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Total lipid and fatty acid composition of seaweeds for the selection of species for oil-based biofuel and bioproducts

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Abstract

We investigated the potential of seaweeds as feedstock for oil-based products, and our results support macroalgae (seaweeds) as a biomass source for oil-based bioproducts including biodiesel. Not only do several seaweeds have high total lipid content above 10% dry weight, but in the brown alga *Spatoglossum macrodontum* 50% of these lipids are in the form of extractable fatty acids. *S. macrodontum* had the highest fatty acid content (57.40 mg g^{-1} dw) and a fatty acid profile rich in saturated fatty acids with a high content of C18:1, which is suitable as a biofuel feedstock. Similarly, the green seaweed *Derbesia tenuissima* has high levels of fatty acids (39.58 mg g^{-1} dw), however, with a high proportion of PUFA (n-3) (31% of total lipid) which are suitable as nutraceuticals or fish oil replacements. Across all species of algae the critical parameter of fatty acid content (measured as fatty acid methyl esters, FAME) was positively correlated (R^2 = 0.67) with total lipid content. However, the proportion of fatty acids to total lipid decreased markedly with total lipid content, generally between 30% and 50%, making it an inaccurate measure of the potential to identify seaweeds suitable for oil-based bioproducts. Finally, we quantified within species variation of fatty acids across locations and sampling periods supporting either environmental effects on quantitative fatty acid profiles, or genotypes with specific quantitative fatty acid profiles, thereby opening the possibility to optimize the fatty acid content and quality for oil production through specific culture conditions and selective breeding.

Keywords: algae, biodiesel, biofuel, *Derbesia*, *Dictyota*, fatty acid methyl ester, macroalgae, nutraceutical, omega-3, *Spatoglossum*

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Introduction

Algal biomass offers an innovative contribution to the challenge of providing sustainable bioenergy resources (Brennan & Owende, 2010). Algae have high productivities, can be cultured on nonarable land and can utilize waste-streams as a nutrient source. Their rapid growth through photosynthesis provides for the capture and recycling of carbon dioxide (CO_2), and algal biomass provides for bioenergy generation through a diversity of processes including direct combustion, gasification, saccharification and fermentation, and thermochemical processing (reviewed by Demirbas, 2011; Nigam & Singh, 2011; Singh & Olsen, 2011). However, the major focus for research, development and commercialization is the cultivation of algae for the production of oil (lipid) based products. While algal oils have applications as nutraceuticals, fish oil replacement and as feedstock for industrial chemicals, the main emphasis is the production of biofuels to replace fossil derived fuels, in particular biodiesel through the transesterification of lipids.

The vast majority of research on algal oils has concentrated on microalgae, where some species contain in excess of 30% total lipid based on dry weight (dw) (Renaud *et al*., 1999; Chisti, 2007; Huerlimann *et al*., 2010). However there are significant technical challenges in the cost and complexity of cultivation and harvesting microalgae at an industrial scale (Ginzburg, 1993; Mata *et al*., 2010a; Stephens *et al*., 2010a,b). In contrast, marine and freshwater macroalgae are simpler to produce and are particularly suited to low technology cultivation methods with a well established multi-billion dollar industry producing more than 15 million tonnes per annum (FAO, 2011). However, none of this production targets oil. This is in part because macroalgae are typically perceived as unsuitable for the production of oil-based products as most species have low total lipid contents of < 5% dw (e.g. Montgomery & Gerking, 1980; McDermid & Stuercke, 2003; Kumari *et al*., 2010). However, this paradigm of low total lipid content for macroalgae is challenged by some species which have a

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total lipid content above 15% dw, in particular within the order Dictyotales which consistently have a total lipid content between 11% and 20% dw (Montgomery & Gerking, 1980; McDermid & Stuercke, 2003).

Although total lipid content has been determined for a number of seaweed (macroalgae) species, this is normally in the context of gross chemical composition (% lipid, carbohydrate, protein etc.) for nutritional profiling of species for use in aquaculture (Viera *et al.*, 2005; Zhang *et al.*, 2010) or human consumption (McDermid & Stuercke, 2003; Hwang *et al.*, 2008). However, quantification of the fatty acid component of the total lipid extract [generally up to 24-carbon (C24) long aliphatic hydrocarbon chains with a carboxylic head group and varying degrees of saturation], is the critical parameter in determining the yield and suitability of oils as biofuel feedstock, or nutraceutical or fish oil replacement. The limited information available for the fatty acid composition of seaweeds shows a similar range of fatty acids to microalgae, with carbon chains between C14 and C24 (Vaskovsky *et al.*, 1996; Khotimchenko *et al.*, 2002; Sánchez-Machado *et al.*, 2004; Kumari *et al.*, 2010; Saito *et al.*, 2010). Notably, the diversity of species investigated for quantitative fatty acid profile is very low, approximated at < 200 of the estimated 8000 species identified to date (Lűning, 1990). This provides significant potential to identify novel high oil species, similar to those already identified within the Dictyotales. Therefore, the first critical step in assessing macroalgae as a feedstock for oil-based products for bioenergy is to establish, qualitatively, and quantitatively, their total lipid content and fatty acid profiles. This includes variation within individuals of the same species to identify the possible effects of genotype and environment on lipid quality and quantity. The subsequent step is to determine the extent to which environmental factors affect total lipid and fatty acid content, such as light (Hotimchenko, 2002), temperature (Graeve *et al.*, 2002; Nelson *et al.*, 2002), and nutrient regime (Livne & Sukenik, 1992; Hu *et al.*, 2008; Rodolfi *et al.*, 2009). This then provides the basis to manipulate and select environments and/or genotypes to optimize lipid quantity and quality under intensive culture. The final step in this process is then to deliver intensive cultivation of elite macroalgae to provide high productivity of oil, based on biomass productivity (g dw m^{-2} day^{-1}) and fatty acid (FA) yield to meet productivity benchmarks [g (FA) m^{-2} day^{-1}] (Stephens *et al.*, 2010a).

As the first step in identifying target seaweed species for culture for oil-based products, we simultaneously quantify total lipid content and fatty acid profiles of a diversity of tropical seaweed species from North Queensland, Australia. The primary objective is to identify species with high total lipid content and suitable fatty acid profiles, primarily for applications as biofuel and alternatively as nutraceuticals, as these are the two key demand drivers for algal oil production. In addition, we evaluate the relationship between total lipid content and fatty acid content for each of the three main taxonomic groups of seaweeds (brown, green, and red). Finally, we quantify conspecific variation in lipid profiles between and within locations and sampling periods to identify the potential for manipulating environment, or selecting genotypes, to optimize yields and initiate selective breeding for high lipid yielding traits.

Materials and methods

Field sites

Four sites were selected in tropical North Queensland, Australia, to maximize species diversity and ensure multiple habitats, from intertidal rocky shores to midshelf coral reefs. Kissing Point (19.23°S, 146.79°E) is an intertidal rocky shore/sedimentary habitat located on the mainland in Townsville. Samples were collected during low tides when seaweeds were exposed, as water at this site was consistently turbid from wind and wave action. Nelly Bay (19.16°S, 146.85°E) is located approximately 8 km from Townsville on Magnetic Island. Samples were collected from the reef flat and fringing reef approximately 100 m from the shoreline. This reef flat is dominated by seaweeds, with increasing coral cover towards the reef slope. Orpheus Island (18.61°S, 146.49°E) is an inshore island 50 km north of Townsville. The island is surrounded by fringing coral reefs and samples were taken from a floating and continuously submerged long line located 50 m from shore. Rib Reef (18.48°S, 146.87°E) is a midshelf coral reef located within the central Great Barrier Reef approximately 50 km east of Orpheus Island. The reef is approximately 5 km^2 in dimension. Seaweeds were mainly found on sandy flats and on rocks between the coral outcrops. Sampling was conducted by snorkel at low tide when water depth was between 1 and 2 m at the sampling areas. In addition to these field collections, some seaweed samples were also evaluated from the culture collections at the Marine and Aquaculture Research Facilities Unit (MARFU) at James Cook University (JCU), Townsville, where seaweeds are maintained in outdoor tank-based recirculation systems.

Sampling procedures

The four sites provided predominantly different flora and the most apparent species at each of the sites were selected, with an effort to sample species of different taxonomic origin and also different morphologies (e.g. foliose and turfing varieties). Kissing Point and Nelly Bay were sampled multiple times while Orpheus Island and Rib Reef were only sampled once. To ensure that a representative measure of both lipid content and quality (i.e. fatty acid profiles) was made for each seaweed species, at least five individual plants per species, depending

on availability, were haphazardly collected during each sampling period at each field site; constituting a sample. An individual was characterized by a single holdfast and was not connected to another plant with rhizomes or other tissue. There were many turfing algae that could not be identified below broader taxonomic groupings. Turfing species were treated as individuals if that seaweed mat was spatially isolated.

Individuals were held in seawater filled plastic bags and transported in an ice box to JCU, Townsville. Each individual was washed in freshwater to remove debris, epiphytes, and animals. A small representative portion of each individual was preserved in 70% ethanol and 4% formalin for taxonomic identification, while the remainder of the material was lyophilized and ground with an analytical mill through a 1 mm sieve. The seaweed powder was sealed in airtight jars and stored at −20 °C.

Total lipid analysis

Total lipid content was analysed for three individuals of a sample that represented a unique species, following Folch *et al.* (1957). Approximately 200 mg (± 0.1 mg) of dried seaweed powder was placed into an 8 mL Teflon capped glass vial. To this, 5 mL chloroform:methanol (2 : 1, v/v) mixture was added. The samples were then heated at 60 °C for 1 h followed by filtration to remove particulate matter using a vacuum pump through a Whatman GF/A filter (Whatman Plc, Maidstone, UK). Additional chloroform-methanol mixture was used to rinse the filter to recover all lipids. The filtered crude extract was then washed with 20% of its volume in 0.9% NaCl solution. After vortexing and standing for several minutes, an upper and lower phase established. The upper phase was siphoned out and the lower phase, containing the lipids, was evaporated under a gentle stream of nitrogen. The total weight of the lipid extract was then determined.

Fatty acid extraction and analysis

Fatty acids were analysed for three individuals of each collected sample. A direct transesterification method, adapted from Cohen *et al.* (1988) and Rodríguez-Ruiz *et al.* (1998), was used to simultaneously extract and esterify the fatty acids to fatty acid methyl esters (FAMEs) for analysis by GC-MS. This method is more efficient and rapid in recovering fatty acids than traditional solvent extraction methods followed by transesterification (Griffiths *et al.*, 2010). Briefly, 2 mL freshly prepared methylation mixture [methanol, acetyl chloride, 20 : 1 (v/v)] and 300 μL internal standard solution [nonadecanoic acid ($C_{19}H_{38}O_2$; >99%, Sigma-Aldrich, Castle Hill, NSW, Australia)], 0.2 mg mL^{-1} in methanol) was added to approximately 50 mg (± 0.1 mg) of dried seaweed powder. The samples were heated at 100 °C for 1 h, then allowed to cool down before 1 mL of hexane was added. To ensure complete partitioning of FAMEs into the hexane phase, samples were heated again to 100 °C for 1 min, during which a single methanol/hexane phase formed. Two millilitre of deionized water was then added to facilitate phase separation. The hexane (upper) phase containing the FAMEs was collected and filtered through a 0.2 μm PTFE syringe filter prior to injection on the GC column. All solvents were HPLC grade.

Fatty acid analysis was carried out on an Agilent 7890 GC equipped with a flame ionization detector (FID) and connected to an Agilent 5975C Electron Ionization (EI) Turbo Mass Spectrometer (Agilent Technologies Australia Pty Ltd, Mulgrave, Vic., Australia), for identification of FAMEs. Separation was achieved on a DB-23 capillary column with a cyanopropyl stationary phase (60 m × 0.55 mm, id 0.15 μm) with helium as carrier gas in constant pressure mode. Injector and FID inlet temperatures were 150 and 250 °C, respectively (split injection, 1/50). Column temperature was programmed as outlined in David *et al.* (2002) which in brief ramped from 50 to 230 °C. Fatty acids were quantified relative to peak areas of standards (Sigma-Aldrich) corrected to the recovery of an internal standard (C19:0). Total fatty acid content was determined as the sum of all FAMEs. Fatty acids are designated as CX:Y(n-z), where X is the total number of carbon, Y is the number of double bonds, and z is the position of the ultimate double bond from the terminal methyl group.

Statistical analysis

The broad variation in fatty acids between the main taxonomic groups (phylum, order, and species) was investigated using Principal Component Analysis (PCA) (SYSTAT 13; Systat software Inc, Chicago, IL, USA) with the mean of three individual plants of a sample as the variables. The outcome was plotted in two dimensions (PCA1, PCA2). The score loading was analysed for each fatty acid, however, only saturated FAs and polyunsaturated FAs (PUFA) (n-3) are identified in the biplot of PCA1 vs. PCA2. The intra and inter spatial (sites) and temporal variability in fatty acid composition of *Dictyota bartayresii* and *Dictyota dichotoma* were subsequently analysed in separate PCAs using the fatty acids of individual plants as variables to assess conspecific variation between and within environments (location, sampling period).

Results

Total lipids

Brown seaweeds (Phaeophyceae) typically had the highest total lipid content, followed by green (Chlorophyta) and red seaweeds (Rhodophyta) (Fig. 1; Table S1). However, at lower taxonomic levels there is considerable variation in total lipid content between orders, and also species (Fig. 1; Table S1). For example, within the brown seaweeds it is predominantly the order Dictyotales (79.8 mg g^{-1} dw ± 14.0 SE) which has a high total lipid content. Similarly, within the green seaweeds, the orders Bryopsidales (68.9 mg g^{-1} dw ± 10.5 SE) and Cladophorales (57.5 mg g^{-1} dw ± 12.4 SE) have the highest total lipid content (Fig. 1). It is therefore these orders which are discussed in detail. There is also considerable variation in total lipid content within these orders. For

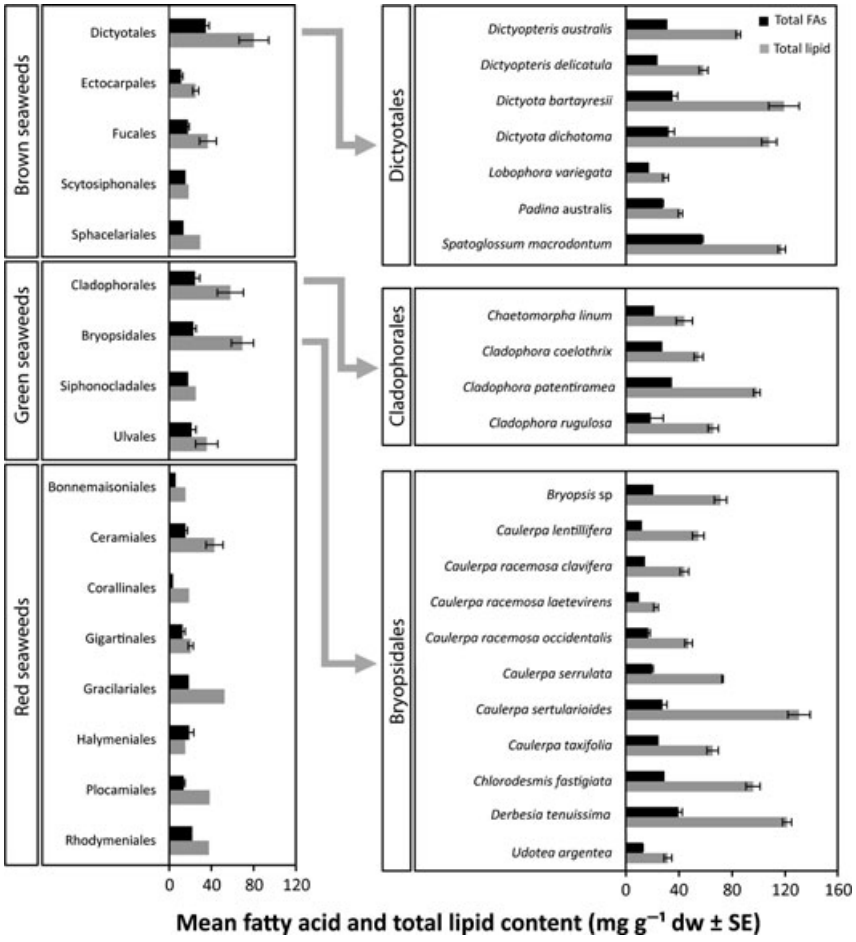

Fig. 1 Mean total lipid (mg g^{-1} dw ± SE) and mean total fatty acid content (mg g^{-1} dw ± SE) of seaweed orders (left) and species (right). All means are the means of samples except total lipid for species is expressed as the mean of a single set of triplicates.

the Dictyotales, *Dictyota bartayresii*, *Dictyota dichotoma*, and *Spatoglossum macrodontum* have a total lipid content > 100 mg g^{-1} dw, whereas *Lobophora variegata* and *Padina australis* have well below 50 mg g^{-1} dw (Fig. 1; Table S1). Within the Bryopsidales, *Caulerpa sertularioides* (130.4 mg g^{-1} dw ± 8.4 SE) and *Derbesia tenuissima* (121.4 mg g^{-1} dw ± 3.4 SE) have the highest total lipid content found in this study. *Cladophora patentiramea* is the only species in the Cladophorales which approaches 100 mg lipid g^{-1} dw (Fig. 1).

Fatty acids

Fatty acid content, measured as FAME for each taxonomic group (brown, green, and red seaweeds), correlates positively with total lipid content with the line of best-fit being a polynomial (2nd order) function ($R^2 = 0.69$, $y = -0.0173\ x^2 + 0.5175\ x$; Fig. 2). Notably, the fatty acid content decreases with increasing total lipid content for all groups. In brown seaweeds the trend is least pronounced while it is strongest in green seaweeds. Red seaweeds have a similar trend to green seaweeds with the lowest levels of both lipid and fatty acid compared to the other groups.

The three seaweed orders with the highest fatty acid content are the Dictyotales followed by the Cladophorales and Bryopsidales (Fig. 1; Table S2). These orders also contain the species with the highest fatty acid content. *Spatoglossum macrodontum* (Dictyotales) (57.40 mg g^{-1} dw ± 0.87 SE) has an exceptionally high fatty acid content, essentially 50% of the total lipid content (Fig. 1). Other Dictyotales species, *Dictyota bartayresii* and *Dictyota dichotoma*, not only have a considerably lower fatty acid content compared to *Spatoglossum macrodontum*, but also only contain 30% fatty acids relative to total lipids. There are, however, high fatty acid to total lipid ratios in *Padina australis* (67%) and the closely related *Lobophora variegata* (58%) (Fig. 1). Within the Bryopsidales, only *Derbesia tenuissima* has a fatty acid content above 30 mg g^{-1} dw, although this corresponds to a low fatty acid to total lipid ratio (32%). *Cladophora patentiramea* is the only species of the Cladophorales with a

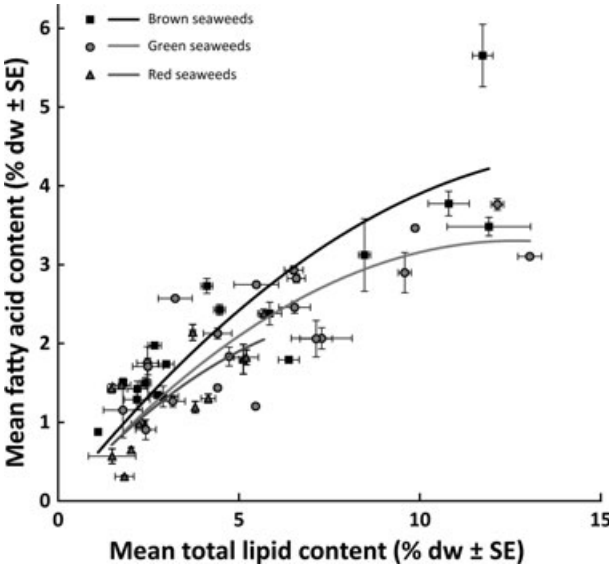

Fig. 2 Polynomial (2nd order) relationships between total lipid content and fatty acid content for different seaweed taxa (brown, green and red seaweed). Individual data points represent mean values (% dw ± SE) for each species. Brown seaweed: $y = -0.0188\ x^2 + 0.5218\ x$, $R^2 = 0.76$; Green seaweed: $y = -0.0206\ x^2 + 0.5218\ x$, $R^2 = 0.64$; Red seaweed: $y = -0.0287\ x^2 + 0.5234\ x$, $R^2 = 0.53$. Functions are only predictive within the shown data range.

fatty acid content above 30 mg g^{-1} dw at 35% of total lipids.

In a similar manner to total lipid and total fatty acid content, there was also considerable variation in fatty acid quality between taxonomic groups and species (Fig. 3a; Table S2). Over 50% of variation between samples (mean of three replicate individuals) can be explained by two principal components. The first component (PCA1) separates the seaweed species primarily based on their total fatty acid content (*x*-axis), and so separates the fatty acid rich species (right) from the fatty acid poor species (left) (Fig. 3a). Fatty acid rich species such as *Dictyota bartayresii*, *Spatoglossum macrodontum*, and *Derbesia tenuissima* therefore have the highest PCA1 scores, with species of the red seaweeds generally have the lowest PCA1 scores, reflecting their lower fatty acid contents. On the second principal component axis (PCA2), the seaweed samples are separated into two main groups based on their fatty acid profiles, with the brown and red seaweeds grouped together while the green seaweeds group out with higher PCA2 scores (Fig. 3a). A second PCA separates the seaweed species primarily based on their saturated and polyunsaturated fatty acids. Green seaweeds are distinguished by their high quantities of polyunsaturated fatty acids, PUFA (n-3), and in particular by their high level of C18:3(n-3) (Fig. 3b). In contrast, brown seaweeds are characterized

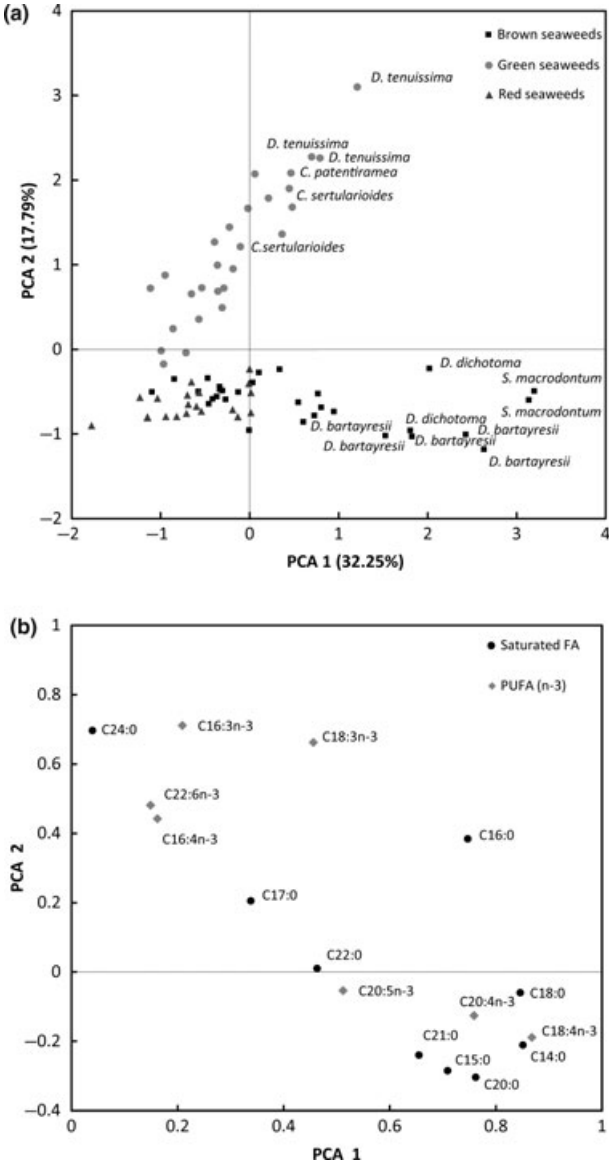

Fig. 3 (a) Principal component analysis (PCA) of seaweed species samples based on fatty acid profiles. (b) Score loading biplot of saturated and PUFA (n-3) fatty acids (FAs).

by their higher amount of saturated fatty acids, in particular C14:0.

A more detailed investigation of fatty acids across taxonomic groups further clarifies the relationship between fatty acid composition and taxonomy (Figs 4 and 5). The most abundant saturated fatty acid (SFA) in these tropical seaweeds was C16:0 across all taxonomic groups. The red seaweeds had the highest proportion of C16:0 relative to total fatty acid content, followed by the green and brown seaweeds (Fig. 4). Within the red seaweeds the Halymeniales (8.36 mg g^{-1} dw ± 0.79 SE) had the highest total C16:0 content. This was followed by the Dictyotales within the brown seaweeds (7.82 mg g^{-1} dw ± 0.78 SE).

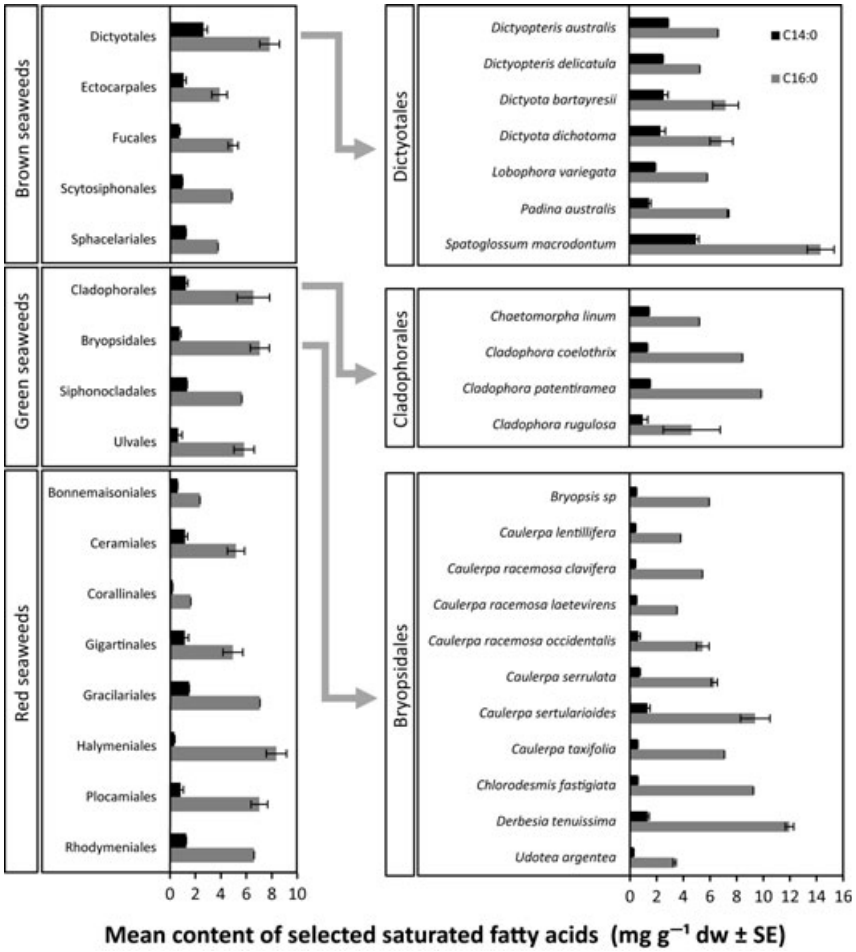

Fig. 4 Mean content (mg g^{-1} dw ± SE) of the saturated fatty acids C14:0 and C16:0 in different seaweed orders (left) and species (right).

On a lower taxonomic level *Spatoglossum macrodontum* (14.30 mg g^{-1} dw ± 1.00 SE) (Dictyotales) had the highest C16:0 content followed by *Derbesia tenuissima* (11.96 mg g^{-1} dw ± 0.31 SE) (Bryopsidales).

C14:0 was generally low in most taxonomic groups rarely exceeding 10% of total SFA content. Exceptions were the Dictyotales where C14:0 reaches 26% of total SFA in *Dictyopteris australis*, 23% in *Spatoglossum* macrodontum, and 21% in *Dictyota bartayresii*. *Spatoglossum macrodontum* (4.95 mg g^{-1} dw ± 0.21 SE) had the highest total C14:0 content. The Dictyotales were also characterized by a high amount of C18:1 which reaches 18% of total fatty acids in *Spatoglossum macrodontum* (Table 1).

The PUFA(n-3) composition of these seaweeds is clearly related to taxonomy (Fig. 5). Most notable are the high amounts of C18:3(n-3) in green seaweeds, particularly the Bryopsidales. *Derbesia tenuissima* (6.14 mg g^{-1} dw ± 1.40 SE) had the highest C18:3(n-3) content amongst all seaweed. The Dictyotales also had high amounts of C18:3(n-3), in particular *Spatoglossum macrodontum* (2.62 mg g^{-1} dw ± 0.04 SE).

The PUFA C20:5(n-3) was well represented in all taxa and was the dominant essential PUFA in the red seaweeds with *Champia parvula* (3.30 mg g^{-1} dw) having the highest amount in the study. In the brown seaweeds, C20:5(n-3) was generally as abundant as C18:3 (n-3) and the most abundant essential PUFA(n-3) in *Dictyota bartayresii*, *Dictyota dichotoma*, and *Dictyopteris delicatula*. *Spatoglossum macrodontum* (2.03 mg g^{-1} dw ± 0.06 SE) had the highest total C20:5(n-3) content within the Dictyotales. The essential PUFA C22:6(n-3) was most common in the green seaweeds and was generally low or below the detection threshold in the brown and red seaweeds.

Conspecific variation

Fatty acid content in *Dictyota bartayresii* varied by up to 50% between samples from different environments (different locations and sampling periods), while fatty acid content within samples (same location and time) varied only between 5% and 17% (Table S3). For example, indi-

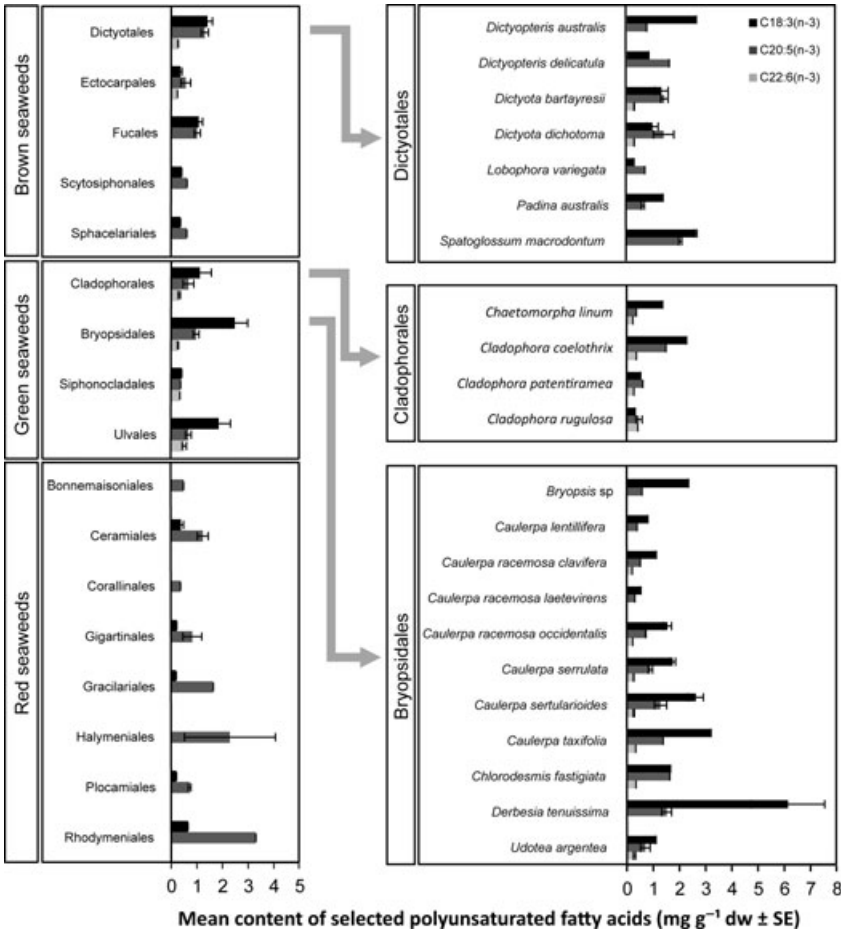

Fig. 5 Mean content (mg g^{-1} dw ± SE) of selected essential PUFA(n-3) in different seaweed orders (left) and species (right).

viduals from Orpheus Island had the lowest fatty acid content (22.46 mg g^{-1} dw ± 1.24 SE) while individuals from Kissing Point had the highest content (42.56 mg g^{-1} dw ± 1.36 SE). Furthermore, the fatty acid content between two locations within Nelly Bay collected on the same day but from opposite ends of the bay, approximately 500m apart, differed by 27% (South Nelly Bay, 8th September 2010: 32.04 mg g^{-1} dw ± 0.50 SE; North Nelly Bay, 8th September 2010: 43.88 mg g^{-1} dw ± 3.45 SE; Table S3). In contrast, fatty acid content between two sets of samples from the same site collected 6 weeks apart differed only by 8% (North Nelly Bay, 21th October 2010: 34.82 mg g^{-1} dw ± 1.17 SE; Table S3).

Principal component analysis also demonstrated a larger variation in fatty acid composition between samples from a different environment than between individual replicate plants (Fig. 6a, c). For *Dictyota bartayresii*, 64.80% of the variation in fatty acid composition between the individual plants can be explained by the first two principal components (Fig. 6a). Differences in total fatty acid content (PCA 1) explained over 40% of the total variation, with the Orpheus Island individuals being clearly lower than individuals from Kissing Point. Furthermore, the Orpheus Island individuals are characterized by a low PUFA(n-3) (PCA 2), and particularly low C18:3(n-3) level, compared to plants from other locations (Fig. 6b).

Fatty acid content of *Dictyota dichotoma* varied by 38% between samples, but only by 8-27% between individual replicate plants within a sample (Table S4). The Kissing Point sample had the lowest total fatty acid content (23.32 mg g^{-1} dw ± 2.49 SE) while the two samples from Nelly Bay collected 7 weeks apart had similar high fatty acid contents of 35.52 mg g^{-1} dw ± 0.95 (8th September 2010) and 37.74 mg g^{-1} dw ± 1.56SE (27th October 2010; Table S4).

Principal component analysis demonstrated that there was no clear pattern between location and fatty acid composition (Fig. 6c). Plants from Kissing Point and Nelly Bay (8th September 2010) had a similar fatty acid composition while the plants from Nelly Bay collected on the 27th October 2010 were distinct. The

Table 1 Fatty acid profiles (mg g^{-1} dw, mean of samples ± STDEV) and total lipid content (mg g^{-1} dw, mean ± STDEV, $n = 3$) of selected seaweed species

	Dictyotales			Cladophorales	Bryopsidales	
	Dictyota bartayresii	*Dictyota dichotoma*	*Spatoglossum macrodontum*	*Cladophora patentiramea*	*Caulerpa sertularioides*	*Derbesia tenuissima*
C14:0	2.54 ± 0.70	2.28 ± 0.65	4.95 ± 0.30	1.53	1.32 ± 0.27	1.34 ± 0.17
C14:1	0.38 ± 0.12	0.30 ± 0.06	0.14 ± 0.10	0.23		0.30 ± 0.03
C15:0	0.34 ± 0.07	0.24 ± 0.08	0.32 ± 0.03	0.23	0.17 ± 0.05	0.24 ± 0.01
C16:0	7.17 ± 2.14	6.85 ± 1.49	14.30 ± 1.42	9.84	9.39 ± 1.56	11.96 ± 0.54
C16:1(n-9)	0.28 ± 0.02	0.21 ± 0.08	0.24 ± 0.00	0.48	0.37 ± 0.06	0.34 ± 0.04
C16:1(n-7)	0.62 ± 0.09	0.82 ± 0.37	1.89 ± 0.16	1.05	1.26 ± 0.28	1.91 ± 0.37
C16:1	2.21 ± 0.65	2.09 ± 1.50	0.38 ± 0.00	0.13	0.07 ± 0.01	0.24 ± 0.02
C16:2(n-6)	0.27 ± 0.06	0.22 ± 0.18	0.17 ± 0.12	0.39	0.61 ± 0.20	0.82 ± 0.38
C16:2(n-4)	0.05 ± 0.07	0.10 ± 0.11	0.11 ± 0.06	3.39		0.13 ± 0.05
C17:0	0.06 ± 0.09	0.14 ± 0.05	0.22 ± 0.01		0.15 ± 0.01	0.21 ± 0.05
C16:3(n-6)	0.42 ± 0.10	0.34 ± 0.09	0.23 ± 0.01	0.22	0.03 ± 0.05	0.16 ± 0.04
C16:3(n-3)	0.16 ± 0.08	0.25 ± 0.28	0.14 ± 0.06	0.07	1.75 ± 0.35	3.34 ± 1.71
C16:4(n-3)	0.31 ± 0.13	0.38 ± 0.28	0.18 ± 0.00	0.74	0.25 ± 0.02	0.43 ± 0.03
C18:0	0.78 ± 0.16	0.60 ± 0.11	0.74 ± 0.05	0.35	0.45 ± 0.06	0.50 ± 0.09
C18:1(n-9)	4.65 ± 1.38	4.43 ± 0.51	10.31 ± 0.91	4.48	1.28 ± 0.05	2.41 ± 0.32
C18:2(n-6) trans	0.42 ± 0.29	0.29 ± 0.04	0.26 ± 0.00			0.02 ± 0.04
C18:2(n-6) cis	0.92 ± 0.33	0.71 ± 0.08	1.54 ± 0.04	3.03	1.97 ± 0.58	2.75 ± 0.39
C18:3(n-6)	0.49 ± 0.17	0.49 ± 0.13	0.65 ± 0.04	3.31	0.39 ± 0.05	0.77 ± 0.19
C18:3(n-3)	1.31 ± 0.57	0.97 ± 0.38	2.62 ± 0.06	0.52	2.62 ± 0.41	6.14 ± 2.42
C18:4(n-3)	3.42 ± 1.21	2.51 ± 0.81	5.21 ± 0.52	0.45	0.52 ± 0.12	0.64 ± 0.13
C20:0	0.39 ± 0.13	0.33 ± 0.10	0.45 ± 0.00			0.10 ± 0.05
C21:0	0.80 ± 0.29	0.60 ± 0.41	0.22 ± 0.16		0.26 ± 0.04	
C20:3(n-6)	0.52 ± 0.33	0.47 ± 0.23	2.15 ± 0.09	0.15	0.26 ± 0.04	0.28 ± 0.09
C20:4(n-6)	2.57 ± 1.58	2.76 ± 1.75	4.47 ± 0.37	2.77	0.33 ± 0.04	1.17 ± 0.23
C20:4(n-3)	0.73 ± 0.24	0.54 ± 0.14	2.21 ± 0.01		0.36 ± 0.05	0.17 ± 0.11
C22:0	0.52 ± 0.11	0.49 ± 0.25	0.65 ± 0.06	0.16	0.45 ± 0.08	0.52 ± 0.14
C20:5(n-3)	1.42 ± 0.32	1.40 ± 0.67	2.03 ± 0.08	0.60	1.28 ± 0.33	1.51 ± 0.33
C24:0	0.01 ± 0.03	0.02 ± 0.04	0.30 ± 0.01	0.08	0.98 ± 0.16	0.90 ± 0.17
C22:6(n-3)	0.20 ± 0.10	0.18 ± 0.01		0.18	0.26 ± 0.03	
Other FAs	1.18 ± 0.47	1.21 ± 0.38	0.29 ± 0.16	0.26	0.81 ± 0.19	0.28 ± 0.24
Total FAs	35.15 ± 8.69	32.19 ± 7.76	57.40 ± 1.23	34.63	27.61 ± 4.85	39.58 ± 5.09
Total SFA	12.60 ± 3.12	11.55 ± 2.41	22.17 ± 1.32	12.19	13.17 ± 2.15	15.76 ± 0.53
Total MUFA	8.14 ± 1.88	7.85 ± 1.61	12.95 ± 1.17	6.37	2.98 ± 0.40	5.19 ± 0.61
Total PUFA	13.23 ± 4.61	11.58 ± 4.41	21.99 ± 1.09	15.82	10.65 ± 2.11	18.35 ± 4.25
PUFA(n-3)	7.57 ± 2.33	6.22 ± 2.28	12.41 ± 0.61	2.57	7.05 ± 1.31	12.23 ± 4.61
PUFA(n-6)	5.62 ± 2.52	5.27 ± 2.08	9.47 ± 0.42	9.86	3.60 ± 0.80	5.98 ± 0.33
Total lipid	119.10 ± 20.0	108.00 ± 9.90	117.30 ± 4.9	98.70 ± 4.3	130.40 ± 14.6	121.40 ± 5.9

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

main distinction was a particularly high PUFA(n-3) content and low C14:0 content in the Nelly Bay individuals sampled on 27th October 2010 (Fig. 6d; Table S4).

Discussion

This study provides clear evidence that some seaweed have high total lipid and fatty acid content and that the paradigm that seaweeds are not suitable for oil-based products, because of their presumably low total lipid and fatty acid content, is clearly challenged with lipid contents exceeding those of many species of microalgae (e.g. Huerlimann *et al.*, 2010; Mata *et al.*, 2010a). Furthermore, the quantitative and qualitative yields of fatty acids of selected species makes them suitable targets for biofuels, or alternatively as nutraceuticals. In general, brown seaweeds are preferred because of their high fatty acid content, followed by green seaweeds, while red seaweeds appear to have little application for

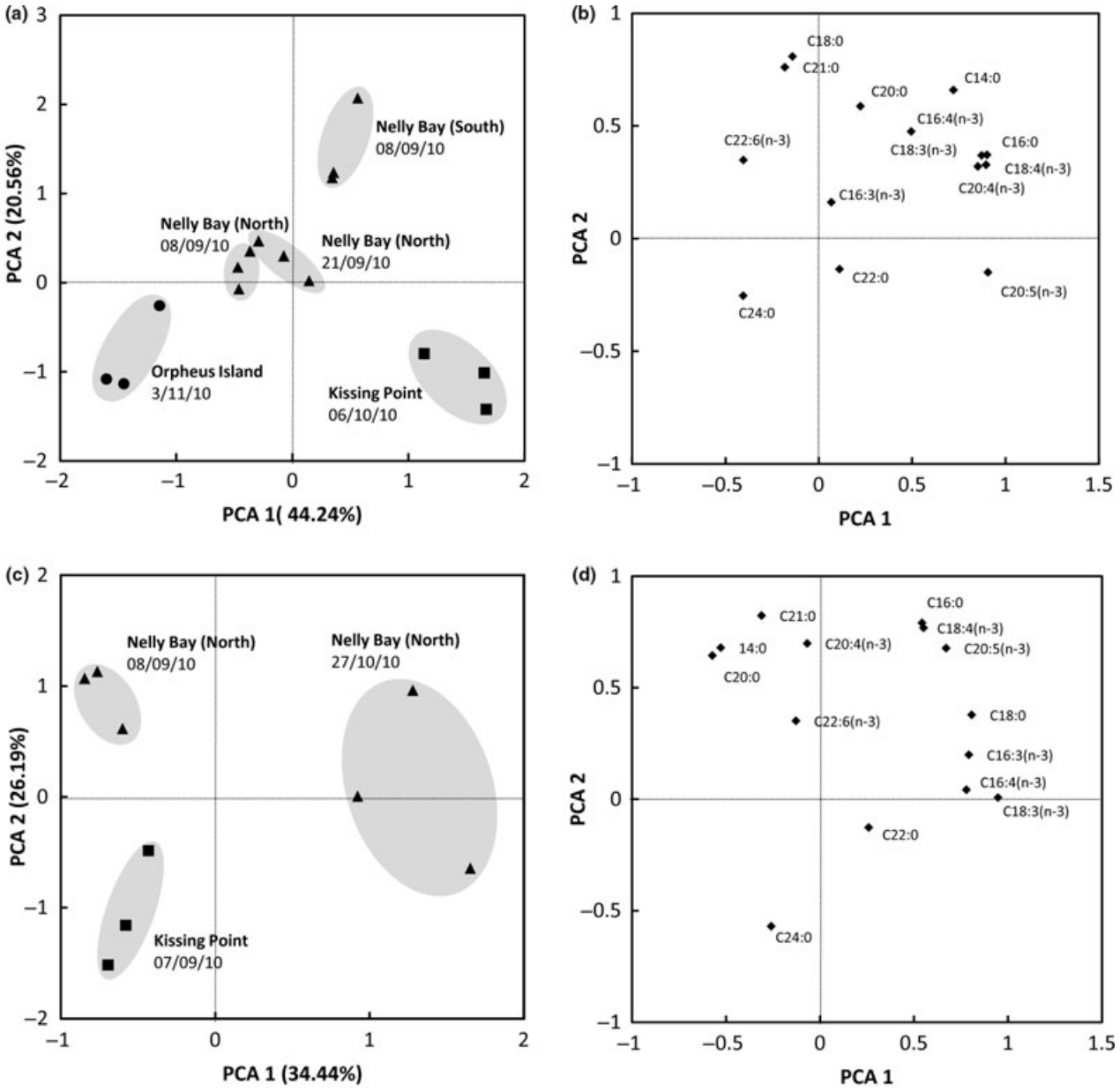

Fig. 6 (a) Principal component analysis of fatty acid composition for *Dictyota bartayresii* triplicate samples from different locations (Kissing Point, Nelly Bay, Orpheus Island) and sampling times. (b) Bi-plot of fatty acid loadings for *Dictyota bartayresii*. (c) Principal component analysis of fatty acid composition of *Dictyota dichotoma* triplicate samples from two different locations (Kissing Point, Nelly Bay) and sampling times. (d) Bi-plot of fatty acid loadings for *Dictyota dichotoma*. Shaded areas only used to visualize the replicates of one sample.

oil-based products. Two species are clearly identified as targets from this study, *Spatoglossum macrodontum* from the Dictyotales and *Derbesia tenuissima* from the Bryopsidales. Notably, there is also important variation in total lipid content and fatty acid profiles within species due to environment and/or genotype. These data provide a basis for the optimisation of environmental culture conditions and selection of high oil-yielding genotypes to further enhance fatty acid yields under culture.

Total lipid content

Although the majority of investigated seaweed species in this study have a total lipid content below 5% dw, there are a number of species with a total lipid content > 10% dw and these are therefore interesting candidates for oil-based products. Within the brown seaweeds, the Dictyotales and in particular *Dictyota bartayresii*, *Spatoglossum macrodontum*, and *Dictyota dichotoma* have the

highest total lipid contents (10–12% dw). *Dictyota* has been previously identified as a lipid rich genus, with consistently high total lipid contents of up to 20% dw (Montgomery & Gerking, 1980; McDermid & Stuercke, 2003). No information on total lipid content is available for *Spatoglossum macrodontum* and its potential for oil-based products, in particular biofuels, is identified here for the first time. This may be a unique species rather than genus, as the congener *Spatoglossum asperum* has a total lipid content of < 5% dw (Kumari *et al*., 2010). *Spatoglossum macrodontun* has a pan-tropical distribution, occurs in large stands, and is in some places an introduced 'pest' species due to its high growth rate and reproductive ability (Skelton *et al*., 2007). These attributes, in conjunction with the high levels of fatty acids reported here, make this species a primary target for low cost, high density culture for oil-based products.

In a similar manner the total lipid content in *Derbesia tenuissima* (12–13% dw) is among the highest found for green seaweeds. While the Bryopsidales are a lipid rich order when compared to other green seaweeds, total lipids rarely exceed 5% dw (McDermid & Stuercke, 2003). This is the first report of lipid and fatty acid content for *Derbesia tenuissima*, and fortuitously it also has rapid growth at high density under intensive culture (unpublished data), providing for optimized production for bio-oils and more broadly for biomass derived energy.

Fatty acids

Although total lipid content is a potential indicator for the suitability of seaweeds for biofuel production, or other oil-based products, it is the fatty acid content and quality that are most relevant to determine applicability for a specific end-use. Although there is a strong correlation between total lipid and fatty acid content in our study, the relative proportion of fatty acid actually decreases with increasing total lipid content. Therefore, high total lipid contents in seaweeds are not necessarily good indicators for screening for high fatty acid content, and by virtue screening for the suitability of oil-based products. However, brown seaweeds have a stronger relationship between total lipid and fatty acid content than either green or red seaweeds.

Fatty acid profiles varied between taxonomic groups and green, red, and brown seaweeds can all be distinguished by their fatty acid profile. Green seaweeds have a high level of PUFA(n-3) and in particular a high amount of C18:3(n-3), whereas red seaweeds have high amounts of saturated fatty acids and some brown seaweeds are particularly rich in C14:0. Red seaweeds also have the highest level of C16:0 confirming previous studies (Vaskovsky *et al*., 1996). In addition, the essential fatty acid C20:5(n-3) is abundant in the red seaweeds and is a common trait (Graeve *et al*., 2002; Khotimchenko *et al*., 2002). Finally, brown seaweeds generally have a lower level of C16:0, with the Dictyotales being an exception. The Dictyotales are unique in also being rich in C14:0 with a high proportion of saturated fatty acids (Khotimchenko, 1995).

For biofuel production, algae with a high proportion of saturated fatty acids are preferred as this leads to higher oxidative stability and higher ignition quality (cetane number), and produces an overall higher quality product (Hu *et al*., 2008; Knothe, 2008). Feedstocks with a high proportion of the monounsaturated fatty acid (MUFA) C18:1 can further enhance biofuel quality by improving the low-temperature properties and kinematic viscosity, and reducing the emissions of hydrocarbon and CO_2 (Knothe, 2008). In contrast, a high content of PUFAs results in less stable and lower quality biofuel. The results from our study suggest that the Dictyotales are a suitable biomass target for biodiesel production because of their high total lipid, high fatty acid level and high proportion of SFA. In addition to having the highest quantities of fatty acids reported for a seaweed, *Spatoglossum macrodontum* also has the highest quality of FAs for biofuel production with a fatty acid profile rich in saturated fatty acids and with C18:1 as the second most abundant fatty acid.

Algae with a high content of the essential PUFA(n-3) are preferred for human consumption or the production of nutraceuticals and fish oil replacements. The red alga *Champia parvula,* has an exceptional level of C20:5(n-3) (3.30 mg g^{-1} dw ± 0.11 SE), while *Derbesia tenuissima* is rich in C20:5(n-3) and has a high level of C18:3(n-3). *Derbesia tenuissima* is also suitable for biofuel production; however, its low saturated fatty acid profile may produce a lower quality product compared to *Spatoglossum macrodontum*.

Conspecific variation

One of the major outcomes of this study is the identification of significant variation in the quantitative fatty acid profile amongst individuals within species for *Dictyota bartayresii* and *Dictyota dichotoma* sampled from different sites, and at different times. The relative contribution of environment and genotype could not be partitioned in this sampling design, however, the potential for these effects are critical for cultivation strategies for seaweeds. The main environmental differences between the locations (inshore vs. offshore) and sampling periods (spring vs. summer) are water temperature and nutrient load and these factors affect the quantitative fatty acid profiles of seaweeds and algae more broadly. For example, seaweeds from cold water environments had higher

PUFA contents compared to seaweeds from warmer waters (Graeve *et al*., 2002; Nelson *et al*., 2002). Experimental data also suggests that chilling prior to harvest can increase the levels of C20:4 and C20:5 in Phaeophyceae and Rhodophyta (Al-Hasan *et al*., 1991). In addition, macroalgae grown in shade have higher total lipid content compared to algae grown in full light (Hotimchenko, 2002). There is also evidence that nitrogen starvation increases lipid synthesis and so improves total lipid content of algae (Livne & Sukenik, 1992; Hu *et al*., 2008). However, most data related to this effect has been obtained from microalgae and limited evidence is available for macroalgae (e.g. Mulbry *et al*., 2008).

In addition to environmental factors directly influencing the fatty acid content and profile of seaweeds, it is also possible that genotypes with specific quantitative fatty acid profiles exist across environments. Relatively little information is available for the heritability of seaweed natural products (see Wright *et al*., 2004). However, if genotype or genotype-environment interactions exist, individual plants of a seaweed species with particularly high total lipid content and favourable fatty acid profile can be identified for selective breeding of seaweed strains with favourable properties for oil production. The next step in our research is to develop this concept for *Spatoglossum macrodontum* and *Derbesia tenuissima*, in combination with intensive culture for high biomass productivities. Combining the selection of traits for high quantitative fatty acid profile with high productivities provides opportunities for delivering macroalgal biomass for oil-based products. While the techno-economic evaluation of macroalgal oil-based production has not reached the levels of sophistication for microalgae (Stephens *et al*., 2010a,b), many macroalgae have productivities that match or exceed those of microalgae on a dry weight per unit area basis (Cappo *et al*., 1999; Mata *et al*., 2010b) and do not require complex and costly harvesting and drying systems (Paul & Tseng, 2012). For example, a conservative macroalgal biomass productivity of 20 g dw m^{-2} day^{-1} with an extractable total lipid (oil) content of 10% gives a total lipid yield of 2 g (total lipid) m^{-2} day^{-1}, which although below the benchmark for microalgae [5 g (oil) m^{-2} day^{-1}] (Stephens *et al*., 2010a) may be achieved economically, with significant scale for improvements in both biomass productivity and lipid content. Furthermore, there are a diversity of viable options to firstly deliver oil-based products including biofuel (biodiesel) from the lipid fraction of algal biomass, with the remaining biomass being converted to a range of bioenergy products (Ross *et al*., 2008; Brennan & Owende, 2010; Demirbas, 2011; Nigam & Singh, 2011; Singh & Olsen, 2011). This includes the production of liquid fuels with seaweed biomass (macroalgae) being investigated for biocrude (bio-oil) production through hydrothermal liquefaction (Zhou *et al*., 2010; Anastasakis & Ross, 2011) and pyrolysis (Budarin *et al*., 2011). Importantly, this approach of lipid extraction and subsequent pyrolysis can enhance total bio-oil yield, above that achieved by pyrolysis of the algal biomass in-toto (Grierson *et al*., 2011).

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Supporting Information

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

Table S1. Total lipid content (mg g^{-1} dw, mean of triplicates of one sample ± STDEV) of all investigated seaweed species

Table S2. Fatty acid profiles (mg g^{-1} dw, mean of samples ± STDEV) of all investigated seaweed species

Table S3. Fatty acid profile (mg g^{-1} dw) of all investigated *Dictyota bartayresii* plants (every replicate from all samples of this species)

Table S4. Fatty acid profile (mg g^{-1} dw) of all investigated *Dictyota dichotoma* plants (every replicate from all samples of this species)

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