) [28]. However, the FRAP reducing potential was substantially lower in *Derbesia* compared to traditional high-antioxidant crops such as blueberries and apples (Table 1) [29].

3.2. Productivity

Biomass productivity was highest in low density-weekly treatment at 25.4 ± 1.4 SE g dw m^{-2} day^{-1} averaged over the three weeks, and this was significantly higher than high density-weekly treatment (14%) and high density-daily treatment (13%) (PERMANOVA, $F_{2,4} = 10.662$, $P = 0.023$) (Fig. 2a). These productivities are similar to those reported for *Derbesia* cultured in the same systems the previous year [15], emphasizing the stable production of this species of up to 91 ton dw ha^{-1} $year^{-1}$ assuming full year-round production. These high productivities are also among the highest reported for green marine macroalgae cultured in land-based systems [30,31]. As previously [15], the differences in productivity between treatments can largely be

Table 1
Comparison of antioxidant content and productivity between *Derbesia tenuissima* cultured at 0.5 g L^{-1} stocking density, other marine macroalgae, and antioxidant rich produce.

Biomass	Type of plant	Content		Productivity[a]			Reference
		Phenol (GAE)	FRAP (TE)	Biomass	Phenol (GAE)	FRAP (TE)	
		[mg g^{-1} dw]		[t dw ha^{-1} $year^{-1}$]	[kg ha^{-1} $year^{-1}$]		
D. tenuissima[b]	Green macroalga	3.8	3.0	91	352	272	This study
Enteromorpha intestinalis	Green macroalga	2.7					[4]
Ulva lactuca	Green macroalga	2.4					[4]
Palmaria palmata	Red macroalga	0.8		26–49	31–39		[4,34]
Porphyra purpurea	Red macroalga	3.0					[4]
Chondrus crispus	Red macroalga	1.1					[4]
Polysiphonia fucoides	Red macroalga	19.2					[4]
Grateloupia filicina	Red macroalga	0.38					[7]
Fucus vesiculosus	Brown macroalga	10.5					[4]
Blueberry	Berry	25.7	41.0	1.2	31	49	[29,35,40,41]
Strawberry	Berry	23.7	44.5	1.7	40	75	[29,36,40,42]
Apple	Fruit	11.9		1.4	168		[29,33,40]

[a] Productivity data [kg dw ha^{-1} $year^{-1}$] are calculated from reported fresh weight productivities and moisture contents (fw:dw), and reported antioxidant contents.
[b] Maximum theoretical yield calculated as 12 months production per year at 25 g dw m^{-2} day^{-1}.

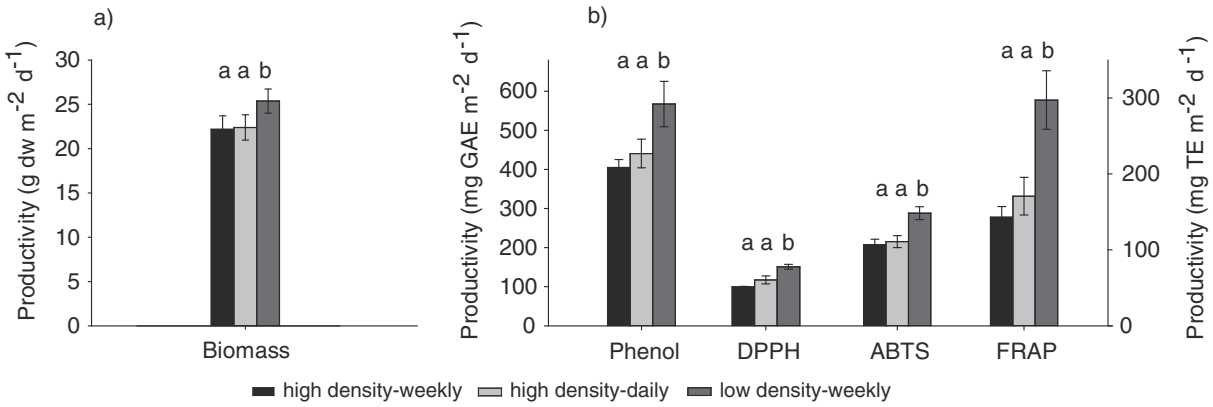

Fig. 2. Productivity of *Derbesia tenuissima* related to culture conditions a) biomass (g dw m^{-2} day^{-1}), and b) antioxidant (mg GAE or TE m^{-2} day^{-1}). High density-weekly = culture stocked at 2 g L^{-1}, high density-daily = continuously maintained at 2 g L^{-1}, low density-weekly = culture stocked at 0.5 g L^{-1}.

attributed to light availability in the tanks (Fig. 3, Section 3.3) as nutrients and carbon dioxide (NO_3^- L^{-1}1–6 mg L^{-1}, PO_4^{3-} > 1 mg L^{-1}, 8.11 < pH < 9.27) were available in excess throughout the experiment.

The differences in biomass productivity combined with the effect of culture conditions on antioxidant content yielded a significantly higher overall antioxidant productivity (expressed as μmol GAE or TE m^{-2} day^{-1}) in low density-weekly treatment compared to high density-weekly and high density-daily treatments (Fig. 2b, PERMANOVA, $F_{2,4} = 17.23$, $P = 0.006$). Specifically, the productivity of Trolox equivalents (mg TE m^{-2} day^{-1}) measured using FRAP was more than double in the low density-weekly treatment compared to the high density-weekly treatment, and 40% higher using the ABTS assay. Productivity of gallic acid equivalents (mg GAE m^{-2} day^{-1}) was also 50% (total phenol assay) and 40% (DPPH assay) higher in the low density-weekly treatment than in the high density-weekly treatment, and 30% higher than in the high density-daily treatment. With such a clear effect of lowering the stocking density it is possible that lowering stocking densities further would yield both higher biomass productivity – as reported previously [15] – and a higher antioxidant content. However, we note that there is an increased risk of contamination at low stocking densities [32; pers. observations for *Derbesia*, unpublished data]. Therefore, we propose an approach of initiating cultivation with a low stocking density to induce oxidative stress, and then allowing a full 7-day production cycle where the biomass spends at least half of the time at a higher density. This would represent a practical scenario for the long-term culture of *Derbesia* with high antioxidant content and productivity in land-based intensive systems.

3.3. Effects on light conditions and photosynthesis

Light availability (μmol photons cm^{-2} s^{-1}) at 10-cm depth intervals in the culture tanks changed over the 7-day culture cycle as hypothesised based on the culture conditions (Fig. 3). Specifically, in high density-weekly treatment tanks only 30% of the surface light (on average 2130 μmol photons cm^{-2} s^{-1} on sunny days) reached 5 cm below the surface in the first few days of culture, and from day 3 to day 7 (final average biomass density was 3.7 g fw L^{-1}, equivalent to ~0.55 g dw L^{-1} based on treatment specific fw:dw ratios) this decreased further from 18 to 12% of surface light. At 15 cm depth, less than 2% of surface light was available at any given stage during the culture cycle, meaning that approximately 80% of the entire biomass in high density-weekly treatments was effectively without light at any given time in the experiment. As anticipated, continuously maintaining the biomass at 2 g fw L^{-1} (~0.32 g dw L^{-1} based on treatment specific fw:dw ratios, high density-daily treatment) successfully maintained a stable light/depth profile in the tanks, with approximately 30% of surface light reaching 5 cm depth throughout each 7-day cycle, and 2% reaching 15 cm (Fig. 3b). Conversely, biomass in the low density-weekly treatment had much higher light availability early in each 7-day growth cycle; with 66% of surface light available at 5 cm depth and less than 20% of the biomass being effectively without light at any given time on the day of stocking (Fig. 3c). By the end of the 7-day cycle with a final average biomass density in low density-weekly treatment tanks of 2.5 g fw L^{-1} (~0.35 g dw L^{-1} based on treatment specific fw:dw ratios), 30% of surface light reached 5 cm depth, and there was measurable light available for photosynthesis

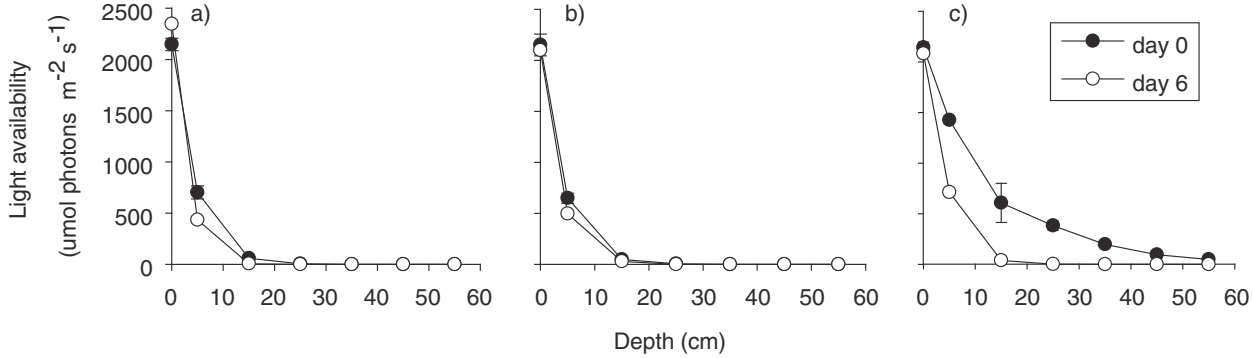

Fig. 3. Weekly variation in light availability (μmol photons m^{-2} s^{-1}) at different depths in *Derbesia tenuissima* 2000 L culture systems related to culture conditions a) high density-weekly = culture stocked at 2 g L^{-1}, b) high density-daily = continuously maintained at 2 g L^{-1}, and c) low density-weekly = culture stocked at 0.5 g L^{-1}.

below 15 cm depth (41 μmol photons cm^{-2} s^{-1}). Altering stocking density and harvest frequency are therefore both effective methods for manipulating and controlling the availability of light to the biomass, and these different levels of light had corresponding responses in potential quantum yield (photosynthetic capacity) as described below.

Potential quantum yield (F_v/F_m) was lowest at midday (12–1 pm) in all treatments, and recovered by each afternoon to a treatment specific maximum (Fig. 4a). There was a significant interaction between culture conditions and day for midday F_v/F_m, (PERMANOVA, $F = 12.057_{12,24}$ $P = 0.001$) driven by lower F_v/F_m in low density-weekly treatment over the first 3 days. Specifically, potential quantum yield of this biomass was 20% lower compared to high density-weekly and high density-daily treatments on day 0 (harvest and re-stocking), and 8–11% lower on days 1–2 (Fig. 4b), recovering to around 4% lower than high density-weekly treatment biomass by day 5. This shows that the biomass was comparatively more light stressed at the 0.5 g L^{-1} stocking density (low density-weekly treatment) during the middle of the day, until such a stage where biomass growth resulted in self-shading. Conversely, the F_v/F_m of high density-daily treatment was similar to high density-weekly treatment in the first few days, and slightly lower (3–5%) in the final three days of each culture cycle as a result of the F_v/F_m of high density-weekly treatment biomass increasing with increasing culture density. There was a significant negative correlation between F_v/F_m early in the culture cycle (days 0–3) and antioxidant content of the biomass on the day of harvest expressed as FRAP ($R^2 = -0.80$, $P = 0.009$), ABTS ($R^2 = -0.72$, $P = 0.026$), and total phenol ($R^2 = -0.76$, $P = 0.017$) (Fig. 5). Together these data demonstrate a clear link between light stress and antioxidant production and that

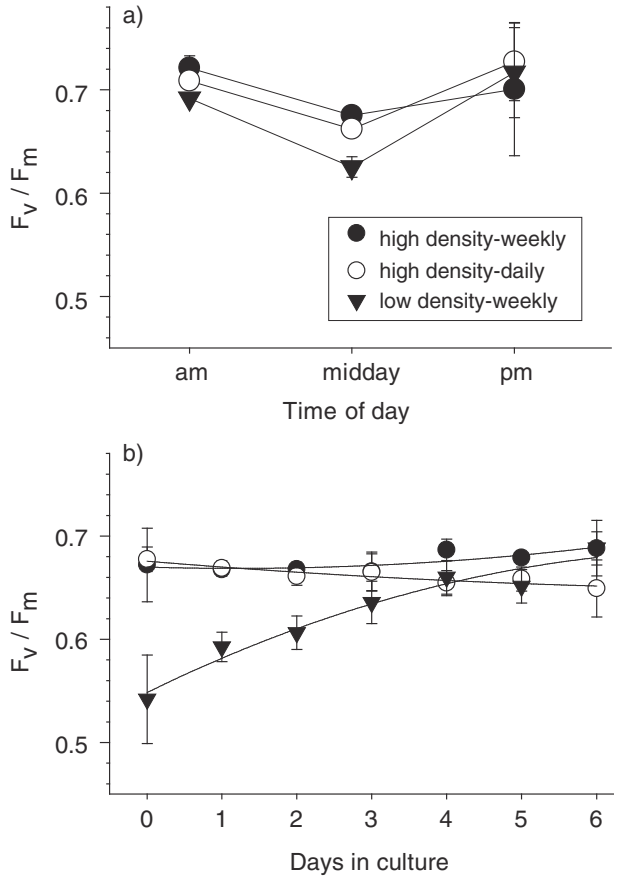

Fig. 4. Potential quantum yield (F_v/F_m) of *Derbesia tenuissima* related to culture conditions a) daily variation, average of measurements over 3 weeks at each time point, b) noon-time F_v/F_m averaged per treatment per day over the three weeks. High density-weekly = culture stocked at 2 g L^{-1}, high density-daily = continuously maintained at 2 g L^{-1}, low density-weekly = culture stocked at 0.5 g L^{-1}.

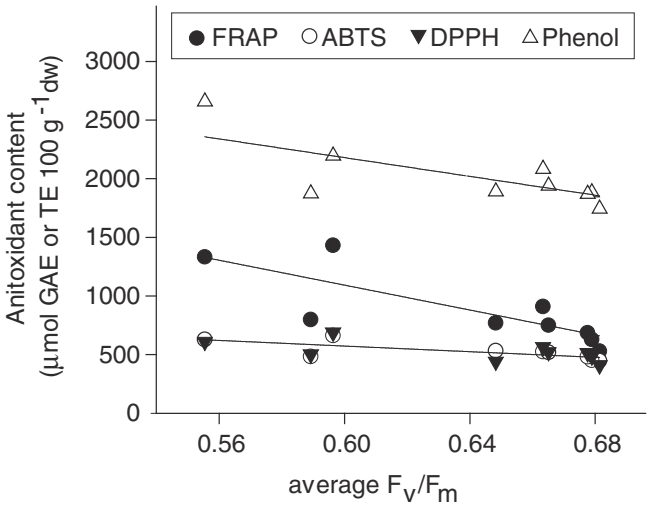

Fig. 5. Pearson's correlations between light stress early in the culture cycle (days 0–3), expressed as F_v/F_m, and antioxidant content in the harvested biomass expressed as total phenol (Pearson's correlation = −0.76, **P = 0.017**), DPPH (Pearson's correlation = −0.62, P = 0.073), ABTS (Pearson's correlation = −0.72, **P = 0.026**), and FRAP (Pearson's correlation = −0.80, **P = 0.009**). Significant correlations are highlighted in bold.

biomass that was more light stressed (i.e. had a lower F_v/F_m) had overall higher antioxidant capacity. This manifests at two scales, firstly, between the three treatments (average F_v/F_m, up to 23% difference) and secondly trends within each culture condition that relates to week to week variation in F_v/F_m (2–8% differences). A 15% decrease in F_v/F_m during the first two days of culture thus correlated with an average increase of total phenol content (as GAE) by approximately 20% after 7 days of culture, regardless of F_v/F_m recovering to higher, non-stressed values in that 7-day period. This also demonstrates a means to manage culture conditions in a land-based, intensive macroalgal crop, relative to feedback from photosynthetic capacity and we propose that this would be at least 2 days with F_v/F_m below 85% of maximum F_v/F_m, followed by 5 days where F_v/F_m is allowed to recover to its species and system specific maximum to follow the pattern described for low density-weekly treatment biomass (Fig. 4b).

3.4. Antioxidant content and productivity comparisons between crops

For land-based intensive production of marine macroalgae, improving both the antioxidant content and productivity can be achieved by changing culture conditions without altering the crop-footprint. This is in stark contrast to many terrestrial crops, where an increased biomass yield and improved crop quality for a given cultivar will often come at a cost of increasing plant spacing [33] and therefore the areal footprint of the crop. Our results highlight the advantages of land-based production of macroalgae for nutraceutical and bioproducts applications, with high crop productivity on an areal basis, and with the opportunity to manipulate the biomass composition through changes in stocking density. As a comparison, productivity of the red marine macroalgae *P. palmata* is estimated at 26–49 ton dw ha^{-1} $year^{-1}$ [34], 30–50% of *Derbesia* (Table 1). Additionally, areal productivities (ton dw ha^{-1} $year^{-1}$) of high-antioxidant crops such as blueberries (1.3% of *Derbesia*) [35], strawberries (1.9% of *Derbesia*) [36] and apples (1.5% of *Derbesia*) [33] (Table 1) reach just a fraction of the production potential of *Derbesia* of over 90 ton dw ha^{-1} $year^{-1}$. These lower biomass productivities for terrestrial crops mean that the capacity for total antioxidant production is also much lower than for *Derbesia*, despite their higher absolute antioxidant capacity measured as total phenol content (mg GAE g^{-1} dw) (3–7-fold higher) or FRAP (mg TE g^{-1} dw) (13–15-fold higher) (Table 1).

However, a serving size of blueberries (assuming 70 g fresh weight) or apples (assuming 100 g fresh weight) will deliver 252 mg GAE and 190 mg GAE, respectively, while 50–66 g dry weight of *Derbesia* would need to be consumed to deliver the same dose. As consumer acceptance for such large portion sizes of algae may be low, this alga could be more suitable as a feedstock for the extraction of the anti-oxidant components for bioproducts or nutraceutical applications [5,29]. Nevertheless, along with its antioxidant content, *Derbesia* has a favourable fatty acid composition [15,37], high protein content (25% dw) and quality (40% essential amino acids), and high content of dietary fibre (15% dw) [38]. The direct nutritional quality, together with the demonstrable functional benefits of inclusion in rat models, particularly in relation to triglyceride and cholesterol functionality [39], highlights the potential for *Derbesia* as an industrial crop for functional food applications.

4. Conclusions

Managing culture conditions has a strong effect on the content of antioxidants of *D. tenuissima* cultured in intensive land-based culture systems under ambient outdoor conditions. Antioxidant content was maximised at lower stocking densities without affecting biomass productivity. This increase in production of antioxidants at lower stocking densities is clearly linked to increased light stress with a 15% decrease in F_v/F_m during the first two days of culture. Early light stress was sufficient to nearly double the antioxidant potential (as FRAP) of the biomass, regardless of F_v/F_m recovering to higher, non-stressed values in that 7-day period. This has positive implications in terms of culture maintenance, as managing initial culture stocking density is a simple and effective method to manipulate culture light conditions. *D. tenuissima* is a high-productivity (91 ton ha^{-1} $year^{-1}$) biomass feed-stock for applications as functional food or other bio-products, compared to antioxidant rich produce such as fruit and berries, and commonly consumed marine macroalgae, emphasizing its potential as an industrial crop for bioproducts.

Author contributions

All authors contributed to the conception and design of the study, or acquisition of data, or analysis and interpretation of data; drafting the article or revising it critically for important intellectual content final, and all authors approve of the version to be submitted.

Acknowledgements

This research is part of the MBD Energy Research and Development program for Biological Carbon Capture and Storage. The project is supported by the Australian Government through the Advanced Manufacturing Cooperative Research Centre (AM-CRC), funded through the Australian Government's Cooperative Research Centre Scheme. The funding bodies had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication. We thank Veronique Mocellin and Kerri-Lee Dyer for post-harvest processing, biochemical analyses and culture production, and Sophie Raillard, Elien Boogaerts, Ana Wegner and Scott Seymour for culture production.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.algal.2015.02.007.

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