GLOBAL CHANGE BIOLOGY
BIOENERGY

GCB Bioenergy (2015) 7, 673–689, doi: 10.1111/gcbb.12171

Comparing the potential production and value of high-energy liquid fuels and protein from marine and freshwater macroalgae

NICOLAS NEVEUX[1], MARIE MAGNUSSON[1], THOMAS MASCHMEYER[2], ROCKY DE NYS[1] and NICHOLAS A. PAUL[1]

[1]*School of Marine & Tropical Biology, Centre for Macroalgal Resources & Biotechnology, James Cook University, Townsville, Queensland 4811, Australia,* [2]*School of Chemistry, The University of Sydney, Sydney, NSW 2006, Australia*

Abstract

The biomass production and biochemical properties of marine and freshwater species of green macroalgae (multicellular algae), cultivated in outdoor conditions, were evaluated to assess the potential conversion into high-energy liquid biofuels, specifically biocrude and biodiesel and the value of these products. Biomass productivities were typically two times higher for marine macroalgae (8.5–11.9 g m^{-2} d^{-1}, dry weight) than for freshwater macroalgae (3.4–5.1 g m^{-2} d^{-1}, dry weight). The biochemical compositions of the species were also distinct, with higher ash content (25.5–36.6%) in marine macroalgae and higher calorific value (15.8–16.4 MJ kg^{-1}) in freshwater macroalgae. Lipid content was highest for freshwater *Oedogonium* and marine *Derbesia*. Lipids are a critical organic component for biocrude production by hydrothermal liquefaction (HTL) and the theoretical biocrude yield was therefore highest for *Oedogonium* (17.7%, dry weight) and *Derbesia* (16.2%, dry weight). Theoretical biocrude yields were also higher than biodiesel yields for all species due to the conversion of the whole organic component of biomass, including the predominant carbohydrate fraction. However, all marine species had higher biomass productivities and therefore had higher projected biocrude productivities than freshwater species, up to 7.1 t of biocrude ha^{-1} yr^{-1} for *Derbesia*. The projected value of the six macroalgae was increased by 45–77% (up to US$7700 ha^{-1} yr^{-1}) through the extraction of protein prior to the conversion of the residual biomass to biocrude. This study highlights the importance of optimizing biomass productivities for high-energy fuels and targeting additional coproducts to increase value.

Keywords: algae, amino acid, biocrude, biodiesel, hydrothermal liquefaction, productivity, protein, seaweed, value

Received 5 July 2013 and accepted 18 December 2013

Introduction

Biomass represents a carbon-neutral renewable resource for the production of biofuels and biomaterials (Perlack *et al.*, 2005; Ragauskas *et al.*, 2006; Farine *et al.*, 2012). However, the expansion of biofuel production requires the development of fast-growing crops that can provide continuous and affordable biomass with a minimal impact on the environment (Fargione *et al.*, 2008; Brennan & Owende, 2010; Frank *et al.*, 2013). Algae, and more specifically both marine and freshwater macroalgae, are now recognized as targets for low-cost feedstocks for biofuels (Rowbotham *et al.*, 2012) and in particular high-energy liquid biofuels (>30 MJ kg^{-1}) for aviation and heavy vehicle transport (ARENA, 2012). Marine macroalgae (seaweeds) are already cultivated at scale (>15 million tonnes per annum) in a well-established and valuable industry for food and phycocolloid production (Chopin & Sawhney, 2009; Paul & Tseng, 2012). More recently, new technologies have been investigated for the conversion of macroalgal biomass to bioenergy (Ross *et al.*, 2008; Rowbotham *et al.*, 2012) and, at the same time, macroalgal proteins are now considered a suitable source for human and animal nutrition (Holdt & Kraan, 2011; Boland *et al.*, 2012).

There are numerous pathways to bioenergy from macroalgae that depend on the biochemical composition of the target species. The key biochemical components of lipid, protein, carbohydrate and ash contents vary substantially between the taxonomic grouping of species, and between marine or freshwater origin (Holdt & Kraan, 2011; Gosch *et al.*, 2012; Jung *et al.*, 2012). There are also effects of seasonal, environmental and culture conditions on the biochemical compositions of species (Fleurence, 1999; Taylor *et al.*, 2005; Adams *et al.*, 2011; Angell *et al.*, 2014). Importantly, the options for the conversion of macroalgal biomass to liquid biofuels vary from the traditional fermentation of carbohydrates to ethanol (Kraan, 2013) and the esterification of fatty acids

Correspondence: Nicolas Neveux, tel. +61 7 4781 4778, fax +61 7 4781 4585, e-mail: nicolas.neveux@my.jcu.edu.au

© 2014 John Wiley & Sons Ltd

for biodiesel production (Gosch *et al*., 2012), to the more recent use of thermochemical conversion, such as pyrolysis and hydrothermal liquefaction (HTL), that yield a liquid biocrude (Rowbotham *et al*., 2012). Of these, the extraction and esterification of fatty acids to biodiesel and the hydrothermal liquefaction of whole biomass to biocrude, with subsequent refining, represent two promising pathways for the production of high-energy liquid fuels from algae for the aviation industry (Aresta *et al*., 2005; Brennan & Owende, 2010; Biller & Ross, 2012; Rowbotham *et al*., 2012; Frank *et al*., 2013). These pathways focus primarily on the lipid and carbohydrate components of the biomass due to the high conversion efficiency of lipids and the high proportion of carbohydrate in macroalgal biomass, respectively (Biller & Ross, 2012; Rowbotham *et al*., 2012). Consequently, the pre-extraction of the protein component of the biomass represents an attractive option to add value to biomass *in-toto* in a biorefinery concept (Lammens *et al*., 2012).

Regardless of the technology and processing opportunities, the development of liquid biofuels from macroalgae inextricably relies on high biomass productivities and the integration of production systems with marine (de Paula Silva *et al*., 2008; Bolton *et al*., 2009; Nobre *et al*., 2010) and freshwater (Mulbry *et al*., 2008) waste water streams. Productivities for land-based cultivated macroalgae (Capo *et al*., 1999; Mulbry *et al*., 2008; Bolton *et al*., 2009; Mata *et al*., 2010) are higher than for many land crops (Kraan, 2013) and are also higher than that of macroalgae cultivated at sea, due to the ability to control both the supply of dissolved carbon and nutrients, and limit the action of epiphytes and grazers (Capo *et al*., 1999; Lüning & Pang, 2003). Furthermore, macroalgae in land-based systems can deliver simultaneous biomass production, CO_2 capture, and the removal of aquatic contaminants including nutrients (Gao & Mckinley, 1994; Israel *et al*., 2005; Mata *et al*., 2010) and more intractable industrial contaminants (Saunders *et al*., 2012; Roberts *et al*., 2013). Given that industrial and agricultural waste streams, including land-based aquaculture, represent the primary resource for intensive macroalgal biomass production, the focus must be on macroalgae that are robust and highly productive in land-based systems within these environments (Paul & de Nys, 2008; de Paula Silva *et al*., 2008; Lawton *et al*., 2013).

In this study, the biochemical features of six selected marine and freshwater green macroalgae were quantified and compared to identify the most promising species for the production of high-energy liquid fuels. These species were selected as they have relatively simple morphologies, are suited to intensive land-based production in nutrient-rich water (Mulbry *et al*., 2008; Bolton *et al*., 2009; Mata *et al*., 2010) and are resistant to contamination with a high tolerance to environmental fluctuations (de Paula Silva *et al*., 2008; Lawton *et al*., 2013). Biomass productivities were quantified per unit area (g m^{-2} d^{-1}, dry weight) and the biochemical profiles of each species analysed. These biochemical data provided the basis to firstly calculate the potential yield of high-energy liquid biofuel from each biomass, using either esterification of fatty acids to obtain biodiesel or HTL of the organic fraction to obtain biocrude, and secondly to calculate the projected productivity and value of these biofuels. Subsequently, we evaluated the potential of extracting protein prior to converting the residual biomass to biocrude, as an option to add value to the production of biocrude. Finally, we used sensitivity analyses for the highest value marine and freshwater species to evaluate the influence of the production parameters on the potential value of feedstocks.

Materials and methods

Study organisms

Six species of green macroalgae were selected from the culture collections at the Marine & Aquaculture Research Facilities Unit, at James Cook University, Townsville (19°33′S; 146°76′E). These included four species of marine green macroalgae (seaweed), *Chaetomorpha linum* (Kutzing), *Cladophora coelothrix* (Kutzing), *Derbesia tenuissima* (Crouan) and *Ulva ohnoi* (Hiraoka and Shimada), hereafter referred to by genus and origin. *Chaetomorpha*, *Cladophora* and *Ulva* were originally collected from the bioremediation pond at Good Fortune Bay Fisheries Ltd. (20°02′S; 148°22′E) in May 2010. *Derbesia* was collected from a shallow coastal rock platform at Rowes Bay, Townsville (19°29′S; 146°83′E) in August 2010. For the two freshwater species, *Cladophora vagabunda* (Hoek) was originally collected from the freshwater ponds at the Townsville Barramundi Fish farm, Kelso (19°36′S; 146°70′E) in March 2011 and *Oedogonium* sp. (Lawton *et al*., 2013) was collected from an irrigation channel in the Brandon sugar cane region (19°55′S; 146°35′E) in April 2011, hereafter also referred to by genus and origin. All macroalgae were maintained in stock cultures in outdoor tanks at James Cook University for at least 3 months prior to the experimental period in August 2011.

Culture experiments

Macroalgae were cultured in an outdoor tank-based system with the same regime of nutrient addition and water exchange. This enabled the biomass productivities of marine and freshwater species to be compared simultaneously. Each species was cultured in triplicate in 50 L batch culture cylindrical tanks (Blyth Enterprise Pty. Ltd., Perth, WA, Australia) stocked at 2 g L^{-1} (fresh weight) with a water exchange rate of 0.25 vol d^{-1} (12.5 L d^{-1}). Each tank had a footprint of 0.16 m^2 and a water depth of 0.36 m. Nutrients and trace elements

were provided with 60 mg L^{-1} of f/2 medium (Guillard & Ryther, 1962) with each water exchange. Water motion in batch cultures was provided through an aeration ring around the base of the tank bottom, ensuring the biomass had an even exposure to sunlight in the water column (Fig. 1).

The experimental conditions for all cultures were maintained for three culture cycles of 6 days with biomass productivities being measured at day 6, 12 and 18. Culture tanks were randomly repositioned every two days in the holding tank. The entire biomass within each culture tank was harvested every six days using an aquarium fish net, placed in a mesh bag, spun to constant fresh weight (fw) in a domestic centrifuge (MW512; Fisher & Paykel), weighed and subsequently restocked at 2 g L^{-1}. After 18 days, all biomass in each tank was harvested using a fish net (2 mm screen). A subsample of each replicate ($n = 3$ tanks) for each of the six species was weighed and oven-dried at 60 °C (Binder, Germany) to a constant weight to determine the fresh to dry weight ratio (fw : dw). Remaining biomass was freeze-dried at −55 °C and 120 μbars for 48 h (VirTis BTK Manifold; Quantum Scientific, Brisbane, QLD, Australia). Dried samples were then ground to a mean particle size of <500 μm and placed in a desiccator for 30 min to reach a stable moisture content (dry weight). Powdered macroalgae were stored in airtight vials under refrigeration and used for all subsequent biochemical analyses.

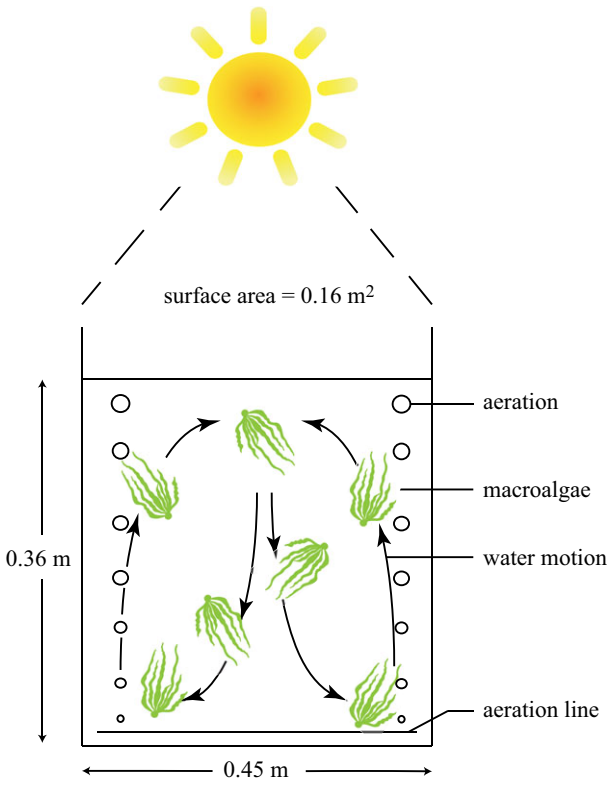

Fig. 1 Schematic of a batch culture tank. Macroalgae move freely within the water column driven by aeration from the base of the tank.

Environmental culture conditions were monitored and adjusted accordingly. Salinity and pH were recorded daily (YSI 63; YSI, Inc., Yellow Springs, OH, USA). Salinity for marine species was adjusted daily to 35 ppt using dechlorinated freshwater. Salinity of freshwater cultures was stable at 0–1 ppt for the duration of the experiment. The pH in batch cultures varied from 8.2 (sunrise) to 9.4 (sunset) for marine species and from 8.4 (sunrise) to 10.5 (sunset) for freshwater species. The culture tanks were placed within a larger holding tank which acted as a water bath to maintain the batch cultures at 25 °C. All cultures were held outdoor under full ambient sunlight. Light (photosynthetically active radiation) was monitored hourly using a data logger (Li-1400; LI-COR, Inc., Lincoln, NE, USA) adjacent to the tanks for the duration of the experiment. Total photons received for the final 6-day culture cycle was 260 mol photons m^{-2} with a peak daily irradiance of 1870 μmol photons m^{-2} s^{-1}.

Biomass productivity

Macroalgae productivity was determined for each culture cycle using Eqn (1):

$$P = (\mathrm{FW}_f - \mathrm{FW}_i)/(t * (\mathrm{fw} : \mathrm{dw}) * S) \quad (1)$$

where P is the macroalgae productivity (g m^{-2} d^{-1}, dry weight), FW_f is the final fresh weight (g), FW_i the initial fresh weight (g), t is the number of cultivation days, fw : dw the fresh to dry weight ratio and S is the surface area (m^2) of the culture tank. Mean biomass productivities for each species ($n = 3$) were analysed by one-factor Analysis of Variance (ANOVA, see Quinn & Keough, 2002 for details) followed by a pairwise comparison for each species combination using Tukey's Honestly Significant Difference (HSD) multiple comparisons (significant differences at $P < 0.05$ are reported) using the SPSS Statistics software (v20, IBM). Biomass productivities of the species were analysed for the final 6-day culture cycle ($n = 3$ replicate tanks) as this was the source of the biomass for all biochemical analyses.

Proximate analysis

Ash (dry inorganic) content was determined after combustion of the macroalgal sample (~100 mg) in a muffle furnace (SEM Ltd., Adelaide, SA, Australia) at 550 °C until constant weight was reached. Moisture content was determined by drying the sample (~1.5 g) at 110 °C in a moisture balance (MS70, A&D Company Ltd., Tokyo, Japan). Total lipids of macroalgal samples were extracted using a mixture of chloroform: methanol (2 : 1, v/v) and quantified by weight (Folch *et al.*, 1957), as described in Gosch *et al.* (2012). Proteinogenic amino acids (protein content) were quantified using the Water AccQTag method at the Australian Proteome Analysis Facility (Sydney, Australia). Total carbohydrates were determined by difference, by subtracting ash, moisture, total lipid and protein contents from 100%. Mean values of ash, moisture, lipid, protein and carbohydrate were analysed separately using one-factor ANOVAs and Tukey's HSD multiple comparisons.

Ultimate analysis

Carbon, hydrogen, oxygen, nitrogen and sulphur contents of macroalgal samples were analysed externally (OEA Laboratory Ltd., Callington, Cornwall, UK) using an elemental analyser. Higher heating values (HHV) were calculated from the ultimate analysis of samples, incorporating the ash content (Channiwala & Parikh, 2002). HHV were analysed using a one-factor ANOVA and Tukey's HSD multiple comparisons.

Biodiesel yield

Biodiesel yield was determined through the conversion of biomass fatty acids (FA) to fatty acid methyl esters (FAME), the components of crude biodiesel (Chisti, 2007), following the relationship (2):

$$Y_{\mathrm{BIODIESEL}} = W_{\mathrm{FAME}} \quad (2)$$

where $Y_{\mathrm{BIODIESEL}}$ is the crude biodiesel yield (wt%), corresponding W_{FAME}, the FAME content (wt%) extracted from the macroalgae.

FA were converted to FAME using a direct esterification method adapted for macroalgae (Gosch *et al.*, 2012). This method simultaneously extracts and esterifies FA to FAME for subsequent separation and quantification by gas chromatography–mass spectrometry (Agilent 7890 GC with FID – Agilent 5975C EI/TurboMS). The FAME profile of macroalgae was used to analyse the quality of biodiesel, through the calculation of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) concentrations.

Theoretical biocrude yield

Although a complex reaction cascade occurs in the production of biocrude through hydrothermal liquefaction (HTL), it has been demonstrated that the conversion of lipids, proteins and carbohydrates is additive and that biocrude yield can be estimated based on the feedstock biochemical content (Biller & Ross, 2011) using the Eqn (3):

$$Y_{\mathrm{BIOCRUDE}} = (Y_{\mathrm{LIPID}} * W_{\mathrm{LIPID}}) + (Y_{\mathrm{PROTEIN}} * W_{\mathrm{PROTEIN}}) + (Y_{\mathrm{CARBOHYDRATE}} * W_{\mathrm{CARBOHYDRATE}}) \quad (3)$$

where Y_{BIOCRUDE}, Y_{LIPID}, Y_{PROTEIN} and $Y_{\mathrm{CARBOHYDRATE}}$ are biocrude, lipid, protein and carbohydrate HTL yields (wt%), and W_{LIPID}, W_{PROTEIN} and $W_{\mathrm{CARBOHYDRATE}}$ are lipid, protein and carbohydrate contents (wt%) of macroalgae. The theoretical biocrude yields were calculated as a range with an upper and a lower limit for each species. The upper limit used the biochemical yield conversion factors of 0.80, 0.18, 0.15 for lipids, proteins and carbohydrates, respectively, and the lower limit used conversion factors of 0.55, 0.11, 0.06 for the same components (Biller & Ross, 2011). These conversion factors are based on the yields of a range of model compounds obtained through HTL performed at 350 °C for 1 h and 10% solids.

Theoretical protein yield

The theoretical protein yield (wt%) was calculated from the sum of all amino acids (AA). The essential amino acids were calculated from the sum of histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine.

Projected areal productivities

The projected productivities of biodiesel, biocrude and protein were determined by multiplying individual yields by the biomass productivity for each species, using Eqn (4):

$$P_{\mathrm{BIOPRODUCT}} = P * Y_{\mathrm{BIOPRODUCT}}/100\% \quad (4)$$

where $P_{\mathrm{BIOPRODUCT}}$ is biodiesel, biocrude or protein productivity (g m^{-2} d^{-1}), P is the biomass productivity (g m^{-2} d^{-1}, dry weight) and $Y_{\mathrm{BIOPRODUCT}}$ is the biodiesel, biocrude or protein yield (wt%).

Projected production values – at scale with sequential extraction

To evaluate the potential value of macroalgal feedstock at scale, $P_{\mathrm{BIOPRODUCT}}$ was converted into t ha^{-1} yr^{-1} and the values of comparable commodities were used to estimate the value per hectare per year of each species in US$. The value of crude diesel ($3.1 gal^{-1}) was converted to $975.0 t^{-1} according to the specific gravity of 0.84 at 15 °C for crude diesel (Tat & Van Gerpen, 2000) and assuming that one US gallon contains 3.785 L. Then, the price of biodiesel ($941.4 t^{-1}) was derived from crude diesel price after adjustment for volume with a biodiesel specific gravity of 0.87 (Miao & Wu, 2006), using the conversion factor of 0.9655 (=0.84/0.87) to account for this difference in quality. Similarly, the value of WTI (West Texas Intermediate) crude oil ($105.3 bbl^{-1}) was converted to $798.1 t^{-1} according to the specific gravity of 0.83 at 15 °C for WTI crude oil (Weaver, 2004) and assuming that one barrel contains 158.987 L. The price of biocrude ($682.5 t^{-1}) was then derived from WTI crude oil price after adjustment for volume with biocrude specific gravity of 0.97 (Jena & Das, 2011), using the conversion factor of 0.8550 (=0.83/0.97) to account for this difference in quality. Soybean meal ($431.9 t^{-1}) was used to estimate the value of the protein in a conservative way, acknowledging that soybean meal is composed of about 50 wt % amino acids (Lywood *et al.*, 2009), whereas the protein extract would theoretically be 100 wt% amino acids. The values of crude diesel, WTI crude oil and soybean meal were based on a two-year average price index sourced from Indexmundi (http://www.indexmundi.com/australia/). Projected values of biodiesel, biocrude and protein were calculated for each product singularly and then sequentially for the extraction of protein prior to conversion of the residual biomass to biocrude. The sequential extraction of lipids (value estimated from soy oil price at $1169.7 t^{-1}, Indexmundi) or fatty acids for biodiesel production (see above for value), prior to the conversion of the residual biomass to biocrude, was also calculated for comparison (Table S1).

Projected production values – sensitivity analysis

Sensitivity analysis was used as a tool to visualize the relative importance of production parameters under a range of

different cases. This tool has recently been used for algal biofuels as it is particularly useful where there are knowledge gaps or uncertainty for the parameters of different systems. For example, sensitivity analysis provides a mechanism to synthesize laboratory, pilot and commercial scale information into a single package while acknowledging the limitations and uncertainties of each parameter to define unfavourable, standard and favourable cases (Yang *et al*., 2011; Ong *et al*., 2012; Liu *et al*., 2013). Sensitivity analyses were used in the present study to provide context for the outcomes of protein extraction prior to biocrude production from the residual biomass for the most valuable marine species (*Derbesia* and *Ulva*) and the most valuable freshwater species (*Oedogonium*), given that this sequential process yielded the highest projected values (see Results and Table S2). It also served to provide additional context for projections, for example, while there is no commercial scale production of *Derbesia* and *Oedogonium*, there are analogous culture systems in place for both marine (*Ulva* – Bolton *et al*., 2009) and freshwater algae (Park *et al*., 2011). Similarly, while there are no reported yields from HTL of macroalgae for large-scale continuous flow reactors, there are laboratory data (batch reactor) yields for the green macroalga *Ulva* (Zhou *et al*., 2010) and a range of microalgae (Tables S3 and S4) that can be used for projections. Full calculations and references for the sensitivity analyses are provided in the supporting information (Tables S2–S4).

Values for biomass productivity were defined as standard (centre, average of the current study), favourable (right of centre, 24.0 g m^{-2} d^{-1} for *Derbesia* from Magnusson *et al*., 2014, 26;.1 g m^{-2} d^{-1} for *Ulva* from Bolton *et al*., 2009 and 16.0 g m^{-2} d^{-1} for *Oedogonium*, A.J. Cole, R. de Nys, N.A. Paul, in review) and unfavourable (left of centre, one standard deviation below the average of the current study). Values for theoretical biocrude conversion yield were defined as standard (centre, upper limit of the current study) with favourable (right of centre, 50% increase from the upper yield) and unfavourable (left of centre, lower limit of the current study). Values for protein content were defined as standard (centre, average of the current study), favourable (right of centre, one standard deviation above the average of the current study) and unfavourable (left of centre, one standard deviation below the average of the current study). Values for biocrude and protein extract, adjusted from the values of WTI crude oil and soybean meal (see above section – Projected production values), were defined as standard (centre, average price for the last two years from Indexmundi), favourable (right of centre, maximum price for the last two years from Indexmundi) and unfavourable (left of centre, minimum price for the last two years from Indexmundi).

Projected values for the sequential extraction of protein and the conversion of the residual biomass for *Derbesia*, *Ulva* and *Oedogonium* (US$ ha^{-1} yr^{-1}) were calculated separately for each species according to Eqn (5):

$$\text{Feedstock value} = 3.65 * P * [(Y_{\text{BIOCRUDE-AA}} * \text{Price-}_{\text{BC}}) + (W_{\text{PROTEIN}} * \text{Price-}_{\text{PE}})]/100\% \quad (5)$$

where the multiplier of '3.65' is derived from the conversion of productivity in g m^{-2} d^{-1} to productivity in t ha^{-1} y^{-1}, P is the biomass productivity (g m^{-2} d^{-1}, dry weight), $Y_{\text{BIOCRUDE-AA}}$ is the biocrude yield (wt%) after protein extraction, Price-$_{\text{BC}}$ is the two year average price (US$ t^{-1}) of biocrude derived from WTI crude oil price, W_{PROTEIN} is the protein (AA) content (wt%) of macroalgae and Price-$_{\text{PE}}$ is the two year average price (US$ t^{-1}) of the protein extract derived from soybean meal price.

Results

Biomass productivity

Biomass productivity (g m^{-2} d^{-1}, dry weight) for outdoor batch cultures was up to two times higher for marine macroalgae than for freshwater macroalgae (Fig. 2; ANOVA, $F_{5,12} = 63.09$, $P < 0.001$). *Derbesia* (11.9 g m^{-2} d^{-1}) and *Ulva* (11.4 g m^{-2} d^{-1}) were the most productive species. *Oedogonium* (5.1 g m^{-2} d^{-1}) had the highest productivity of the two freshwater species, and freshwater *Cladophora* (3.4 g m^{-2} d^{-1}) the lowest productivity of all species. These biomass productivities are for the final 6-day cycle and were consistent with the previous two cycles, for example, ranging from 11.5 to 12.7 g m^{-2} d^{-1} for *Derbesia*, 10.8 to 11.9 g m^{-2} d^{-1} for *Ulva* and 4.9 to 5.5 g m^{-2} d^{-1} for *Oedogonium*.

Proximate analysis

The proximate and biochemical composition of macroalgae, expressed as the percentage of the dry weight of samples, varied substantially between species (Table 1). Ash content ranged from 17.8 to 36.6% and freshwater macroalgae typically had lower ash contents than marine macroalgae (ANOVA, $F_{5,12} = 15.43$, $P < 0.001$). Marine *Chaetomorpha* (36.6%) and *Derbesia* (34.7%) had the highest ash content, and the freshwater *Cladophora*

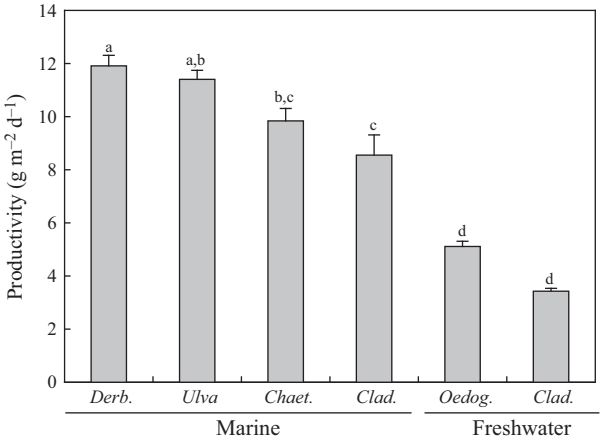

Fig. 2 Biomass productivity of macroalgae. Data show productivity means ($n = 3 \pm$ SE, in g m^{-2} d^{-1}, dry weight) of each marine (M) and freshwater (FW) macroalgae. Species sharing the same letter above the bars are not significantly different (Tukey's HSD, $P < 0.05$).

Table 1 Proximate and biochemical analysis of macroalgae. Data show means ($n = 3 \pm$ SE, in wt%, dry weight) of ash, moisture, lipid, protein and carbohydrate contents of marine (M) and freshwater (FW) macroalgae. Carbohydrate content was determined by difference. Protein equals the sum of amino acids. Species sharing the same letter in superscript are not significantly different (ANOVA, Tukey's HSD, $P < 0.05$)

		Proximate		Biochemical		
Species	Source	Ash	Moisture	Lipid	Protein	Carbohydrate
Derbesia	M	$34.7^{a} \pm 0.4$	$6.4^{a,b} \pm 0.5$	$10.4^{a} \pm 0.1$	$21.6^{b} \pm 0.2$	$26.9^{b} \pm 0.6$
Ulva	M	$30.7^{a,b} \pm 0.5$	$7.2^{a} \pm 0.4$	$1.9^{d} \pm 0.1$	$16.3^{c} \pm 0.2$	$43.9^{a} \pm 0.8$
Chaetomorpha	M	$36.6^{a} \pm 1.0$	$5.1^{b} \pm 0.4$	$3.3^{c} \pm 0.1$	$11.1^{d} \pm 0.4$	$43.9^{a} \pm 0.8$
Cladophora	M	$25.5^{b,c} \pm 1.0$	$6.7^{a,b} \pm 0.3$	$4.6^{b} \pm 0.2$	$17.8^{c} \pm 1.1$	$45.4^{a} \pm 1.7$
Oedogonium	FW	$20.6^{c} \pm 4.2$	$6.5^{a,b} \pm 0.4$	$9.4^{a} \pm 0.3$	$22.5^{b} \pm 0.3$	$41.0^{a} \pm 4.0$
Cladophora	FW	$17.8^{c} \pm 1.5$	$5.7^{a,b} \pm 0.3$	$5.3^{b} \pm 0.3$	$26.8^{a} \pm 0.4$	$44.4^{a} \pm 0.5$

(17.8%) the lowest. The organic component varied widely across species as well, in many cases by a factor of two. Lipid content ranged from 1.9 to 10.4% and varied independently from macroalgae marine or freshwater origin. Marine *Derbesia* (10.4%) and freshwater *Oedogonium* (9.4%) had the highest lipid content and marine *Ulva* (1.9%) had the lowest (ANOVA, $F_{5,12} = 276.58$, $P < 0.001$). Variation in protein content was primarily driven by the difference between marine and freshwater species, ranging from 11.1 to 26.8% (ANOVA, $F_{5,12} = 97.70$, $P < 0.001$). Protein contents were above 20% for three species and highest for freshwater *Cladophora* (26.8%) and *Oedogonium* (22.5%). *Derbesia* (21.6%) had the third highest protein content, which was the highest of all marine species and was double that of *Chaetomorpha* (11.1%), which had the overall lowest protein content. Carbohydrates were the main organic component of all species, ranging from 26.9 to 45.4% (ANOVA, $F_{5,12} = 14.11$, $P < 0.001$). Marine *Cladophora* (45.4%) and freshwater *Oedogonium* (44.4%) had the highest carbohydrate contents, ~75% higher than *Derbesia* (26.9%), which had the lowest content.

Ultimate analysis

The carbon content of macroalgae ranged from 26.5 to 37.5% on a dry weight basis (Table 2). Freshwater *Cladophora* (37.5%) and *Oedogonium* (36.6%) had the highest carbon content of all species. Marine *Cladophora* (30.9%) and *Derbesia* (29.2%) had the highest carbon content of the marine species, whereas marine *Chaetomorpha* (26.5%) had the lowest. Carbon content correlated with higher heating values (HHV) that ranged from 10.3 to 16.4 MJ kg^{-1} (ANOVA, $F_{5,12} = 39.88$, $P < 0.001$). Freshwater *Cladophora* (16.4 MJ kg^{-1}) and *Oedogonium* (15.8 MJ kg^{-1}) had the highest HHV of all species. Marine *Cladophora* (12.7 MJ kg^{-1}) and *Derbesia* (12.4 MJ kg^{-1}) had the highest HHV of the marine species and marine *Chaetomorpha* (10.3 MJ kg^{-1}) had the lowest. Nitrogen content was species dependent and ranged from 3.4 to 6.5%. Both freshwater *Cladophora* (6.5%) and marine *Cladophora* (5.2%) had the highest nitrogen content and marine *Chaetomorpha* (3.4%) had the lowest.

Biodiesel yield

Yields of crude biodiesel ranged from 1.6 to 4.9% on a dry weight basis (Table 3). Freshwater *Cladophora* (4.9%) and *Oedogonium* (4.7%) had the highest biodiesel yields of all species (ANOVA, $F_{5,12} = 119.23$, $P < 0.001$). The third highest biodiesel yield was obtained from marine *Derbesia* (4.2%), which was more than 2.5 times higher than the lowest biodiesel yield of marine *Ulva* (1.6%). The quality of biodiesel (FA concentrations, measured as FAME) also differed between species (Table 4). The quantity of saturated fatty acids (SFA) in all species was

Table 2 Ultimate analysis of macroalgae. Data show means ($n = 3 \pm$ SE) of C, H, O, N, S (in wt%, dry weight) and higher heating value (HHV, in MJ kg^{-1}) of marine (M) and freshwater (FW) macroalgae. HHV is calculated from Channiwala & Parikh (2002). Species sharing the same letter in superscript are not significantly different (Tukey's HSD, $P < 0.05$)

Species	Source	C	H	O	N	S	HHV
Derbesia	M	29.2 ± 0.3	4.8 ± 0.1	27.4 ± 0.3	4.5 ± 0.0	2.8 ± 0.1	12.4 ± 0.2^{b}
Ulva	M	27.7 ± 0.3	5.5 ± 0.1	41.1 ± 0.4	3.5 ± 0.1	5.0 ± 0.1	$11.7 \pm 0.2^{b,c}$
Chaetomorpha	M	26.5 ± 0.6	4.1 ± 0.1	31.0 ± 1.0	3.4 ± 0.1	2.1 ± 0.1	10.3 ± 0.3^{c}
Cladophora	M	30.9 ± 0.3	5.0 ± 0.1	34.9 ± 0.8	5.2 ± 0.1	2.3 ± 0.1	12.7 ± 0.1^{b}
Oedogonium	FW	36.6 ± 1.9	5.7 ± 0.2	30.9 ± 1.9	4.8 ± 0.2	0.4 ± 0.0	15.8 ± 0.8^{a}
Cladophora	FW	37.5 ± 1.2	5.9 ± 0.1	32.9 ± 0.5	6.5 ± 0.1	1.8 ± 0.1	16.4 ± 0.6^{a}

Table 3 Theoretical biodiesel, biocrude and protein yields. Data show yield means ($n = 3 \pm$ SE, in wt%, dry weight) of biodiesel, SFA, MUFA, PUFA, biocrude (upper and lower limits), total protein (amino acids) and essential amino acids of marine (M) and freshwater (FW) macroalgae. Species sharing the same letter in superscript are not significantly different (Tukey's HSD, $P < 0.05$)

Species	*Derbesia*	*Ulva*	*Chaetomorpha*	*Cladophora*	*Oedogonium*	*Cladophora*
Source	M	M	M	M	FW	FW
Biodiesel						
Total	4.2 ± 0.2^{b}	1.6 ± 0.1^{d}	$2.1 \pm 0.1^{c,d}$	2.6 ± 0.1^{c}	$4.7 \pm 0.1^{a,b}$	4.9 ± 0.2^{a}
SFA	1.5 ± 0.0^{a}	0.7 ± 0.0^{c}	0.7 ± 0.0^{c}	1.0 ± 0.1^{b}	1.1 ± 0.0^{b}	1.5 ± 0.0^{a}
MUFA	0.5 ± 0.0^{b}	$0.4 \pm 0.0^{b,c}$	0.3 ± 0.0^{c}	0.6 ± 0.0^{b}	0.5 ± 0.0^{b}	1.1 ± 0.1^{a}
PUFA	2.2 ± 0.2^{c}	0.5 ± 0.1^{d}	1.1 ± 0.0^{d}	0.9 ± 0.0^{d}	3.1 ± 0.1^{a}	2.3 ± 0.1^{b}
Biocrude						
Upper	$16.2 \pm 0.0^{a,b}$	11.1 ± 0.1^{d}	11.2 ± 0.2^{d}	13.7 ± 0.1^{c}	17.7 ± 0.6^{a}	15.7 ± 0.4^{b}
Lower	9.7 ± 0.0^{a}	5.5 ± 0.1^{d}	5.7 ± 0.1^{d}	7.2 ± 0.1^{c}	10.1 ± 0.3^{a}	8.5 ± 0.2^{b}
Amino acids						
Total	21.6 ± 0.2^{b}	16.3 ± 0.2^{c}	11.1 ± 0.4^{d}	17.8 ± 1.1^{c}	22.5 ± 0.3^{b}	26.8 ± 0.4^{a}
Essential	9.1 ± 0.1^{b}	6.4 ± 0.1^{c}	4.4 ± 0.1^{d}	7.1 ± 0.1^{c}	$9.7 \pm 0.3^{a,b}$	10.1 ± 0.1^{a}

primarily driven by palmitic acid (C16 : 0) content. The proportion of SFA was highest in marine *Ulva* (43.0%) and marine *Cladophora* (38.7%), and lowest in freshwater *Oedogonium* (23.5%). The same species, *Ulva* (25.2%) and marine *Cladophora* (25.0%), had the highest monounsaturated fatty acid (MUFA) content. This was driven primarily by high concentrations of oleic acid (C18 : 1) for *Ulva* (1.6 mg g^{-1}) and for marine *Cladophora* (3.4 mg g^{-1}) relatively to their total FA content. The two species with the highest proportion of PUFA were *Oedogonium* (66.4%) and *Derbesia* (53.2%), for which the concentrations of hexadecatrienoic acid (C16 : 3) and α-linolenic acid (C18 : 3) were particularly high, with 6.1 and 12.8 mg g^{-1}, respectively for *Oedogonium*, and 4.9 and 9.5 mg g^{-1}, respectively for *Derbesia*. However, the FA content of macroalgae differed from the total lipid content and the lipid:FA ratio ranged from 1.1 to 2.5 across all species, and was highest for marine *Derbesia* (2.5) and freshwater *Oedogonium* (2.0) and lowest for freshwater *Cladophora* (1.1). This high ratio shows that *Derbesia* and *Oedogonium* had the highest proportions of non-FA lipids.

Theoretical biocrude yield

The theoretical yields of biocrude from macroalgae through HTL yielded 2–7 times more biocrude than the esterification of fatty acids (FA) yielded biodiesel (Table 3, ANOVA, $F_{5,12} = 75.27$, $P < 0.001$). Overall, theoretical biocrude yields ranged from 5.5% to 17.7% on a dry weight basis. For each species, the theoretical biocrude yields calculated as a range with lower and upper limits, were highest for freshwater *Oedogonium* (10.1–17.7%) and marine *Derbesia* (9.7–16.2%), which were ~75% higher than the lowest yields for marine *Ulva* (5.5–11.1%).

Theoretical protein yield

The theoretical protein yield (sum of individual amino acids) ranged from 11.1% to 26.8% (dry weight) and was highest for freshwater *Cladophora* and *Oedogonium* and marine *Derbesia* (Table 3, ANOVA, $F_{5,12} = 97.70$, $P < 0.001$). The quality of the protein also differed between species (Table 5). Both aspartic and glutamic acids – and their respective amides – were the main amino acids in all species and were highest in freshwater *Cladophora* (37.9 and 41.3 mg g^{-1}, respectively) and lowest in marine *Chaetomorpha* (17.7 and 15.7 mg g^{-1}, respectively). The essential amino acids content, expressed as a proportion of total amino acids, was highest for freshwater *Oedogonium* (43%), marine *Derbesia* (42%), and lowest for freshwater *Cladophora* (38%). The quantity of the essential amino acid methionine, expressed as a relative amount of total amino acids, and the ratio of methionine to lysine were highest in marine *Derbesia* (2.1% and 0.31%, respectively), *Ulva* (1.6% and 0.30%, respectively) and freshwater *Oedogonium* (1.9% and 0.28%, respectively), and lowest in marine *Chaetomorpha* (1.0% and 0.12%, respectively). The protein : N ratio for green macroalgae ranged from 3.3 to 4.8, highest for marine *Derbesia* (4.8) and freshwater *Oedogonium* (4.7) and lowest for marine *Chaetomorpha* (3.3).

Projected areal productivities

The projected areal productivities of biodiesel, biocrude and protein, calculated by integrating biomass productivity and biochemical composition (Eqn 4), demonstrated that biocrude productivity was consistently higher (by 40–80%) than biodiesel productivity across all species on a dry weight basis (Fig. 3a). Marine species had a higher productivity of biocrude than

Table 4 Biodiesel (FAME) profiles of macroalgae. Data show means (n = 3 ± SE) of fatty acid methyl esters (FAME, mg g^{-1}, dry weight) of each marine (M) and freshwater (FW) macroalgae. Chemical properties of biodiesel including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are expressed as a proportion (wt%) of total fatty acid content

Species	*Derbesia*	*Ulva*	*Chaetomorpha*	*Cladophora*	*Oedogonium*	*Cladophora*
Source	M	M	M	M	FW	FW
C14 : 0	1.02 ± 0.04	0.30 ± 0.01	1.66 ± 0.00	2.18 ± 0.22	0.76 ± 0.20	3.27 ± 0.14
C14 : 1 (n−5)	0.40 ± 0.01	0.29 ± 0.01	0.27 ± 0.00	0.29 ± 0.00	0.43 ± 0.01	0.37 ± 0.01
C15 : 0	0.46 ± 0.02	0.32 ± 0.01	0.30 ± 0.01	0.32 ± 0.00	0.50 ± 0.01	0.45 ± 0.01
C15 : 1 (n−5)	0.73 ± 0.03	0.43 ± 0.02	0.40 ± 0.01	0.46 ± 0.01	0.83 ± 0.02	0.68 ± 0.02
C16 : 0	9.84 ± 0.15	5.08 ± 0.10	4.09 ± 0.23	6.51 ± 0.36	8.59 ± 0.17	10.19 ± 0.3
C16 : 1 (n−9)	0.28 ± 0.01	0.21 ± 0.01	0.24 ± 0.00	0.55 ± 0.10	0.30 ± 0.01	0.58 ± 0.05
C16 : 1 (n−7)	1.74 ± 0.05	1.26 ± 0.03	1.03 ± 0.11	1.59 ± 0.14	1.68 ± 0.42	2.02 ± 0.09
C16 : 2 (n−6)	0.41 ± 0.03	0.22 ± 0.01	0.30 ± 0.01	0.60 ± 0.02	0.93 ± 0.02	0.44 ± 0.05
C16 : 2 (n−4)	0.22 ± 0.01	0.24 ± 0.01	1.46 ± 0.05	0.36 ± 0.02	0.66 ± 0.25	1.62 ± 0.15
C17 : 0	0.24 ± 0.01	0.22 ± 0.01	0.22 ± 0.00	0.20 ± 0.00	0.27 ± 0.02	0.31 ± 0.07
C16 : 3 (n−6)	0.26 ± 0.02	0.21 ± 0.01	0.21 ± 0.01	0.22 ± 0.00	0.84 ± 0.38	0.46 ± 0.11
C16 : 3 (n−3)	4.92 ± 0.52	0.32 ± 0.02	0.22 ± 0.01	0.24 ± 0.00	6.05 ± 0.85	0.24 ± 0.01
C16 : 4 (n−3)	0.40 ± 0.02	0.77 ± 0.21	1.33 ± 0.04	1.25 ± 0.17	1.43 ± 0.16	3.70 ± 0.12
C18 : 0	0.53 ± 0.00	0.28 ± 0.01	0.25 ± 0.00	0.27 ± 0.02	0.36 ± 0.00	0.33 ± 0.01
C18 : 1 (n−9)	1.76 ± 0.06	1.61 ± 0.05	1.36 ± 0.04	3.39 ± 0.25	1.24 ± 0.09	6.64 ± 0.66
C18 : 2 (n−6)	1.93 ± 0.09	0.39 ± 0.03	4.35 ± 0.06	1.99 ± 0.06	2.17 ± 0.12	7.45 ± 0.57
C18 : 3 (n−6)	0.87 ± 0.04	0.27 ± 0.01	0.29 ± 0.01	0.25 ± 0.01	1.39 ± 0.06	0.59 ± 0.04
C18 : 3 (n−3)	9.46 ± 0.62	0.97 ± 0.18	0.63 ± 0.18	2.64 ± 0.17	12.84 ± 1.21	3.98 ± 0.15
C18 : 4 (n−3)	0.96 ± 0.10	1.18 ± 0.36	0.35 ± 0.08	0.41 ± 0.05	2.58 ± 0.04	0.28 ± 0.02
C20 : 0	0.24 ± 0.01				0.21 ± 0.01	
C20 : 1 (n−9)	0.22 ± 0.00			0.21 ± 0.00	0.21 ± 0.00	0.46 ± 0.02
C20 : 2 (n−6)			0.23 ± 0.00	0.21 ± 0.00	0.30 ± 0.01	0.29 ± 0.01
C20 : 4 (n−6)	0.38 ± 0.01		0.23 ± 0.00		0.32 ± 0.03	0.30 ± 0.01
C20 : 3 (n−6)	1.46 ± 0.06	0.24 ± 0.01	0.60 ± 0.02	0.50 ± 0.01	0.43 ± 0.13	1.15 ± 0.05
C20 : 5 (n−3)	1.15 ± 0.10	0.30 ± 0.02	0.32 ± 0.04	0.79 ± 0.05	1.13 ± 0.52	1.84 ± 0.03
C22 : 0	0.91 ± 0.03	0.49 ± 0.01		0.24 ± 0.01		
C24 : 0	1.38 ± 0.01	0.22 ± 0.00	0.38 ± 0.05	0.33 ± 0.01	0.31 ± 0.09	0.51 ± 0.04
C22 : 6 (n−3)		0.25 ± 0.01	0.26 ± 0.02			0.48 ± 0.03
Total FAME	42.2 ± 1.7	16.1 ± 1.1	21.0 ± 0.7	26.0 ± 0.9	46.8 ± 1.0	48.6 ± 1.9
Biodiesel chemical profile [wt%]						
SFA	34.6	43.0	32.9	38.7	23.5	31.0
MUFA	12.2	25.2	17.0	25.0	10.0	23.1
PUFA	53.2	31.8	50.2	36.4	66.4	45.9
Ratio						
lipid : FA	2.5	1.2	1.6	1.8	2.0	1.1

freshwater species due to their higher growth rates, for which *Derbesia* (1.15–1.93 g m^{-2} d^{-1}) and *Ulva* (0.63–1.26 g m^{-2} d^{-1}) had the maximum projected biocrude productivity of the marine species, and *Oedogonium* (0.52–0.90 g m^{-2} d^{-1}) the highest of the freshwater species. Freshwater *Cladophora* (0.29–0.54 g m^{-2} d^{-1}) had the lowest overall biocrude productivity even though it had the third highest theoretical biocrude yield. The most productive species in terms of protein were marine *Derbesia* (2.57 g m^{-2} d^{-1}) and *Ulva* (1.86 g m^{-2} d^{-1}), and the least productive species was freshwater *Cladophora* (0.92 g m^{-2} d^{-1}) (Fig. 3b).

Projected production values – at scale with sequential extraction

To assess the potential value of macroalgae at scale, the projected value of biodiesel, biocrude and protein was calculated per unit hectare of production (Eqn 5) by scaling biomass productivities and bioproduct yields (Table 6; values rounded to the nearest $100). With a starting point of a single product use for the entire biomass, the conversion into biocrude was the most valuable option for five of the six species. Marine *Derbesia* had the highest projected productivity of biocrude at

Table 5 Amino acids profiles of macroalgae. Data show means (n = 3 ± SE) of α-amino acids (mg g^{-1}, dry weight, tryptophan and cysteine not included) of each marine (M) and freshwater (FW) macroalgae. Chemical properties including essential and nonessential amino acids, lysine and methionine contents are expressed as a proportion (wt%) of total amino acid content. Data also include methionine : lysine and protein : N ratios

Species	*Derbesia*	*Ulva*	*Chaetomorpha*	*Cladophora*	*Oedogonium*	*Cladophora*
Source	M	M	M	M	FW	FW
Aspartic acid/asparagine	23.0 ± 0.4	22.7 ± 0.3	17.7 ± 0.8	26.6 ± 3.5	25.3 ± 0.5	37.9 ± 0.8
Glutamic acid/glutamine	33.0 ± 0.4	20.0 ± 0.4	15.7 ± 0.6	26.9 ± 1.6	29.4 ± 0.6	41.3 ± 1.4
Histidine*	4.7 ± 0.1	2.8 ± 0.0	1.6 ± 0.1	2.8 ± 0.1	4.6 ± 0.1	3.7 ± 0.1
Serine	11.2 ± 0.2	9.4 ± 0.1	5.1 ± 0.3	8.4 ± 0.9	11.4 ± 0.1	14.3 ± 0.1
arginine	12.6 ± 0.2	10.3 ± 0.1	6.0 ± 0.1	10.3 ± 0.5	13.2 ± 0.2	21.1 ± 1.4
glycine	12.4 ± 0.2	9.5 ± 0.1	6.5 ± 0.1	10.7 ± 0.5	12.4 ± 0.0	14.9 ± 0.1
threonine*	11.2 ± 0.2	9.1 ± 0.1	4.2 ± 0.3	8.0 ± 1.3	12.3 ± 0.1	14.1 ± 0.1
alanine	14.7 ± 0.2	13.7 ± 0.3	6.3 ± 0.4	11.6 ± 0.4	16.2 ± 0.3	13.9 ± 0.2
proline	10.0 ± 0.1	8.5 ± 0.1	7.6 ± 0.2	9.4 ± 0.3	11.5 ± 0.2	14.3 ± 0.3
lysine*	14.8 ± 0.2	8.8 ± 0.1	9.8 ± 0.3	10.8 ± 0.3	15.2 ± 0.5	21.1 ± 0.4
tyrosine	8.4 ± 0.1	5.7 ± 0.0	2.8 ± 0.2	3.7 ± 0.8	8.0 ± 0.1	8.9 ± 0.3
methionine*	4.6 ± 0.0	2.6 ± 0.1	1.2 ± 0.1	1.8 ± 0.4	4.3 ± 0.1	3.7 ± 0.2
valine*	14.3 ± 0.2	10.7 ± 0.1	6.8 ± 0.2	12.5 ± 0.3	14.6 ± 0.2	15.7 ± 0.0
isoleucine*	10.2 ± 0.1	7.4 ± 0.1	5.1 ± 0.2	8.7 ± 0.2	10.7 ± 0.0	10.5 ± 0.1
leucine*	18.1 ± 0.2	12.0 ± 0.1	8.5 ± 0.4	15.7 ± 0.3	21.8 ± 0.3	19.6 ± 0.2
phenylalanine*	13.1 ± 0.2	10.2 ± 0.1	6.4 ± 0.2	10.5 ± 0.3	14.0 ± 0.1	12.8 ± 0.2
Total AA[a]	216.2 ± 2.3	163.2 ± 2.0	111.3 ± 4.1	178.5 ± 11.4	224.8 ± 2.9	267.9 ± 4.4
Protein chemical properties [wt%]						
essential	42.1	38.9	39.2	39.7	43.4	37.8
nonessential	57.9	61.1	60.8	60.3	56.6	62.2
lysine	6.8	5.4	8.8	6.1	6.7	7.9
methionine	2.1	1.6	1.0	1.0	1.9	1.4
Ratio						
methionine : lysine	0.31	0.30	0.12	0.16	0.28	0.18
protein : N	4.8	4.6	3.3	3.4	4.7	4.1

[a]Total α-amino acids (tryptophan and cysteine not included).
*Essential amino acid.

7.1 t biocrude ha^{-1} yr^{-1}. Notably, *Derbesia* was the most valuable biomass in each scenario of biodiesel ($1700 ha^{-1} yr^{-1}), biocrude ($4800 ha^{-1} yr^{-1}) and protein ($4100 ha^{-1} yr^{-1}) production. Marine *Ulva* was the second most valuable species for biocrude ($3100 ha^{-1} yr^{-1}) and protein ($2900 ha^{-1} yr^{-1}) production. *Oedogonium* was the most valuable of the freshwater species, however, biomass productivities were half that of *Derbesia* and correspondingly the projected value per ha was also proportionally lower for biodiesel ($800 ha^{-1} yr^{-1}), biocrude ($2300 ha^{-1} yr^{-1}) and protein ($1800 ha^{-1} yr^{-1}). Freshwater *Cladophora* was an anomaly in that it had a higher projected value per unit hectare for protein ($1400 ha^{-1} yr^{-1}) compared to biocrude ($1300 ha^{-1} yr^{-1}).

In the scenario where protein is extracted prior to HTL of residual biomass to biocrude, the projected value of the feedstock increased by 45 to 77% (Table 6, scenario 5). The pre-extraction of protein followed by the production of biocrude was the most valuable option for all species and was highest for marine *Derbesia* ($7700 ha^{-1} yr^{-1}) and *Ulva* ($5200 ha^{-1} yr^{-1}), and *Oedogonium* was the highest of the freshwater species ($3500 ha^{-1} yr^{-1}). In this instance, each product generated by *Derbesia, Ulva* and *Oedogonium* – protein ($4100 ha^{-1} yr^{-1}, $2900 ha^{-1} yr^{-1} and $1800 ha^{-1} yr^{-1}, respectively) and biocrude ($3700 ha^{-1} yr^{-1}, $2300 ha^{-1} yr^{-1} and $1700 ha^{-1} yr^{-1}, respectively) – accounted for approximately half of the projected value of the feedstock. *Derbesia* had the highest protein productivity (9.4 t ha^{-1} yr^{-1}) of all species, and *Oedogonium* had the highest protein productivity (4.2 t ha^{-1} yr^{-1}) of the freshwater species. *Derbesia* and *Ulva* had the highest projected biocrude productivity postextraction of protein (5.4 and 3.4 t ha^{-1} yr^{-1}, respectively), again corresponding to the highest value ($3700 ha^{-1} yr^{-1} and $2300 ha^{-1} yr^{-1}, respectively), while *Oedogonium* had a projected biocrude productivity postextraction of 2.5 t ha^{-1} yr^{-1} corresponding to a value of $1700 ha^{-1} yr^{-1}. Given the highest projected values for *Derbesia and Ulva* for marine species

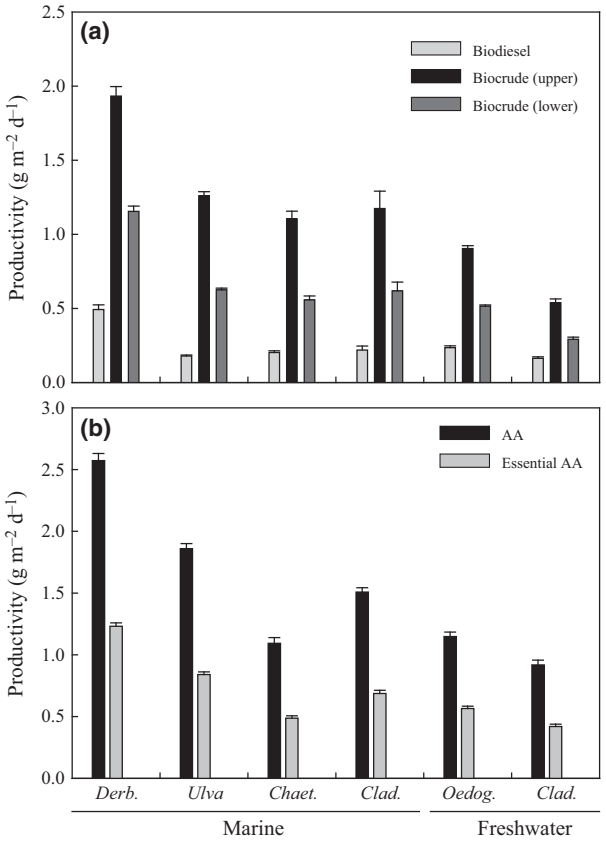

Fig. 3 Projected areal productivities of biofuels and bioproducts from macroalgae. Data show the theoretical productivities means (n = 3 ± SE, in g m^{-2} d^{-1}, dry weight) of biodiesel and biocrude – upper and lower limits (a); AA and essential AA (b) of marine (M) and freshwater (FW) macroalgae.

and *Oedogonium* for freshwater species, these species were further considered using sensitivity analysis.

Projected production values – sensitivity analysis

Sensitivity analyses were used to predict the relative influence of different parameters on the value of the feedstock (US$ ha^{-1} yr^{-1}) for the most valuable marine species, *Derbesia* and *Ulva*, (Fig. 4a and b) and the most valuable freshwater species, *Oedogonium* (Fig. 4c). The most valuable processing scenario, the sequential pre-extraction of proteins and subsequent HTL of residual biomass to biocrude (scenario 5 in Table 6), was used for each species. Therefore, the parameters for each sensitivity analysis were biomass productivity, protein content of the biomass, theoretical biocrude yield and the commodity prices for biocrude and protein (Table S2).

Under standard conditions (centre lines, Fig. 4), *Derbesia* had a higher projected value ($7700 ha^{-1} yr^{-1}) than *Ulva* ($5200 ha^{-1} yr^{-1}) and *Oedogonium* ($3500 ha^{-1} yr^{-1}). The influence of each parameter was also assessed in both favourable and unfavourable conditions to assess the potential range of the feedstock value relative to the empirical values in the literature or potential fluctuations in market prices. Biomass productivity was the most influential parameter that could potentially double the value of *Derbesia* and *Ulva*, and triple the value of *Oedogonium* when higher biomass productivities of >15 g m^{-2} d^{-1} (dry weight) are achieved at larger scale (Table S2). Theoretical biocrude yield was the second most influential parameter that could increase the value of *Derbesia* by 24%, of *Ulva* by 22% and of *Oedogonium* by 25%, assuming that HTL optimization translates to maximum yields of 12.2% to 20.6% using the residual biomass after protein extraction. The other parameters – protein content, biocrude and protein prices – had a lesser impact on the projected feedstock value. Notably, if all favourable conditions were summed for each parameter, the projected ceiling value per ha per year of *Derbesia* would reach $23600 ha^{-1} yr^{-1}, *Ulva* would reach $18100 ha^{-1} yr^{-1}and *Oedogonium* would reach $17100 ha^{-1} yr^{-1}.

Discussion

Of the two theoretical pathways considered in this study to convert biomass to high-energy biofuel, the hydrothermal liquefaction (HTL) of biomass to biocrude was more attractive than the extraction and esterification of fatty acids to biodiesel. Higher theoretical yields were achieved through HTL as the whole organic fraction of biomass is used in the conversion, including proteins, carbohydrates and the entire lipid component (Frank *et al.*, 2013). Importantly, the sequential extraction of proteins and subsequent conversion of the residual biomass by HTL could add significant value to the feedstock. This multiple or sequential product approach is considered to be critical for the viability of biofuel applications for microalgae (Vardon *et al.*, 2011; Chakraborty *et al.*, 2012; Miao *et al.*, 2012). To date, there have been no empirical analyses of coproducts from macroalgae and, morespecifically, no analysis of the sequential extraction of protein followed by conversion into biocrude. However, this option needs to be considered on a species by species basis as protein content generally varies substantially between species (Lourenço *et al.*, 2002) as exemplified by the significant differences between related green macroalgae in this study. Although freshwater macroalgae had a higher theoretical yield of biocrude and higher protein content, marine macroalgae had higher projected productivities of both biocrude and protein per unit area of production. The importance of this 'areal' metric is highlighted in the sensitivity analyses for marine *Derbesia* and *Ulva* and

Table 6 Projected productivity and value of commodities produced by macroalgae. Data show macroalgae projected productivities (P, in metric t ha^{-1} yr^{-1}) and values (V, in US$ ha^{-1} yr^{-1}) of commodities generated by marine (M) and freshwater (FW) macroalgae through different scenarios including conversion into biodiesel (1), to biocrude (2), extraction of protein (3), and HTL conversion of residual biomass to biocrude after protein extraction (4). Theoretical values of protein extract plus biocrude from residual biomass (5) was also calculated. Products prices are derived from equivalent commodities prices (see Methods). Note that theoretical values (V) are rounded to the nearest $100 for each scenario

Scenario		1.	2.	3.	4.	5.
Commodity		Biodiesel	Biocrude	Protein	Biocrude - Protein	3 + 4
Species/Price (US$ t^{-1})	Source	941	682	432	682	
Derbesia	M					
	P	1.8	7.1	9.4	5.4	
	V	$1700	$4800	$4100	$3700	$7700
Ulva	M					
	P	0.6	4.6	6.8	3.4	
	V	$600	$3100	$2900	$2300	$5200
Chaetomorpha	M					
	P	0.7	4.0	4.0	3.3	
	V	$700	$2700	$1700	$2300	$4000
Cladophora	M					
	P	0.8	4.3	5.5	3.3	
	V	$700	$2900	$2400	$2200	$4600
Oedogonium	FW					
	P	0.8	3.3	4.2	2.5	
	V	$800	$2300	$1800	$1700	$3500
Cladophora	FW					
	P	0.6	2.0	3.4	1.4	
	V	$600	$1300	$1400	$900	$2400

freshwater *Oedogonium*, in which biomass productivity is the single most influential parameter for feedstock value for macroalgal cultivation at scale.

Biomass productivity

Of the six species of green macroalgae considered in this study, marine macroalgae had higher biomass productivities than freshwater macroalgae under identical culture conditions. The biomass productivity of marine *Derbesia* (43 t ha^{-1} yr^{-1}, dry weight) was similar to sugar beet (Renouf *et al*., 2008) and twice that of the promising industrial biomass crop *Miscanthus* (20 t ha^{-1} yr^{-1}, Mckendry, 2002). Furthermore, the carbon productivity of *Derbesia* equated to 13 t C ha^{-1} yr^{-1}, which is similar or higher than most land crops (Stephens *et al*., 2013), irrespective of the higher ash content in macroalgae. In contrast, freshwater macroalgae had lower biomass productivities (12–18 t ha^{-1} yr^{-1}), yet were typically twice the average annual biomass productivity of soybean (6–8 t ha^{-1} yr^{-1}) (Salvagiotti *et al*., 2008). Most importantly, however, marine *Derbesia* and *Ulva* cultured at scale have biomass productivities that exceed 20 g m^{-2} d^{-1} (dry weight), or effectively >73 t ha^{-1} yr^{-1} (Bolton *et al*., 2009; Magnusson *et al*., 2014), while freshwater *Oedogonium* at scale has values twice that of the present study exceeding 15 g m^{-2} d^{-1} (dry weight), or effectively >55 t ha^{-1} yr^{-1} (Cole *et al*., 2013). These high biomass productivities at scale highlight the conservative nature of the data presented in this study, and justify the use of higher favourable values in the sensitivity analyses. Biomass productivities contrast with terrestrial crops due, in part, to the filamentous or leaf-like structure of green macroalgae that provides a uniform morphology with no differentiation of tissues and, therefore, all cells within the biomass are photosynthetic. Furthermore, this homogeneity of cells within marine and freshwater filamentous green macroalgae translates into a homogenous feedstock for biomass applications.

High-energy liquid fuels

Notably, the potential applications for macroalgal biomass are a direct function of the biomass productivities and their biochemical profiles. As an outcome, the species with the highest lipid content, specifically the marine *Derbesia* and freshwater *Oedogonium*, had the highest theoretical yields of biocrude (16–18%, dry weight). The

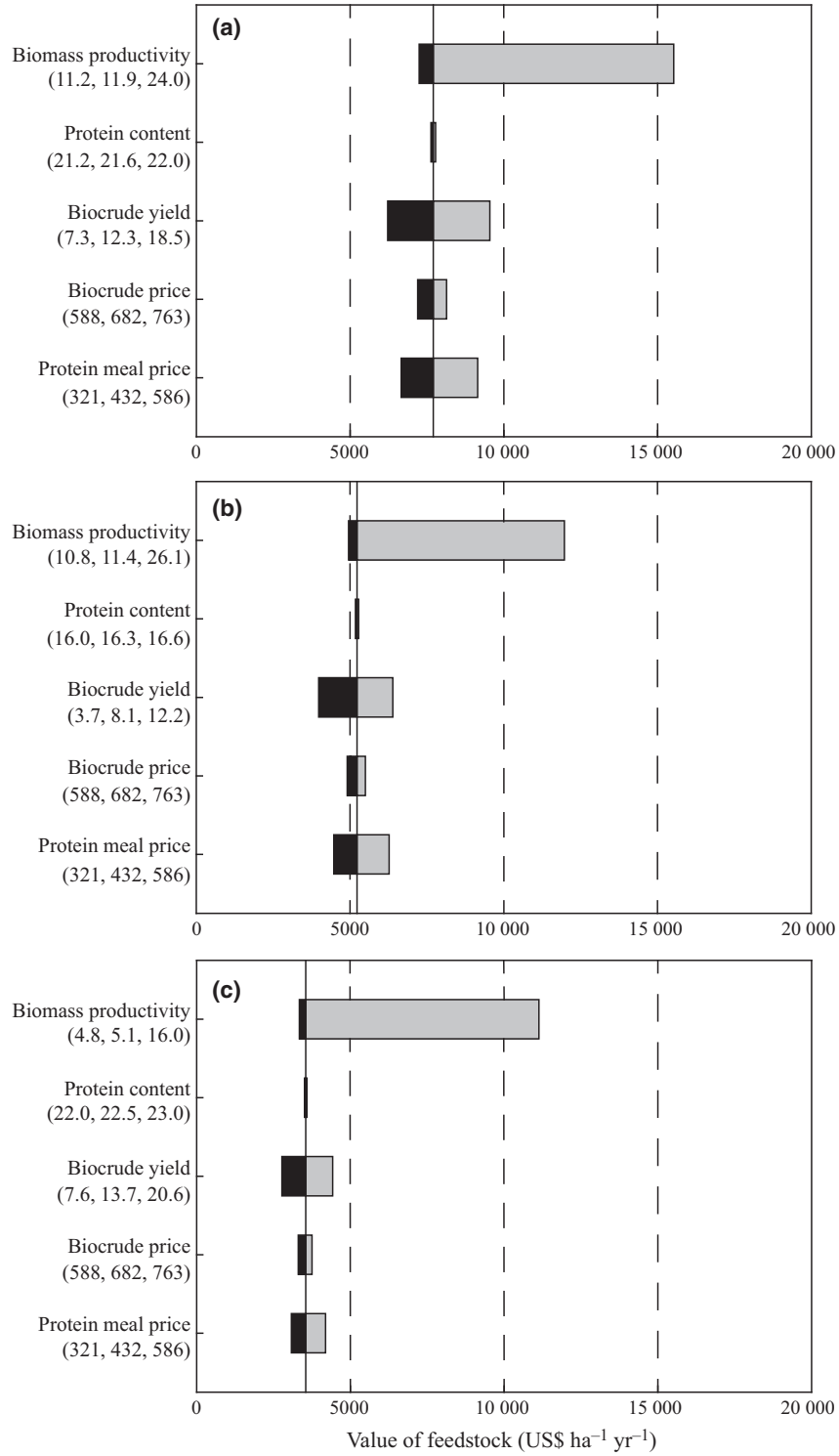

Fig. 4 Sensitivity analysis. Sequential protein extraction followed by conversion of residual biomass to biocrude for marine *Derbesia* (a), marine *Ulva* (b) and freshwater *Oedogonium* (c). Variation in the value of selected feedstock (US$ ha^{-1} yr^{-1}) is associated to the variation in each parameter while the other parameters remain the same. Values for each parameter are indicated in brackets (unfavourable, standard and favourable).

biochemical profiles of the selected macroalgae were similar in composition to the model compounds used by Biller & Ross (2011) for determining the individual conversion factors of lipid, protein and carbohydrate. In particular, carbohydrates as the major biochemical component in green macroalgae correspond with the model compounds of starch and glucose used in the equation (Biller & Ross, 2011). In addition, these theoretical yields were comparable to the yields obtained from the HTL of green and brown macroalgae (Zhou *et al*., 2010; Anastasakis & Ross, 2011), but noticeably lower than the yields obtained from a range of microalgae (26–57%; Table S3; López Barreiro *et al*., 2013). The projected biodiesel yields were less attractive than for biocrude due to the generally lower fatty acid contents of green macroalgae compared to other seaweeds (Gosch *et al*., 2012). Although HTL represents a more efficient utilization of all organic components of the biomass, a number of hurdles remain for the commercialization of this technology including a reduction in the energy requirements to operate at high temperature, a reduction in the hydrogen demand for biocrude upgrading and an efficient method for nitrogen recycling (Frank *et al*., 2013). In contrast, while biodiesel production is a less-effective process for deriving high-energy fuels from macroalgae, this technology is commercial and can be integrated with alternative bioenergy production including, for example, anaerobic digestion of residual biomass after fatty acid extraction (Chisti, 2007; Krohn *et al*., 2011). However, biodiesel derived from green macroalgae will likely contain a higher oxygen content than biocrude, further increasing the hydrogen demand required for upgrading (Frank *et al*., 2013). It also appears that the high proportions of PUFA, that are detrimental to the quality of biodiesel due to increased rates of oxidation during storage (Chisti, 2007), represent a major hurdle to the production of biodiesel from green macroalgae. In a similar way, biocrude from algae, while consistent in quality (see typical elemental composition in Table S3), contains high amounts of nitrogen compared to conventional crude oil, which represents an issue for refining (Jazrawi *et al*., 2013). However, the pre-extraction of protein from biomass would facilitate the removal of the majority of nitrogenous organic compounds that would otherwise influence the nitrogen content of the resulting crude (Peterson *et al*., 2008; Toor *et al*., 2011). Therefore, the sequential extraction of protein followed by HTL conversion of the residual biomass could ensure the highest quality of the respective products in a way that would not otherwise be achieved through the single use of the biomass for either biofuel or protein meal. In this scenario, the higher proportion of carbohydrates and lipids compared to the original feedstock could also enable fine-tuning of the HTL settings, for example, through the use of catalysts such as Na_2CO_3 that could double the yield of biocrude (Biller & Ross, 2011). Furthermore, the HTL coproducts of this process (biochar, aqueous and gas products) may offer additional opportunities to increase the value of macroalgal feedstock in commercial production (Biller & Ross, 2012).

Protein

The development of efficient separation technology for multiple product streams will be critical for algae (Chakraborty *et al*., 2012). However, this could potentially be achieved in the same facility, for example, using mild HTL conditions to extract proteins and then altering conditions to process the remaining organic material to biocrude (Yoshida *et al*., 1999; Biller & Ross, 2012). The protein extracts of green macroalgae could potentially complement terrestrial plant protein (soybean) meal in food and animal feed industries (Lammens *et al*., 2012). All six species of green macroalgae had a high proportion of the two most limiting amino acids in livestock diets, methionine and lysine (Boland *et al*., 2012). The protein extract of *Derbesia*, *Ulva* and *Oedogonium* contained 2.1%, 1.6% and 1.9% of methionine and 6.8%, 5.4%, 6.7% of lysine, respectively (Table 5). This is comparable to soybean meal at 0.9% methionine and 2.8% lysine (Glencross *et al*., 2007), assuming that soybean meal contains ~50% crude protein (Glencross *et al*., 2007; Lywood *et al*., 2009). Furthermore, the relative proportion of methionine to lysine for *Derbesia* (0.31), *Ulva* (0.30) and *Oedogonium* (0.28) is within the range of 0.27 to 0.38 and is therefore suitable for humans, pigs and poultry (Boland *et al*., 2012).

Alternative bioproducts for biorefinery

The strategy of sequential treatment of biomass to derive multiple coproducts (the biorefinery concept) is arguably the most important aspect for the development of biofuels more broadly, including from microalgae and terrestrial biomass crops (Fatih Demirbas, 2009; Foley *et al*., 2011). It is also notable that thermochemical conversion such as HTL could yield additional 'niche' products rather than just commodities that would enable higher returns for the same biomass, for example, by targeting valuable polysaccharides (Chakraborty *et al*., 2012). Green macroalgae have high proportions of carbohydrates, mostly in the form of glucose-based cellulose and starch that are involved in cell wall formation and energy storage, respectively (Lobban & Harrison, 1996). However, there are also high-value polysaccharides unique in form and function that could be recovered from the biomass prior to HTL, the most

prominent examples being sulphated polysaccharides such as ulvans in *Ulva* (Lahaye & Robic, 2007). Similarly, nonfatty acid lipids such as pigments, sterols and free alcohols could be recovered from biodiesel production and be used as feedstock for further HTL processing or targeted specifically for high-value nutraceuticals (Krohn *et al.*, 2011; see also Table S1). These niche-market nutraceutical products offer the opportunity to bridge the technology gap for biomass production by justifying the development of larger culture systems and fast-tracking the expected economies of scale to compete with commodity biomass (ARENA, 2012).

Limitations and perspectives

There are considerable limitations for the development of algal-based biofuels, including the technical

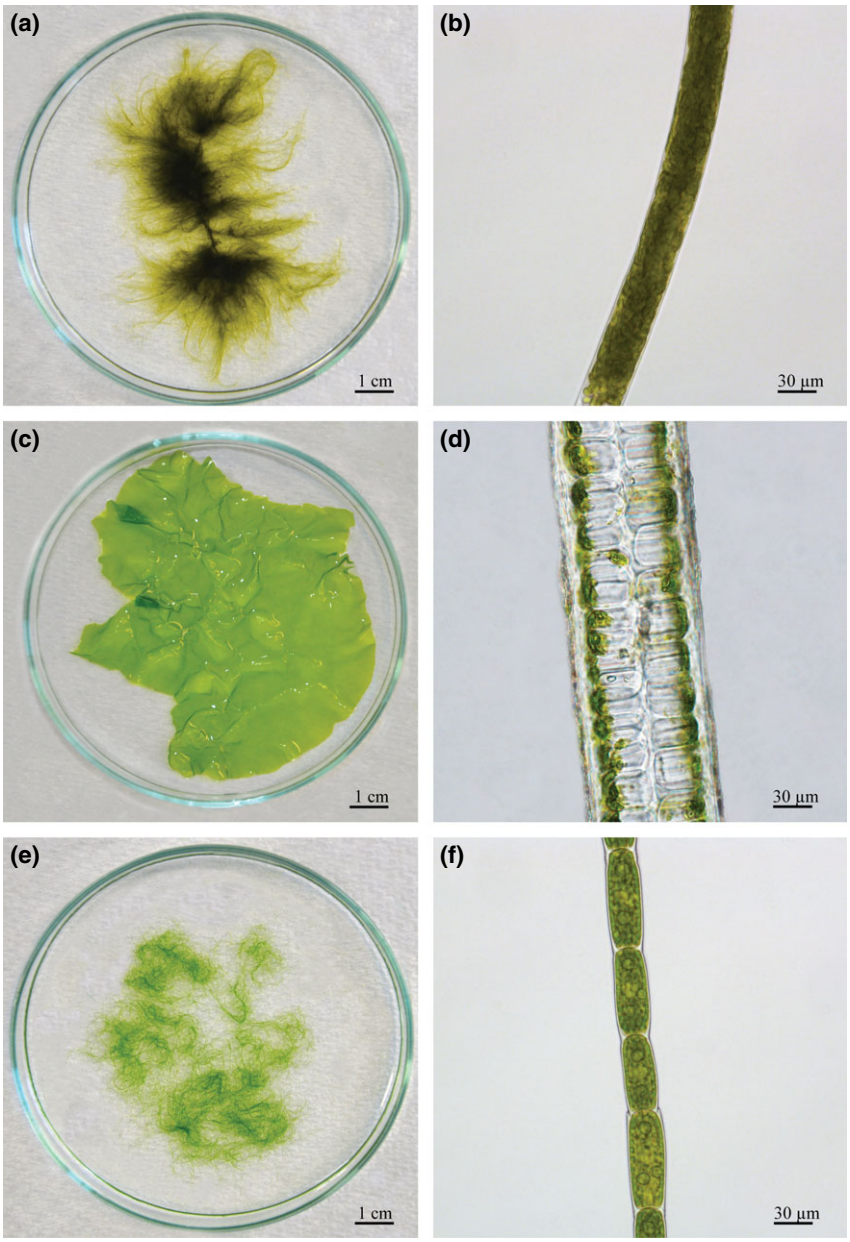

Fig. 5 Specimen photos of *Derbesia tenuissima* (a and b), *Ulva ohnoi* (c and d) and *Oedogonium* sp.(e and f) showing growth habit in culture (Nikon D7000) (a, c, e) and cellular detail at 400x magnification (Olympus DP73 camera connected to Olympus BX53 microscope) (b, d, f; note that *Ulva* is a transverse section).

developments for efficiencies in conversion and refining (Biller & Ross, 2012; Rowbotham *et al*., 2012). However, this and recent studies highlight that biomass production is a key limiting step, which includes the selection of robust species and the scale-up of operations on non-arable land (Lawton *et al*., 2013; Stephens *et al*., 2013). There are both benefits and problems associated with land-based production of marine and freshwater macroalgae. Marine macroalgae are typically larger than freshwater macroalgae and therefore simpler to handle (see images of *Derbesia*, *Ulva* and *Oedogonium* in Fig. 5), but they may require the removal of salts through freshwater rinsing, which is an additional process cost. In contrast, freshwater macroalgae are relatively low in salt and higher in carbon than marine macroalgae, and can be cultured on marginal land or in freshwater waste streams (Mulbry *et al*., 2008; Pittman *et al*., 2011; Saunders *et al*., 2012; Lawton *et al*., 2013). However, freshwater macroalgae have consistently lower biomass productivities than marine macroalgae. Notably, strain selection and selective breeding offer clear opportunities to deliver tailored crops, with the added benefit that production of macroalgae is a continuous process in comparison to the fixed cycles of terrestrial crops. In conclusion, a major outcome of this study is the identification of two novel species of filamentous macroalgae, marine *Derbesia* and freshwater *Oedogonium*, alongside the well-established marine *Ulva*, for the production of biocrude. While we highlight the sequential production of protein and biocrude as an important driver to increase feedstock value, it is clear from the sensitivity analyses that key drivers to deliver high value per unit area are biomass productivity and HTL technology optimization.

Acknowledgements

The project is supported by the Australian Government through the Australian Renewable Energy Agency (ARENA), and the Advanced Manufacturing Cooperative Research Centre (AMCRC), funded through the Australian Government's Cooperative Research Centre Scheme. This research is part of the MBD Energy Research and Development program for Biological Carbon Capture and Storage. We thank Good Fortune Bay Fisheries Ltd, Kelso, and the Barramundi Fishing Farm, Townsville, for allowing collection of algae from their ponds. We gratefully acknowledge Margaret Brownjohn and Bogdan Skomra for assistance and review of modelled data.

References

Adams JMM, 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. *Bioresource Technology*, **102**, 226–234.

Anastasakis K, Ross AB (2011) Hydrothermal liquefaction of the brown macro-alga *Laminaria saccharina*: effect of reaction conditions on product distribution and composition. *Bioresource Technology*, **102**, 4876–4883.

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). *Journal of Phycology*, doi: 10.1111/jpy.12154-13-071.

ARENA (2012) Advanced Biofuels Study, Strategic Directions for Australia. Australian Renewable Energy Agency. Available at: http://www.arena.gov.au/_documents/abir/Advanced-Biofuels-Study-Appendix.pdf (accessed 15 August 2013).

Aresta M, Dibenedetto A, Carone M, Colonna T, Fragale C (2005) Production of biodiesel from macroalgae by supercritical CO_2 extraction and thermochemical liquefaction. *Environmental Chemistry Letters*, **3**, 136–139.

Biller P, Ross AB (2011) Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content. *Bioresource Technology*, **102**, 215–225.

Biller P, Ross AB (2012) Hydrothermal processing of algal biomass for the production of biofuels and chemicals. *Biofuels*, **3**, 603–623.

Boland MJ, Rae AN, Vereijken JM *et al*. (2012) The future supply of animal-derived protein for human consumption. *Trends in Food Science & Technology*, **29**, 62–73.

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

Brennan L, Owende P (2010) Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable & Sustainable Energy Reviews*, **14**, 557–577.

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

Chakraborty M, Miao C, Mcdonald A, Chen S (2012) Concomitant extraction of bio-oil and value added polysaccharides from *Chlorella sorokiniana* using a unique sequential hydrothermal extraction technology. *Fuel*, **95**, 63–70.

Channiwala SA, Parikh PP (2002) A unified correlation for estimating HHV of solid, liquid and gaseous fuels. *Fuel*, **81**, 1051–1063.

Chisti Y (2007) Biodiesel from microalgae. *Biotechnology advances*, **25**, 294–306.

Chopin T, Sawhney M (2009) Seaweeds and their mariculture. In: *The Encyclopedia of Ocean Sciences* (eds Steele JH, Thorpe SA, Turekian KK), pp. 4477–4487. Elsevier, Oxford.

Cole AJ, Mata L, Paul NA, de Nys R (2013) Using CO_2 to enhance carbon capture and biomass applications of freshwater macroalgae. *Global Change Biology Bioenergy*, doi:10.1111/gcbb.12097

Fargione J, Hill J, Tilman D, Polasky S, Hawthorne P (2008) Land clearing and the biofuel carbon debt. *Science*, **319**, 1235–1238.

Farine DR, O'connell DA, John Raison R *et al*. (2012) An assessment of biomass for bioelectricity and biofuel, and for greenhouse gas emission reduction in Australia. *Global Change Biology Bioenergy*, **4**, 148–175.

Fatih Demirbas M (2009) Biorefineries for biofuel upgrading: a critical review. *Applied Energy*, **86**, S151–S161.

Fleurence J (1999) Seaweed proteins: biochemical, nutritional aspects and potential uses. *Trends in Food Science & Technology*, **10**, 25–28.

Folch J, Lees M, Sloane-Stanley G (1957) A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry*, **226**, 497–509.

Foley PM, Beach ES, Zimmerman JB (2011) Algae as a source of renewable chemicals: opportunities and challenges. *Green Chemistry*, **13**, 1399–1405.

Frank ED, Elgowainy A, Han J, Wang Z (2013) Life cycle comparison of hydrothermal liquefaction and lipid extraction pathways to renewable diesel from algae. *Mitigation and Adaptation Strategies for Global Change*, **18**, 137–158.

Gao K, Mckinley KR (1994) Use of macroalgae for marine biomass production and CO_2 remediation: a review. *Journal of Applied Phycology*, **6**, 45–60.

Glencross B, Booth M, Allan G (2007) A feed is only as good as its ingredients–a review of ingredient evaluation strategies for aquaculture feeds. *Aquaculture Nutrition*, **13**, 17–34.

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. *Global Change Biology Bioenergy*, **4**, 919–930.

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. *Canadian Journal of Microbiology*, **8**, 229–239.

Holdt SL, Kraan S (2011) Bioactive compounds in seaweed: functional food applications and legislation. *Journal of Applied Phycology*, **23**, 543–597.

Israel A, Gavrieli J, Glazer A, Friedlander M (2005) Utilization of flue gas from a power plant for tank cultivation of the red seaweed *Gracilaria cornea*. *Aquaculture*, **249**, 311–316.

Jazrawi C, Biller P, Ross AB, Montoya A, Maschmeyer T, Haynes BS (2013) Pilot plant testing of continuous hydrothermal liquefaction of microalgae. *Algal Research*, **2**, 268–277.

Jena U, Das K (2011) Comparative evaluation of thermochemical liquefaction and pyrolysis for bio-oil production from microalgae. *Energy & Fuels*, **25**, 5472–5482.

Jung KA, Lim S-R, Kim Y, Park JM (2012) Potentials of macroalgae as feedstocks for biorefinery. *Bioresource Technology*, **135**, 186–193.

Kraan S (2013) Mass-cultivation of carbohydrate rich macroalgae, a possible solution for sustainable biofuel production. *Mitigation and Adaptation Strategies for Global Change*, **18**, 27–46.

Krohn BJ, McNeff CV, Yan B, Nowlan D (2011) Production of algae-based biodiesel using the continuous catalytic Mcgyan process. *Bioresource Technology*, **102**, 94–100.

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

Lammens T, Franssen M, Scott E, Sanders J (2012) Availability of protein-derived amino acids as feedstock for the production of bio-based chemicals. *Biomass and Bioenergy*, **44**, 168–181.

Lawton RJ, de Nys R, Paul NA (2013) Selecting reliable and robust freshwater macroalgae for biomass applications. *PLoS ONE*, **8**, e64168.

Liu X, Saydah B, Eranki P, Colosi LM, Greg Mitchell B, Rhodes J, Clarens AF (2013) Pilot-scale data provide enhanced estimates of the life cycle energy and emissions profile of algae biofuels produced via hydrothermal liquefaction. *Bioresource Technology*, **148**, 163–171.

Lobban CS, Harrison PJ (1996) Light and photosynthesis. In: *Seaweed Ecology and Physiology*, pp. 146–150. Cambridge University Press, Cambridge. ISBN: 9780521408974.

López Barreiro D, Prins W, Ronsse F, Brilman W (2013) Hydrothermal liquefaction (HTL) of microalgae for biofuel production: state of the art review and future prospects. *Biomass and Bioenergy*, **53**, 113–127.

Lourenço SO, Barbarino E, de Paula JC, Pereira LOS, Marquez UML (2002) Amino acid composition, protein content and calculation of nitrogen to protein conversion factors for 19 tropical seaweeds. *Phycological Research*, **50**, 233–241.

Lüning K, Pang S (2003) Mass cultivation of seaweeds: current aspects and approaches. *Journal of Applied Phycology*, **15**, 115–119.

Lywood W, Pinkney J, Cockerill S (2009) Impact of protein concentrate coproducts on net land requirement for European biofuel production. *Global Change Biology Bioenergy*, **1**, 346–359.

Magnusson M, Mata L, de Nys R, Paul NA (2014) Biomass, lipid and fatty acid production in large-scale cultures of the marine macroalga *Derbesia tenuissima* (Chlorophyta). *Marine Biotechnology*, doi: 10.1007/s10126-014-9564-1.

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

Mckendry P (2002) Energy production from biomass (part 1): overview of biomass. *Bioresource Technology*, **83**, 37–46.

Miao X, Wu Q (2006) Biodiesel production from heterotrophic microalgal oil. *Bioresource Technology*, **97**, 841–846.

Miao C, Chakraborty M, Chen S (2012) Impact of reaction conditions on the simultaneous production of polysaccharides and bio-oil from heterotrophically grown *Chlorella sorokiniana* by a unique sequential hydrothermal liquefaction process. *Bioresource Technology*, **110**, 617–627.

Mulbry W, Kondrad S, Buyer J (2008) Treatment of dairy and swine manure effluents using freshwater algae: fatty acid content and composition of algal biomass at different manure loading rates. *Journal of Applied Phycology*, **20**, 1079–1085.

Nobre A, Robertson-Andersson D, Neori A, Sankar K (2010) Ecological–economic assessment of aquaculture options: comparison between abalone monoculture and integrated multi-trophic aquaculture of abalone and seaweeds. *Aquaculture*, **306**, 116–126.

Ong HC, Mahlia TMI, Masjuki HH, Honnery D (2012) Life cycle cost and sensitivity analysis of palm biodiesel production. *Fuel*, **98**, 131–139.

Park J, Craggs R, Shilton A (2011) Wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology*, **102**, 35–42.

Paul NA, de Nys R (2008) Promise and pitfalls of locally abundant seaweeds as biofilters for integrated aquaculture. *Aquaculture*, **281**, 49–55.

Paul NA, Tseng CK (2012) Seaweed. In: *Aquaculture: Farming Aquatic Animals and Plants*, 2nd edn (eds Lucas JS, Southgate PC), pp. 268–284. Blackwell Publishing Ltd, Oxford.

de Paula Silva PH, Mcbride S, de Nys R, Paul NA (2008) Integrating filamentous 'green tide' algae into tropical pond-based aquaculture. *Aquaculture*, **284**, 74–80.

Perlack RD, Wright LL, Turhollow AF, Graham RL, Stokes BJ, Erbach DC (2005) Biomass as feedstock for a bioenergy and bioproducts industry: the technical feasibility of a billion-ton annual supply. DOE GO-102005-2135, Oak Ridge National Laboratory. Available at: www.esd.ornl.gov/eess/FinalBillionOnVisionReport2.pdf (accessed 15 June 2013).

Peterson AA, Vogel F, Lachance RP, Fröling M, Antal MJ Jr, Tester JW (2008) Thermochemical biofuel production in hydrothermal media: a review of sub-and supercritical water technologies. *Energy & Environmental Science*, **1**, 32–65.

Pittman JK, Dean AP, Osundeko O (2011) The potential of sustainable algal biofuel production using wastewater resources. *Bioresource Technology*, **102**, 17–25.

Quinn GP, Keough MJ (2002) *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.

Ragauskas AJ, Williams CK, Davison BH *et al.* (2006) The path forward for biofuels and biomaterials. *Science*, **311**, 484–489.

Renouf M, Wegener M, Nielsen L (2008) An environmental life cycle assessment comparing Australian sugarcane with US corn and UK sugar beet as producers of sugars for fermentation. *Biomass and Bioenergy*, **32**, 1144–1155.

Roberts DA, de Nys R, Paul NA (2013) The effect of CO2 on algal growth and metal bioremediation of industrial waste water. *PLoS ONE*, **8**, e81631.

Ross A, Jones J, Kubacki M, Bridgeman T (2008) Classification of macroalgae as fuel and its thermochemical behaviour. *Bioresource Technology*, **99**, 6494–6504.

Rowbotham J, Dyer P, Greenwell H, Theodorou M (2012) Thermochemical processing of macroalgae: a late bloomer in the development of third-generation biofuels? *Biofuels*, **3**, 441–461.

Salvagiotti F, Cassman KG, Specht JE, Walters DT, Weiss A, Dobermann A (2008) Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. *Field Crops Research*, **108**, 1–13.

Saunders RJ, Paul NA, Hu Y, de Nys R (2012) Sustainable sources of biomass for bioremediation of heavy metals in waste water derived from coal-fired power generation. *PLoS ONE*, **7**, e36470.

Stephens E, de Nys R, Ross IL, Hankamer B (2013) Algae fuels as an alternative to petroleum. *Journal of Petroleum & Environmental Biotechnology*, **4**, 148.

Tat ME, Van Gerpen JH (2000) The specific gravity of biodiesel and its blends with diesel fuel. *Journal of the American Oil Chemists' Society*, **77**, 115–119.

Taylor R, Fletcher RL, Raven JA (2005) Preliminary studies on the growth of selected 'green tide' algae in laboratory culture: effects of irradiance, temperature, salinity and nutrients on growth rate. *Botanica Marina*, **44**, 327–336.

Toor SS, Rosendahl L, Rudolf A (2011) Hydrothermal liquefaction of biomass: a review of subcritical water technologies. *Energy*, **36**, 2328–2342.

Vardon DR, Sharma BK, Scott J *et al.* (2011) Chemical properties of biocrude oil from the hydrothermal liquefaction of *Spirulina* algae, swine manure, and digested anaerobic sludge. *Bioresource Technology*, **102**, 8295–8303.

Weaver JW (2004) Characteristics of spilled oils, fuels, and petroleum products: 3a. simulation of oil spills and dispersants under conditions of uncertainty, US EPA. *Ecosystems Research Division National Exposure Research Laboratory, Athens, Georgia*, **30605**, 648–654.

Yang J, Xu M, Zhang X, Hu Q, Sommerfield M, Chen Y (2011) Life-cycle analysis on biodiesel production from microalgae: water footprint and nutrients balance. *Bioresource Technology*, **102**, 159–165.

Yoshida H, Terashima M, Takahashi Y (1999) Production of organic acids and amino acids from fish meat by sub-critical water hydrolysis. *Biotechnology progress*, **15**, 1090–1094.

Zhou D, Zhang L, Zhang S, Fu H, Chen J (2010) Hydrothermal liquefaction of macroalgae *Enteromorpha prolifera* to bio-oil. *Energy & Fuels*, **24**, 4054–4061.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Alternative biorefinery options with sequential extraction of proteins, lipids or fatty acids (FA) and conversion of residual biomass to biocrude. Data show macroalgae projected productivities (P, in metric t ha^{-1} yr^{-1}) and values (V, in US \$ ha^{-1} yr^{-1}) of commodities generated by marine (M) and freshwater (FW) macroalgae through different scenarios. Products prices are derived from equivalent commodities prices (see Methods). Note that theoretical values (V) are rounded to the nearest \$100 for each scenario.
Table S2. Sensitivity analyses of marine *Derbesia* and *Ulva* and freshwater *Oedogonium* for parameters influencing the value of feedstock (US\$ ha^{-1} yr^{-1}) for sequential extraction of protein from biomass and hydrothermal liquefaction of the residual biomass to biocrude. Values (A); Parameters (B); References (C) 'Best Case' scenarios (D).
Table S3. Biocrude yield from several studies on hydrothermal liquefaction of macroalgae and microalgae. M = marine origin, FW = freshwater origin, dw = dry weight, afdw = ash-free dry weight.
Table S4. References cited in supporting information.