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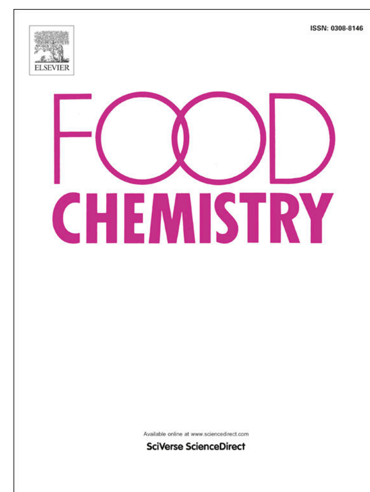

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Halophyte plants from sustainable marine aquaponics are a valuable source of omega-3 polar lipids

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Abstract

Marine aquaponics is a promising sustainable approach for the production of profitable crops such as halophytes. However, the effect of this culture approach on the lipid composition of halophytes remains unknown. In this work, we contrasted the polar lipidome of *Salicornia ramosissima* and *Halimione portulacoides* when produced in marine aquaponics (effluent from a super-intensive flatfish aquaculture production), with that of conspecifics from donor wild populations. Phospholipids and glycolipids were identified and quantified by LC-MS and their profile statistically analysed. Halophytes produced in aquaponics have higher levels of glycolipids with *n*-3 fatty acids (DGDG 36:3; SQDG 36:3; MGDG 36:6) compared with the donor wild populations, . In the case of *H. portulacoides,* a significant increase of phospholipids bearing *n*-3 fatty acids (most in PC and PE) was also recorded. These lipids have potential applications in food, feed and pharmaceutical industries, contributing to the valorization of halophytes produced under sustainable aquaculture practices.

Keywords: *Salicornia ramosissima* (Chenopodiaceae); *Halimione portulacoides* (Amaranthaceae); Lipidomics; Glycolipids; Phospholipids; omega-3 fatty acids; Marine aquaponics; Integrated multi-trophic aquaculture

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1. Introduction

The growing population worldwide demands the development of sustainable food production systems with minimal environmental impact and in line with the United Nations 2030 Agenda for Sustainable Development, "*a plan of action for people, planet and prosperity*". To face this huge challenge, new production approaches are mandatory. Aquaponic systems are emerging as promising sustainable approaches for the combined production of two profitable crops, fish and plants (König et al., 2018).

Halophyte species are vegetable crops that can cope with variable concentrations of salinity and thrive in environments that are commonly unsuitable for conventional crops (Brown et al., 1999; Marques et al., 2017), such as the case of marine aquaponics. These plants are attractive sources of *n*-3 polyunsaturated fatty acids (PUFA) and are even richer in these valuable nutrients when compared with other conventional crops commonly used for human consumption (Y Ventura & Sagi, 2013). These properties, allied to their salty taste when consumed fresh (e.g., in salads) lead to the growing interest in the use of these new ingredients in gourmet cuisine and the creation of new markets for the commercialization of these "sea veggies".

Commercial halophytes can be collected from wild populations (salt marshes habitats) but can also be farmed outdoors or in greenhouses, as a suitable and environmental-friendly alternative to their harvesting from natural salt marshes. It is well established that the grow-out environment and exposure to different abiotic factors can promote major shifts in plant metabolism and, consequently, in their chemical composition (Sampaio, Edrada-Ebel, & Da Costa, 2016). The fatty acid (FA) composition and the membrane lipids can shift according to grow-out conditions and thus, promoting significant differences in the nutritional value of these "sea veggies" (Sui et al., 2010; Sui and Han, 2014a; Ventura et al., 2011; Ramani et al., 2004). Sui

and Han, 2014b demonstrated that under salt stress digalactosyldiacylglycerol (DGDG)/ monogalactosyldiacylglycerol (MGDG) increased in *Suaeda salsa,* while phosphatidylglycerol (PG) content increased in *S. salsa* and in *Thellungiella halophila.* In addition, Ramani et al., 2004 also observed a rise in the content of sulfolipids in *Aster tripolium* L., Compositae, and *Sesuvium portulacastrum* L., Aizoaceaeduring during salt stress. The lipidome of *Salicornia ramosissima* (Hook. f.) J. Woods and *Halimione portulacoides* (L.) Aellen [Syn. Atriplex portulacoides L.], has been recently identified (Maciel et al., 2018), and revealed the presence of valuable lipids with nutritional and bioactive value. The present study aims to understand if the production in marine aquaponics affects the lipid profile of these two halophyte species when compared with their conspecifics from wild donor sites. In order to achieve this goal, we tested the following null hypothesis: the FA-containing polar lipids of halophytes culture in marine aquaponics does not present any major shift from that of conspecifics grown in their natural environment (salt marshes). These two species were chosen because they are both occurring in salt marsh habitats classified under EU Environmental legislation (Habitats Directive and Natura 2000 network), meaning that harvesting of wild specimens is limited, and both can be used for human consumption or pharma applications. Therefore, marine aquaponics could be an alternative to reduce the potential impact and dependency from wild populations. In the wild, the two species do not form mixed populations: *S. ramosissima* is an annual pioneer plant occurring in low marsh areas, whilst *H. portulacoides* is a perennial plant occurring in higher elevated sites in middle marsh areas. This also makes the two species very interesting as potential cash crops, because *S. ramosissima* reproduces by seeds, whilst *H. portulacoides* can be cultured through vegetative propagation (all year around) or through seeds germination. Having as starting point the differences in habitat (low and

middle marsh) and life cycle, and the same potential as cash crop, the motivation for the present study was the need to gather scientific evidence that sustainable marine aquaponics does not cause a loss in nutritional value, neither of bioactive compounds, on "sea veggies" farmed under aquaponics environmentally friendly approach.

2. Materials and Methods

2.1. Reagents

Chloroform, methanol and acetonitrile were purchased from Fisher scientific (Leicestershire, UK); all the solvents were of high-performance liquid chromatography (HPLC) grade and were used without further purification. All the other reagents and chemicals used were of the highest grade of purity commercially available. The water was of Milli-Q purity (Synergy1, Millipore Corporation, Billerica, MA).

Lipid internal standards mixture 1,2-dimyristoyl-sn-glycero-3-phosphocholine (dMPC) (0.005 μg μL^{-1}), 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (dMPE) (0.005 μg μL^{-1}), 1,2-dimyristoyl-sn-glycero-3-phospho-(10-rac-glycerol) (dMPG) (0.003 μg μL^{-1}), , 1,2-dipalmitoyl-sn-glycero-3-phosphatidylinositol (dPPI) (0.02 μg μL^{-1}) , and 1,2-dimyristoyl-sn-glycero-3-phosphate (DMPA) (0.02 μg μL^{-1})were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL).

2.2. Plant materials

The studied halophytes (*Salicornia ramosissima* (Hook. f.) J. Woods and *Halimione portulacoides* (L.) Aellen [Syn. Atriplex portulacoides L.]) in Ria de Aveiro coastal lagoon salt marshes (40º38´N 08º44´W) were collected at the doner sites of the plants produced in marine aquaponics. Plants were cultured in aquaponics tanks (6 m long x 2 m wide x 0.3 m deep; water wenewal ≅ 12h; N=3). Each aquaponics tank was stocked

with circa 800 plants equally distributed over 9 Styrofoam trays floating in the tank water and they were cultured using the nutrient-rich effluent water originating from a super-intensive marine RAS system producing Senegalese sole (*Solea senegalensis*). The effluent had an average concentration of dissolved inorganic nitrogen (DIN) was 8.9 ± 1.3 mg L^{-1} (maximum 10.0 mg L^{-1} and a minimum 6.8 mg L^{-1}) and average concentration of dissolved inorganic phosphorus (DIP) was 0.32 ± 0.11 mg L^{-1} (maximum 0.50 mg L^{-1} and mini- mum 0.21 mg L^{-1}) (Marques et al. 2017). Under both growing conditions (wild habitat or in aquaponics) plants were not limited by nutrients, light, or stressed by any other environmental factor.

Wild plants were collected in healthy populations (donor sites) in May and were cultivated (salinity, light availability and air temperature similar to donor sites) under aquaponics conditions for five months. (for a detailed description please see Marques et al. 2017). For the study of the lipidome profile wild and cultivated plants were collected during the summer season (July) ensuring that plants were not flowering. At each site (salt marsh / aquaponics) eighteen healthy plants were collected randomly, covering a representative area of the marsh population and of the aquaponics system. Sampling from each aquaponics tank was performed by randomly selecting 2 plants from each of the 9 floating Styrofoam trays. In the laboratory, plants were carefully washed and edible organs separated. These were then grouped into five composed samples to be analysed.

2.3. Lipid extraction from leaves

Total lipids were extracted according to Bligh and Dyer (Bligh & Dyer, 1959) with slight modifications (Maciel et al., 2018). Three mL of chloroform: methanol (1:2, *v*/*v*) were added to 100 mg of dried plant material followed by vigorous stirring on a

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vortex mixer and incubated on ice during 1h. Then, the homogenate was centrifuged at 626× *g* for 5 min. This procedure was repeated twice to improve extraction efficiency and after 1.8 mL of water were mixed with supernatant, stirred on a vortex mixer followed by centrifugation at 626× *g* for 5 min. The lower chloroform phase containing lipids were recovered and dried under nitrogen current. The lipid extracts were stored at −20 °C before analysis by LC−MS. Five technical replicates were performed using the plant material.

2.4. Quantification of phospholipids

The phospholipids were quantified from lipid extract by measuring the phosphorous amount (Bartlett & Lewis, 1970). The lipid hydrolysis was performed by adding 125 µL of 70% perchloric acid to the dried extracts followed incubation for 1h at 180 °C. After cooling to room temperature, 825 µL of Milli-Q water, 125 µL of ammonium molybdate (2.5 g/100 mL), and 125 µL of ascorbic acid (0.1 g/1 mL) were added to each sample, with vortex mixing between each addition. The samples were incubated in a water bath at 100 °C for 10 min, and then placed in cold water. Phosphate standards with different concentrations (from 0.1 to 2 µg of phosphorus) were prepared from sodium dihydrogen phosphate dihydrate (NaH2PO4·2H2O, 100 µg/ mL of P). These underwent the same procedures, without the incubation in the heating block, and adjusting for the total water volume added. Absorbance was measured at 897 nm at room temperature in a Multiskan GO 1.00.38 Microplate Spectrophotometer (Thermo Scientific, Hudson, NH, USA) using SkanIT software, version 3.2 (Thermo Scientific). The phosphorus content was calculated by linear regression, and this value was multiplied by 25 to obtain the phospholipid amount.

2.5. Quantification of glycolipids

The glycolipids were quantified from lipid extracts by calculating the hexose content using the orcinol colorimetric method (da Costa et al., 2017). Briefly, 1 mL of orcinol solution (0.2% in 70% H2SO4) was added to the dried lipid extracts and incubated at 80 ºC during 20 min. The absorbance was measured at 505 nm at room temperature in a Multiskan GO 1.00.38 Microplate Spectrophotometer (Thermo Scientific, Hudson, NH, USA) using SkanIT software, version 3.2 (Thermo Scientific). The amount of sugar was determined from a calibration curve prepared by performing the reaction on known amounts of glucose (up to 40 µg, from an aqueous solution containing 5 mg/mL of sugar). The mount of glycolipids was estimated using the conversion factor 100/35 (Bell, Daniels, Fearn, & Stewart, 1987).

2.6. Polar lipid analysis by HILIC−LC−Q−Exactive-MS

Lipid extracts from *S. ramosissima* and *H. portulacoides* were analyzed by high High−performance LC (HPLC) system (Thermo scientific Accela^{TM}) with an autosampler coupled online to the high resolution Q-Exactive® mass spectrometer with Orbitrap® technology, as reported in our previous work (Maciel et al., 2018). The solvent system consisted of two mobile phases as follows: mobile phase A [acetonitrile:methanol:water 50:25:25 (*v*/*v*/*v*) with 1 mM ammonium acetate] and mobile phase B [acetonitrile:methanol 60:40 (*v*/*v*) with 1 mM ammonium acetate]. Initially, 0% of mobile phase A was held isocratically for 8 min, followed by a linear increase to 60% of 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 50 µg of lipophilic extract, 4 µL of internal standard (IS) mixture and 91 µL of mobile phase B was introduced into the Ascentis®Si column (15 cm × 1 mm, 3 µm, Sigma-Aldrich) with a flow rate of 40 µL min^{-1} and at 30 °C. The mass spectrometer with Orbitrap®

technology was operated in simultaneous positive (electrospray voltage 3.0 kV) and negative (electrospray voltage −2.7 kV) modes at a resolution of 70,000 and AGC target of 1E6, the 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 1E5 was used, and the cycles consisted of one full scan mass spectrum and ten data-dependent MS/MS scans, repeated continuously throughout the experiments with a dynamic exclusion of 60 seconds and intensity threshold of 1e4. 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, USA). Two analytical replicates were performed for each sample

2.7. Data and Statistical analysis

Lipid species peak integration and assignments were performed using MZmine 2.30 (Pluskal, Castillo, Villar-Briones, & Orešič, 2010). The software allows for filtering and smoothing, peak detection, peak processing, and assignment against an in-house database. During the processing of the raw data acquired in full MS mode, all the peaks with raw intensity lower than 1E4 were excluded. For normalization of the data, the peak areas of the extracted ion chromatogram (XICs) of each lipid species precursors of each class were divided for the peak area of the IS selected for the class.

All statistical analyses were performed using R version 3.5.1 (Team R Core, 2018) in Rstudio Version 1.1.456 (RStudio Team, 2016). Data were glog transformed and then autoscaled prior to statistical analyses using the Metaboanalyst R package (Xia, Sinelnikov, Han, & Wishart, 2015). Lipid profile data were analyzed by principal component analysis (PCA), with the R function prcomp() and ellipses were drawn using the R package ellipse (Murdoch, Duncan and Chow, E. D., 2018), assuming a

multivariate normal distribution and a level of 0.95. Mann-Whitney was performed with R command wilcox.test with the Benjamin–Hochberg correction (R function p.adjust) for the false discovery rate (FDR, q-value). Graphics were created using the R package ggplot2 (H. Wickham, 2016)(Hadley Wickham, 2016). Other r packages used for data management and graphics include plyr (Hadley Wickham, 2011), dplyr (Hadley Wickham, François, Henry, & Müller, 2018), tidyr (Hadley Wickham & Henry, 2018) and ggrepel (Slowikowski, 2018).

3. Results and Discussion

3.1. Total amount of lipid glycolipids and phospholipids

Lipid amount in both plants *S. ramosissima* and *H. portulacoides* was estimated by gravimetry and no differences were observed between samples from wild (*S. ramosissima* yielded 19.6±12.8 and *H. portulacoides* yielded 5.6±2.2 of leaves dry weight) and aquaponic (*S. ramosissima* yielded 9.7±4.6 and *H. portulacoides* yielded 5.6±0.9 of leaves dry weight) for both plants.

The polar lipids, GL and PL levels were also estimated in lipid extracts, using colourimetric methods. In the case of wild *S. ramosissima,* the GL was 37.6±4.7 % and PL 24.6±3.6 % of total lipid, while the aquaponic *S. ramosissima* the percentage of GL was 62.7±9.5 % and PL was 27.7 ±1.7 % of total lipid extracts. In *H. portulacoides* the GL contend increased from 42.4±1.9 % in the wild to 66.8±3.9 % in aquaponics, and the PL contend increased from 7.6±2.2 % in the wild to 23.1±0.4 % from in aquaponic plants. The results showed that the percentage of GL and PL increased in lipid extracts in both aquaponic plants. These findings are in line with a previous study, which reported that polar lipids, glycolipids and phospholipids, are the major lipids found in halophyte plants and can represent up to 80% of total lipids (Rozentsvet, Nesterov, &

Bogdanova, 2014). The increase of polar lipids, specially the glycolipids was described to be related with the increase of salt concentration (Wu, Seliskar, & Gallagher, 1998), and this increase was related to their stabilization function during membrane permeabilization (Kobayashi, 2016). Thus, the higher % glycolipid of total lipids can be explained with increase of the salinity in IMTA systems. More recently studies regarding variation in lipid composition in halophytes showed that both the development and the biochemical composition of halophytes do not depend only on salt concentration, but also on the availability of water and nutrients factors. For example Rozentsvet et al, conclude in their study with ten halophyte species that the variations of the GL content do not depend on characteristics related to salt accumulation but depend on the ecological regime related to the water factor (Rozentsvet et al., 2014). Studies regarding the impact of nutrients (N and P) on halophyte plants have been performed on aquaponic system (Pinheiro et al., 2017) or mimicking the aquaculture regimes (A. K. Buhmann, Waller, Wecker, & Papenbrock, 2015), but most of the studies were related with growth and the biomass production and less extended to their biochemical composition and even less about the lipid profile.

3.2. Polar lipidome plasticity

The lipid profile of both halophytes *S. ramosissima* and *H. portulacoides* were analyzed by high-resolution LC-MS and MS/MS. Lipid species belonging to 5 different PL classes: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI) and phosphatidic acid (PA); belonging to 3 different GL classes: Digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG), sulfoquinovosyldiacylglycerol (SQDG); and a class of glycosphingolipid: hexosylceramide (HexCer) (Maciel et al., 2018) were

identified and semi-quantified. The semi-quantification of all lipid species was obtained using the peak areas of the extracted ion chromatograms (XICs) of each lipid species, normalized using the peak area of the IS selected for the class.

The dataset obtained for both plant species grown in two distinct conditions (wild and aquaponics) were glog-transformed and autoscaled and then subjected to a principal component analysis (PCA). Univariate analysis (Mann-Whitney) was performed to test for significant molecular species differences between groups.

3.2.1. Lipidome profile of *Salicornia* cultivated in aquaponics

Multivariate analysis by Principal component analysis (PCA) showed that the two groups, wild and aquaponic, were discriminated in a two-dimensional score plot, expressing 85.1 % of the total variance in the dataset, including principal component 1 (67.8 %) and principal component 2 (17.3 %), as can be seen in Figure 1. The contributions of variables in accounting for the variability in the first principal component (>1%) included 5 DGDG, 2MGDG, 8 SQDG, 2 HexCer, 24 PC, 13 PE, 7 PG and 6 PI molecular species (supplementary Table S1). The contribution of variables in accounting for the variability in second principal component (>1%) included 3 DGDG, 3 MGDG, 3 SQDG, 1 HexCer, 1 PC, 5 PE and 10 PA molecular species (supplementary Table S1). Most features were more abundant in the aquaponic group, except the PC (37:4) species, which decreased in the aquaponics group.

Univariate analysis was performed to test for significant lipid species differences between both groups. Mann-Withney test showed that 61 of 125 lipid molecular species (49%) were significantly different between groups (q-value<0.05). These lipids included 5 DGDG, 3 MGDG, 6 SQDG, 23 PC, 17 PE, 5 PG, 1 PI and 1 HexCer species and are summarized in Table 1. The volcano plot representation of data (Figure S1)

Journal Pre-proofs

highlights the most significant differences among lipid species and the positive or negative fold change seen in aquaponics *S. ramosissima* compared to the wild group. Volcano plot with following criteria: *p*-value <0.05, fold change >2 showed 77 significant features, where 76 molecular lipid species were detected at higher concentration in the aquaponics group and only one was found in a lower amount in this group (PC 37:4). The molecular species with significant *p*-value and high fold-change in aquaponics groups were DGDG 36:3; 36:4; 34:1; 34:3 and 36:6, the SQDG 34:1 and 36:3 and the MGDG 36:6 and 35:2. Some PC and PE with 36 and 38 carbons and polyunsaturated fatty acyl chains, such as PC 36:5, PC 36:6, PC 38:8 and PE 36:4, PE 36:5 and PE 36:6 were also found with high scores in aquaponics group.

We further looked to the percentage of major polar lipids according to their total carbon chain length (C32, C34, C36, C38, C40 and C42) (Figure 2A) and the number of unsaturation (DB-0 up to DB-8, (DB: double bond)) (Figure 2B) within each group (aquaponics and wild groups). In both groups, the lipids with a total carbon chain length of C34 and C36 were the most abundant, but in aquaponics halophytes, there was a higher percentage of C36 while in wild halophytes, there was a higher percentage of C34 carbons lipids. Concerning the unsaturation, the most abundant lipid species in both cases were those with DB-3, DB-6, DB-2 and DB-4. Halophytes from aquaponics had a higher percentage of lipids with DB-6, while wild halophytes had a higher percentage of lipid species with DB-2.

The in-depth comparison of the halophytes lipidome at the molecular level, comparing conspecifics from the wild and farmed in marine aquaponics, showed that the same major lipids (higher than 1E4 in LC-MS data) were present under the two grow-out conditions. The main differences observed were related to lipid molecular species abundances. Most shifts in molecular lipid species abundance were due to the

increase recorded in *S. ramosissima* grown in marine aquaponics, namely GL classes carrying *n*-3 FA (e.g. α-Linolenic acid (ALA)).

3.2.2. Lipidome profile of *Halimione* cultivated in aquaponics

The PCA of all molecular lipid species identified in *H. portulacoides* showed that both groups, wild and aquaponics, were separated from each other in a two-dimensional score plot, expressing 93 % of the total variance in the dataset, including principal component 1 (80.7 %) and principal component 2 (12.3 %), where principal component 1 was the major discriminating component (Figure 3). The analysis of the PCA contributions of variables (supplementary Table S2) showed that the species that most contributed for the separation of the two groups were mostly polyunsaturated glycolipids (3 DGDG, 3 MGDG, 7 SQDG) containing α-linolenic acid (18:3*n*-3), that were in higher amounts in aquaponics samples, and four classes of phospholipids (31 PC, 16 PE, 10 PG and 5 PI) containing a large variety of molecular species.

The univariate analysis showed statistically significant differences in 82 of 104 (78%) lipid molecular species between groups. These lipids included 4 DGDG, 4 MGDG, 7 SQDG, 32 PC, 18 PE, 11 PG, 5 PI and 1 PA (Table 2). Most of these lipid molecular species are represented in the volcano plot (71 of 82 features) (Figure S2) and had high fold-change in aquaponics group. The glycolipids statistically different were esterified to *n*-3 and *n*-6 polyunsaturated FA, particularly with a major contribution of 18:3*n*-3. The significant phospholipids molecular species are from 5 classes (PC, PE, PG, PI and PA), with the majority of molecular species belonging to PC and PE classes.

The features of major lipids concerning their total carbon chain length (C32, C34, C36 and C38) (Figure 4A) and their unsaturation (DB-0 up to DB-6) (Figure 4B) were also examined. Lipid species with C34 are the most abundant ones in both groups

Journal Pre-proofs

(wild and aquaponic) followed by lipids with C36. In what concern lipid unsaturation, the lipids that contain DB-2, DB-3 and DB-6 were more abundant in both groups. Although the lipids with DB-6 were in a higher percentage in aquaponics than in the wild group, lipids withDB-2 and DB-3 were predominant in the wild group. Similarly to the observed in S. ramosissima, the *n*-3 lipids increase in H. portulacoides grown in marine aquaponics, although in this case the n-3 FA were distributed between GL and PL classes.

Results showed that halophyte production in IMTA systems featuring outdoors marine aquaponics enhanced their nutritional value through the increase of *n*-3 lipids, as well as their potential to be a source for bioactivate compounds. To our best knowledge, only a few studies addressed the effect of aquaponics production on halophytes, with these mostly focusing phenolic compounds and/or fatty acids. Pinheiro et al., 2017, analyzed the number of phenolic compounds and their antioxidant activity for *Sarcacornia ambigua* grown in aquaponics as well as Bertin et al., 2014 that compared the proximate composition, phenolic compounds and fatty acid profile of *Sarcocornia ambigua* collected from salt marshes and grown in marine aquaponics, reporting similar fatty acid pools for wild and farmed specimens. Our results seem to contradict these findings although it must be highlighted that the level of detail and quantification of polar lipids of the present study is unprecedented, it was possible using modern lipidomic tools with high throughput and high-resolution analysis. Indeed, modern mass spectrometry has been an ally in the evolution of lipidomics, allowing to fully unravel complex lipidomes, such as those of marine macrophytes (which include halophyte plants) (Maciel et al., 2016).

Journal Pre-proofs

The polyunsaturated polar lipids found in halophyte plants grown under marine aquaponics are those most likely to give origin to value-added products. The results from our work show that marine aquaponics production of *S. ramosissima* and *H. portulacoides,* under similar salinity, light availability and air temperature to donor sites, and no nutrients limitations, holds the potential to increase the commercial value of these halophyte species. Beyond their value as a source of PUFA, important in modern-day healthy food markets, these halophytes displayed highly valued novel-lipids with health beneficial effects and potential applications in nutraceutical and pharmaceutical industries. As an example, PL represents the major structural lipid classes of extraplatidial cell membranes and have been shown to support brain health and healthy cell function (Küllenberg, Taylor, Schneider, & Massing, 2012). Additionally, GL are mainly found in chloroplasts and thylakoid membranes, playing important regulatory and signalling roles (Hölzl et al., 2009). This class of lipids usually contains polyunsaturated fatty acids with recognized health benefits (Christensen, 2012). Bioactive properties such as anti-inflammatory, anti-microbial, anti-proliferative and antioxidant, have been attributed to GL. These properties are dependent on the fatty acyl composition of lipids and also on the polar head group (da Costa et al., 2017; Lopes et al., 2014; Plouguerné et al., 2013; Tsai and Sun Pan, 2012). In addition, some studies have already shown that the polar structure allows for an easier digestion and absorption of *n*-3 FA, enhanced bioavailability and uptake into tissues than other lipids (Kagan, West, Zante, & Calder, 2013), as well as increased bioavailability of other important nutrients, particularly vitamins and minerals (Zúñiga & Troncoso, 2012).

Currently, commercial products displaying polar lipids can be found in different markets, being traded as supplements and claiming to promote the beneficial effects described above, such as LIPOGEN PSPA™ and Almega PL™. These products are

usually obtained from cabbage, soy or microalgae (Hellhammer, Vogt, Franz, Freitas, & Rutenberg, 2014)(Kagan et al., 2013). Halophyte plants cultivated in marine aquaponics are a good source of these valuable lipids and can be explored using more sustainable and secure practices, such as IMTA, as long as they obey all EU regulatory framework on food safety. While halophytes collected from wild also display high levels of valuable lipids, their production in marine aquaponics provides a set of environmental and socioecomic benefits, namely: i) decrease dependency from wild populations, which enables reducing potential environmental impact and disables the risk from uncontrolled environmental variables that induce changes in the lipidome profile (e.g., natural inter-annual and seasonal variation and human impact factors, such as contaminats or extreme weather events), therefore safeguarding food safety and more stable lipid profile; ii) enhance conservation of salt marshes, which are aknowledge as vulnerable ecosystems, and promotes sustainable use of natural resources in line with the principles of circular economy (e.g., designing out waste and reducing the risk of eutrophication due to nutrients pollution); iii) contribute in an integrated way to the implementation of the United Nations 2030 Agenda for Sustainable Development.

In both cases (cultured/harvested in their natural environmental or cultured in aquaponics) any commercial use of these “sea veggies” must strictly obey by all EU regulatory framework on food safety, that covers all substances that may put at risk human health.

4. Conclusions

Marine aquaponics using organic-rich effluents of a super-intensive marine fish farm combined with similar wild habitat abiotic factors like salinity, light availability and air temperature, does not shift the lipidome of *S. ramosissima* and *H. portulacoides*

Journal Pre-proofs

but enhances the abundance of key lipids of halophytes farmed under these conditions when compared to conspecifics from the wild. These shifts are more pronounced in the increase of glycolipids with *n*-3 FA present in halophytes grown under marine aquaponics and can be used to advocate the trade of these “sea veggies” in health food markets that value sustainable production practices.

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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Figure Captions

Figure 1: Principal component analysis (PCA) scores plot of total lipidome acquired by LC-MS of *Salicornia ramosissima* cultured in marine aquaponics (Sr.Aq) and collected from the wild (Sr.W).

Figure 2: A) Percentage of lipid species from *Salicornia ramosissima* comprised of the same number of carbon atoms on the hydrocarbon chains. B) Lipid species comprised of the same number of unsaturation on the fatty acyl chains. Sr.W: wild *Salicornia ramosissima;* Sr.Aq: *Salicornia ramosissima* cultured in marine aquaponic. * ρ<0.05

Figure 3: Principal component analysis (PCA) scores plot of total lipidome acquired by LC-MS of *Halimione portulacoides* cultured in marine aquaponics (Sr.Aq) and collected from the wild (Sr.W).

Figure 4: A) Percentage of lipid species from *Halimione portulacoides* comprised of the same number of carbon atoms on the hydrocarbon chains. B) Lipid species comprised of the same number of unsaturation on the fatty acyl chains. Hp.W: wild *Halimione portulacoides;* Hp.Aq: *Halimione portulacoides* cultured in marine aquaponics. * ρ<0.05

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Tables

Table 1: Significant differences of lipid molecular species between *Salicornia ramosissima* cultured in marine aquaponics and collected from the wild (q-value <0.05). Mann-Whitney was performed with R command wilcox.test with the Benjamin–Hochberg correction (R function p.adjust) for the false discovery rate (FDR q-value). DGDG – Digalactosyldiacylglycerol; HexCer – Hexosylceramide; MGDG – Monogalactosyldiacylglycerol; PC – Phosphatidylcholine; PE - Phosphatidylethanolamine, PG - Phosphatidylglycerol, PI – Phosphatidylinositol; SQDG – Sulfoquinovosyldiacylglycerol;

Identification (C:DB)	*m/z*	Fatty acyl composition	q-value
DGDG 34:1	936.653	18:1/16:0	0.019
DGDG 34:3	932.627	18:3/16:0	0.019
DGDG 36:3	960.656	18:3/18:0; 18:2/18:1	0.019
DGDG 36:4	958.644	18:3/18:1; 18:2/18:2	0.019
DGDG 36:6	954.612	18.3/18:3	0.019
HexCer d18:1/h16:1	714.549	d18:1/h16:1	0.033
MGDG 35:2	786.525	17:0/18:2; 16:0/19:2	0.019
MGDG 36:6	954.612	18:3/18:3	0.019
MGDG 35:3	784.513	18:3/17:0; 19:3/16:0	0.019
PC 32:1	732.550	16:0/16:1	0.019
PC 34:2	758.567	16:0/18:2	0.033
PC 34:3	756.551	16:0/18:3	0.019
PC 36:1	788.608	*	0.019
PC 36:2	786.597	18:0/18:2;18:1/18:1	0.019
PC 36:3	784.579	*	0.019
PC 36:4	782.566	18:2/18:2;18:1/18:3	0.019
PC 36:5	767.483	18:3/18:2	0.019
PC 36:6	778.538	18:3/18:3	0.019
PC 37:4	796.547	18:4/19:0; 18:3/19:1; 18:2/19:2	0.019
PC 38:1	816.618	18:1/20:0	0.019
PC 38:2	814.630	18:2/20:0;18:1/20:1;19:1/19:1;19:0/19:2	0.019
PC 38:3	812.613	18:3/20:0;18:2/20:1;18:1/20:2	0.019
PC 38:4	810.597	18:3/20:1; 18:2/20:2	0.019
PC 38:5	808.583	18:3/20:2; 18:2/20:3	0.019
PC 38:6	806.569	16:0/22:6	0.019
PC 38:7	804.548	*	0.019
PC 38:8	802.532	18:4/20:4	0.019
PC 40:2	842.629	18:2/22:0	0.019
PC 40:3	840.617	18:3/22:0; 18:2/22:1	0.019
PC 40:4	838.601	18:3/22:1; 18:2/22:2	0.019

Journal Pre-proofs

PC 40:5	836.616	20:2/20:3	0.019
PC 42:2	870.666	18:2/24:0	0.033
PE 34:2	714.490	16:0/18:2	0.033
PE 34:3	712.490	16:0/18:3	0.019
PE 36:2	742.537	18:0/18:2;18:1/18:1;19:2/17:0	0.019
PE 36:3	740.517	18:0/18:3;18:2/18:1	0.019
PE 36:4	738.505	18:2/18:2	0.019
PE 36:5	736.489	18:3/18:2	0.019
PE 36:6	734.474	18:3/18:3	0.019
PE 37:0	760.584	16:0/21:0	0.033
PE 38:3	768.551	18:2/20:1; 18:3/20:0; 18:1/20:2	0.019
PE 38:4	738.505	18:2/18:2	0.019
PE 39:2	784.474	18:2/21:0; 19:2/20:0	0.019
PE 39:3	782.459	18:3/21:0; 19:2/20:1	0.033
PE 40:2	798.598	18:2/22:0	0.019
PE 41:3	810.493	18:3/23:0; 18:2/23:1	0.019
PE 42:2	826.630	18:2/24:0	0.019
PE 36:1	744.450	18:0/18:1; 16:0/20:1	0.019
PE 42:3	826.630	18:2/24:0	0.033
PG 34:2	745.499	16:0/18:2	0.033
PG 34:3	743.484	*	0.019
PG 36:2	773.530	18:0/18:2; 16:1/20:1; 16:0/20:2; 18:1/18:1	0.019
PG 36:5	780.550	18:3/18:2	0.019
PG 36:6	765.471	18:3/18:3	0.019
PI 36:2	861.546	18:0/18:2; 18:1/18:1	0.019
SQDG 32:0	793.511	16:0/16:0	0.019
SQDG 34:1	819.529	18:1/16:0	0.019
SQDG 34:3	815.496	16:0/18:3	0.019
SQDG 36:3	843.526	18:3/18:0	0.019
SQDG 36:4	841.511	18:1/18:3; 18:2/18:2	0.019
SQDG 36:6	837.479	18:3/18:3	0.019

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Table 2: Significant differences of lipid molecular species between *Halimione portulacoides* cultured in marine aquaponics and collected from the wild (q-value <0.05). Mann-Whitney was performed with R command wilcox.test with the Benjamin–Hochberg correction (R function p.adjust) for the false discovery rate (FDR q-value). DGDG – Digalactosyldiacylglycerol; HexCer – Hexosylceramide; MGDG – Monogalactosyldiacylglycerol; PC – Phosphatidylcholine; PE - Phosphatidylethanolamine, PG - Phosphatidylglycerol, PI – Phosphatidylinositol; SQDG – Sulfoquinovosyldiacylglycerol;

Identification (C:DB)	m/z	Fatty acyl composition	FDR
DGDG 34:3	932.626	18:3/16:0	0.011
DGDG 34:6	926.580	18:3/16:3	0.011
DGDG 36:6	954.611	18:3/18:3	0.011
DGDG 34:0	938.665	18:0/16:0	0.021
MGDG 34:1	774.601	18:1/16:0	0.021
MGDG 34:6	764.527	18:3/16:3	0.011
MGDG 36:6	792.558	18:3/18:3	0.011
MGDG 37:5	808.553	18:3/19:2	0.011
PA 34:4	667.433	16:1/18:3; 16:0/18:4; 16:2/18:2; 16:3/18:1	0.021
PC 32:1	732.554	16:0/16:1; 14:0/18:1	0.011
PC 34:1	760.585	16:0/18:1	0.011
PC 34:2	758.571	16:0/18:2; 16:1/18:1	0.011
PC 34:3	756.556	16:0/18:3; 16:1/18:2	0.011
PC 35:0	804.552	16:0/19:0	0.011
PC 35:1	774.601	17:0/18:1	0.040
PC 35:3	770.569	17:0/18:3; 17:1/18:2; 17:2/18:1	0.011
PC 35:4	768.554	17:1/18:3; 17:2/18:2; 17:3/18:1	0.011
PC 36:1	788.606	18:0/18:1; 16:1/20:0	0.011
PC 36:3	784.586	18:1/18:2; 18:0/18:3	0.011
PC 36:4	782.566	18:1/18:3; 18:2/18:2	0.011
PC 36:5	780.554	18:2/18:3; 16:1/20:4	0.011
PC 36:6	778.538	18:3/18:3	0.011
PC 37:1	802.538	18:1/19:0	0.011
PC 37:2	800.522	18:2/19:0; 18:1/19:1; 18:0/19:2	0.011
PC 37:4	796.585	18:3/19:1; 18:2/19:2	0.011
PC 37:5	794.569	18:3/19:2	0.011
PC 38:2	814.632	18:1/20:1; 18:2/20:0	0.011
PC 38:3	812.616	18:3/20:0; 18:2/20:1; 18:1/20:2	0.011
PC 38:4	810.596	18:3/20:1; 18:2/20:2; 18:1/18:3	0.011
PC 38:5	808.583	18:3/20:2; 18:2/20:3	0.011
PC 38:6	806.569	16:0/22:6	0.011
PC 39:2	828.553	18:2/21:0	0.011

PC 40:1	844.647	18:1/22:0	0.011
PC 40:2	842.631	18:2/22:0; 18:1/22:1	0.011
PC 40:3	840.617	18:3/22:0; 18:2/22:1	0.011
PC 40:4	838.601	18:3/22:1; 18:2/22:2	0.011
PC 40:5	836.616	20:2/20:3	0.011
PC 42:0	874.725	18:0/24:0	0.011
PC 35:2	772.585	17:0/18:2; 17:1/18:1; 17:2/18:0; 16:0/19:2	0.011
PC 37:3	798.601	18:1/19:2; 18:2/19:1; 18:3/19:0	0.011
PC 36:2	786.601	18:1/18:1; 18:0/18:2	0.011
PE 34:2	714.505	16:0/18:2; 16:1/18:1	0.011
PE 34:3	712.489	16:0/18:3; 16:1/18:2	0.011
PE 35:2			0.011
PE 35:3	726.505	17:1/18:2; 17:0/18:3; 16:0/19:3	0.040
PE 36:2	742.535	18:0/18:2	0.011
PE 36:3	740.520	18:1/18:2; 18:0/18:3	0.011
PE 36:4	738.505	18:2/18:2; 18:1/18:3	0.011
PE 36:5	736.489	18:3/18:2	0.011
PE 36:6	734.474	18:3/18:3	0.011
PE 37:0	760.474	16:0/21:0	0.011
PE 38:3	768.552	18:3/20:0; 18:2/20:1; 18:1/20:2	0.011
PE 38:4	766.536	18:3/20:1; 18:2/20:2; 18:1/20:3	0.011
PE 39:2	784.474	18:2/21:0; 19:0/20:2; 19:1/20:1	0.011
PE 39:3	782.459	18:3/21:0; 19:1/20:2; 18:2/21:1; 16:0/23:3	0.011
PE 40:2	798.522	18:2/22:0; 18:1/22:1	0.011
PE 40:3	768.552	18:3/20:0; 18:2/20:1; 18:1/20:2	0.011
PE 40:4	794.491	18:2/22:2	0.011
PE 41:2	812.506	17:2/24:0; 18:2/23:0; 19:1/22:1	0.011
PG 32:0	721.499	16:0/16:0; 14:0/18:0	0.011
PG 32:1	719.485	16:0/16:1; 14:0/18:1	0.011
PG 34:0	749.525	16:0/18:0	0.011
PG 34:1	747.516	16:0/18:1	0.011
PG 34:2	745.499	16:0/18:2; 16:1/18:1	0.011
PG 34:3	743.485	16:0/18:3; 16:1/18:2	0.011
PG 34:4	741.468	16:1/18:3; 16:0/18:4	0.011
PG 36:3	771.515	18:0/18:3; 18:1/18:2	0.011
PG 36:5	767.485	18:2/18:3	0.011
PG 36:6	765.475	18:3/18:3; 16:1/20:5	0.011
PG 36:2	773.532	18:1/18:1; 18:0/18:2	0.021
PI 34:2	833.515	16.0/18:2	0.011
PI 34:3	831.499	16:0/18:3	0.011
PI 36:2	861.545	18:0/18:2; 18:1/18:1	0.011
PI 36:4	857.513	18:1/18:3; 18:2/18:2	0.011
PI 36:5	855.498	18:2/18:3	0.011

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SQDG 32:0	793.511	16:0/16:0	0.011
SQDG 32:3	787.465	18:3/14:0; 16:3/16:0	0.011
SQDG 33:3	801.480	18:3/15:0	0.011
SQDG 34:2	817.512	18:1/16:1; 20:0/14:2	0.011
SQDG 34:3	815.497	18:3/16:0	0.011
SQDG 34:4	813.481	18:3/16:1	0.011
SQDG 36:5	839.496	18:3/18:2	0.011

CRediT author statement

Elisabete Maciel: Conceptualization, methodology, formal analysis, Investigation, Writing – Original Draft, Writing- Review and Editing, Visualization. **Pedro Domingues**: Formal analysis, Visualization, Writing- Review and Editing. M**. Rosário M. Domingues:** Supervision, Validation, Writing- Review and Editing. **Ricardo Calado**: Conceptualization, Funding acquisition, Supervision, Writing- Review and Editing. **Ana Lillebø**: Conceptualization, Methodology, Funding acquisition, Supervision, Writing- Review and Editing.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Highlights

- Lipidome of two halophytes from aquaponics and donor wild populations were compared
- Lipidomics revealed high levels of *n*-3 lipids in halophytes from marine aquaponics
- *Salicornia* and *Haliminone* displayed distinct adaptations to marine aquaponics
- Highly-valued *n*-3 lipids can promote these “sea veggies” in health food markets

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