Lipidomic signature of the green macroalgae *Ulva rigida* farmed in a sustainable integrated multi-trophic aquaculture

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Abstract

*Ulva* species, green macroalgae, are widely distributed across the globe, being one of the most heavily traded edible seaweeds. Nonetheless, although this genus has been largely used in scientific studies, its lipidome remains rather unexplored. The present study sheds light over the lipid profile of *Ulva rigida* produced in a land-based integrated multi-trophic aquaculture (IMTA) system using liquid chromatography coupled to high-resolution mass spectrometry for molecular lipid species identification. The lipidome of *U. rigida* revealed the presence of distinct beneficial n-3 fatty acids for human health, namely alpha-linoleic acid (ALA) and docosapentaenoic acid (DPA). A total of 87 molecular species of glycolipids, 58 molecular species of betaine lipids, and 57 molecular species of phospholipids were identified in the lipidome of *U. rigida* including some species bearing PUFA and with described bioactive properties. Overall, the present study contributes to the valorization and quality validation of sustainably farmed *U. rigida*.

Keywords Chlorophyta · Edible · Lipidome · Mass spectrometry · Seaweed · *Ulva rigida*

Introduction

Edible macroalgae are a good source of beneficial compounds for human health that display distinct functional properties that stimulate interest to number of high-value chains (e.g., medical, nutraceutical, and cosmeceutical) (Holdt and Kraan 2011; Leal et al. 2013; Abreu et al. 2014; Rajauria 2015; Roohinejad et al. 2016). *Ulva* spp. have long been listed in FAO as one of the main macroalgae for commercial use (Naylor 1976). These popular green seaweeds can be used...
fresh, dried, or in liquid extracts, either for direct or processed consumption worldwide (McHugh 2003; Barriga et al. 2017). Popularly known in the human food market as sea lettuce, Ulva belongs to class Ulvophyceae and can be found in marine and brackish waters, being widely distributed across the globe. Ulva species are well adapted to aquaculture production and can be successfully cultured by using an integrated multitrophic aquaculture (IMTA) framework (Bolton et al. 2008; Msuya and Neori 2008; Marinho et al. 2013; Shpigel et al. 2017). This innovative and sustainable culture approach mimics the natural ecosystem of species from different trophic levels, associating the production of fed species (e.g., finfish) with other extractive organisms, namely marine invertebrates and/or algae, that incorporate organic and inorganic compounds resulting from the metabolism of fed species, as well as from uneaten feed. Overall, IMTA promotes a balanced production framework that is environmentally sustainable and viable from an economic point of view (Barrington et al. 2009; Chopin et al. 2012). The culture of seaweeds under an IMTA approach allows the removal of excess nutrients, namely phosphorus and nitrogen, from wastewater (Neori 2008; Lawton et al. 2013), while enhancing quality and stability of seaweeds biomass and their biochemical profile (Abreu et al. 2014).

Ulva species are consumed directly as “sea vegetables” and used as a food and feed ingredient. They are also recognized as an important source of valuable polysaccharides (such as ulvans) and oligosaccharides rich in functional groups that bind important microelements for human and animal nutrition (Lahaye and Robic 2007; Stengel et al. 2011; Berri et al. 2016; Wijesekara et al. 2017). However, to date, the lipid profile of Ulva spp. is still poorly studied at molecular level and few articles have reported their lipid characterization (Takahashi et al. 2002; Rozentsvet and Nesterov 2012; Ragonese et al. 2014), with most studies solely describing their fatty acid (FA) profile (van Ginneken et al. 2011; Ragonese et al. 2014; Kendel et al. 2015). While lipids may represent from 1 to 3% of the whole algal dry matter, they do display an important nutritional value, with emphasis into polyunsaturated fatty acids (PUFAs) from the n-3 (e.g., alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid) and n-6 (linoleic acid, gamma-linolenic acid, and arachidonic acid) (Kumari et al. 2010). As essential PUFAs are not synthesized by humans, they need to be obtained through diet to provide energy and other health benefits (e.g., reduce the risk of coronary disease and blood cholesterol) (Ginzberg et al. 2000; Simopoulos 2008; Kendel et al. 2015). Furthermore, PUFAs are also precursors of important mediators that play a key-role in inflammation and regulation of immunity (Calder 2001). These biomolecules mostly occur in their esterified form in polar lipids, namely phospholipids (PLs) and glycolipids. This feature enhances the nutritional properties of these classes of polar lipids. Additionally, glycolipids isolated from macroalgae have already been described as displaying bioactive proprieties, namely antitumoral (Ohta et al. 1998; Eitsuka et al. 2004), anti-inflammatory (Banskota et al. 2013, 2014), antimicrobial (El Baz et al. 2013; Parveez Ahamed et al. 2017), and antiviral activity (Wang et al. 2007).

The potential added value of macroalgal polar lipids has received a new momentum with the advent of mass spectrometry-based approaches which have already been employed to provide an in-depth characterization of lipidomic signatures of different macroalgae, namely Chondrus crispus (Melo et al. 2015), Codium tomentosum, Gracilaria sp., and Porphyra dioica (da Costa et al. 2015, 2017, 2018). The aim of the present study is to analyze the lipidome of Ulva rigida from a land-based IMTA system using liquid chromatography-high-resolution mass spectrometry-based approach. The data presented will contribute to promote ongoing efforts in the responsible, controlled, and sustainable production of high-value macroalgae.

**Material and methods**

**Reagents**

HPLC grade chloroform (CHCl3) and methanol (CH3OH) were from Fisher Scientific Ltd. (UK). All other reagents were purchased from major commercial sources. Milli-Q water was obtained from a water purification system (Synergy, Millipore Corporation, USA). Phospholipid internal standards 1,2-dimyristoyl-sn-glycero-3-phosphocholine (dMPC), 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (dMPE), 1,2-dimyristoyl-sn-glycero-3-phospho-(10-rac-glycerol) (dMPG), 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine (dMPS), 1,2-di-palmitoyl-sn-glycero-3-phosphatidylinositol (dPPI), N-palmitoyl-d-erythro-sphingosylphosphorylcholine (NPSM), and 1-nonadecanoyl-2-hydroxy-sn-glycero-3-phosphocholine (LPC) were from Avanti Polar Lipids, Inc. (Alabaster, AL; USA).

**Biomass**

The fresh biomass of Ulva rigida was produced by ALGAplus (production site located at Ria de Aveiro coastal lagoon, mainland Portugal, 40° 36’ 43” N, 8° 40’ 43” W) in an IMTA system, harvested in November 2016 (batch U1.46L16). The ALGAplus IMTA system is composed of fish organic certified production units (seabass and seabream) and the seaweed land-based tank system. The water flows from the fish units, to the seaweed tanks, and then to the exit channel that discharges clean water into the coastal lagoon. Seaweeds are cultivated using exclusively water input from the fish farm (nothing is added to the water). Stocking densities and water flows are manipulated in each season to achieve optimal biomass yields and/or specific biomass quality traits (i.e., chemical composition, color). After being harvested, all biological samples were cleaned to remove epiphytic foreign matters.
washed with seawater that is sequentially filtered up to 25 μm, and then sterilized by UV and Ozone treatment. The samples were then frozen at −80 °C, freeze-dried, and stored at −80 °C until lipid extraction.

**Moisture and ash determination**

Moisture was determined by drying freeze-dried samples (250 mg × five replicates) in crucibles on an oven at 105 °C for 15 h. For ash determination, the dried biomass in the crucibles was first pre-incinerated for 20 min using a heating plate and then placed in a muffle furnace at 575 °C for 6 h.

**Nitrogen determination and protein estimation**

Nitrogen content of freeze-dried samples (2 mg × five replicates) was obtained by elemental analysis on a Leco Truspec-Micro CHNMS 630-200-200 elemental analyzer at combustion furnace temperature 1075 °C and afterburner temperature 850 °C. Nitrogen was detected using thermal conductivity. The protein content was estimated from the nitrogen determination using two nitrogen-protein conversion factors, 6.25 and 5 (Angell et al. 2016).

**Total lipid extraction**

Freeze-dried samples were homogenized in a mortar and pestle to obtain small-sized flakes. A biomass of 250 mg of macroalgae was mixed with 2.5 mL of CH3OH and 1.25 mL of CHCl3 in a glass PYREX tube and homogenized by vortexing for 2 min. After incubation in ice on rocking platform shaker (Stuart equipment, Bibby Scientific, UK) for 2.5 h, the mixture was centrifuged (Selecta JP Mixtasel, Spain) for 10 min at 2000 rpm and the organic phase was collected in a new glass tube. The biomass residue was re-extracted twice with 2 mL of MeOH and 1 mL of CHCl3. To wash the lipid extract and induce phase separation, 2.3 mL of Milli-Q water was added to the final organic phase, following by centrifugation for 10 min at 2000 rpm. The organic lower phase was collected in a new glass tube, dried under nitrogen stream. Lipid extracts were then transferred to amber vials, dried again, weighed, and stored at −20 °C for 15 cm × 1 mm, 3 μm, Sigma-Aldrich) on a high-performance LC (HPLC) system (Thermo Scientific AccelaTM) with a autosampler coupled online to a Q-Exactive mass spectrometer (Thermo Scientific AccelaTM) with a autosampler coupled online to a Q-Exactive mass spectrometer with Orbitrap technology. Mobile phase A consisted of 25% water, 50% acetonitrile, and 25% methanol, with 1 mM ammonium acetate in relation to the water volume, and mobile phase B consisted of 60% acetonitrile and 40% methanol, with the same amount of ammonium acetate in mobile phase A. The solvent gradient, flow rate through column, and conditions used for acquisition of full scan LC-MS spectra and LC-MS/MS spectra in both positive and negative ion modes were the same as previously described (da Costa et al. 2015; Melo et al. 2015). Initially, 0% of mobile phase A was held
isocratically for 8 min, followed by a linear increase to 60% of mobile phase A within 7 min, and a maintenance period of 15 min, returning to the initial conditions in 10 min. A volume of 5 μL of each sample, containing 10 μg (10 μL) of lipid extract in CHCl3, 4 μL of phospholipid standards mix (dMPC—0.02 μg, dMPE—0.02 μg, NPSM—0.02 μg, LPC—0.02 μg, dPPI—0.08 μg, dMPG—0.012 μg, dMPS—0.04 μg), and 86 μL of eluent B, was introduced into the Ascentis Si column HPLC Pore column (15 cm × 1 mm, 3 μm, Sigma-Aldrich) with a flow rate of 40 μL min⁻¹ at 30 °C. The mass spectrometer with Orbitrap technology was operated in simultaneous positive and negative (electrospray voltage 3.0 kV) modes with high resolution with 70,000 and AGC target of 1 × 10⁶, capillary temperature was 250 °C, and the sheath gas flow was 15 U. In MS/MS experiments, a resolution of 17,500 and AGC target of 1 × 10⁵ was used and the cycles consisted in one full scan mass spectrum and ten data-dependent MS/MS scans were repeated continuously throughout the experiments with the dynamic exclusion of 60 s and intensity threshold of 1 × 10⁴. Normalized collision energy (CE) ranged between 25, 30, and 35 eV. Data acquisition was performed using the Xcalibur data system (V3.3, Thermo Fisher Scientific). The identification of molecular species of polar lipids was based on the assignment of the molecular ions observed in LC-MS spectra, typical retention time, mass accuracy, and LC-MS/MS spectra interpretation that allows to confirm the identity of the polar head group and the fatty acyl chains for most of the molecular species.

### Results

The total lipid content of the *U. rigida* was estimated by gravimetry of the lipid extracts. Also, samples were analyzed for the contents of moisture and ash, proteins, and carbohydrates and other compounds (estimated by difference). The mean moisture content (expressed as percentage of freeze-dried sample weight) of *U. rigida* was 6.41 ± 0.84, which was

#### Table 1 Fatty acid profile of *U. rigida* sustainably produced under IMTA conditions, expressed as relative abundance (%). Values are means of seven samples ± standard deviation (SD)

| Fatty acids | Relative abundance (%) ± SD |
|-------------|----------------------------|
| 14:0        | 1.33 ± 0.21                |
| 16:0        | 43.41 ± 0.75               |
| 16:1 (n-7)  | 1.39 ± 0.12                |
| 16:1 (n-9)  | 1.76 ± 0.16                |
| 16:4 (n-3)  | 3.76 ± 0.17                |
| 18:0        | 19.30 ± 1.64               |
| 18:1        | 8.56 ± 1.21                |
| 18:2 (n-6)  | 1.21 ± 0.10                |
| 18:3 (n-6)  | 0.29 ± 0.04                |
| 18:3 (n-3)  | 4.45 ± 0.22                |
| 18:4 (n-3)  | 8.82 ± 0.40                |
| 20:4 (n-3)  | 0.65 ± 0.06                |
| 20:5 (n-3)  | 0.84 ± 0.10                |
| 22:0        | 0.46 ± 0.08                |
| 22:5 (n-3)  | 3.76 ± 0.54                |
| Σ SFAs      | 64.50 ± 2.10               |
| Σ MUFAs     | 11.71 ± 0.78               |
| Σ PUFAs     | 23.78 ± 1.33               |
| Σ (n-3)     | 22.28 ± 1.22               |
| Σ (n-6)     | 1.50 ± 0.13                |

#### Table 2 Molecular species of SQDGs and SQMGs identified by HILIC-ESI-MS as negative [M – H]⁻ ions. Identification as sulfoglycolipids and fatty acyl composition was confirmed by the analysis of the LC-MS/MS spectra of each [M – H]⁻ ion. C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains. The most abundant species are marked in italic

| [M – H]⁻ m/z | Lipid species (C:N) | Fatty acyl chains |
|--------------|---------------------|------------------|
| 527.3        | SQMG (14:0) a        | 16:1             |
| 553.3        | SQMG (16:1)         | 18:1             |
| 555.3        | SQMG (16:0)         | 18:0             |
| 577.3        | SQMG (18:3)         | 18:3             |
| 581.3        | SQMG (18:1)         | 18:1             |
| 737.5        | SQDG (28:0)         | 14:0/14:0 and 12:0/16:0 |
| 763.5        | SQDG (30:1)         | 14:0/16:1        |
| 765.5        | SQDG (30:0)         | 14:0/16:0        |
| 785.5        | SQDG (32:4)         | 16:4/16:0 and 14:0/18:4 |
| 787.5        | SQDG (32:3)         | 14:0/18:3 and 16:3/16:0 |
| 789.5        | SQDG (32:2)         | 18:2/14:0        |
| 791.5        | SQDG (32:1)         | 16:1/16:0 and 18:1/14:0 |
| 793.5        | SQDG (32:0)         | 16:0/16:0        |
| 805.5        | SQDG (33:1)         | 17:1/16:0        |
| 807.5        | SQDG (34:7)         | 18:1/16:0 and 14:0/18:4 |
| 811.4        | SQDG (34:5)         | 20:5/14:0        |
| 813.5        | SQDG (34:4)         | 18:4/16:0        |
| 815.5        | SQDG (34:3)         | 18:3/16:0        |
| 819.5        | SQDG (34:2)         | 18:1/16:0        |
| 839.5        | SQDG (36:5)         | 20:5/16:0        |
| 841.5        | SQDG (36:4)         | 20:4/16:0 and 18:1/18:3 |
| 843.5        | SQDG (36:3)         | 20:3/16:0        |
| 845.5        | SQDG (36:2) a       | 20:1/16:0 and 18:0/18:1 |
| 847.5        | SQDG (36:1)         | 20:1/16:0 and 18:0/18:1 |
| 867.5        | SQDG (38:5)         | 22:5/16:0        |

a Molecular species identified only by retention time and mass accuracy calculation

b Molecular species identified only by retention time, mass accuracy calculation and typical product ion at m/z 225.0
considered to express the content of ash and other components as percentage of dry weight (DW). The content (%DW) of ash and lipids was 26.47 ± 0.51 and 2.53 ± 0.22, respectively. Although the factor 6.25 is the most commonly used indirect nitrogen-to-protein conversion factor, studies have shown that the protein content of seaweed is overestimated by applying factor 6.25 (Hardouin et al. 2016). Angell et al. (2016) proposed the use of a universal nitrogen-to-protein conversion factor of 5 for determination of the protein content of seaweeds. Thus, both factors were used. Using factor 6.25 for protein estimation, the protein content (%DW) was 17.75 ± 0.492, and the content of carbohydrates and other compounds (%DW) was 53.25. Considering factor 5, the protein content decreased to 14.20 ± 0.393, while the content of carbohydrates and other compounds increased to 56.80.

The fatty acids (FAs) profile of *U. rigida* revealed the presence of saturated FAs (SFAs) such as 14:0, 16:0, 18:0, and 22:0; monounsaturated FAs (MUFAs) such as 16:1 and 18:1; and PUFAs such as 16:4, 18:3, 18:4, 20:4, 20:5, and 22:5, as detailed in Table 1. The FA profile showed 16:0 and 18:0 as the most abundant with relative abundance of 43.41 and 19.30%, respectively. It is also noteworthy the abundance of the PUFAs 16:4 (n-3) (3.76%), 18:3 (n-3) (4.45%), 18:4 (n-3) (8.82%), and 22:5 (n-2) (3.76%).

Polar lipid profile evaluated by HILIC-LC-MS and HILIC-LC-MS/MS allowed the identification at molecular level of glycolipids, betaine lipids, and phospholipids in *U. rigida*. This lipidomic approach allowed the identification, in the case of glycolipids, of the acidic glycolipid sulfoquinovosyl diacylglycerol (SQDG) and it lyso form sulfoquinovosyl monoacylglycerol (SQMG), as well as the neutral glycolipid digalactosyldiacylglycerol (DGDG) and monogalactosyldiacylglycerol (MGDG). SQDGs and SQMGs were identified as negative [M − H] ions in the LC–MS spectra. Overall, 20 molecular species of SQDG and 5 molecular species of SQMG (Table 2 and Fig. 1) were identified. The most abundant SQDG was assigned as SQDG (34:1) at m/z 819.5, identified as SQDG (18:1/16:0), while the most abundant SQMG was detected at m/z 555.3 and corresponded to SQMG (16:0) (Fig. 1). Typical fragmentation of SQMG and SQDG species observed in LC-MS/MS spectra as [M − H] ions showed the product ion at m/z 225.0, corresponding to the anion of the sulfoquinovosyl polar head group that confirmed the presence of sulfoglycolipids, as seen in the LC-MS/MS spectra of SQMG at m/z 555.3 (Fig. 1b) and SQDG at m/z 819.5 (Fig. 1d). Furthermore, product ions corresponding to the neutral loss of fatty acyl chains as carboxylic acid (RCOOH) can be identified and confirm the composition of fatty acyl chains. SQMG species exhibit only one neutral loss of one fatty acid R1COOH (El Baz et al. 2013; da Costa et al. 2015; Melo et al. 2015). LC-MS/MS spectrum of SQMG (16:0) at m/z 555.3 shows the neutral loss of palmitic acid (~16:0 R1COOH, 256 Da) that leads to the formation of the product ion at m/z 299.0 (Fig. 1b). LC-MS/MS spectrum at m/z 819.5, corresponding to SQDG (18:1/16:0), shows the loss of two fatty acyl chains R1COOH and R2COOH, that correspond to the neutral loss of 18:1 RC1OOH (~282 Da) and the neutral loss of palmitic acid 16:0 R2COOH (~256 Da) with formation of the product ions at m/z 537.3 and 563.3, respectively (Fig. 1d).

![Fig. 1](image_url) LC-MS spectra in negative ion mode of SQMG (a) and SQDG (c) classes identified as [M − H] ions. LC-MS/MS spectra of the [M − H] ions of the most abundant species of SQMG at m/z 555.3 (b) and SQDG at m/z 819.5 (d).
The neutral molecular species monogalactosyldiacylglyceride (MGDG), digalactosyldiacylglyceride (DGDG), and their lyso forms, monogalactosylmonoacylglyceride (MGMG) and digalactosylmonoacylglyceride (DGMG), were identified in the positive LC-MS spectra as [M + NH₄]+ ions. Overall, 27 molecular species of MGDG, 13 of MGMG, 13 of DGDG, and 9 of DGMG were identified (Table 3 and Fig. 2). The representative LC-MS spectra of MGDG and DGDG classes are shown in Fig. 2, as well as the LC-MS/MS spectra of the most abundant species of each class. The predominant MGDG were detected at m/z 760.5. The DGDG were similarly predominant at m/z 932.6 and 936.7, representative spectrum in Fig. 2 concerns DGDG at m/z 932.6. The MGDG at m/z 760.5 corresponds to MGDG (34:8) and was identified as MGDG (16:4/18:4), while the DGDG at m/z 932.6 refers to DGDG (34:3) and was identified as DGDG (18:3/16:0). The typical fragmentation observed in the LC-MS/MS spectra of MGDG and DGDG species as [M + NH₄]+ ions allows to confirm the presence of these neutral glycolipids. LC-MS/MS spectrum of MGDG (34:8) at m/z 760.5 (Fig. 2b) indicate the product ion at m/z 563.4, assigned as [M + NH₄−197]+, that results from combined loss of NH₃ (−17 Da) and loss of a hexose (−180 Da) formed due to the cleavage of the sugar bond near the hemiacetal oxygen bond with proton transfer to render a diacylglycerol structure. Similarly, in the LC-MS/MS spectrum of DGDG (34:3) at m/z 932.6 (Fig. 2d), we can observe the loss of the carbohydrate moiety (loss of 180 + 162 Da) combined with loss of NH₃ (−17 Da), leading to

### Table 3

| [M + NH₄]+ m/z | Lipid species (C:N) | Fatty acyl chains |
|----------------|---------------------|------------------|
| 502.3          | MGMG (16:4)         | 16:4             |
| 504.3          | MGMG (16:3)         | 16:3             |
| 506.3          | MGMG (16:2)         | 16:2             |
| 508.3          | MGMG (16:1)         | 16:1             |
| 510.4          | MGMG (16:0)         |                  |
| 530.3          | MGMG (18:4)         |                  |
| 532.4          | MGMG (18:3)         |                  |
| 534.4          | MGMG (18:2)         |                  |
| 536.4          | MGMG (18:1)         |                  |
| 556.4          | MGMG (20:5)         |                  |
| 558.4          | MGMG (20:4)         |                  |
| 584.4          | MGMG (22:5)         |                  |
| 592.4          | MGMG (22:1)         |                  |
| 712.5          | MGDG (30:4)         |                  |
| 714.4          | MGDG (30:3)         |                  |
| 732.5          | MGDG (32:8)         | 16:4/16:4        |
| 734.5          | MGDG (32:7)         | 16:3/16:4        |
| 736.5          | MGDG (32:6)         | 16:2/16:4 and 16:3/16:3 |
| 738.5          | MGDG (32:5)         |                  |
| 740.5          | MGDG (32:4)         | 16:4/16:0 and 16:1/16:3 |
| 742.5          | MGDG (32:3)         | 16:3/16:0 and 18:3/14:0 |
| 748.6          | MGDG (32:0)         | 16:0/16:0        |
| 760.5          | MGDG (34:8)         | 18:4/16:4        |
| 764.5          | MGDG (34:6)         |                  |
| 766.6          | MGDG (34:5)         | 18:1/16:4        |
| 768.6          | MGDG (34:4)         | 18:4/16:0 and 18:3/16:1 |
| 770.6          | MGDG (34:3)         | 18:3/16:0 and 18:2/16:1 |
| 774.6          | MGDG (34:1)         | 18:1/16:0        |
| 786.5          | MGDG (36:9)         |                  |
| 788.5          | MGDG (36:8)         | 18:4/18:4 and 20:5/16:3 |
| 790.5          | MGDG (36:7)         | 18:4/18:3 and 20:3/16:4 |
| 800.6          | MGDG (36:2)         |                  |
| 792.5          | MGDG (36:6)         | 18:3/18:3 and 18:4/18:2 |
| 796.6          | MGDG (36:4)         |                  |
| 826.6          | MGDG (38:3)         |                  |
| 828.7          | MGDG (38:2)         |                  |
| 830.7          | MGDG (38:1)         |                  |
| 854.7          | MGDG (40:3)         |                  |
| 856.7          | MGDG (40:2)         |                  |
| 858.7          | MGDG (40:1)         |                  |
| 644.4          | DGMG (14:0)         |                  |
| 664.4          | DGMG (16:4)         |                  |
| 666.4          | DGMG (16:3)         |                  |
| 668.4          | DGMG (16:2)         |                  |
| 670.4          | DGMG (16:1)         | 16:1             |

* Molecular species identified only by retention time and mass accuracy calculation.

The neutral molecular species monogalactosyldiacylglyceride (MGDG), digalactosyldiacylglyceride (DGDG), and their lyso forms, monogalactosylmonoacylglyceride (MGMG) and digalactosylmonoacylglyceride (DGMG), were identified in the positive LC-MS spectra as [M + NH₄]+ ions. Overall, 27 molecular species of MGDG, 13 of MGMG, 13 of DGDG, and 9 of DGMG were identified (Table 3 and Fig. 2). The representative LC-MS spectra of MGDG and DGDG classes are shown in Fig. 2, as well as the LC-MS/MS spectra of the most abundant species of each class. The predominant MGDG were detected at m/z 760.5. The DGDG were similarly predominate at m/z 932.6 and 936.7, representative spectrum in Fig. 2 concerns DGDG at m/z 932.6. The MGDG at m/z 760.5 corresponds to MGDG (34:8) and was identified as MGDG (16:4/18:4), while the DGDG at m/z 932.6 refers to DGDG (34:3) and was identified as DGDG (18:3/16:0). The typical fragmentation observed in the LC-MS/MS spectra of MGDG and DGDG species as [M + NH₄]+ ions allows to confirm the presence of these neutral glycolipids. LC-MS/MS spectrum of MGDG (34:8) at m/z 760.5 (Fig. 2b) indicate the product ion at m/z 563.4, assigned as [M + NH₄−197]+, that results from combined loss of NH₃ (−17 Da) and loss of a hexose (−180 Da) formed due to the cleavage of the sugar bond near the hemiacetal oxygen bond with proton transfer to render a diacylglycerol structure. Similarly, in the LC-MS/MS spectrum of DGDG (34:3) at m/z 932.6 (Fig. 2d), we can observe the loss of the carbohydrate moiety (loss of 180 + 162 Da) combined with loss of NH₃ (−17 Da), leading to
the formation of the product ion at $m/z$ 573.5, indicated as [M + NH$_4$−359]$^+$. The fatty acyl chains composition can be inferred by the presence of product ions corresponding to each fatty acyl group as an acylium ion plus 74 (RCO + 74). These ions can be seen at $m/z$ 305.2 and 333.2 in MGDG spectrum (Fig. 2b) and correspond to 16:4 and 18:4, respectively. In the case of DGDG spectrum (Fig. 2d), the [RCO + 74]$^+$ ions can be seen at $m/z$ 313.3 and 335.3 and correspond to 16:0 and 18:3, respectively (Murphy 2015).

Betaine lipids identified in _U. rigida_ included the diacylglyceroltrimethylhomoserine (DGTS) and its lyso form monoacylglyceroltrimethylhomoserine (MGTS). The DGTS and MGTS were identified in the LC-MS spectra as positive [M + H]$^+$ ions. Overall, 40 molecular species of DGTS and 17 molecular species of MGTS were identified (Table 4 and Fig. 3). The structural features of betaine lipids were confirmed through the identification of the typical product ions and fragmentation pathways observed in the LC-MS/MS spectra. A representative LC-MS/MS spectrum of MGTS and DGTS is shown in Fig. 3b, c, corresponding to the MGTS (18:4) at $m/z$ 494.3 and DGTS (34:4), identified as DGTS (18:4/16:0) at $m/z$ 732.6. Both LC-MS/MS spectra of MGTS (Fig. 3b) and DGTS (Fig. 3d) showed the typical reported ion of this class at $m/z$ 236.1 corresponding to the combined loss of both fatty acids as keto derivatives (R$_1$CO and R$_2$CO) (Melo et al. 2015; da Costa et al. 2018). The fatty acyl composition can be deduced by the losses of fatty acyl chains as acid (-RCOOH) and ketene (-R=C=O) derivatives. The ion at $m/z$ 236.1 in LC-MS/MS spectrum of MGTS (18:4) (Fig. 3b) also represents the loss of 18:4 fatty acyl chain as keto derivative (−258 Da). In its turn, the LC-MS/MS spectrum of DGTS (18:4/16:0) (Fig. 3d) showed the ions at $m/z$ 474.4 and 494.3 corresponding to the loss of fatty acyl chains as keto derivatives (−258 and −238 Da), matching to 18:4 and 16:0 fatty acids. Moreover, the ion at $m/z$ 456.4 confirmed the presence of the fatty acid 18:4 since it corresponds to the loss of this fatty acyl chain as an acid derivative (−276 Da).

PL classes identified in _U. rigida_ included phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylyethanolamine (PE), phosphatidylcholine (PC), and their lyso forms LPG, LPI, LPE, and LPC. They were identified in negative mode [M − H]$^-$ ions. Overall, 5 molecular species of LPG, 17 of PG, 6 of PI, and 1 of LPI were recognized (Table 5). The LC-MS/MS spectra of PG (Fig. 4a) and LPG species allowed to confirm their polar head by the presence of the product ion at $m/z$ 171.0, corresponding to [C$_3$H$_7$O$_2$OPO$_3$H]$^-$.

On the other hand, the polar head of PI (Fig. 4b) and LPI is observed at $m/z$ 241.0, corresponding to an inositol-1,2-cyclic phosphate anion (C$_6$H$_{10}$O$_5$PO$_3$)$^-$. The carboxylate anions R$_1$COO$^−$ and R$_2$COO$^−$ allowed the identification of fatty acyl chains (Murphy 2015).

LPE, PE, LPC, and PC molecular species were identified in positive mode as [M + H]$^+$ ions. Overall, 7 molecular species of LPE, 3 of PE, 3 of LPC, and 15 of PC were identified (Table 6). Typical loss of 141 Da was noted in LC-MS/MS spectra of [M + H]$^+$ ions of LPE and PE, while the acyl chains
were identified in negative mode by the presence of carboxylate \( \text{RCOO}^- \) anions observed in the LC-MS/MS spectra of the respective \([M - \text{H}]^-\) ions. The LC-MS/MS spectra of \([M + \text{H}]^+\) ions of LPC and PC showed the typical product ion of the polar head at \( m/z \) 184.0, while the carboxylate \( \text{RCOO}^- \) anions that allowed the identification of fatty acyl composition were observed in the LC-MS/MS spectra of the respective \([M - \text{CH}_3\text{COO}]^-\) ions (Murphy 2015).

### Discussion

To the best knowledge of the authors, the present study represents the first in-depth characterization of lipidomic signature of the green macroalga \textit{U. rigida}. \textit{Ulva rigida} screened in the present work was produced in a land-based IMTA system, with this culture approach being considered as a sustainable and environmentally friendly approach to produce seaweeds and provide high-grade safe biomass. When compared to the harvesting of seaweeds from the wild, this production system has as main advantages the production of high biomass loads under controlled and replicable conditions, a less variable biochemical profile that allows product standardization, as well as the implementation of mandatory traceability protocols for seaweeds and seaweed-based-products targeting premium markets (Ridler et al. 2007; Chopin et al. 2012). Fatty acids profile identified was similar with that reported for the same species (Ak et al. 2014) and for other species belonging to the genus \textit{Ulva}, namely \textit{Ulva lactuca}, \textit{Ulva rotundata}, \textit{Ulva clathrata}, and \textit{Ulva intestinalis} (Fleurence et al. 1994; Peña-Rodríguez et al. 2011; van Ginneken et al. 2011; Rozentsvet and

| [M + H]$^+$ m/z | Lipid species (C:N) | Fatty acyl chains |
|------------------|---------------------|------------------|
| 446.3            | MGTS (14:0)         | 14:0             |
| 464.3            | MGTS (16:3)         | 16:3             |
| 466.3            | MGTS (16:4)         | 16:4             |
| 470.3            | MGTS (16:2)         | 16:2             |
| 472.4            | MGTS (16:1)         | 16:1             |
| 474.4            | MGTS (16:0)         | 16:0             |
| 492.3$^b$        | MGTS (18:5)         | 18:5             |
| 494.3            | MGTS (18:4)         | 18:4             |
| 496.4            | MGTS (18:3)         | 18:3             |
| 498.4            | MGTS (18:2)         | 18:2             |
| 500.4            | MGTS (18:1)         | 18:1             |
| 502.4            | MGTS (18:0)         | 18:0             |
| 520.4            | MGTS (20:5)         | 20:5             |
| 522.4            | MGTS (20:4)         | 20:4             |
| 524.4            | MGTS (20:3)         | 20:3             |
| 530.4            | MGTS (20:2)         | 20:2             |
| 536.5            | MGTS (22:5)         | 22:5             |
| 558.5            | MGTS (22:0)         | 22:0             |
| 606.5            | DGTS (28:0)         | 14:0/14:0        |
| 626.5$^a$        | DGTS (30:4)         | 14:0/14:0        |
| 682.6            | DGTS (30:1)         | 16:0/16:1        |
| 684.6            | DGTS (30:0)         | 16:0/16:0        |
| 700.6            | DGTS (32:6)         | 16:2/16:4        |
| 702.6$^b$        | DGTS (32:5)         | 16:0/16:4        |
| 704.5            | DGTS (32:4)         | 14:0/18:4        |
| 706.6            | DGTS (32:3)         | 16:1/16:2        |
| 708.6$^a$        | DGTS (32:2)         | 16:0/16:2 and 16:1/16:1 |
| 710.6            | DGTS (32:1)         | 16:0/16:1 and 14:0/18:1 |
| 712.6$^b$        | DGTS (32:0)         | 16:0/16:0        |
| 724.6            | DGTS (34:8)         | 16:4/18:4        |
| 726.6            | DGTS (34:7)         | 16:4/18:3        |
| 728.5            | DGTS (34:6)         | 16:2/18:4        |
| 730.6            | DGTS (34:5)         | 16:1/18:4 and 16:2/18:3 |
| 732.6$^a$        | DGTS (34:4)         | 16:0/18:4        |
| 734.6$^b$        | DGTS (34:3)         | 16:0/18:3        |
| 736.6$^b$        | DGTS (34:2)         | 16:0/18:2 and 16:1/18:1 |
| 738.6            | DGTS (34:1)         | 16:0/18:1        |
| 746.6            | DGTS (35:4)         | 17:0/18:4        |
| 750.6            | DGTS (36:9)         | 18:4/18:5        |
| 752.5$^b$        | DGTS (36:8)         | 18:4/18:4        |
| 754.6$^b$        | DGTS (36:7)         | 18:3/18:4        |
| 756.6$^b$        | DGTS (36:6)         | 18:3/18:3 and 18:2/18:4 |
| 758.6$^b$        | DGTS (36:5)         | 18:1/18:4        |
| 760.6$^b$        | DGTS (36:4)         | 18:1/18:2        |
| 762.6            | DGTS (36:3)         | 18:1/18:2        |
| 764.6            | DGTS (36:2)         | 18:1/18:1        |
| 776.6            | DGTS (38:10)$^a$    | 20:5/18:4        |
| 778.6            | DGTS (38:9)         | 20:4/18:4        |
| 780.6$^b$        | DGTS (38:8)         | 20:4/18:3 and 20:3/18:4 |
| 784.6            | DGTS (38:6)         | 20:2/18:4 and 16:1/22:5 |
| 808.6$^b$        | DGTS (40:8)         | 22:5/18:3        |
| 812.6            | DGTS (40:6)         | 22:5/18:1        |
| 816.7            | DGTS (40:4)         | 22:0/18:4        |
| 830.6$^b$        | DGTS (42:11)$^a$    | 22:5/20:5        |
| 832.6            | DGTS (42:10)        | 22:5/20:5        |
| 860.6            | DGTS (44:10)        | 22:5/20:5        |

$^a$ Molecular species identified only by retention time and mass accuracy calculation

$^b$ Ion with contribution of sodium adduct \([M + \text{Na}]^+\) of DGTS observed as \([M + \text{H}]^+\) with mass difference of 22 Da

were identified in negative mode by the presence of carboxylate \(\text{RCOO}^-\) anions observed in the LC-MS/MS spectra of the respective \([M - \text{H}]^-\) ions. The LC-MS/MS spectra of \([M + \text{H}]^+\) ions of LPC and PC showed the typical product ion of the polar head at \( m/z \) 184.0, while the carboxylate \(\text{RCOO}^-\) anions that allowed the identification of fatty acyl composition were observed in the LC-MS/MS spectra of the respective \([M - \text{CH}_3\text{COO}]^-\) ions (Murphy 2015).
As the PUFAs reported in the present study are essential FAs for humans, *U. rigida* can be an affordable dietary source of these FAs (Li et al. 2009; Cottin et al. 2011). There are several studies that defend an ideal n-6/n-3 ratio. While n-3 PUFAs exhibit anti-inflammatory and antioxidant activity, improve the cardiac system, and prevent breast cancer (Mozaffarian et al. 2005; Siriwardhana et al. 2012; Fabian et al. 2015), n-6 PUFAs tend to promote tumor growth and inflammatory processes (Patterson et al. 2011). One of the important dietary factors in the obesity prevention is a balanced n-6/n-3 ratio of 1−2/1 (Simopoulos 2016). Therefore, the consumption of n-6 FAs should be lower than n-3, in order to avoid several diseases including depressive disorder (Okuyama et al. 1997; Husted and Bouzinova 2016). In addition, lower n-6/n-3 ratio was associated with decreased risk of breast cancer in women (Simopoulos 2008). In this context, *U. rigida* had a relative abundance of n-6 and n-3 PUFAs of 1.51 and 21.77%, respectively. Therefore, its n-6/n-3 ratio is lower than 1, highlighting the potential health-promoting properties of this macroalgae for human consumption. Although n-6/n-3 ratios are known to vary between species and growth condition, to the authors best knowledge, *U. rigida* farmed using a sustainable land-based IMTA approach described in the present study displayed the lowest n-6/n-3 ratio report so far for *Ulva* spp. (van Ginneken et al. 2011; Kendel et al. 2015). This finding confirms the added value of algal biomass originating from land-based IMTA, as a higher contents in n-3 fatty acids are commonly associated with health-promoting benefits for consumers (Simopoulos 2002).

Identified FAs are esterified into lipid molecules such as glycolipids, betaine lipids, and phospholipids (PLs). The glycolipids detected include sulfolipids and galactolipids which together represented the most abundant structural compounds of chloroplast membranes (Hölzl and Dörmann 2007) with up to 87 molecular species being identified in *U. rigida*.

There are several studies that demonstrated glycolipids bioactivity from different algae species, such as antiviral, antibacterial, and antitumoral activity (Plouguerné et al. 2014; Blunt et al. 2016). Wang et al. (2007) described the antiviral activity attributed to SQDG (32:0) from the green macroalgae *Caulerpa racemosa*. Furthermore, El Baz et al. (2013) analyzed the SQMG (16:0) as antitumor and antimicrobial activity. Other authors demonstrated the inhibitory effect of SQDG and DGDG from the brown macroalgae *Sargassum horneri* suggesting the use of these compounds like chemotherapy agents (Hossain et al. 2005). It is also reported that seaweeds with an abundant presence of PUFAs in their composition proved to display anti-inflammatory activity by inhibiting nitric oxide release by macrophages (Banskota et al. 2013; Lopes et al. 2014). Betaine lipids (DGTS and MGTS) represent a group of polar lipids low studied to date and few studies have characterized their profile in seaweeds (da Costa et al. 2015, 2017; Mello et al. 2015). Some species of DGTS identified in *U. rigida* have already been reported in green microalgae like *Chlamydomonas reinhardtii* and chlorarachniophytes (Vieler et al. 2007; Roche and Leblond 2010). It has been suggested that DGTS has the same function...
as PC due to their similar zwitterionic structure. Moreover, they are interchangeable with each other in their roles within the cell (Riekhof et al. 2005). Organisms that contain a high level of DGTS display either an absence of PC or its presence is very low (Dembitsky and Rezanka 1995; Kunzler and Eichenberger 1997). Furthermore, van Ginneken et al. (2017) revealed that *Ulva* sp. uses a mechanism rarely reported in eukaryotes, as it applies the biochemical pathway to produce DGTS that can replace PC in seaweed cell wall (Klug and Benning 2001). It was suggested that the high DGTS/PC ratio occurs commonly in species of the genus *Ulva*.

Regarding PLs, their beneficial effects have been studied since the early 1900s (Küllenberg de Gaudry et al. 2012). The positive effect of PLs is supported by several studies that showed an improvement of the pharmacokinetics of some drugs when associated with PLs compounds, and a reduction of side effects of some drugs when administered together, namely indomethacin (NSAID) (Dial et al. 2006; Lichtenberger et al. 2009). Their cytoprotective effects and anti-fibrogenic potential have already been highlighted (Gundermann et al. 2011). Moreover, PLs from marine organisms have shown a remarkable effect in the regulation of the blood lipid profile in patients suffering from hyperlipidemia (Bunea et al. 2004). PLs beneficial dietary effect is the result of their interaction with cellular membranes influencing a vast number of signaling processes and also the effect of their fatty acid composition. The great advantage of these molecules is related with the ability of their esterified ω-3 FAs to compensate ω-3 FA deficiency in a more efficient way than other ω-3 FA supplements (e.g., as triacylglycerides or as free FAs). Thus, PLs from foodstuff are major supplies of ω-3 PUFAs for living systems (Jannace et al. 1992). Furthermore, the antioxidant potential of PG found in *U. rigida* could be explored (Banskota et al. 2014).

Traditionally, the study of algal lipids has targeted fatty acids analysis through GC-MS or GC-FID (Marshall et al. 2002). However, the overall information acquired through these techniques is limited and solely refers to fatty acids, which in living systems are mostly linked to polar lipids. In the last decade, with the advent of mass spectrometry, the commercialization of new devices with higher sensitivity,

Table 5 Molecular species of LPG, PG, LPI, PI identified by HILIC-ESI-MS as negative [M − H]− ions. Identification of different PL classes and fatty acyl composition was confirmed by the analysis of the LC-MS/MS spectra of each [M − H]− ion. C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains. The most abundant species are marked in italic

| [M − H]− | m/z  | Lipid species (C:N) | Fatty acyl chains |
|---------|-----|---------------------|------------------|
| 481.3   | LPG (16:1) | 16:1                |
| 483.3   | LPG (16:0) | 16:0                |
| 505.3   | LPG (18:3)* |                    |
| 507.3   | LPG (18:2)* |                    |
| 509.3   | LPG (18:1) | 18:1                |
| 691.5   | PG (30:1) | 14:0/16:1           |
| 693.5   | PG (30:0) | 14:0/16:0           |
| 711.5   | PG (32:5) | 16:1/16:4           |
| 713.5   | PG (32:4) | 16:0/16:4 and 16:1/16:3 |
| 717.5   | PG (32:2) | 16:1/16:1           |
| 719.5   | PG (32:1) | 16:1/16:0 and 14:0/18:1 |
| 739.5   | PG (34:5) | 16:1/18:4           |
| 741.5   | PG (34:4) | 16:1/18:3           |
| 743.5   | PG (34:3) | 18:3/16:0 and 16:1/18:2 |
| 745.4   | PG (34:2) | 16:1/18:1 and 18:2/16:0 |
| 747.5   | PG (34:1) | 18:1/16:0 and 16:1/18:0 |
| 749.5   | PG (34:0) | 18:0/16:0           |
| 765.5   | PG (36:6) | 16:1/20:5           |
| 767.5   | PG (36:5) | 20:5/16:0 and 18:1/18:4 |
| 769.5   | PG (36:4) | 18:1/18:3 and 18:2/18:2 |
| 771.5   | PG (36:3) | 18:1/18:2           |
| 773.5   | PG (36:2) | 18:1/18:1           |
| 571.3   | LPI (16:0) | 16:0                |
| 781.5   | PI (30:0) | 14:0/16:0           |
| 829.5   | PI (34:4) | 16:0/18:4           |
| 831.5   | PI (34:3) | 16:0/18:3           |
| 833.5   | PI (34:2) | 16:0/18:2           |
| 835.5   | PI (34:1) | 16:0/18:1           |
| 873.5   | PI (38:10) |                   |

*Molecular species identified only by retention time and mass accuracy calculation*

![Fig. 4](image-url) LC-MS/MS spectrum in negative mode of PG (34:4) specie at m/z 741.5 (a) and PI (34:3) specie at m/z 831.5 (b) identified as [M − H]− ions
resolution, and sample screening speed, such as Orbitrap ant Q-TOF instruments, allowed to gain a more in-depth knowledge of lipids. The used of liquid chromatography (LC) online with mass spectrometry is nowadays an advanced and promising approach to study lipids in living systems. The LC-MS platforms allows to identify and quantify molecular structural details in one single run over very short periods of time (Maciel et al. 2016). In 1 LC-MS run, more than 200 lipid species from different lipid classes are routinely identified and quantified. Lipid species identification is based on the ions in MS and, in the case of high-resolution MS, through confirmation of mass accuracy. The structural details are confirmed by MS/MS data of each molecular species, namely through the analysis of typical ion fragments. In recent years, this lipidomic approach has been successfully used to unravel the lipidome of seaweeds (da Costa et al. 2015, 2017, 2018; Melo et al. 2015) and has become a powerful tool to screen for high value lipid species with potential biotechnological applications.

### Table 6

| [M + H]⁺ m/z | Lipid species (C:N) | Fatty acyl chains |
|--------------|---------------------|------------------|
| 496.3        | LPC (16:0)ᵃ         |                  |
| 542.3        | LPC (20:5)ᵇ         |                  |
| 568.3        | LPC (22:6)ᵇ         |                  |
| 706.5        | PC (30:0)ᵇ          |                  |
| 728.5        | PC (32:3)ᵇ          |                  |
| 730.5        | PC (32:2) 16:1/16:1 |
| 730.6        | PC (34:4)ᵇ          |                  |
| 756.6        | PC (34:3)ᵇ          |                  |
| 758.6        | PC (34:2) 16:1/18:1 |
| 760.6        | PC (34:1) 16:0/18:1 |
| 780.6        | PC (36:5)ᵇ          |                  |
| 784.6        | PC (36:3) 18:1/18:2 |
| 786.6        | PC (36:2)ᵇ          |                  |
| 804.6        | PC (38:7)ᵇ          |                  |
| 806.6        | PC (38:6)ᵇ          |                  |
| 808.6        | PC (38:5)ᵇ          |                  |
| 828.6        | PC (40:9)ᵇ          |                  |
| 830.6        | PC (40:8)ᵇ          |                  |
| 452.3        | LPE (16:1)ᵇ         |                  |
| 454.3        | LPE (16:0)          | 16:0             |
| 478.3        | LPE (18:2)          | 18:2             |
| 480.3        | LPE (18:1)ᵇ         |                  |
| 500.3        | LPE (20:5)          | 20:5             |
| 502.3        | LPE (20:4)          | 20:4             |
| 528.3        | LPE (22:5)          | 22:5             |
| 688.5        | PE (32:2)ᵇ          |                  |
| 690.5        | PE (32:1)ᵇ          |                  |
| 716.5        | PE (34:2) 16:1/18:1 |
|              |                     | 16:0/18:2        |

ᵃ Molecular species identified only by retention time and mass accuracy calculation
ᵇ Molecular species of PC identified by retention time, mass accuracy calculation, and typical product ion observed at m/z 184 in the LC-MS/MS spectrum of [M + H]⁺ ion
ᶜ Molecular species of PE identified by retention time, mass accuracy calculation, and typical neutral loss of 141 in the LC-MS/MS spectrum of [M + H]⁺ ion

### Conclusion

The mass spectrometry-based approach employed in the present study allowed the identification of 202 molecular species of polar lipids shared between glycolipids, betaine lipids, and phospholipids, most of them confirmed by their fatty acids composition. The knowledge of lipid composition of *U. rigida* from a sustainable land-based IMTA system comes to inspire future studies of valorization of this seaweed, as its aquaculture production under controlled conditions will continue to increase as it offers consumers a safer and more standardized product, from an organoleptically (industry communication) and biochemical point of view. Moreover, the present study may also serve to stimulate the consumption of *U. rigida* produced under controlled conditions, as its lipidome displays a number of molecular species with beneficial bioactive properties that may also foster new biotechnological applications.

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References

Abreu M, Pereira R, Sassi J-F (2014) Marine algae and the global food industry. In: Pereira L, Neto J (eds) Marine algae: biodiversity, taxonomy, assessment and biotechnology. CRC Press, Boca Raton, pp 300–319

Ak I, Örtaşkent C, Özüdoğru Y, Göksan T (2014) Effect of sodium acetate and sodium nitrate on biochemical composition of green algae Ulva rigida. Aquac Int 23:1–12

Angell AR, Mata L, de Nys R, Paul NA (2016) The protein content of green macroalgae: a universal nitrogen-to-protein conversion factor of five. J Appl Phycol 28:511–524

Banskota AH, Stefanova R, Sparker S, Melanson R, Osborne JA, O’Leary SJ (2013) Five new galactolipids from the freshwater macroalga Porphyridium aerugineum and their nitric oxide inhibitory activity. J Appl Phycol 25:951–960

Banskota AH, Stefanova R, Sparker S, Lall SP, Craigie JS, Hafting J, Critchley AT (2014) Polar lipids from the marine macroalga Palmaria palmata inhibit lipopolysaccharide-induced nitric oxide production in RAW264.7 macrophage cells. Phytochemistry 101:101–108

Barriga LGC, Ruvalcaba FS, Carmona GH, Briones ER, Herrera RMH (2017) Effect of seaweed liquid extracts from Ulva lactuca on seedling growth of mung bean (Vigna radiata). J Appl Phycol 29:2479–2488

Barrington K, Chopin T, Robinson S (2009) Integrated multi-trophic aquaculture (IMTA) in marine temperate waters. Integrated Marine Culture - A Global Review - FAO Fish Aquac Tech Pap No 46

Berri M, Slugocki C, Olivier M, Helioin E, Jacques I, Salmon H, Demais H, Le Goff M, Collen PN (2016) Marine-sulfated polysaccharides extract of Ulva armoricana green algae exhibits an antimicrobial activity and stimulates cytokine expression by intestinal epithelial cells. J Appl Phycol 28:2999–3008

Blunt JW, Copp BR, Keyzers RA, Munro MHG, Prinsen MR (2016) Marine natural products. Nat Prod Rep 33:382–431

Bolton J, Robertson-Andersson D, Shulukha D, Kandjengo L (2008) Growing Ulva (Chlorophyta) in integrated systems as a commercial crop for abalone feed in South Africa: a SWOT analysis. J Appl Phycol 20:575–583

Bunea R, El Farrah K, Deutsch L (2004) Evaluation of the effects of Neptune krill oil on the clinical course of hyperlipidemia. Altern Med Rev 9:420–428

Calder PC (2001) Polysaturated fatty acids, inflammation, and immunity. Lipids 36:1007–1024

Chopin T, Cooper JA, Reid G, Cross S, Moore C (2012) Open-water integrated multi-trophic aquaculture: environmental bio-mitigation and economic diversification of fed aquaculture by extractive aquaculture. Rev Aquac 4:209–220

Cottin SC, Sanders TA, Hall WL (2011) The differential effects of EPA and DHA on cardiovascular risk factors. Proc Natl Soc 70:215–231

da Costa E, Melo T, Moreira ASP, Alves E, Domingues P, Calado R, Abreu MH, Domingues MR (2015) Decoding bioactive polar lipid profile of the macroalga Codium tomentosum from a sustainable IMTA system using a lipidomic approach. Algal Res 12:388–397

da Costa E, Melo T, Moreira ASP, Bernardo C, Helguero L, Ferreira I, Cruz MT, Rego AM, Domingues P, Calado R, Abreu MH, Domingues MR (2017) Valorization of lipids from Gracilaria sp. through lipidomics and decoding of antiproliferative and anti-inflammatory activity. Mar Drugs 15:1–17

da Costa E, Azevedo V, Melo T, Rego AM, Evtuguin DV, Domingues P, Calado R, Pereira R, Abreu MH, Domingues MR (2018) High-resolution lipoproteins of the early life stages of the red seaweed Porphyra dioica. Molecules 23:1–20

Dembitsky VM, Rezanka T (1995) Distribution of acetylenic acids and polar lipids in some aquatic bryophytes. Phytochemistry 40:93–97

Dial EJ, Doyen JR, Lichtenberger LM (2006) Phosphatidylcholine-associated nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit DNA synthesis and the growth of colon cancer cells in vitro. Cancer Chemother Pharmacol 57:295–300

Eitsuka T, Nakagawa K, Igarashi M, Miyazawa T (2004) Telomerase inhibition by sulfoquinovosyldiacylglycerol from edible purple laver (Porphyra yezoensis). Cancer Lett 212:15–20

El Baz FK, El Baroty GS, Abd El Baky HH, Abd El Salam OI, Ibrahim EA (2013) Structural characterization and biological activity of sulfolipids from selected marine algae. Grassas Aceites 64:561–571

Fabian CJ, Klimler BF, Hurstding SD (2015) Omega-3 fatty acids for breast cancer prevention and survivorship. Breast Cancer Res 17:1–11

Florence J, Gubier G, Mabeau S, Leray C (1994) Fatty acids from 11 marine macroalgae of the French Brittany coast. J Appl Phycol 6:527–532

Ginzberg A, Cohen M, Sod-Moriah UA, Shany S, Rosenshtrauch A, Arad SM (2000) Chickens fed with biomass of the red microalga Porphyridium sp. have reduced blood cholesterol level and modified fatty acid composition in egg yolk. J Appl Phycol 12:325–330

Gundermann KJ, Kunter E, Drozdzik M (2011) Activity of essential phospholipids (EPL) from soybean in liver diseases. Pharmacol Reports 63:634–659

Hardouin K, Bedoux G, Burlot AS, Donnay-Moreno C, Bergé JP, Neyvall-Collen P, Bourougougn N (2016) Enzyme-assisted extraction (EAE) for the production of antiviral and antioxidant extracts from the green seaweed Ulva armoricana (Ulvales, Ulvophyceae). Algal Res 16:233–239

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

Hölzl G, Dörmann P (2007) Structure and function of glycoconjugates in plants and bacteria. Prog Lipid Res 46:225–243

Hossain Z, Kurhiara H, Hosokawa M, Takahashi K (2005) Growth induction and induction of differentiation and apoptosis mediated by sodium butyrate in Caco-2 cells with algal glycolipids. Vit Cell Dev Biol 41:154–159

Husted KS, Bouzinov EV (2016) The importance of n-6/n-3 fatty acids ratio in the major depressive disorder. Med 52:139–147

Jannace PW, Lerman RH, Santos JJ, Vitale JJ (1992) Effects of oral soy phosphatidylcholine on phagocytosis, arachidonate concentrations, and killing by human polymorphonuclear leukocytes. Am J Clin Nutr 56:599–603

Kendel M, Wielgosz-collin G, Bertrand S, Roussakis C, Bourougougn N, Bedoux G (2015) Lipid composition, fatty acids and sterols in the seaweeds Ulva armoricana, and Solieria chordalis from Brittany (France): an analysis from nutritional, chemotaxonomic, and anti-proliferative activity perspectives. Mar Drugs 13:5606–5628

Klug RM, Benning C (2001) Two enzymes of diacylglycerol-O-4′-(N,N,-trimethyl)homoserine biosynthesis are encoded by btaA and btaB in the purple bacterium Rhodobacter sphaeroides. Proc Natl Acad Sci U S A 98:5910–5915

Kü llenberg de Gaudry D, Taylor LA, Schneider M, Massing U (2012) Health effects of dietary phospholipids. Lipids Health Dis 11:1–16

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

Kunzler K, Eichenberger W (1997) Betaine lipids and zwitterionic phospholipids in plants and fungi. Phytochemistry 46:883–892

Kulhary M, Robic A (2007) Structure and function properties of ulvan, a polysaccharide from green seaweeds. Biomacromolecules 8:1767–1774

Lawton RJ, Mata L, de Nys R, Paul NA (2013) Algal biomitigation of waste waters from land-based aquaculture using Ulva. J Appl Phycol 25:697–708

Li MH, Robinson EH, Tucker CS, Manning BB, Khoo L (2009) Effects of dried algae Schizochytrium sp., a rich source of docosahexaenoic acid.
acid, on growth, fatty acid composition, and sensory quality of channel catfish *Ictalurus punctatus*. Aquaculture 292:232–236

Lichtenberger LM, Romero JJ, Dial EJ (2009) Gastrointestinal safety and therapeutic efficacy of parenterally administered phosphatidylcholine-associated indomethacin in rodent model systems. Br J Pharmacol 157:252–257

Lopes G, Daletos G, Proksch P, Andrade PB, Valentão P (2014) Anti-inflammatory potential of monogalactosyldiacylglycerols and a monogalcylycerol from the edible brown seaweed *Fucus spiralis* Linnaeus. Mar Drugs 12:1406–1418

Maciel E, Leal MC, Lillebo AI, Domingues MR, Calado R (2016) Bioprospecting of marine macrophytes using MS-based lipidomics as a new approach. Mar Drugs 14:1–28

Marinho G, Nunes C, Sousa Pinto L, Pereira R, Rema P, Valente L (2013) The IMTA-cultivated Chlorophylla *Ulva* spp. as a sustainable ingredient in Nile tilapia (*Oreochromis niloticus*) diets. J Appl Physiol 25:1359–1367

Marshall JA, Nichols PD, Hallengaef G (2002) Chemotaxonomic survey of sterols and fatty acids in six marine raphidophyte algae. J Appl Physiol 14:255–265

McHugh DJ (2003) A guide to the seaweed industry. FAO Fisheries Technical Paper. Rome, p 105

Melo T, Alves E, Azevedo V, Martins AS, Neves B, Domingues P, Calado R, Abreu H, Domingues MR (2015) Lipidomics as a new approach for the bioprospecting of marine macroalgae—unraveling the polar lipid and fatty acid composition of *Chondrus crispus*. Algal Res 8:181–191

Mozaffarian D, Ascherio A, Hu FB, Stampfer MJ, Willett WC, Siscovick DS, Rimm EB (2005) Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. Circulation 111:157–164

Msaya FE, Neori A (2008) Effect of water aeration and nutrient load level on biomass yield, U, uptake and protein content of the seaweed *Ulva lactuca* cultured in seawater tanks. J Appl Physiol 20:1021–1031

Murphy RC (2015) Tandem mass spectrometry of lipids. The Royal Society of Chemistry, Cambridge

Naylor J (1976) Production, trade and utilization of seaweeds and seaweed products. FAO Fisheries Technical Paper 1–73

Neori A (2008) Essential role of seaweed cultivation in integrated multi-trophic aquaculture farms for global expansion of mariculture: an analysis. J Appl Physiol 20:567–570

Ohita K, Mizushina Y, Hirata N, Takemura M, Sugawara F, Matsukage A, Yoshida S, Sakaguchi K (1998) Sulfoquinovosyldiacylglycerol, KM043, as a novel potent inhibitor of eukaryotic DNA polymerases and HIV-reverse transcriptase type 1 from a marine red alga, *Gigartina tenella*. Chem Pharm Bull (Tokyo) 46:684–686

Okuyama H, Kobayashi T, Watanabe S (1997) Carcinogenesis and metastasis are affected by dietary n-6/n-3 fatty acids. In: Ohigashi H, Osawa T, Terao J, Watanabe S, Yoshikawa T (eds) Food factors for cancer prevention. Springer, Tokyo, pp 509–512

Parveez Ahamed AA, Rashid UM, Peer Muhamed Noonani K, Reehana N, Santoshkumar S, Mohamed Imran YM, Alharbi SN, Arunachalam C, Alharbi AS, Akbarsha MA, Thajuddin N (2012) In vitro antibacterial activity of MGDG-palmitoyl from the diatom *Cyclotella meneghiniana* NTAPC05 against extended-spectrum β-lactamase producing. J Antibiot (Tokyo) 70:754–762

Patterson RE, Flatt SW, Newman VA, Natarajan L, Rock CL, Thomson CA, Caan BJ, Parker BA, Pierce JP (2011) Marine fatty acid intake is associated with breast cancer prognosis. J Nutr 141:201–206

Péna-rodriguez A, Mawhinney TP, Ricque-marie D, Cruz-suárez LE (2011) Chemical composition of cultivated seaweed *Ulva lactuca* (Roth) C. Agardh. Food Chem 129:491–498

Plouguerné E, da Gama BAP, Pereira RC, Barreto-Bergter E (2014) Glycolipids from seaweeds and their potential biotechnological applications. Front Cell Infect Microbiol 4:1–5

Ragonese C, Tedone L, Beccaria M, Torre G, Cicchello F, Cacciola F, Dugo P, Mondello L (2014) Characterisation of lipid fraction of marine macroalgae by means of chromatography techniques coupled to mass spectrometry. Food Chem 145:932–940

Rajauria G (2015) Seaweeds: a sustainable feed source for livestock and aquaculture. In: seaweed sustainability: food and non-food applications. Elsevier Inc., University College Dublin, Lyons Research Farm, Newcastle, Co. Dublin, Ireland, pp 389–420

Ridler N, Wowchuk M, Robinson B, Barrington K, Chopin T, Robinson S, Page F, Reid G, Szemereda M, Sewuster J, Boyne-Travis S (2007) Integrated multi-trophic aquaculture (IMTA): a potential strategic choice for farmers. Aquac Econ Manag 11:99–110

Riekhof WR, Andre C, Benning C (2005) Two enzymes, BtaA and BtaB, are sufficient for betaine lipid biosynthesis in bacteria. Arch Biochem Biophys 441:96–105

Roche SA, Leblond JD (2010) Betaine lipids in chlorarachniophytes. Phycol Res 58:298–305

Roohinejad S, Koubaa M, Barba FJ, Saljoughian S, Amid M, Greiner R (2016) Application of seaweeds to develop new food products with enhanced shelf-life, quality and health-related beneficial properties. Food Res Int 99:1066–1083

Rozenstvet OA, Nesterov VN (2012) Lipids and fatty acids from *Ulva lactuca* from an integrated multi-trophic aquaculture (IMTA) biofilter system as a protein supplement in gilthead seabream (*Sparus aurata*) diet. Aquaculture 481:112–118

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

Simopoulos AP (2008) The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med 233:674–688

Simopoulos AP (2016) An increase in the Omega-6/Omega-3 fatty acid ratio increases the risk for obesity. Nutrients 8:1–17

Siriwardhana N, Kalupahana NS, Moustaid-Moussa N (2012) Health benefits of n-3 polysaturated fatty acids. Eicosapentaenoic acid and docosahexaenoic acid. Adv Food Nutr Res 65:211–222

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

Takahashi Y, Ishi K, Iishi M, Itabashi Y (2002) Induction of larval settlement and metamorphosis of the sea urchin *Strongylocentrotus intermedius* by glycolylglycerolipids from the green alga *Ulvella lens*. Mar Biol 140:763–771

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

van Ginneken V, Gittenberger A, Rensing M, de Vries E, Peeters ETHM, Verheij E (2017) Seaweed competition: *Ulva* sp. has the potential to produce the betaine lipid diacylglyceryl-O-4-(N,N,N-trimethyl) homoserine (DGTS) in order to replace phosphatidylcholine (PC) under phosphate-limiting conditions in the P-limited Dutch Wadden Sea and outcompete an aggressive non-indigenous *Gracilaria vermiculophylla* red drift algae out of this unique UNESCO world heritage coastal area. Oceanogr Fish 2:55596

Vieler A, Wilhelm C, Goss R, StÜ R, Schiller J (2007) The lipid composition of the unicellular green alga *Chlamydomonas reinhardtii* and the diatom *Cyclotella meneghiniana* investigated by MALDI-TOF MS and TLC. Chem Phys Lipids 150:143–155

Wang H, Li YL, Shen WZ, Rui W, Ma XI, Cen YZ (2007) Antiviral activity of a sulfoquinovosyldiacylglycerol (SQDG) compound isolated from the green alga *Caulerpa racemosa*. Bot Mar 50:185–190

Wijesekara I, Lang M, Marté C, Gemin M-P, Boullé R, Douzenel P, Wickramasinghe I, Bedoux G, Bourgougnon N (2017) Different extraction procedures and analysis of protein from *Ulva* sp. in Brittany, France. J Appl Physiol 29:2503–2511

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