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Selecting Australian marine macroalgae based on the fatty acid composition and anti-inflammatory activity

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Abstract Increasingly, macroalgae are being recognised as a growth opportunity for functional foods and nutritional security in the future. Dominating traits of interest are metabolites that function as anti-inflammatories and are antiproliferative. However, seaweeds from the northern hemisphere dominate this field of research. Australia has a unique flora of macroalgae, and it is poorly understood which species should be targeted for cultivation towards food and health markets. Here, six Australian marine macroalgae were selected for screening of one anti-inflammatory group; n-3 polyunsaturated fatty acids (PUFA). PUFA profiles were determined using gas chromatography-mass spectrometry and multivariate analysis. Thirty-one fatty acids (FA) were identified across the six macroalgal species with C16:0 the dominant FA in all samples, variations across taxa in the saturated FA C10:0, C14:0, C16:0, C18:0 and C20:0 and variations in monounsaturated FA attributed to C16:1 n-7 and C18:1 n-9. For PUFA profiles, all six species had significantly different n-6/n-3 ratios, while the green seaweed *Ulva* species possessed the lowest n-6/n-3 ratio of 0.4, along with a 2-fold higher C18:3 n-3 to C18:2 n-6 content. *Ulva* sp. was the only species that contained docosahexaenoic acid. Extracts of both the *Ulva* sp. and *Hormosira banksii* showed selective cytotoxicity towards a human pancreatic cancer cell line, while the nonpolar extracts of all six algae species strongly inhibited production of the inflammatory-mediator nitric oxide.

Keywords Seaweeds · Fatty acid profiles · PUFA · Omega-3 · Inflammation · Functional foods

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Introduction

Macroalgae are a large and growing multibillion dollar food and biotechnology industry (FAO 2012; Bixler and Porse 2011; Mazarrasa et al. 2013). Increasingly, they are being recognised as a rich source of biologically active metabolites for utilisation in functional foods, nutritional supplements and/or pharmaceuticals (Stengel et al. 2011). This is due to important functional roles of these metabolites in a range of biological processes including cell mediation and immune responses (Warude and Joshi 2006; Rubio-Rodríguez et al. 2010; Calder and Yaqoob 2009; Kumari et al. 2010; Mohamed et al. 2012; Smit 2004). Research in medical and health applications of seaweed metabolites now dominate the publication of phycology-related scientific literature (Winberg et al. 2014). There is particular promise for chronic inflammatory diseases and cancers of the digestive system (Vo et al. 2012; O'Sullivan et al. 2010) (Fig. 1). Metabolites that contribute to anti-inflammatory function include essential polyunsaturated fatty acids (PUFA) such as alpha-linolenic acid

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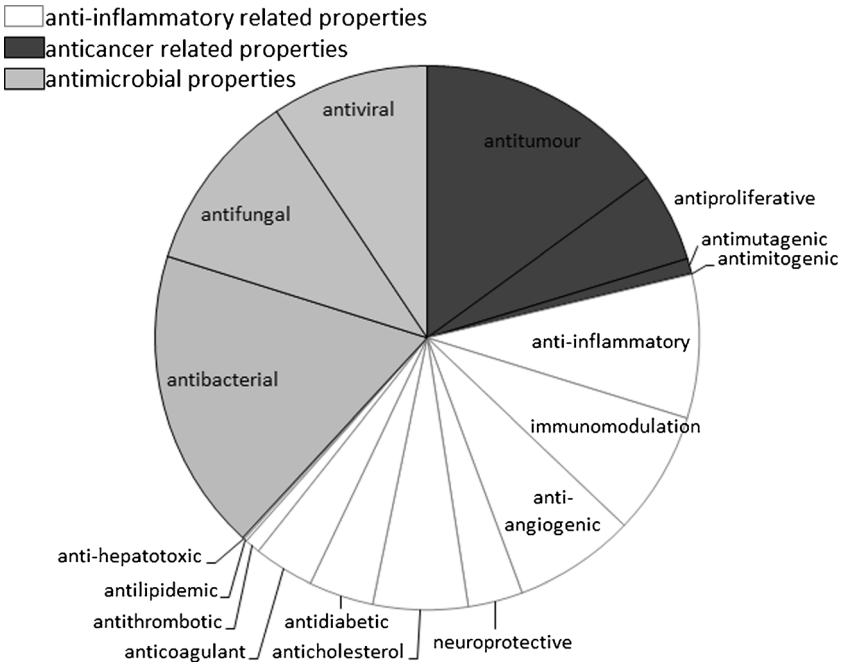

Fig. 1 The distribution of published research effort in terms of reference to macroalgae and each of the health application terminologies (e.g. anti-inflammatory) as found in Google Scholar database of scientific literature

(ALA, C18:3n-3) that cannot be synthesised by humans and therefore must be obtained from dietary sources, as well as the essential n-3 long-chain PUFA such as eicosapentaenoic acid (EPA, C20:5n-3), docosapentaenoic acid (DPA, C22:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) (Glick and Fischer 2013). These PUFA function as competitors to the pro-inflammatory metabolism of linoleic acid (LA, C18:2n-6) as the parent fatty acids (FA) for the pro-inflammatory metabolism of the n-6 PUFA arachidonic acid (AA). n-6 AA is a biosynthetic precursor to prostaglandin E2 (PGE2) and leukotriene B4 (LTB4) inflammation pathways and inflammatory eicosanoids such as prostaglandins and leukotrienes (Glick and Fischer 2013). Conversely, n-3 PUFA can be enzymatically converted to bioactive autacoids with inflammatory-resolving properties that compete with and offset inflammation status (Zhang and Spite 2012; Jaswir and Monsur 2011). Thus, a reduced ratio of n-6/n-3 PUFA is considered important to suppress chronic inflammatory disease triggered by a higher intake of n-6 PUFA in modern diets (Meyer et al. 2003; Simopoulos 2002). The pathogenesis of diseases includes cardiovascular disease, cancer and other inflammatory disorders (Simopoulos 2002; Grenon et al. 2011). Thus, a greater consumption of long chain n-3 FA may offer protective effects by suppressing pro-inflammatory activity (Grenon et al. 2011; Harper and Jacobson 2001).

Of the n-3 PUFA, ALA is predominately found in vegetable oils and nuts and EPA and DHA, in marine fish and fish oils (Meyer et al. 2003). Conversion of ALA to EPA and DHA is limited, and therefore, dietary consumption of these two long-chain PUFA is highly recommended (Goyens et al. 2006; Burdge and Wootton 2002). The proposed health benefits of n-3 PUFA have led to increased demand for their use as animal feed additives to promote bioaccumulation and for direct human consumption (Mansour et al. 2005; Nettleton 1995). Because of increasing costs and availability of fish-derived PUFA from fish oil, there is a need to develop alternative sources.

Marine algae are primary producers of PUFA that fish obtain through trophic bioaccumulation (Sijtsma and de Swaaf 2004; Guschina and Harwood 2006) and represent a promising new PUFA source. This presents an opportunity to target whole seaweed as a food and animal feed, not only for trace elements like iron and iodine but also for lipids that are deficient in the Western diet (Lucas 2006; Meyer et al. 2003). However, a challenge remains to understand which species contain optimal PUFA profiles that could balance current dietary practice and the profile variability between taxa.

Inflammation is a complex and multifactorial network of chemical signals (Coussens and Werb 2002). Nitric oxide (NO), an important biological mediator, is one chemical molecule that is generated by the enzyme inducible nitric oxide synthase (iNOS) during immune and inflammatory responses (Coleman 2001). Elevated and chronic NO production, as seen in persistent inflammatory disorders, is associated with dysregulation of signalling events and immune response resulting in cell and tissue injury (Cross and Wilson 2003) and subsequently cancer (Coussens and Werb 2002). Suppression of NO production has been observed with n-3 PUFA via inhibition of iNOS (Ohata et al. 1997). Further anti-inflammatory compounds are diverse across fractions of

metabolites and function in different inflammation pathways including iNOS, cyclooxygenase (COX) and reactive oxygen species (ROS) metabolism (Vo et al. 2012). For algae, these fractions include both nonpolar and polar extracts and have been shown to function differently across assays for these different inflammatory processes. For example, nonpolar algal extracts include phlorotannins that have been shown to reduce the production of NO and reduce edema, along with anti-inflammatory PUFA, carotenoids, terpenoids and xanthophylls (Jaswir and Monsur 2011).

The metabolic profiles and biological activity of extracts from northern hemisphere macroalgae species have been well described including both genetic and spatial/temporal variations (Holdt and Kraan 2011; Schmid et al. 2014). However, in Australia, which is a macroalgae biodiversity hotspot (Womersley 1990) with high species richness and endemism of benthic flora (Phillips 2001), a legacy of little use in food has resulted in a paucity of knowledge of Australian species with the potential for applications in health. Australia has been seeking to grow its aquatic production industries, and macroalgae have been selected based on existing market (colloid) or bioremediation potential, but little has been done to select species based on the potential applications in targeted health markets. Here, six Australian macroalgae species were screened for their FA profiles, and organic extracts of varying polarity from each species were evaluated for both anti-inflammatory activity and selective cytotoxicity for prioritising candidates from a human health context.

Materials and methods

Collection Macroalgae species for biological assessment and fatty acid profiling were collected seasonally from a number of locations along the southeast coast of New South Wales (NSW), Australia (Table 1). Samples were cleaned of epiphytes and sand and dried (70 °C), pulverised and vacuum-sealed until analysis. For fatty acid profiling, fresh samples were collected, cleaned and freeze-dried immediately prior to analysis. The *Ulva* species used was obtained from culture, kept and maintained for several months (Table 1).

Extraction and transesterification of fatty acids Lipids were extracted using the method of Folch et al. (1957) with modifications by Kumari et al. (2010). In brief, 250 mg dry weight was extracted with 3 × 3 mL of chloroform/methanol (2/1, *v*/*v*) with 0.001 % butylated hydroxytoluene (BHT) by vortexing (~1 min) and centrifugation (3000 rpm, RT, 15 min). Combined supernatants were washed with 2 mL of Milli-Q water (3000 rpm, RT, 5 min). The extract was evaporated to dryness in preweighed 9-mL Teflon-lined screw-top glass culture tubes under a stream of nitrogen. Crude lipid extracts were transesterified according to the method of Lepage and Roy (1986) with added BHT (0.01 %*w*/*v*). Extractions performed in triplicate using homogenised powder of the whole algae sample.

FA analysis FA were analysed by flame-ionisation GC (model GC-17A; Shimadzu, Australia) using a 50 m × 0.25 mm internal diameter capillary column. One microliter of the transesterified sample was auto-injected into the column, and individual FA were quantified using the Shimadzu analysis software (Class-VP 7.2.1 SP1). FA were identified by comparison with known standards (Nu-Chek or Sigma, Australia). Heneicosanoic acid (0.2 mg mL^{-1} in toluene) was used as an internal standard for quantification, and samples were analysed in triplicate. Relative compositional data is represented as molar % composition of total FA taking into account the molecular weight of the identified FA. Quantified data is represented as milligrams per gram dry weight (d.w.).

Biological assays For biological assays, samples were dried at 70 °C for 48 h, pulverised to a fine powder and extracted in a 1:1 dichloromethane (DCM):methanol solution using a modified version of Ebada et al. (2008). The crude extract was partitioned into DCM, ethyl acetate (EtOAc) and butanol (BuOH) and organic phases evaporated to dryness. Stock solutions were dissolved in 100 % dimethyl sulfoxide (DMSO) (10 mg mL^{-1}) and diluted with culture medium to obtain testing concentrations of 100 μg mL^{-1} in 1 % DMSO.

Extracts were assessed for cytotoxicity at 100 μg mL^{-1} on human pancreatic carcinoma (MIA-PaCa-2) cell line, Abelson murine leukaemia virus-induced tumour macrophage (RAW 264.7) and wild-type murine embryo fibroblast cell lines (WT-MEF). Cytotoxicity was determined as % inhibition of cell proliferation, measured by colorimetric markers (MTS reagent) of dehydrogenase enzyme activity, relative to controls (24–48 h incubations) (McCauley et al. 2013). RAW 264.7 macrophage cells were either pre- or co-incubated with lipopolysaccharides (LPS) (1 μg mL^{-1}) and the extracts (100 μg mL^{-1}) for 24 h (37 °C, 5 % CO_2), including controls.

The quantity of nitrite (NO_2^-) accumulated in the supernatant, as a measure of positive inflammation response (NO production), was measured by mixing equal volumes of supernatant with Griess reagent (1 % sulphanilamide and 1 % N-(1-naphthyl)ethylenediamine dihydrochloride solution). After 10 min incubation at room temperature, absorbance was measured. NO_2^- concentrations were calculated using a standard curve. NO inhibition was determined as % of LPS-induced inflammatory controls (Shanmugam et al. 2008).

Statistical analysis Multivariate FA profiles across macroalgal taxa were visualised in multidimensional scaling (MDS) plots using Euclidean distances due to a large number of zeros in the data. Apparent groupings were tested using

Table 1 Summary of samples collected seasonally from various locations along the southeast coast of NSW Australia, including species, phylum, order, collection site (latitude and longitude) and/or date of harvest or collection

Species	Phylum	Order	Location	Latitude	Longitude	Date
Ecklonia radiata (C. Agardh) J. Agardh[ǂ]	Ochrophyta	Laminariales	Jervis Bay	150.7347	35.0653	10 Apr. 2014
Hormosira banksii (Turner) Decaisne[ǂ]	Ochrophyta	Fucales	Jervis Bay	150.7347	35.0653	10 Apr. 2014
Phyllospora comosa (Labillardière) C. Agardh[ǂ]	Ochrophyta	Fucales	Jervis Bay	150.7347	35.0653	10 Apr. 2014
Solieria robusta (Greville) Kylin[ǂ]	Rhodophyta	Gigartinales	Jervis Bay	150.7347	35.0653	10 Apr. 2014
Ecklonia radiata	Ochrophyta	Laminariales	Gerringong	150.8271	34.7423	4 Apr. 2012
Hormosira banksii	Ochrophyta	Fucales	Gerringong	150.8271	34.7423	4 Apr. 2012
Phyllospora comosa	Ochrophyta	Fucales	Gerringong	150.8271	34.7423	4 Apr. 2012
Solieria robusta	Rhodophyta	Gigartinales	Callala Beach	150.6899	35.0086	29 Apr. 2012
Myriogloea sciurus (Harvey) Kuckuck ex Oltmanns[♭]	Ochrophyta	Ectocarpales	Manyana	150.5167	35.2500	1 Nov. 2012
Ulva sp.[♭]	Chlorophyta	Ulvales	Cultivated	–	–	–

For biological assessment, the algal species *Hormosira banksii*, *Phyllospora comosa* and *Ecklonia radiata* were collected from Gerringong; *Solieria robusta* was collected from Callala Beach; and *Myriogloea sciurus* was collected from Manyana

[ǂ] Fresh samples collected and immediately freeze-dried for species fatty acid profiling; [♭] Freeze-dried sample prepared from frozen voucher sample

[♭] Species name withheld; species maintained in culture

Analysis of similarity (ANOSIM) with PRIMER-E v6 software (Plymouth Marine Laboratories (Clarke and Gorley 2006). Data were analysed in both untransformed raw format to compare the relative abundance of different FA to chemical profile differences, as well as presence/absence transformation (either a 1 or a 0 value) to compare the overall FA composition. This is a rapid method to objectively identify key groups of or specific FA that differ across taxa, which could then be tested in univariate analyses. Identification of the FA or groups of FA that contributed most to differences between taxa was done using the PRIMER-E similarity percentages (SIMPER) analysis tool in PRIMER-E. One-way ANOVA, Tukey's multiple comparison test and Student's *t* test were used to determine significantly different means for individual fatty acids of interest using GraphPad Prism version 6.00, GraphPad Software.

Results

Key differences in FA composition across species A total of 31 fatty acids were identified across the six algal species (Table 2). The highest numbers of FA (26) were identified in both the brown alga *Hormosira banksii* and the green alga *Ulva* sp.; followed by the three brown algae species with 18 FA identified in both *Myriogloea sciurus* and *Phyllospora comosa*, 15 in *Ecklonia radiata*; and the lowest number (14 FA) in the lipid extract of the red alga *Solieria robusta*.

Key differences in abundance across species Analysis of the quantified fatty acid data (mg g^{-1} d.w. whole biomass) showed significant differences that were observed between phyla (analysis of similarity, $R<0.9$, $p<0.01$) and between species within the Ochrophyta (analysis of similarity $R<0.9$, $p<0.02$) (Fig. 2). Transformation of the data to reduce the effect of abundance indicated that phyla differences are significantly distinct in terms of the composition of fatty acid profiles (analysis of similarity $R<0.9$, $p<0.01$). However, species differences were not significant within the Ochrophyta ($p=0.10$) despite some evident clustering (data not shown).

SIMPER analysis revealed that C18:0, C20:0 and C22:0 were the three main SFA that determined species variation in terms of both composition and abundance (Fig. 2). C18:0 is most abundant in *S. robusta*, followed by *Ulva* sp. along with higher C20:0 content for the latter species. C22:0 was not present in *E. radiata* or *S. robusta* and was most abundant in *H. banksii*. In regards to PUFA, SIMPER analysis showed that species variations were primarily due to C18:2n-6 (LA), C18:3 n-3 (ALA), C20:4n-6 (AA), C20:3n-3 (ETA) and C20:5n-3 (EPA). Oleic acid (C18:1n-9) was most abundant in *H. banksii* and *M. sciurus* with trace amounts in *S. robusta. Ulva* sp. showed the highest abundance of essential ALA (C18:3n-3) as a proportion of total significantly identified PUFA. This was followed by *H. banksii*, which had the second highest ALA content along with a significantly higher proportion of AA (C20:4n-6) and EPA (C20:5n-3).

On a molar % basis, palmitic acid (C16:0) was the predominant FA in all six samples ranging from 20.5 % in the *Ulva* sp. to more than double that amount (46.3 %) in *M. sciurus*. The second most dominant FA was oleic acid (C18:1n-9) for *E. radiata*, *M. sciurus* and *H. banskii* (20.2, 19.7 and 18.7 %, respectively); AA (C20:4n-6) for *P. comosa*

Table 2 Fatty acid composition of six Australian macroalgal species

Fatty acid	Ochrophyta				Rhodophyta	Chlorophyta
% molar area	*E. radiata*	*M. sciurus*	*H. banksii*	*P. comosa*	*S. robusta*	*Ulva* sp.
10:0	8.5^{b}	4.3^{ad}	3.6^{a}	5.3^{ad}	17.1^{c}	5.6^{d}
11:0	0.0	0.0	0.0	0.0	0.0	0.1
12:0	0.0	0.0	0.0	0.0	0.0	0.7
14:0	10.7^{a}	9.4^{a}	4.8^{b}	7.5^{d}	1.0^{c}	3.1^{b}
14:1 n-7	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	2.5^{b}
15:0	0.6	0.6	0.6	0.5	0.0	0.6
15:1	0.2	0.3	0.1	0.9	0.0	0.5
16:0	27.9^{b}	46.3^{a}	27.5^{b}	25.4^{c}	26.4^{bc}	20.5^{d}
16:1 n-7	11.4^{a}	6.5^{cd}	3.7^{b}	6.1^{cd}	9.9^{ae}	9.1^{e}
17:0	0.0	0.2	0.2	0.1	0.0	1.2
17:1 n-7	0.0^{a}	<0.1^{a}	0.1^{a}	0.2^{a}	3.5^{b}	0.6^{a}
18:0	1.8^{a}	3.8^{b}	1.5^{a}	1.7^{a}	16.7^{c}	6.6d
18:1 n-9	20.2^{a}	19.7^{a}	18.7^{a}	13.7^{b}	1.5^{c}	10.6^{d}
18:1 n-7	0.0^{a}	0.0^{a}	0.3^{ac}	0.0^{a}	2.3^{b}	1.9^{bc}
18:2 n-6 (*LA*)	3.0^{a}	3.7^{ab}	5.5^{bc}	7.0^{c}	0.2^{d}	5.4^{b}
18:3 n-6	0.5	<0.1	0.1	0.6	0.0	0.6
18:3 n-3 (*ALA*)	1.8^{a}	1.9^{a}	7.4^{b}	3.9^{d}	0.1^{a}	12.2^{c}
20:0	2.4^{b}	1.2^{b}	1.5^{b}	1.8^{b}	2.6^{b}	5.8^{a}
20:1 n-9	0.0	0.1	0.1	0.0	0.0	0.0
20:2 n-6	0.0	0.0	0.8	0.0	0.0	0.0
20:3 n-6	0.0	0.0	1.3	0.7	0.1	0.1
20:4 n-6 (*AA*)	8.7^{d}	1.3^{b}	13.2^{c}	21.1^{a}	20.6^{a}	0.8^{b}
20:3 n-3 (*ETA*)	0.0	0.0	0.7	0.0	0.0	0.0
20:5 n-3 (*EPA*)	2.3^{acd}	0.9^{a}	5.8^{b}	3.9^{de}	0.9^{a}	5.3^{be}
22:0	0.0	0.3	1.3	0.1	0.0	0.3
22:1 n-9	1.2	0.0	1.7	0.0	0.0	0.3
22:2 n-6	0.0	0.0	0.1	0.0	0.0	0.0
22:5 n-6	0.0	0.0	<0.1	0.0	0.0	0.1
24:0	0.0	0.0	0.1	0.0	0.0	0.0
22:6 n-3 (DHA)	0.0	0.0	0.0	0.0	0.0	0.5
24:1 n-9	0.0	0.0	0.0	0.0	0.0	0.6
Σ SFA	50.7^{a}	65.5^{b}	40.6^{c}	42.0^{c}	60.8^{b}	47.4^{a}
Σ MUFA	33.0^{b}	26.7^{ac}	24.6^{cd}	20.8^{def}	17.2^{e}	26.0^{f}
Σ PUFA	16.3^{d}	7.8^{c}	34.8^{a}	37.2^{a}	22.0^{b}	26.5^{b}
n-6/n-3	3.0^{a}	1.8^{b}	1.5^{c}	3.8^{d}	15.7^{e}	0.4^{f}
LA/*ALA*	1.7	1.94	0.74	1.8	2	0.44

Data presented as % molar area of total FA. Means with different letters are significantly different ($p<0.05$)

AA arachidonic acid, *ALA* alpha-linolenic acid, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *ETA* eicosatrienoic acid, *LA* linoleic acid

and *S. robusta* (21.2 and 20.6 %, respectively); and ALA (18:3n-3) for *Ulva* sp. (12.2 %). The third most abundant FA ranging from 9.4 to 17.1 % differed for each species from saturated FA for *M. sciurus* and *S. robusta* (myrisitic acid (C14:0) and capric acid (C10:0), respectively); to MUFA for *E. radiata* (C16:1n-7), *P. comosa* and *Ulva* sp. (C18:1n-9) and AA (C20:4n-6) for *H. banksii*. The latter species also had a relatively high abundance of other desirable FA such as ALA, LA and EPA (7.4, 5.5 and 5.8 % respectively), identifying this as a key species to pursue further.

Along with high C16:0, *S. robusta* was characterised by a high C18:0 and C10:0 SFA, the latter 2- to 5-folds greater than

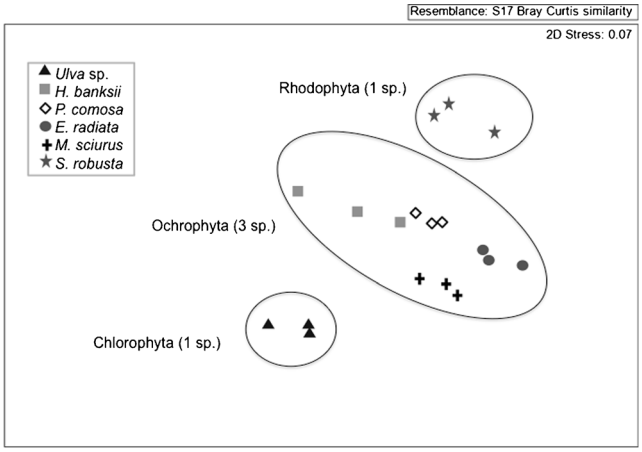

Fig. 2 Multivariate dimensional scaling plot comparing similarities of species fatty acids profiles (mg g^{-1} d.w.). Samples are from triplicate lipid extracts of six Australian native and endemic macroalgae. Data is untransformed

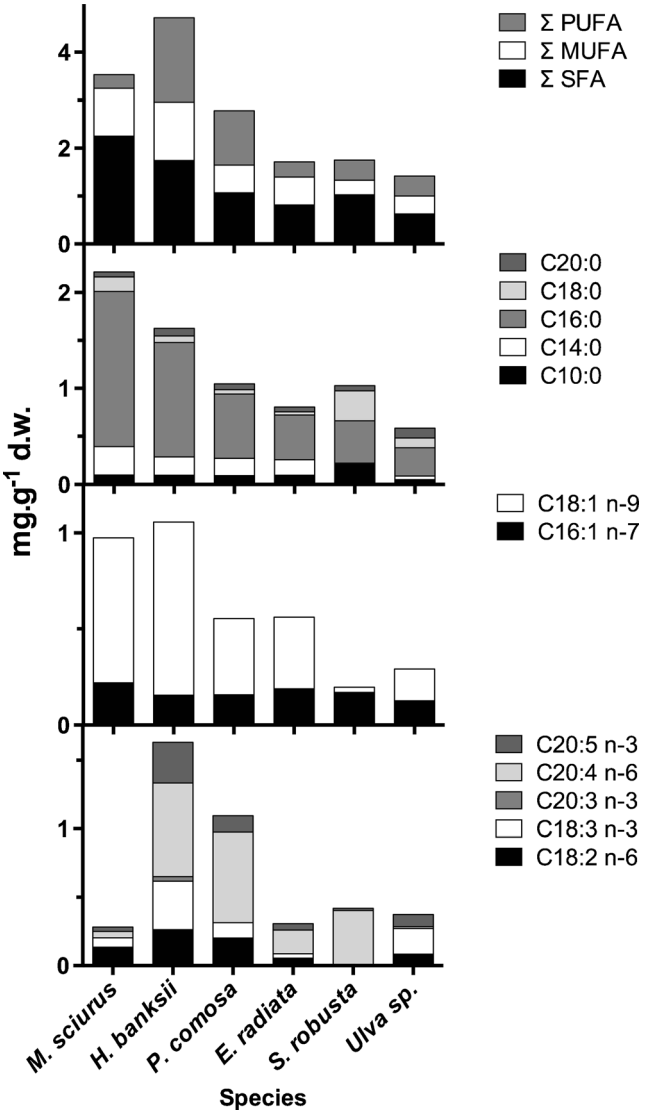

Fig. 3 Key differences in fatty acid content (mg g^{-1} d.w.) across the six Australian marine macroalgae of both total and individual SFA, MUFA and PUFA

for all other species, coupled with low oleic acid (C18:1 n-9) levels (Fig. 3). *Ulva* sp. was characterised by a high proportion of C16:0, oleic acid (C18:1 n-9) and C20:0 SFA and moderate, essentially equivalent palmitoleic (C16:1 n-7) levels (Fig. 3). Conversely, all four Ochrophyta species were characterised by moderate C14:0 and low C18:0 SFA content, instead dominated by a high proportion of oleic acid (C18:1n-9), 10-folds greater than *S. robusta* and 2- to 3-fold greater than *Ulva* sp.

The predominant PUFA across all six species were the n-3 FAs, ALA (C18:3n-3) and EPA (C20:5n-3), and the n-6 FAs LA (C18:2n-6) and AA (C20:4n-6) (Fig. 3). The most desirable ALA to LA ratio was observed for the *Ulva* sp. with a 2-fold higher ALA content compared to LA; followed by *H. banksii* with 1.3-fold higher ALA than LA; and then conversely, *P. comosa* showing a 2-fold higher LA to ALA content. AA (C20:4n-6) was the second most abundant FA for *S. robusta* and *P. comosa* and the third most abundant for *H. banksii* and lowest in the *Ulva* sp. The DHA precursor EPA (C20:5n-3) was highest in *H. banksii* (5.8 %), followed by *Ulva sp*. (5.3 %) and *P. comosa* (3.9 %). *H. banksii* also showed traces of eicosatrienoic acid (ETA; C20:3n-3), while the long-chain n-3 DHA (C22:6n-3) was only detected in the green *Ulva* sp., albeit in trace amounts (0.5 %).

The lowest ratio of n-6/n-3 PUFA was observed for the *Ulva* sp. (0.4), with the ratio ranging from 1.5 to 3.8 for the four brown species and 5-folds higher for the red seaweed *S. robusta* (15.7).

Anti-inflammatory activity A qualitative summary of the percent reduction of NO production in LPS stimulated macrophage RAW 264.7 cells relative to controls (Table 3), showed a significant reduction in NO production across almost all of the 18 extracts. The order of activity was greatest in the nonpolar, lipid-rich dichloromethane (DCM) extracts (>76 % activity for all species), followed by the intermediate polarity ethyl acetate (EtOAc) extracts (>50 % activity for all species except *H. banksii*), with the lowest activity observed in the polar butanol (BuOH) extracts (Table 3). Although the nonpolar DCM extracts were the most active, those of *P. comosa* and *S. robusta* also exhibited high cytotoxicity towards all three cell lines investigated, including the normal (WT-MEF) cell line. In general, the EtOAc extracts, in particular *P. comosa*, *M. sciurus* and *Ulva* sp. showed promising NO reducing activity, coupled with low toxicity towards the normal cell line, with the exception of *M. sciurus*.

Cytotoxicity A qualitative summary of 24 and 48 h cytotoxicity data for three cell lines is presented in Table 3. The nonpolar DCM extracts of *P. comosa* and *S. robusta* showed high cytotoxicity (>76 %) across all cell lines investigated (i.e.

Table 3 Cytotoxicity and anti-inflammatory activity (via inhibition of nitric oxide (NO)) of 18 algal extracts from six macroalgae

Species	Extract	RAW 264.7	WT MEF	MIA PaCa-2	% ↓ NO
E. radiata	DCM	+	−	−	+++
	EtOAc	−	−	−	++
	BuOH	+	−	−	−
M. sciurus	DCM	−	−	−	+++
	EtOAc	−	+	−	+++
	BuOH	−	−	−	++
H. banksii	DCM	−	+	−	+++
	EtOAc	−	−	+++	+
	BuOH	+	−	−	−
P. comosa	DCM	+++	+++	+++	+++
	EtOAc	−	−	−	+++
	BuOH	−	−	−	−
S. robusta	DCM	+++	+++	+++	+++
	EtOAc	nt	nt	+++	++
	BuOH	+	−	−	+
Ulva sp.	DCM	+	+	+++	+++
	EtOAc	+	−	+++	+++
	BuOH	−	−	−	+

Data are presented as % activity at 100 μg mL^{-1} from two independent trials done in duplicate

Key: (−) 0–24 % activity; (+) 25–50 %; (++) 51–75 %; (+++) 76–100 % activity; (nt) not tested, no available extract. Investigated cell lines include human pancreatic carcinoma (MIA-PaCa-2), Abelson murine leukaemia virus-induced tumour macrophage (RAW 264.7) and wild-type murine embryo fibroblast cell lines (WT-MEF)

macrophage RAW 264.7, wild-type murine embryonic fibroblast WT-MEF and human pancreatic carcinoma MIA-PaCa-2 cell lines). In addition to strongly reduced NO production, the DCM and EtOAc extracts of *Ulva* sp. showed preferential cytotoxicity towards the MIA-PaCa-2 human pancreatic carcinoma cell line. The EtOAc extract of *H. banksii* showed the greatest selectivity towards the pancreatic cancer cell line, along with a moderate reduction in NO production.

Discussion

This study presents key differences from the screening process of six species of Australian macroalgae for prioritising future work towards anti-inflammatory applications. To this end, consistent anti-inflammatory activity was observed for all taxa in terms of decreased NO production, predominantly in the DCM and EtOAc extracts. However, the species were highly diverse in the abundance of FA, with approximately 30 % more types of FA in *Ulva* sp. and *H. banksii* than in the other taxa. The highest abundance of PUFA was in *H. banksii* and the fourth lowest in *Ulva*, while the more preferable n-6/n-3 ratio and LA/ALA ratio were found for *Ulva*. In addition, the selective cancer cell toxicity was greatest for *Ulva* and *H. banksii* (Table 4).

With the exception of the well-studied green macroalgae genus *Ulva* (Floreto et al. 1993; Alamsjah et al. 2008; Ortiz et al. 2006), the five other macroalgae species described here have been studied in other contexts such as their protein, trace element and carbohydrate content (Edmonds and Francesconi 1981) but rarely in terms of their FA profiles and anti-inflammatory activity. Investigation of a different sample of *P. comosa* from southeastern Australia has been reported by us previously with an analogous profile to that described here (Zivanovic and Skropeta 2012). The FA profile of a red seaweed belonging to the genus *Solieria* has been described as dominated by C16:0 and C20:5 n-3, with the EPA content differing from our results (Bert et al. 1991).

Apart from palmitic acid (C16:0), which was the dominant FA in all six species and is consistent with other macroalgae (Schmid et al. 2014; Zivanovic and Skropeta 2012; Holdt and Kraan 2011), the remainder of the FA profiles were significantly different for the three phyla investigated herein. This is consistent with previous reports where FA signatures have been used to distinguish taxonomic groups up to the family level but not at the level of species (Galloway et al. 2012; Kumari et al. 2013).

From the four Ochrophyta species, the two Fucales species showed comparable total SFA, MUFA and PUFA levels (Kumari et al. 2013; Khotimchenko et al. 2002). *M. sciurus* (order Ectocarpales) and *E. radiata* (order Laminariales) reported here however showed reduced levels of total PUFA when compared to similar species previously reported from the same order (Khotimchenko et al. 2002; Kumari et al. 2013). Overall, the four Ochrophyta species were characterised by high C16:0, moderate C14:0 and low C18:0 SFA; the highest levels of oleic acid, particularly for *E. radiata*, *M. sciurus* and *H. banksii* but varying PUFA profiles and abundance with *H. banksii* and *P. comosa* containing amongst the highest amounts of the long-chain PUFAs AA (C20:4n-6) and EPA (C20:5n-3) and *M. sciurus* and *E. radiata* containing the lowest abundance of PUFA of all the six species. The dominant C18:1n-9, C20:4n-6 and C20:5n3 is consistent with average abundances reported for the Phaeophyceae (Kelly and Scheibling 2012).

The red seaweed *S. robusta* (order Gigartinales) was characterised by the highest amount of capric acid (C10:0), along with other SFA in high amounts (C16:0 and C18:0); the lowest abundance of MUFA; and a PUFA profile comprised almost entirely of AA, consistent with other reports that Rhodophyta are dominated by the presence of C20 PUFA (Kelly and Scheibling 2012; Graeve et al. 2002). EPA (C20:5 n-3) levels herein were comparable to results reported for a number of species belonging to the Gigartinales from the Senegalese coast by Aknin et al. (1990). However, EPA has

Table 4 Summary of key differences from the screening process of six taxa of Australian macroalgae for prioritising future work towards anti-inflammatory applications with a focus on PUFA content

	PUFA content	Abundance	Low n-6/n-3 ratios	LA/ALA, <1	Selective cancer cell toxicity	iNOS inflammatory response
Ecklonia radiata		Yes				Yes
Myriogloea sciurus						Yes
Hormosira banksii	High				Yes	Yes
Phyllospora comosa	Med					Yes
Solieria robusta						Yes
Ulva sp.		Yes	Yes	Yes	Yes	Yes

been reported in higher amounts in other red algae (order Gigartinales) from the Pacific coast of northern California (Khotimchenko et al. 2002). In terms of total SFA, MUFA, PUFA, the results obtained were comparable to the reported values for Rhodophyta species (Kumari et al. 2013). On the other hand, the green *Ulva* sp. was characterised by a high proportion of longer chain SFA such as C20:0; higher amounts of oleic (C18:1n-9) (cf. to C18:0) and equivalent amounts of palmitoleic acid (C16:1n-7), both dominant MUFA; and a PUFA profile rich in ALA (C18:3n-3), the highest proportion of all species, LA (C18:2n-6) and EPA (C20:5n-3), with the LA and ALA content a defining feature of Chlorophyta species (Kelly and Scheibling 2012).

The species differences in PUFA composition contributed to significantly different n-6/n-3 ratios for each of the six species investigated. With the exception of *S. robusta*, the n-6/n-3 ratios were between 0 and 4, which is consistent with previous reports for the FA profiles of diverse species of macroalgae (Schmid et al. 2014; van Ginneken et al. 2011; Colombo et al. 2006). As a desirable ratio for human health is around 1 (Meyer et al. 2003; Simopoulos 2002), this further highlights the potential health benefits available from large-scale cultivation of macroalgae such as *Ulva* sp. as a source of beneficial PUFA with n-6/n-3<1 along with a ratio of LA/ALA <1.

It is important to note that chemical composition, including FA profiles can exhibit clear seasonal and within plant variation, particularly in regards to total lipid content rather than the lipid profile (Gosch et al. 2014; Black 1954; Stengel et al. 2011). Thus, any future algal cultivation would need to consider cultivation conditions that best enhance PUFA profiles for target algae species and total lipid content, along with rigorous measures to ensure quality control.

Biological evaluation of the organic extracts generated from oven-dried biomass of all six species was also performed. All 18 extracts exhibited a degree of inhibition of production of the inflammatory mediator NO, whereby the activity followed the order: nonpolar extracts>intermediate polarity extracts>polar extracts. Nonpolar (DCM) extracts are the FA-rich fraction and also known to contain phenolic compounds as well as pigments; all of which have unique functions in inflammatory metabolism (Jaswir and Monsur 2011; Lee et al. 2013). Interestingly, n-3 PUFA has been reported as modulators of the activity of nitric oxide synthase (NOS) (Radosinska et al. 2011), one of the primary enzymes involved in NO production. MUFA derivatives have also been isolated from *Ulva lactuca* which act as activators of the cytoprotective Nrf2–ARE pathway (Wang et al. 2013), linked to suppression of inflammatory responses (Chen and Kunsch 2004). Furthermore, the brown alga *Eisenia bicyclis* (Laminariales) was evaluated for anti-inflammatory activity via inhibition against LPS-induced NO and other inflammatory mediators in RAW 264.7 cells as in this study. Similar to our findings, the activity was found to be greatest in the nonpolar DCM extract (Jung et al. 2013). In their case, the activity was attributed to fucosterol, which has also been shown to suppress production of NO and the inflammatory cytokines TNF-α and IL-6 in the brown alga *Undaria pinnatifida* (Laminariales) (Yoo et al. 2012).

Anti-inflammatory activity has been reported for a range of other algal metabolites isolated from nonpolar and DCM extracts including the chromene sargachromanol G from the Korean brown alga *Sargassum siliquastrum* (Fucales) (Yoon et al. 2012); halogenated compounds from the red alga *Laurencia snackeyi* (Vairappan et al. 2013) and the porphyrin derivatives, pheophorbide a and pheophytin a, along with the xanthophyll fucoxanthin, from the edible brown alga *S. japonica* (order Laminariales) (Islam et al. 2013). Other bioactive algal metabolites isolated from nonpolar extracts include cytotoxic, antibacterial linear sesquiterpenoids from the green alga *Penicillus capitatus* (Ulvophyceae, Bryopsidales) (Paul and Fenical 1984), antibacterial labdane diterpenoids from *Ulva fasciata* (Chakraborty et al. 2010), and antiatherosclerotic phytosterols from the brown alga *Sargassum fusiforme* (Fucules) (Chen et al. 2014).

Most extracts displayed minimal toxicity to the cell lines investigated. However, six algal extracts showed high cytotoxicity towards a human pancreatic cancer cell line, with the EtOAc extracts of *Ulva* sp. and *H. banksii* showing the greatest selectivity with no toxicity towards a normal murine

cell line. Marine algae are well known as rich sources of unique, structurally diverse, biologically active natural products with a range of reported activities including anticancer, antiviral, antibacterial, antifungal and immunomodulating activities (Gupta and Abu-Ghannam 2011; Brown et al. 2014; Murphy et al. 2014; Mohamed et al. 2012). In particular, a number of promising anticancer compounds have been isolated from algae including the anti-mitotic stypoldione from the brown alga *Stypodium zonale* and the cytotoxic dehydrothysiferol from the red alga *Laurencia viridis* (Brown et al. 2014). Thus, further investigation to identify the active component of the selectively cytotoxic extracts is needed.

In summary, six Australian macroalgal species were evaluated for their FA composition and anti-inflammatory activity. From these studies, the green seaweed *Ulva* sp. emerged with one of the highest number of different FA identified and exhibited a favourable n-6/n-3 ratio of 0.4, as well as a 2-fold higher ALA (C18:3n-3) to LA (C18:2n-6) content. *Ulva* was also the only species to contain the desirable long-chain n-3 DHA with the lowest amounts of AA. The brown seaweed *H. banksii* was also of interest in terms of its rich FA profile with the highest EPA and ETA content of all six species, the latter known to have anti-inflammatory properties (Calder 2009), the second lowest n-6/n-3 ratio of 1.5, the second best ALA to LA ratio of 1.3:1, although accompanied by the highest abundance of AA. These results have implications in product development by highlighting target species such as *Ulva* sp. and *H. banksii* with both desirable n-3 PUFA and biological activity profiles.

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References

Aknin M, Miralles J, Kornprobst J-M (1990) Sterol and fatty acid distribution in red algae from the Senegalese coast. Comp Biochem Physiol 96B:559–563

Alamsjah MA, Hirao S, Ishibashi F, Oda T, Fujita Y (2008) Algicidal activity of polyunsaturated fatty acids derived from *Ulva fasciata* and *U. pertusa* (Ulvaceae, Chlorophyta) on phytoplankton. J Appl Phycol 20(5):713–720

Bert JJ, Dauguet JC, Maume D, Bert M (1991) Fatty acids and sterols of two red algae—*Calliblepharis jubata* and *Solieria chordalis* (Gigartinales). Cryptogam Algol 12:157–162

Bixler HJ, Porse H (2011) A decade of change in the seaweed hydrocolloids industry. J Appl Phycol 23:321–335

Black WAP (1954) Concentration gradients and their significance in *Laminaria saccharina* (L.) Lamour. J Mar Biol Assoc UK 33:49–60

Brown EM, Allsopp PJ, Magee PJ, Gill CIR, Nitecki S, Strain CR, McSorley EM (2014) Seaweed and human health. Nutr Rev 72: 205–216

Burdge GC, Wootton SA (2002) Conversion of α-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. Br J Nutr 88:411–420

Calder PC (2009) Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale. Biochimie 91:791–795

Calder PC, Yaqoob P (2009) Omega-3 polyunsaturated fatty acids and human health outcomes. BioFactors 35:266–272

Chakraborty K, Lipton AP, Raj PR, Vijayan KK (2010) Antibacterial labdane diterpenoids of *Ulva fasicata* Delile from southwestern coast of the Indian Peninsula. Food Chem 119:1399–1408

Chen X-L, Kunsch C (2004) Induction of cytoprotective genes through Nrf2/antioxidant response element pathway: a new therapeutic approach for the treatment of inflammatory diseases. Curr Pharm Des 10:879–891

Chen Z, Liu J, Fu Z, Ye C, Zhang R, Song Y, Zhang Y, Li H, Ying H, Liu H (2014) 24(*S*)-saringosterol from edible marine seaweed Sargassum fusiforme is a novel selective LXRβ agonist. J Agric Food Chem 62:6130–6137

Clarke KR, Gorley RN (2006) PRIMER V6: user manual/tutorial. PRIMER-E Ltd, Plymouth

Coleman JW (2001) Nitric oxide in immunity and inflammation. Int Immunopharmacol 1:1397–1406

Colombo ML, Risé P, Giavarini F, De Angelis L, Galli C, Bolis CL (2006) Marine macroalgae as sources of polyunsaturated fatty acids. Plant Foods Hum Nutr 61:67–72

Coussens LM, Werb Z (2002) Inflammation and cancer. Nature 420:860–867

Cross RK, Wilson KT (2003) Nitric oxide in inflammatory bowel disease. Inflamm Bowel Dis 9:179–189

Ebada SS, Edrada RA, Lin W, Proksch P (2008) Methods for isolation, purification and structural elucidation of bioactive secondary metabolites from marine invertebrates. Nature Protoc 3:1820–1831

Edmonds JS, Francesconi KA (1981) Arseno sugars from brown kelp (*Ecklonia radiata*) as intermediates in cycling of arsenic in a marine ecosytem. Nature 289:602–604

FAO (2012) The state of world fisheries and aquaculture. UN Fisheries and Aquacultural Organisation, Rome

Floreto EAT, Hirata H, Ando S, Yamasaki S (1993) Fatty acid composition of *Ulva pertusa* Kjellman (Chlorophyta) and *Gracilaria incurvata* Okamura (Rhodophyta) in Japanese coastal waters. Bot Mar 36:217–222

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

Galloway AWE, Britton-Simmons KH, Duggins DO, Gabrielson PW, Brett MT (2012) Fatty acid signatures differentiate marine macrophytes at ordinal and family ranks. J Phycol 48:956–965

Glick NR, Fischer MH (2013) The role of essential fatty acids in human health. J Evid Based Complement Alternat Med 18:268–289

Gosch BJ, Paul NA, de Nys R, Magnusson M (2014) Seasonal and within-plant variation in fatty acid content and composition in the brown seaweed *Spatoglossum macrodontum* (Dictyotales, Phaeophyceae). J Appl Phycol. doi:10.1007/s10811-014-0308-4

Goyens PLL, Spilker ME, Zock PL, Katan MB, Mensink RP (2006) Conversion of α-linolenic acid in humans is influenced by the absolute amounts of α-linolenic and linoleic acid in the diet and not by their ratio. Am J Clin Nutr 84:44–53

Graeve M, Kattner G, Wiencke C, Karsten U (2002) Fatty acid composition of arctic and antarctic macroalgae: indicator of phylogenetic and trophic relationships. Mar Ecol Prog Ser 231:67–74

Grenon SM, Hughes-Fulford M, Rapp J, Conte MS (2011) Polyunsaturated fatty acids and peripheral artery disease. Vasc Med 17:51–63

Gupta S, Abu-Ghannam N (2011) Bioactive potential and possible health effects of edible brown seaweeds. Trends Food Sci Technol 22:315–326

Guschina IA, Harwood JL (2006) Lipids and lipid metabolism in eukaryotic algae. Prog Lipid Res 45:160–186

Harper CR, Jacobson TA (2001) The fats of life. Arch Int Med 161:2185–2192

Holdt SL, Kraan S (2011) Bioactive compounds in seaweed: functional food applications and legislation. J Appl Phycol 23:543–597

Islam MN, Ishita IJ, Jin SE, Choi RJ, Lee CM, Kim YS, Jung HA, Choi JS (2013) Anti-inflammatory activity of edible brown alga *Saccharina japonica* and its constituents pheophorbide a and pheophytin a in LPS-stimulated RAW 264.7 macrophage cells. Food Chem Toxicol 55:541–548

Jaswir I, Monsur HA (2011) Anti-inflammatory compounds of macroalgae origin: a review. J Med Plant Res 5:7146–7154

Jung HA, Jin SE, Ahn BR, Lee CM, Choi JS (2013) Anti-inflammatory activity of edible brown alga Eisenia bicyclis and its constituents fucosterol and phlorotannins in LPS-stimulated RAW 264.7 macrophages. Food Chem Toxicol 59:199–206

Kelly JR, Scheibling RE (2012) Fatty acids as dietary tracers in benthic food webs. Mar Ecol Prog Ser 446:1–22

Khotimchenko SV, Vaskovsky VE, Titlyanova TV (2002) Fatty acids of marine algae from the Pacific Coast of North California. Bot Mar 45: 17–22

Kumari P, Kumar M, Gupta V, Reddy CRK, Jha B (2010) Tropical marine macroalgae as potential sources of nutritionally important PUFAs. Food Chem 120:749–757

Kumari P, Bijo AJ, Mantri VA, Reddy CRK, Jha B (2013) Fatty acid profiling of tropical marine macroalgae: an analysis from chemotaxonomic and nutritional perspectives. Phytochemistry 86:44–56

Lee JC, Hou MF, Huang HW, Chang FR, Yeh CC, Tang JY, Chang HW (2013) Marine algal natural products with anti-oxidative, anti-inflammatory, and anti-cancer properties. Cancer Cell Int 13:55. doi: 10.1186/1475-2867-13-55

Lepage G, Roy CC (1986) Direct transesterification of all classes of lipids in a one-step reaction. J Lipid Res 27:114–120

Lucas I (2006) Omega-3 EPA/DHA and the major dietary deficiency in the Western Diet. Cereal Foods World 51:264–267

Mansour MP, Frampton DMF, Nichols PD, Volkman JK (2005) Lipid and fatty acid yield of nine stationary-phase microalgae: applications and unusual C24-C28 polyunsaturated fatty acids. J Appl Phycol 17: 287–300

Mazarrasa I, Olsen YS, Mayol E, Marbà N, Duarte CM (2013) Rapid growth of seaweed biotechnology provides opportunities for developing nations. Nature Biotechnol 31:591–592

McCauley J, Zivanovic A, Skropeta D (2013) Bioassays for anticancer activities. In: Roessner U, Dias DA (eds) Metabolomics tools for natural product discovery methods and protocols. Humana Press, pp 191–205

Meyer BJ, Mann NJ, Lewis JL, Milligan GC, Sinclair AJ, Howe PRC (2003) Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. Lipids 38:391–398

Mohamed S, Hashim SN, Rahman HA (2012) Seaweeds: a sustainable functional food for complementary and alternative therapy. Trends Food Sci Technol 23:83–96

Murphy C, Hotchkiss S, Worthington J, McKeown S (2014) The potential of seaweed as a source of drugs for use in cancer chemotherapy. J Appl Phycol 26:2211–2264

Nettleton JA (1995) Omega-3 fatty acids and health. Chapman & Hall, New York

Ohata T, Fukuda K, Takahashi M, Sugimura T, Wakabayashi K (1997) Suppression of nitric oxide production in lipopolysaccharide-stimulated macrophage cells by ω3 polyunsaturaed fatty acids. Jpn J Cancer Res 88:234–237

Ortiz J, Romero N, Robert P, Araya J, Lopez-Hernandez J, Bozzo C, Navarrete E, Osorio A, Rios A (2006) Dietary Fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. Food Chem 99:89–104

O'Sullivan L, Murphy B, McLoughlin P, Duggan P, Lawlor PG, Hughes H, Gardiner GE (2010) Prebiotics from marine macroalgae for human and animal health applications. Mar Drugs 8:2038–2064

Paul VJ, Fenical W (1984) Bioactive terpenoids from Caribbean marine algae of the genera *Penicillus* and *Udotea* (Chlorophyta). Tetrahedron 40:2913–2918

Phillips JA (2001) Marine macroalgae biodiversity hotspots: why is there high species richness and endemism in southern Australian marine benthic flora. Biodivers Conserv 10:1555–1577

Radosinska J, Bacova B, Bernatova I, Navarova J, Zhukovska A, Shysh A, Okruhlicova L, Tribulova N (2011) Mycocardial NOS activity and connexin-43 expression in untreated and omega-3 fatty acids-treated spontaneously hypertensive and hereditary hypertriglyceridemic rats. Mol Cell Biochem 347:163–173

Rubio-Rodríguez N, Beltrán S, Jaime I, de Diego SM, Sanz MT, Carballido JR (2010) Production of omega-3 polyunsaturated fatty acid concentrates: a review. Innov Food Sci Emerg Technol 11:1–12

Sijtsma L, de Swaaf ME (2004) Biotechnological production and applications of the w-3 polyunsaturated fatty acid docosahexaenoic acid. Appl Microbiol Biotechnol 64:146–153

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

Shanmugam K, Holmquist L, Steele M, Stuchbury G, Berbaum K, Schulz O, García OB, Castillo J, Burnell J, Rivas VG, Dobson G, Münch G (2008) Plant-derived polyphenols attenuate lipopolysaccharide-induced nitric oxide and tumour necrosis factor production in murine microglia and macrophages. Mol Nutr Food Res 52:427–438

Simopoulos AP (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 56:365–379

Smit AJ (2004) Medicinal and pharmaceutical uses of seaweed natural products: a review. J Appl Phycol 16:245–262

Stengel DB, Connan S, Popper ZA (2011) Algal chemodiversity and bioactivity: sources of natural variability and implications for commercial application. Biotechnol Adv 29:483–501

Vairappan CS, Kamada T, Lee WW, Jeon YJ (2013) Anti-inflammatory activity of halogenated secondary metabolites of *Laurencia snackeyi* (Weber-van Bosse) Masuda in LPS-stimulated RAW 264.7 macrophages. J Appl Phycol 25:1805–1813

van Ginneken VJT, Helsper JPFG, de Visser W, van Keulen H, Brandenburg WA (2011) Polyunsaturated fatty acids in various macroalgal species from north Atlantic and tropical seas. Lipids Health Dis 10:1–8

Vo T-S, Ngo D-H, Kim S-K (2012) Potential targets for anti-inflammatory and anti-allergic activities of marine algae: an overview. Inflamm Allergy Drug Targets 11:90–101

Wang R, Paul VJ, Luesch H (2013) Seaweed extracts and unsaturated fatty acid constituents from the green alga *Ulva lactuca* as activators of the cytoprotective Nrf2-ARE pathway. Free Radic Biol Med 57: 141–153

Warude D, Joshi K (2006) Polyunsaturated fatty acids: biotechnology. Crit Rev Biotechnol 26:83–93

Winberg PC, Fitton HJ, Stringer D, Karpiniec SS, Gardiner V (2014) Controlling seaweed biology, physiology and metabolic traits in production for commercially relevant bioactives in glycobiology. Adv Bot Res 71:221–252

Womersley HBS (1990) Biogeography of Australasian marine macroalgae. In: Clayton MN, King RJ (eds) Biology of marine plants. Longman Cheshire, Melbourne, pp 367–381

Yoo M-S, J-S S, Choi H-E, Bang M-H, Baek N-I, Lee K-Y (2012) Fucosterol isolated from *Undaria pinnatifida* inhibits lipopolysaccharid-induced-

production of nitric-oxide and pro-inflammatory cytokines via the inactivation of nuclear factor- B and p38 mitogen-activated protein kinase in RAW264.7 macrophages. Food Chem 135:967–975

Yoon WJ, Heo SJ, Han SC, Lee HJ, Kang GJ, Kang HK, Hyun JW, Koh YS, Yoo ES (2012) Anti-inflammatory effect of sargachromanol G isolated from *Sargassum siliquastrum* in RAW 264.7 cells. Arch Pharm Res 35:1421–1430

Zhang MJ, Spite M (2012) Resolvins: anti-inflammatory and proresolving mediators derived from omega-3 polyunsaturated fatty acids. Annu Rev Nutr 32:203–227

Zivanovic A, Skropeta D (2012) c-AMP dependent protein kinase A inhibitory activity of six algal extracts from southeastern Australia and their fatty acid composition. Nat Prod Commun 7:923–926