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Effects of ocean warming and acidification, combined with nutrient enrichment, on chemical composition and functional properties of *Ulva rigida*

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ABSTRACT

Ulva is increasingly viewed as a food source in the world. Here, *Ulva rigida* was cultured at two levels of temperature (14, 18 °C), pH (7.95, 7.55, corresponding to low and high pCO_2), and nitrate conditions (6 μmol L^{-1}, 150 μmol L^{-1}), to investigate the effects of ocean warming, acidification, and eutrophication on food quality of *Ulva* species. High temperature increased the content of each amino acid. High nitrate increased the content of all amino acids except aspartic acid and cysteine. High temperature, pCO_2, and nitrate also increased the content of most fatty acids. The combination of high temperature, pCO_2, and nitrate increased the swelling capacity, water holding capacity, and oil holding capacity by 15.60%, 7.88%, and 16.32% respectively, compared to the control. It seems that the future ocean environment would enhance the production of amino acid and fatty acid as well as the functional properties of *Ulva* species.

1. Introduction

Humans began to consume seaweeds as food as early as the late Pleistocene (between 14,220 and 13,980 years ago) at Monte Verde in southern Chile (Dillehay et al., 2008). Currently there is growing interest in using seaweeds as food because they are rich sources of dietary fibre, protein, vitamins and minerals (Bolton, Cyrus, Brand, Joubert, & Macey, 2016). *Ulva*, a genus of green seaweeds, is particularly rich in a rare cell-wall polysaccharide termed ulvan which is an important source of dietary fibre and is also a potential source of vitamins A, B2, B12, C and tocopherol (Bolton et al., 2016). Further, *Ulva* species are rich in omega-3 (n3) fatty acids (McCauley, Meyer, Winberg, & Skropeta, 2016). Accordingly, *Ulva* species have been used in salads, soups, cookies, meals and condiments as well as a mixed product with other green seaweeds (Peña-Rodríguez, Mawhinney, Ricque-Marie, & Cruz-Suárez, 2011).

Due to greenhouse gas emissions and other anthropogenic activities, the globally averaged combined land and ocean surface temperature has risen by 0.85 °C (0.65–1.06 °C), over the period 1880–2012 and it will continue to warm during the 21st century (IPCC, 2013). The global mean sea surface temperatures for the months of February and August are projected to increase by 1.9 °C by the end of the 21st century (Bartsch, Wiencke, & Laepple, 2012). The maximum warming of around 4 °C is predicted for high latitudes of the northern hemisphere in summer (Bartsch et al., 2012). In addition, as more atmospheric CO_2 dissolves in seawater the pH of ocean decreases, moving towards a less alkaline and therefore more acidic state—termed ocean acidification. The mean surface ocean pH has decreased by 0.1 units since the beginning of the industrial era, corresponding to a 26% increase in hydrogen ion concentration (IPCC, 2013).

Apart from ocean warming and acidification, eutrophication is another inescapable environmental issue. Inevitable urbanization of a growing human population, increased use of coastal areas, and rising fertilizer use for agricultural intensification have led to accelerated nutrient inputs from land-water to coastal waters (Smith et al., 2003). These changes in nutrient availability result in eutrophication, an increasing threat for coastal ecosystems (Bricker et al., 2008).

The majority of research on *Ulva* species' chemical composition has focused on natural stocks collected from coastal or lagoon waters (Ortiz, et al., 2006; Trigui, Gasmi, Zouari, & Tounsi, 2013; Tsubaki, Oono, Hiraoka, Ondab, & Mitanid, 2016). It has been found that the chemical composition *Ulva* varies with species, geographical

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distribution and season (McCauley et al., 2016; Ortiz et al., 2006; Trigui et al., 2013). To meet the increasing demand for seaweed, *Ulva* cultivation seems to be a sustainable option. However, there is limited information on chemical composition of specimens cultivated under various environmental conditions, and particularly how ocean warming, acidification and eutrophication, three of the most environmentally significant global change factors, will affect the chemical properties of *Ulva*. The present research aims to determine how chemical properties of *U. rigida* will be altered, if the thalli are grown under simulated global change conditions focusing on temperature, CO_2 and nitrogen supply. This provides insight into how predicted future environmental change will affect the quality and potential use of *U. rigida* for food.

2. Materials and methods

2.1. Sample preparation

Adult vegetative *U. rigida* fronds, 50–60 mm long, were collected from the low intertidal reaches of Cullercoats beach, UK (55.03 N, 1.43 W) after a spring tide in May 2014. The fronds were placed in a plastic bag and were transferred to Newcastle University within one hour. They were rinsed in sterile seawater to remove any sediment, epiphytes or small grazers. Afterwards, 720 healthy individuals were haphazardly assigned to 24 tanks, each with a volume of 13.5 L. In order to investigate the interactive effects of ocean acidification, warming and eutrophication, these tanks were then incubated under combinations of two pH levels (7.95, 7.55), two temperatures (14, 18 °C), and two nitrate levels (6, 150 μmol L^{-1}). Phosphate concentration was set as 50 μmol L^{-1} to ensure no phosphorus limitation. Three replicate tanks were set up for each treatment. A computer-controlled pH-regulating system (Aqua-medic™, Loveland, Colorado) was used to maintain the pH of the cultures by injecting CO_2 into the air stream via solenoid valves. Temperature was controlled by incubators with a photoperiod of 16 h light: 8 h dark. Light intensity was 80 μmol photons m^{-2} s^{-1}. The ambient pH (7.95), nitrate concentration (6 μmol L^{-1}) and the summer average temperature (14 °C) of seawater off the coast of the North Sea were set as the control. The decreased pH and elevated temperature represent the predicted levels by the year 2100 (Baede et al., 2001) and the elevated nitrate level is based on the studies referring to eutrophication (Kang & Chung, 2017; Ménesguen et al., 2018). Nitrate concentration was monitored using a rapid spectrophotometer method (Gao, Clare, Rose, & Caldwell, 2017) and adjusted to the experimental level daily. Seawater was renewed every 3 days. After a 12-day cultivation period, *U. rigida* fronds were collected and dried in an oven at 50 °C until completely dry (for 24 h). The dried biomass was then ground to a powder, sieved and placed into tubes that were kept in a desiccator pending further analyses.

2.2. Amino acids analysis

Amino acids were analysed using a high-performance liquid chromatography (Ultimate 3000, USA)-tandem mass spectrometry (API 3200 Q-TRAP, USA) (HPLC-MS/MS) system. *Ulva* powder (100 mg) was transferred to an ampoule and hydrolysed with 4 ml of 6 N hydrochloric acid at 110 °C for 24 h. Fifty μl of this solution was dried under nitrogen and then re-dissolved in 200 μl of deionized water. Eighty μl of borate buffer (0.1 M, pH 8.8) was added to 40 μl of sample solution and mixed. The solution was derivatised with 40 μl of diethyl ethoxymethylenemalonate at 55 °C for 15 min. Samples were transferred to insert vials and 20 μl were injected into the HPLC. The HLPC system consisted of a SRD-3600 Solvent Rack with analytical 6-channel vacuum degasser, a DGP-3600A pump, WPS-3000TSL analytical autosampler, and a tcc-3200 column compartment. Chromatographic separations were performed on a MSLab HP-C18 column (150 × 4.6 mm, 5 μm). The mobile phase consisted of water (A) and acetonitrile (B). The solvent was delivered to the column at a flow rate of 0.8 ml min^{-1} as follows: 0–0.1 min from A-B (95:5) to A-B (95:5); 1–8 min from A-B (95:5) to A-B (40:60); 8–8.1 min from A-B (40:60) to A-B (0:100); 8.1–10 min from A-B (0:100) to A-B (0:100); 10–10.1 min from A-B (0:100) to A-B (95:5); 10.1–15 min from A-B (95:5) to A-B (95:5).

The conditions for MS-MS detection were optimized to obtain the highest signal intensity and were as follows: mode, positive-ion mode; ion spray voltage, 5500 V; nebulizer gas pressure, 55 psi; curtain gas pressure, 20 psi; collision gas pressure, medium; turbo gas temperature, 500 °C; entrance potential, 10 V; collision cell exit potential, 2 V. Nitrogen gas was used as the collision gas in a multiple reaction monitoring mode. The data were obtained using Analyst software version 1.5.1 (Applied Biosystems). Amino acid standard solution (Aladdin, China) was injected for the system's calibration and amino acid quantification. Content of amino acid was expressed as g 100 g^{-1} DW.

2.3. Fatty acid analysis

Homogenized samples (3 g) were extracted with 60 ml chloroform: methanol (2:1) solution vortexed for 20 min at room temperature, and 12 ml NaCl (0.88%) were added to aid stratification. Then, samples were centrifuged for five minutes at 1000*g* and the upper phase was removed. Sixty ml methanol: water (1:1) was used to rinse the tubes, the extraction was repeated once more and the lower lipid phase was dried under a steady stream of nitrogen. The total lipid content was then measured gravimetrically.

Fatty acid methyl ester (FAME) preparation was carried out according to Joseph and Ackman (1992) with some modification. A mixture of 100 mg of lipid and 1.5 ml 0.5 N methanolic NaOH was heated at 100 °C for five minutes. After reaching room temperature, 2 ml boron trifluoride 12% in methanol was added to the solution, which was then heated at 100 °C for 30 min. After cooling for five minutes at room temperature, 1.0 ml isooctane and 5 ml saturated NaCl solution were added. The solution was centrifuged for five minutes at 859*g* and the upper layer was transferred to a new tube. One ml isooctane was added to the first solution and the procedure was repeated. The two layers of isooctane were dried under a steady stream of nitrogen and the residue was re-dissolved in 1.0 ml of hexane before being stored at -20 °C until analysis.

Analysis of FAME was carried out with a gas chromatograph (Shimadzu, GC-2014, Kyoto, Japan) using an Agilent CP-SIL 88 fused silica capillary column (100 m × 0.25 mm ID × 0.2 μm film thickness). Purified helium was used as a carrier gas with a head pressure of 210 kPa and a column flow of 1 ml min^{-1}. A split injection system was used with an auto injector (Shimadzu, AOC-20i) with a split ratio of 50.0 and an injector temperature of 255 °C. FAME peaks were detected by flame ionisation detection at 260 °C. Samples of 1 μl were injected at an initial column temperature of 70 °C, which was held for one minute. The temperature was then raised at 5 °C min^{-1} to 100 °C, where it was held for two minutes, and then increased at 10 °C min^{-1} to 160 °C, where it was held for 61 min. Finally, the temperature was increased to 240 °C at a rate of 5 °C min^{-1}, where it was held for 21 min, thus giving a final gradient of 113 min total runtime. Peaks were identified using a commercial 52 FAME standard (GLC463, Nu-Chek Prep Inc., Elysian, MN, USA). Quantification of FA expressed as a percentage of the total peak are (% of total FA) was based on peak areas of individual identified and non-identified FA. Content of FA (mg g^{-1} DW) was determined by total lipid content × 0.55 × % of total FA, where 0.55 is the proportion of fatty acids to total lipid in the order Ulvales (Gosch, Magnusson, Paul, & Nys, 2012).

2.4. Swelling capacity (SWC)

SWC of *U. rigida* was estimated by a slightly modified method of bed volume after equilibrating in excess solvent (Yaich et al., 2011). *Ulva* powders (0.2 g) were placed into a 10 ml measuring cylinder. Ten ml of

distilled water was added and immediately mixed vigorously. The measuring cylinder was left to stand for 18 h at 37 °C. The swelling volume was recorded and SWC was expressed as ml of swollen sample per gram of sample.

2.5. Water holding capacity (WHC)

WHC of *U. rigida* was analysed by the centrifugation method (Yaich et al., 2011). *Ulva* powder (0.2g) was placed into a pre-weighed centrifuge tube. Five ml of distilled water was added and the mixture was stirred vigorously. The dispersion was centrifuged for 25 min at 3000*g* after standing at 37 °C for one hour. The supernatant was removed and the residue was oven-dried at 50 °C for 25 min. The water holding capacity was expressed as grams of water bound per gram of the sample.

2.6. Oil holding capacity (OHC)

OHC of *U. rigida* was measured by the method described by Yaich et al. (2011). *Ulva* powder (0.5 g) was dispersed in 5 ml of food-grade corn oil and then placed in a centrifuge tube. The mixture was stirred and left at 37 °C for one hour, followed by centrifugation at 3000*g* for 25 min. The oil supernatant was then transferred to a 10 ml measuring cylinder and measured. The OHC of *U. rigida* was expressed as grams of oil held by 1g of sample (DW). The density of the corn oil was 0.92g ml^{-1}.

2.7. Statistical analysis

Results were expressed as means of three replicates ± standard deviation. Data were analysed using SPSS v.21. All data sets conformed to a normal distribution (Shapiro-Wilk, $P > 0.05$) and the variances of all samples could be considered equal (Levene's test, $F < 2.303$, $P > 0.05$) except glycine (Levene's test, $F = 2.786$, $P = 0.042$), proline (Levene's test, $F = 2.817$, $P = 0.041$), cysteine (Levene's test, $F = 2.840$, $P = 0.040$), C17:0 (Levene's test, $F = 4.519$, $P = 0.005$), C20:4(n-6) (Levene's test, $F = 8.180$, $P < 0.001$), and C22:5(n-3) (Levene's test, $F = 2.747$, $P = 0.045$). Three-way multivariate ANOVA was conducted to assess the effects of temperature, pCO_2, and nitrate on amino acid and fatty acid contents of *U. rigida*. Bonferroni adjustment was used to reduce the chance of Type I error. New alpha levels of 0.0024 (0.05 divided by 21) and 0.0017 (0.05 divided by 30) were set for amino acid and fatty acid analysis respectively. Three-way ANOVA was used to assess the effects of temperature, pCO_2, and nitrate on SWC, WHC, and OHC of *U. rigida*. A confidence interval of 95% was set for three-way ANOVA.

3. Results and discussion

3.1. Amino acids

The amino acid composition of *U. rigida* grown under various conditions is shown in Table 1. Glutamic acid (Glu) and alanine (Ala) were the most abundant amino acids, followed by arginine (Arg) and aspartic acid (Asp). All samples were rich in essential amino acids and the ratios of essential to non-essential amino acids were all above 1 except under HTLC, indicating that those samples contained more essential than non-essential amino acids.

High temperature increased the content of every amino acid and thus the total amino acids (Tables 1 and S1). Metabolic theory predicts that the metabolic rates of organisms generally rise exponentially with temperature within a certain range (Iken, 2012), leading to higher rates for most physiological processes, including nitrate assimilation. In the present study, the high temperature (18 °C) significantly increased amino acid content of *U. rigida* compared with the low temperature (14 °C). This is consistent with Mohsen, Nasr, and Metwalli (1973)'s finding in which amino acid content of *U. fasciata* increased with culture temperature (15–25 °C). Although optimal temperature for amino acids synthesis in *U. rigida* was not determined, the finding in the present study combined with the literature mentioned above indicates ocean warming would enhance the amino acids content of *Ulva* species in cold areas.

In contrast, high pCO_2 decreased the content of amino acids, which was particularly significant for aspartic acid (Asp) and glutamic acid (Glu) (Tables 1 and S1). This finding was similar to Gordillo, Jiménez, Goutx, & Niell 's (2001) study in which a high CO_2 level led to lower soluble protein content in *U. rigida* collected from the Mediterranean Sea. CO_2 is also a key factor affecting the synthesis of amino acids, as the amino acid carbon skeleton derives from CO_2 assimilation. In addition, nitrate reduction, reduction of nitrite, and fixation of NH^{4+} depends largely on photosynthesis that provides reducing equivalents and ATP (Heldt & Piechulla, 2005). In theory, high CO_2 could promote photosynthesis and CO_2 assimilation, and thus amino acid synthesis. The mechanisms that high CO_2 reduce content of amino acid or protein in the literature and present study are unclear and may be affected by the synthesis of lipid and carbohydrate.

High nitrate increased the content of all amino acids except aspartic acid (Asp) and cysteine (Cys) (Tables 1 and S1). Nitrogen is an essential element required for amino acid synthesis. The general pathway of nitrate assimilation in plant cells is that nitrate is reduced to nitrite in the cytosol and then nitrite is reduced further to NH^{4+} in the chloroplasts, from which amino acids are synthesised (Heldt & Piechulla, 2005). Consequently, an increase in nitrogen availability commonly enhances the amino acid content. For instance, an increase of 13 μM (from 7 μM to 20 μM) could cause a rise in protein content of *U. lactuca* from 14.2 ± 9.6% DW to 36.6 ± 9.1% DW (Msuya & Neori, 2008). Naldi and Wheeler's study (1999) demonstrated that very high nitrate concentration (1000 μM) still stimulated the nitrogen pool of protein in *U. fenestrata* from 43% to 54% of total nitrogen. Furthermore, the total amino acid content of *U. ohnoi* increased linearly with internal nitrogen content ($r = 0.987$) (Angell, Mata, Nys, & Paul, 2014). In the present study, the high nitrate level (150 μM) enhanced nearly all the amino acid contents of *U. rigida* compared with the low nitrate level. The findings from the present study and the literature indicate eutrophication can increase amino acid and protein content of *Ulva* regardless of the intensity of eutrophication and *Ulva* species. From a human nutritional perspective, this is advantageous as it shows that *Ulva* has the potential to be cultured as a high amino acid and protein food.

Interactive effects of temperature, pCO_2, and nitrate were found in phenylalanine (Phe) and cysteine (Cys) (Table S1). For instance, high temperature (HTLNLC) and high nitrate (LTHNLC) alone increased phenylalanine (Phe) content by 26.74% and 11.63% respectively and pCO_2 did not have any effect on it. Nevertheless, high temperature, high pCO_2 and high nitrate together (HTHNHC) increased phenylalanine (Phe) content by 96.51%. Temperature and nitrate had interactive effects on arginine (Arg), glutamic acid (Glu), tyrosine (Tyr), phenylalanine (Phe), and the ratio of EAA to NEAA (Table S1). For instance, high temperature (HTLNLC) and high nitrate (LTHNLC) alone increased arginine (Arg) by 18.58% and 8.85% respectively, whilst the combination of the two factors (HTHNLC) increased it by 89.38% (Tables 1 and S1). The findings above demonstrate that high temperature, high pCO_2, and high nitrate or two of them could enhance the content of some amino acids synergistically, indicating future ocean environment may promote product of amino acids in *Ulva* species.

3.2. Fatty acids

Fatty acid (FA) composition of *U. rigida* grown under various conditions is shown in Table 2. Thirty-three FA were identified, of which 10 that accounted for less than 0.01 mg g^{-1} DW are not shown. The most abundant FA was palmitic acid (C16:0). In addition, *U. rigida* had a high C18 unsaturated FA content, such as C18:3(n-3) and C18:1(n-7).

High temperature increased C14:0, C15:0, C16:0, C16:1(n-7),

Table 1
Amino acid composition of *U. rigida* grown under different conditions (g 100 g^{-1} DW). Values are means of three replicates ± standard deviation. An alpha level of 0.0024 (0.05 divided by 21) was set since there were 21 dependent variables.

Amino acids	LTLNLC[a]	LTLNHC[b]	LTHNLC[c]	LTHNHC[d]	HTLNLC[e]	HTLNHC[f]	HTHNLC[g]	HTHNH[h]
His	0.21 ± 0.03	0.20 ± 0.02	0.21 ± 0.01	0.22 ± 0.01	0.31 ± 0.02	0.27 ± 0.01	0.36 ± 0.01	0.34 ± 0.03
Ser	0.78 ± 0.10	0.70 ± 0.11	0.90 ± 0.12	0.82 ± 0.12	1.10 ± 0.05	0.99 ± 0.02	1.27 ± 0.08	1.39 ± 0.17
Arg	1.13 ± 0.07	1.02 ± 0.06	1.23 ± 0.04	1.18 ± 0.10	1.34 ± 0.05	1.30 ± 0.08	1.57 ± 0.20	2.14 ± 0.10
Gly	0.63 ± 0.05	0.57 ± 0.06	0.76 ± 0.04	0.72 ± 0.03	0.80 ± 0.02	0.79 ± 0.04	0.83 ± 0.10	1.12 ± 0.09
Asp	1.04 ± 0.06	0.85 ± 0.07	1.18 ± 0.04	1.19 ± 0.03	1.99 ± 0.13	1.46 ± 0.14	1.94 ± 0.24	1.44 ± 0.05
Ala	1.25 ± 0.05	1.10 ± 0.04	1.45 ± 0.08	1.41 ± 0.10	2.05 ± 0.17	1.60 ± 0.05	1.98 ± 0.11	1.92 ± 0.25
Glu	1.22 ± 0.05	1.06 ± 0.04	1.39 ± 0.04	1.41 ± 0.06	2.26 ± 0.03	1.80 ± 0.05	2.21 ± 0.05	1.70 ± 0.05
Thr	0.75 ± 0.07	0.64 ± 0.07	0.83 ± 0.10	0.80 ± 0.12	1.08 ± 0.03	0.95 ± 0.01	1.15 ± 0.15	1.34 ± 0.16
Pro	0.52 ± 0.09	0.46 ± 0.04	0.61 ± 0.06	0.58 ± 0.09	0.69 ± 0.02	0.64 ± 0.03	0.96 ± 0.10	0.91 ± 0.07
Lys	0.76 ± 0.04	0.68 ± 0.05	0.94 ± 0.07	0.93 ± 0.06	1.11 ± 0.06	1.08 ± 0.03	1.24 ± 0.11	1.11 ± 0.11
Tyr	0.39 ± 0.05	0.35 ± 0.06	0.41 ± 0.03	0.39 ± 0.07	0.50 ± 0.03	0.49 ± 0.04	0.58 ± 0.05	0.80 ± 0.05
Met	0.30 ± 0.05	0.27 ± 0.01	0.34 ± 0.03	0.33 ± 0.01	0.38 ± 0.01	0.37 ± 0.00	0.42 ± 0.04	0.56 ± 0.09
Val	0.90 ± 0.08	0.79 ± 0.06	1.00 ± 0.11	0.99 ± 0.11	1.37 ± 0.08	1.16 ± 0.03	1.39 ± 0.05	1.46 ± 0.12
Ile	0.55 ± 0.06	0.51 ± 0.04	0.63 ± 0.08	0.60 ± 0.01	0.81 ± 0.08	0.75 ± 0.02	0.81 ± 0.02	0.94 ± 0.08
Phe	0.86 ± 0.10	0.82 ± 0.04	0.96 ± 0.14	0.91 ± 0.04	1.09 ± 0.04	1.03 ± 0.05	1.17 ± 0.13	1.69 ± 0.08
Cys	0.11 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.07 ± 0.02	0.27 ± 0.03	0.15 ± 0.01	0.18 ± 0.02	0.27 ± 0.02
Leu	0.94 ± 0.04	0.86 ± 0.05	1.09 ± 0.05	1.05 ± 0.06	1.36 ± 0.05	1.15 ± 0.05	1.12 ± 0.06	1.44 ± 0.08
Total	12.33 ± 0.70	10.95 ± 0.46	14.01 ± 0.77	13.61 ± 0.80	18.49 ± 0.59	15.97 ± 0.66	19.18 ± 0.54	20.56 ± 0.79
EAA[i]	6.40 ± 0.37	5.78 ± 0.24	7.23 ± 0.51	7.02 ± 0.44	8.84 ± 0.31	8.06 ± 0.18	9.22 ± 0.49	11.01 ± 0.55
NEAA[j]	5.94 ± 0.30	5.17 ± 0.24	6.78 ± 0.26	6.59 ± 0.36	9.65 ± 0.36	7.91 ± 0.09	9.95 ± 0.17	9.55 ± 0.32
EAA/NEAA	1.08 ± 0.02	1.12 ± 0.03	1.07 ± 0.04	1.06 ± 0.01	0.92 ± 0.03	1.02 ± 0.03	0.93 ± 0.05	1.15 ± 0.05

[a] LTLNLC, low temperature, low nitrate and low pCO_2.
[b] LTLNHC, low temperature, low nitrate and high pCO_2.
[c] LTHNLC, low temperature, high nitrate and low pCO_2.
[d] LTHNHC, low temperature, high nitrate and high pCO_2.
[e] HTLNLC, high temperature, low nitrate and low pCO_2.
[f] HTLNHC, high temperature, low nitrate and high pCO_2.
[g] HTHNLC, high temperature, high nitrate and low pCO_2.
[h] HTHNHC, high temperature, high nitrate and high pCO_2.
[i] EAA, essential amino acids: His, Arg, Thr, Lys, Met, Val, Ile, Phe, and Leu.
[j] NEAA, non-essential amino acids: Ser, Gly, Asp, Ala, Glu, Pro, Tyr, and Cys.

C18:0, C18:1(n-7), C20:0, C18:3(n-6), C18:3(n-3), C22:1(n-9), C20:5(n-3), C22:5(n-3), total FA, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and n3-FA, but decreased C18:2(n-6), C20:3(n-6), n6-FA, and n-6/n-3 (Tables 2 and S2). Temperature is one of the most important and best-studied environmental factors affecting the lipid content and fatty acid composition of photosynthetic tissues or organisms (Guschina & Harwood, 2009 and references therein). The effect of temperature on total lipid varies with species. The total lipid content of *Isochrysis galbana* was higher when grown at 30 °C than at 15 °C regardless of the growth phase (Zhu, Lee, & Chao, 1997). On the other hand, the high temperature of 25 °C decreased the total lipid of *U. pertusa* from 3.6% DW to 2.6% DW at low light intensity and from 4.9% DW to 2.7% DW at high light intensity, compared with the low temperature of 15 °C (Floreto, Hirata, Ando, & Yamasaki, 1993). In the present study, the high temperature enhanced total lipid content. Apart from the difference of species, the opposite effects of temperature on total lipid might be due to different temperature ranges used in different studies. The temperature range was 4 °C in present study while it was 10 °C in Floreto et al. (1993). Mohsen et al. (1973) reported that the largest lipid yield in *U. fasciata* was at 20 °C, followed by 15 °C, and 25 °C. This finding was consistent with both Floreto et al. (1993) and the present studies since the high temperature in the present study is close to 20 °C and the low temperature is near to 15 °C. This indicates that there is an optimal temperature for total lipid content and the temperatures on both sides of this optimal point would lead to a decrease in total lipid. The reasons that high temperature reduced the content of C18:2(n-6), C20:3(n-6), and thus n6-FA in the present study remain to be answered.

High pCO_2 increased C14:0, C16:0, C18:0, C18:1(n-9), C18:1(n-7), C18:2(n-6), C18:3(n-6), C18:3(n-3), C20:5(n-3), C22:5(n-3), total FA, SFA, MUFA, FA(n-6), FA(n-3), and n-6/n-3 (Tables 2 and S2). Synthesis of fatty acids depends on CO_2 assimilation to a large extent because photosynthesis can supply the necessary materials, such as acetyl CoA, ATP, NADPH, etc. (Heldt & Piechulla, 2005). In the present study, high CO_2 showed a positive effect on fatty acid synthesis. Similar findings were reported for *Scenedesmus obliquus* (Tang, Han, Li, Miao, & Zhong, 2011), *Chlorella pyrenoidosa* (Tang et al., 2011), and *Dunaliella viridis* (Gordillo, Jiménez, Figueroa, & Niell, 1998). Furthermore, high CO_2 increased MUFA in the present study. This is consistent with Tang et al. (2011) in which high CO_2 (5%) increased unsaturated fatty acids from 65.78% to 71.39% of total fatty acids in *S. obliquus* and from 59.01% to 71.50% of total fatty acids in *C. pyrenoidosa* compared with 0.03% CO_2. On the other hand, elevated (2–5%) CO_2 concentrations resulted in a general reduction in the degree of unsaturation of fatty acids in *Chlorella vulgaris* compared with ambient (0.04%) CO_2, of which the particularly significant decrease was in C18:3, from 38.1% of total fatty acids down to 17.8% of total fatty acids (Tsuzuki, Ohnuma, Sato, Takaku, & Kawaguchi, 1990). Therefore, the response of algal fatty acid content and composition to CO_2 enrichment may be heterogeneous.

High nitrate increased C14:0, C15:0, C16:0, C16:1(n-7), C18:1(n-7), C18:3(n-6), C18:3(n-3), C22:0, C20:3(n-6), C22:1(n-9), C20:5(n-3), C24:0, C22:5(n-3), total FA, SFA, MUFA, PUFA, FA(n-3), but decreased C18:2(n-6) and n-6/n-3 (Tables 2 and S2). Nitrogen is an important factor that can affect lipid content in algae and nitrogen deficiency commonly enhances lipid accumulation in microalgae (Brennan & Owende, 2010). Generally, there are two hypotheses behind this conclusion. One is that nitrogen deficiency almost invariably causes a steady decline in cell division rate. Meanwhile, biosynthesis of fatty acids can continue under such conditions. Consequently, the synthesized fatty acids are converted to triacylglycerides (TAGs) that are stored within existing cells thereby increasing the content per cell (Brennan & Owende, 2010). The other hypothesis is that when nitrogen is limited, the flow of fixed carbon is diverted from protein to either lipid or carbohydrate synthesis. TAGs composed primarily of saturated and monounsaturated fatty acids can be efficiently packed into the cell and generate more energy than carbohydrates upon oxidation, thus

Table 2
Fatty acid composition of *U. rigida* grown under different conditions (mg g^{-1} DW). Values are means of three replicates ± standard deviation. An alpha level of 0.0017 (0.05 divided by 30) was set since there were 30 dependent variables.

Fatty acid	LTLNLC[a]	LTLNHC[b]	LTHNLC[c]	LTHNHC[d]	HTLNLC[e]	HTLNHC[f]	HTHNLC[g]	HTHNH[h]
C14:0	0.10 ± 0.01	0.11 ± 0.01	0.09 ± 0.00	0.13 ± 0.02	0.20 ± 0.00	0.29 ± 0.01	0.26 ± 0.01	0.32 ± 0.01
C14:1(n-5)	0.01 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.04 ± 0.02
C15:0	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00
C16:0	4.09 ± 0.34	4.57 ± 0.37	3.95 ± 0.08	5.51 ± 0.29	4.94 ± 0.31	6.55 ± 0.58	6.33 ± 0.48	7.97 ± 0.21
C16:1(n-7)	0.17 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.26 ± 0.05	0.26 ± 0.01	0.36 ± 0.02	0.36 ± 0.04	0.44 ± 0.01
C17:0	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01
C17:1(n-8)	0.04 ± 0.00	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.00	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.00
C18:0	0.08 ± 0.01	0.08 ± 0.02	0.06 ± 0.00	0.08 ± 0.01	0.09 ± 0.01	0.12 ± 0.00	0.09 ± 0.00	0.11 ± 0.00
C18:1(n-9)	0.11 ± 0.00	0.14 ± 0.01	0.09 ± 0.00	0.13 ± 0.01	0.08 ± 0.00	0.15 ± 0.02	0.13 ± 0.02	0.19 ± 0.02
C18:1(n-7)	2.03 ± 0.06	2.07 ± 0.08	1.96 ± 0.04	2.56 ± 0.18	2.29 ± 0.04	3.12 ± 0.16	3.08 ± 0.13	3.79 ± 0.08
C18:1(n-6)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
C18:2(n-6)	1.07 ± 0.04	1.23 ± 0.09	0.60 ± 0.09	0.87 ± 0.18	0.39 ± 0.05	0.79 ± 0.03	0.49 ± 0.04	0.75 ± 0.02
C20:0	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
C18:3(n-6)	0.07 ± 0.00	0.06 ± 0.00	0.07 ± 0.01	0.09 ± 0.01	0.06 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.11 ± 0.00
C18:3(n-3)	2.71 ± 0.06	2.59 ± 0.01	2.86 ± 0.10	3.96 ± 0.03	2.84 ± 0.09	3.85 ± 0.07	3.75 ± 0.05	4.50 ± 0.06
C22:0	0.21 ± 0.02	0.22 ± 0.02	0.26 ± 0.01	0.30 ± 0.05	0.18 ± 0.04	0.25 ± 0.04	0.32 ± 0.09	0.41 ± 0.06
C20:3(n-6)	0.04 ± 0.00	0.04 ± 0.01	0.05 ± 0.00	0.06 ± 0.01	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
C22:1(n-9)	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
C20:4(n-6)	0.06 ± 0.01	0.05 ± 0.00	0.07 ± 0.01	0.09 ± 0.04	0.05 ± 0.01	0.08 ± 0.01	0.07 ± 0.00	0.09 ± 0.00
C20:5(n-3)	0.14 ± 0.01	0.13 ± 0.00	0.15 ± 0.00	0.19 ± 0.03	0.23 ± 0.00	0.31 ± 0.01	0.31 ± 0.01	0.36 ± 0.01
C24:0	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.03 ± 0.00	0.03 ± 0.00
C22:4(n-6)	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
C22:5(n-3)	0.58 ± 0.05	0.55 ± 0.04	0.58 ± 0.01	0.73 ± 0.07	0.57 ± 0.01	0.71 ± 0.03	0.77 ± 0.05	0.84 ± 0.02
Total FA	11.60 ± 0.21	12.18 ± 0.44	11.15 ± 0.24	15.13 ± 0.26	12.35 ± 0.60	16.86 ± 0.27	16.30 ± 0.63	20.18 ± 0.19
SFA[i]	4.53 ± 0.36	5.03 ± 0.37	4.42 ± 0.09	6.09 ± 0.28	5.46 ± 0.36	7.29 ± 0.55	7.08 ± 0.53	8.92 ± 0.17
MUFA[j]	2.39 ± 0.08	2.49 ± 0.09	2.33 ± 0.05	3.04 ± 0.22	2.72 ± 0.06	3.71 ± 0.17	3.68 ± 0.19	4.55 ± 0.13
PUFA[k]	4.68 ± 0.15	4.66 ± 0.09	4.40 ± 0.21	6.00 ± 0.24	4.17 ± 0.13	5.86 ± 0.15	5.54 ± 0.11	6.72 ± 0.08
FA(n-6)[l]	1.27 ± 0.05	1.40 ± 0.09	0.83 ± 0.11	1.14 ± 0.18	0.55 ± 0.06	1.01 ± 0.04	0.71 ± 0.04	1.03 ± 0.03
FA(n-3)[m]	3.42 ± 0.10	3.27 ± 0.06	3.58 ± 0.10	4.87 ± 0.07	3.64 ± 0.10	4.86 ± 0.11	4.84 ± 0.10	5.71 ± 0.06
n-6/n-3[n]	0.37 ± 0.00	0.43 ± 0.03	0.23 ± 0.03	0.23 ± 0.03	0.15 ± 0.02	0.21 ± 0.00	0.15 ± 0.01	0.18 ± 0.00

[a] LTLNLC, low temperature, low nitrate and low pCO_2.
[b] LTLNHC, low temperature, low nitrate and high pCO_2.
[c] LTHNLC, low temperature, high nitrate and low pCO_2.
[d] LTHNHC, low temperature, high nitrate and high pCO_2.
[e] HTLNLC, high temperature, low nitrate and low pCO_2.
[f] HTLNHC, high temperature, low nitrate and high pCO_2.
[g] HTHNLC, high temperature, high nitrate and low pCO_2.
[h] HTHNHC, high temperature, high nitrate and high pCO_2.
[i] SFA, saturated fatty acids.
[j] MUFA, monounsaturated fatty acids.
[k] PUFA, polyunsaturated fatty acids.
[l] FA(n-6), total Omega-6 fatty acids.
[m] FA(n-3), total Omega-3 fatty acids.
[n] n-6/n-3, the ratio of total Omega-6 fatty acids to total Omega-3 fatty acids.

constituting the best reserve for rebuilding the cell after the stress (Rodolfi et al., 2009).

However, high nitrate increased total FA in *U. rigida* in the present research. This is in inconsistent with the previous hypotheses and studies since nitrogen limitation enhanced total lipid of *U. rigida* at 350 ppm CO_2 concentration in Gordillo et al. (2001). The possible reason for this positive effect of high nitrate on total lipid content might be due to the stage of *U. rigida* when it was harvested. *U. rigida* was harvested at the end of 12 days of culture for biochemical analysis, when high nitrate could induce more reproduction of *U. rigida* (Gao et al., 2017). When vegetative cells transit into reproductive cells followed by the formation of swarmers, more lipid might be required. Apart from the massive synthesis of lipid during mitosis and meiosis, swarmers may contain more lipid than vegetative cells since they are in great need of energy sources to support swimming and following settlement of swarmers. The total lipid content was 56.7% normalised to carbon in spores of *U. intestinalis* and could be 84.0% in spores of *Zonaria farlowii* (Reed, Brzezinski, Coury, Graham, & Petty, 1999). In addition, it has been found that the lipid content in cells decreased from 176.0 to 123.5 μg 10^{-7} per spore during 10 days of development from spores to gametophytes in *Saccharina latissima* (Steinhoff, Graeve, Wiencke, Wulff, & Bischof, 2011). In Gordillo et al. (2001), *U. rigida* was harvested after 10 days of culture when no reprocution was found. The current study indicates the chemical composition of *Ulva* would change with culture stage and it would be essential to point out in which stage the samples are harvested when presenting data.

Temperature, pCO_2, and nitrate had interactive effects on C18:3(n-3), C22:1(n-9), C22:5(n-3), total FA, PUFA, and FA(n-3) (Table S2). For instance, none of these three factors affected C18:3(n-3) but the combination of these three factors increased it by 66.41% (Tables 2 and S2). Temperature and pCO_2 had interactive effects on C14:0, C18:1(n-7), C18:3(n-6), C18:3(n-3), C22:1(n-9), C20:5(n-3), total FA, MUFA, PUFA, and FA(n-6) (Table S2). For instance, high temperature (HTLNLC) increased C14:0 by 94.87% and pCO_2 (LTLNHC) did not affect it while the combination of these two factors (HTLNHC) increased it by 184.98%. Temperature and nitrate interacted with C14:0, C18:1(n-9), C18:1(n-7), C18:2(n-6), total FA, MUFA, PUFA, FA(n-6), the ratio of FA(n-6) to FA(n-3) (Table S2). For instance, high nitrate did not change C18:1(n-9) at low temperature but increased it by 30.40% at high temperature (Tables 2 and S2). Nitrate and pCO_2 had interactive effects on C18:3(n-3), total FA, PUFA, and FA(n-3) (Table S2). For instance, high nitrate (LTHNLC) or high pCO_2 (LTLNHC) alone did not affect C18:3(n-3) while the combination of them (LTHNHC) increased it by 46.26% (Table 2 and S2). The findings above show high temperature, pCO_2, and

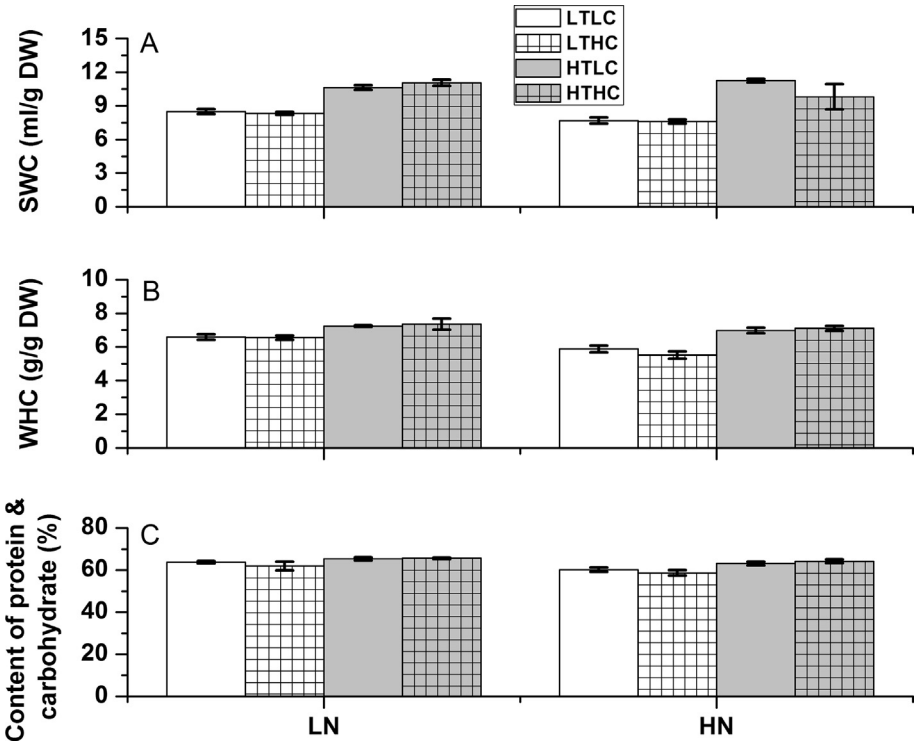

Fig. 1. Swelling capacity (A), water holding capacity (B) and content of protein and carbohydrate (C) in *U. rigida* grown under different conditions. LTLC: low temperature and low pCO_2; LTHC: low temperature and high pCO_2; HTLC: high temperature and low pCO_2; HTHC: high temperature and high pCO_2; LN: low nitrate; HN: high nitrate. Data are the means ± SD (n = 3). Panel C was drawn based on the original data in Gao et al. (2017).

nitrate could increase content of fatty acids synergistically, suggesting that the production of fatty acids in *Ulva* species may benefit from global climate change.

3.3. SWC, WHC and OHC

The swelling capacity (SWC) of thalli varied from 7.59 ± 0.17 ml g^{-1} DW to 11.24 ± 0.17 ml g^{-1} DW (Fig. 1A). Both temperature and nitrate had a significant effect on SWC (Table 3). The high temperature treatment enhanced SWC by 33.21% compared to the low temperature, while high nitrate reduced SWC by 5.56%. pCO_2 did not affect SWC (Table 3). Temperature, pCO_2, and nitrate had an interactive effect on SWC (Table 3). High pCO_2 (LNLTHC) and high nitrate (HNLTLC) alone did not affect SWC, high (LNHTLC) temperature increased it by 25.12% but the combination of these three factors (HNHTHC) increased SWC by 15.60% compared to LNLTLC.

The swelling capacity of *U. rigida* in the present study was slightly lower than that reported in *U. lactuca* by Wong and Cheung (2000) (13.0 ± 0.70 ml g^{-1} DW) whilst Yaich et al. (2011) observed an even lower SWC (0.3 ml g^{-1} DW) in *U. lactuca*. The swelling capacity of seaweed could be mainly determined by the content of protein and carbohydrate as both of them play a role in hydration properties (Yaich et al., 2011) High temperature or low nitrate increased the SWC of *U. rigida* in this study, and the pattern was similar for the total content of protein and carbohydrate (Fig. 1A, C and Table 3). The SWC of *Ulva* can enhance satiety and reduce calorie intake (i.e. carbohydrate, sugar, fat, saturated fat and protein intake), and hence can be applied in an adjunctive therapy for obesity (Dettmar, Strugala, & Richardson, 2011).

As far as water holding capacity (WHC) is concerned, the range was 5.22 ± 0.10–7.24 ± 0.04 g g^{-1} DW when *U. rigida* were grown under different conditions (Fig. 1B). Both temperature and nitrate had a main effect on WHC (Table 3). The high temperature enhanced WHC by 16.88% while high nitrate reduced WHC by 8.11% (Fig. 1B). The pCO_2 treatments were non-significant (Table 3). Temperature and nitrate had interactive effects on WHC (Table 3) since high temperature enhanced WHC more under high nitrate (23.51%) than under low nitrate (10.96%) (Fig. 1B). In addition, the combination of high temperature, pCO_2, and nitrate increased WHC by 7.88%.

The WHC (ranging from 5.22 ± 0.10 to 7.24 ± 0.04 g g^{-1} DW) in this study was noticeably lower than that reported in *U. lactuca* by Wong and Cheung (2000) (9.71 ± 0.11 g g^{-1} DW) but comparable to Yaich et al. (2011) (6.66–7.00 g g^{-1} DW). Furthermore, the WHC of *U. rigida* was also comparable to some commercial dietary fibre-rich supplements (6.60–9.00 g g^{-1} DW) (Goñi & Martin-Carrón, 1998). According to Robertson and Eastwood (1981), water can be associated with fibre either as bound or trapped water. The amount of bound

Table 3
Three-way analysis of variance of the effects of nitrate, temperature (Temp), and pCO_2 on swelling capacity (SWC), water holding capacity (WHC) and protein & carbohydrate (PC) of *U. rigida*. The confidence interval was 95%.

Source	SWC		WHC		PC	
	F	Sig.	F	Sig.	F	Sig.
Temperature	215.470	< 0.001	181.803	< 0.001	60.454	< 0.001
pCO_2	3.073	0.099	0.289	0.598	1.325	0.267
Nitrate	8.675	0.010	53.586	< 0.001	33.762	< 0.001
Temperature*pCO_2	1.023	0.327	4.079	0.061	7.043	0.057
Temperature*Nitrate	1.570	0.228	16.425	0.001	3.009	0.102
pCO_2*Nitrate	6.081	0.055	1.083	0.313	0.324	0.577
Temperature*pCO_2*Nitrate	7.208	0.016	1.277	0.275	0.095	0.762

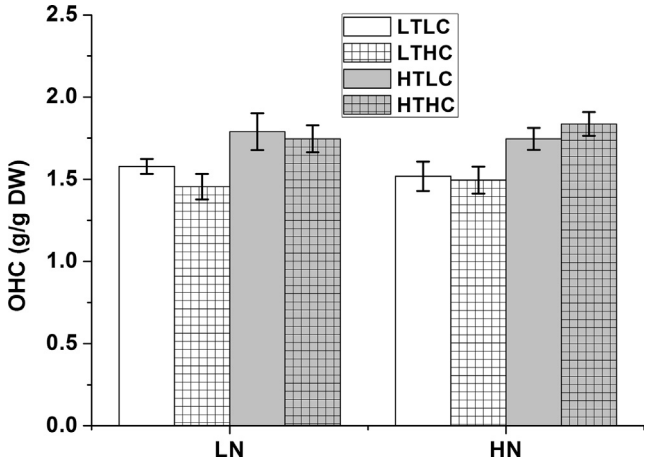

Fig. 2. Oil holding capacity of *U. rigida* grown under different conditions. LTLC: low temperature and low pCO_2; LTHC: low temperature and high pCO_2; HTLC: high temperature and low pCO_2; HTHC: high temperature and high pCO_2; LN: low nitrate; HN: high nitrate. Data are the means ± SD (n = 3).

Table 4
Three-way analysis of variance of the effects of nitrate, temperature, and pCO_2 on oil holding capacity of *U. rigida*. The confidence interval was 95%.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temperature	0.429	1	0.429	66.168	< 0.001
pCO_2	0.004	1	0.004	0.579	0.458
Nitrate	< 0.001	1	< 0.001	0.041	0.841
Temperature*pCO_2	0.014	1	0.014	2.147	0.162
Temperature*Nitrate	0.002	1	0.002	0.269	0.611
pCO_2*Nitrate	0.021	1	0.021	3.176	0.094
Temperature*pCO_2*Nitrate	< 0.001	1	0.000	0.065	0.801
Error	0.104	16	0.006		

water depends on the chemical composition whereas trapped water refers to the structure of the fibre. WHC, as determined by the centrifugation method used in this study, represented both types of water-fibre association. Apart from fibre, protein may also play a role in water holding that involves the protein conformations and nature of the water binding sites in the protein molecules (Chou & Morr, 1979). In this study, there was an explicit correlation between WHC and total content of protein plus carbohydrate in the seaweed samples, in which the high temperature and low nitrate increased both WHC and total content of protein plus carbohydrate (Fig. 1B, C and Table 3). Therefore, the hydration properties could be determined by both chemical components. The water-holding capacity can avoid syneresis and modify the viscosity and texture of formulated food. Furthermore, the increased viscosity due to water absorption could lead to slower rates of intestinal absorption. This property could make *Ulva* clinically useful in reducing blood cholesterol and postprandial glycaemia, decreasing risks of obesity and Type II diabetes (Willett, Manson, & Liu, 2002).

The OHC of *U. rigida* in this study ranged from 1.46 ± 0.08 to 1.84 ± 0.07 g g^{-1} DW (Fig. 2). Temperature had a main effect on OHC (Table 4). The high temperature (HT) treatment enhanced the oil holding capacity (OHC) by 17.69% (Fig. 2) while no significant effects of pCO_2 or nitrate were found (Table 4). There were no interactive effects of temperature, pCO_2 and nitrate on OHC and the combination of these three factors increased OHC by 16.32%.

The OHC in this study was much higher than the value reported in *U. lactuca* by Wong and Cheung (2000) (0.65 ± 0.03 g g^{-1} DW). This may be due to different treatment temperature. The OHC was determined at 37 °C in this study while it was determined at 25 °C in Wong and Cheung's study (2000). The effect of temperature on OHC was investigated by Yaich et al. (2011). The present results were similar to their findings (around 1.60 g g^{-1} DW at 40 °C). In general, protein plays a major role in fat absorption by hydrophobic bonding (Voutsinas & Nakai, 1983). Furthermore, the OHC of seaweeds is also related to the particle size, overall charge density and hydrophilic nature of fibres. Therefore, a high correlation (r = 1.00) between OHC and total amount of protein and dietary fibre was reported (Wong & Cheung, 2000). The high temperature increased OHC of *U. rigida* in this study which can be down to the increased content of total protein and carbohydrate at the high temperature. Due to their OHC, *Ulva* species can be used as stabilizers in formulate food products. Moreover, they can reduce blood lipid level, obesity and coronary heart disease risk and thus can be a valuable functional food. The combination of high temperature and nitrate increased OHC in *U. rigida* by 16.32% in the present study suggests that future ocean conditions may promote the value of *Ulva* species as a healthy food.

4. Conclusion

Little research has been done to study the effects of global change variables on food quality of seaweeds. The present study is the first document to investigate the effects of ocean warming, ocean acidification and eutrophication on chemical composition and functional properties in *Ulva* species. All three factors increased total fatty acids in *U. rigida*. Ocean warming and eutrophication increased total amino acids as well. In terms of functional properties, the combination of ocean warming, acidification, and eutrophication increased SWC, WHC, and OHC. The findings above indicate that the future ocean environment may enhance the content of amino acids, fatty acids, as well as SWC, WHC, and OHC, in *Ulva* species. These data also provide a foundation for how to set cultivation conditions in order to obtain specific chemical and functional properties of *Ulva* species.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2018.03.040.

References

Angell, A. R., Mata, L., Nys, R., & Paul, N. A. (2014). Variation in amino acid content and its relationship to nitrogen content and growth rate in *Ulva ohnoi* (Chlorophyta). *Journal of Phycology, 50*(1), 216–226.

Baede, A. P. M., Ahlonsou, E., Ding, Y., Schimel, D., Bolin, B., & Pollonais, S. (2001). The climate system: An overview. In J. T. Houghton (Ed.). *Climate change 2001: The scientific basis* (pp. 87–98). Cambridge: Cambridge University Press.

Bartsch, I., Wiencke, C., & Laepple, T. (2012). Global seaweed biogeography under a changing climate: The prospected effects of temperature. In C. Wiencke, & K. Bischof (Eds.). *Seaweed biology* (pp. 383–406). Berlin: Springer.

Bolton, J. J., Cyrus, M. D., Brand, M. J., Joubert, M., & Macey, B. M. (2016). Why grow *Ulva*? Its potential role in the future of aquaculture. *Perspectives in Phycology, 3*(3), 113–120.

Brennan, L., & Owende, P. (2010). Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews, 14*(2), 557–577.

Bricker, S. B., Longstaff, B., Dennison, W., Jones, A., Boicourt, K., Wicks, C., & Woerner, J. (2008). Effects of nutrient enrichment in the nation's estuaries: A decade of change. *Harmful Algae, 8*(1), 21–32.

Chou, D. H., & Morr, C. V. (1979). Protein-water interactions and functional properties. *Journal of the American Oil Chemists' Society, 56*(1), A53–A62.
Dettmar, P. W., Strugala, V., & Richardson, J. C. (2011). The key role alginates play in health. *Food Hydrocolloids, 25*(2), 263–266.
Dillehay, T. D., Ramírez, C., Pino, M., Collins, M. B., Rossen, J., & Pino-Navarro, J. D. (2008). Monte Verde: Seaweed, food, medicine, and the peopling of South America. *Science, 320*(5877), 784–786.
Floreto, E. A. T., Hirata, H., Ando, S., & Yamasaki, S. (1993). Effects of temperature, light intensity, salinity and source of nitrogen on the growth, total lipid and fatty acid composition of *Ulva pertusa* Kjellman (Chlorophyta). *Botanica Marina, 36*(2), 149–158.
Gao, G., Clare, A. S., Rose, C., & Caldwell, G. S. (2017). Eutrophication and warming-driven green tides (*Ulva rigida*) are predicted to increase under future climate change scenarios. *Marine Pollution Bulletin, 114*(1), 439–447.
Goñi, I., & Martin-Carrón, N. (1998). In vitro fermentation and hydration properties of commercial dietary fiber-rich supplements. *Nutrition Research, 18*(6), 1077–1089.
Gordillo, F. J., Jiménez, C., Figueroa, F. L., & Niell, F. X. (1998). Effects of increased atmospheric CO_2 and N supply on photosynthesis, growth and cell composition of the cyanobacterium *Spirulina platensis* (Arthrospira). *Journal of Applied Phycology, 10*(5), 461–469.
Gordillo, F. J. L., Jiménez, C., Goutx, M., & Niell, X. (2001). Effects of CO_2 and nitrogen supply on the biochemical composition of *Ulva rigida* with especial emphasis on lipid class analysis. *Journal of Plant Physiology, 158*(3), 367–373.
Gosch, B. J., Magnusson, M., Paul, N. A., & Nys, R. (2012). Total lipid and fatty acid composition of seaweeds for the selection of species for oil-based biofuel and bio-products. *Global Change Biology Bioenergy, 4*(6), 919–930.
Guschina, I. A., & Harwood, J. L. (2009). Algal lipids and effect of the environment on their biochemistry. In M. T. Arts, Brett, & M. J. Kainz (Eds.). *Lipids in aquatic ecosystems* (pp. 1–24). Dordrecht: Springer.
Heldt, H. W., & Piechulla, B. (2005). Nitrate assimilation is essential for the synthesis of organic matter. In H. W. Heldt, & B. Piechulla (Eds.). *Plant biochemistry* (pp. 273–306). San Diego: Academic Press.
Iken, K. (2012). Grazers on benthic seaweeds. In C. Wienche, & K. Bischof (Eds.). *Seaweed biology* (pp. 157–175). Berlin: Springer.
IPCC (2013). Climate change 2013: The physical science basis. In T. F. Stocker, D. Qin, G.-. K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, & P. M. Midgley (Eds.). *Working group I contribution to the fifth assessment report of the intergovernmental panel on climate change* (pp. 6–10). New York: Cambridge Univ Press.
Joseph, J. D., & Ackman, R. G. (1992). Capillary column gas chromatographic method for analysis of encapsulated fish oils and fish oil ethyl esters: Collaborative study. *Journal of AOAC International, 75*(3), 488–506.
Kang, J. W., & Chung, I. K. (2017). The effects of eutrophication and acidification on the ecophysiology of *Ulva pertusa* Kjellman. *Journal of Applied Phycology, 29*(5), 2675–2683.
McCauley, J. I., Meyer, B. J., Winberg, P. C., & Skropeta, D. (2016). Parameters affecting the analytical profile of fatty acids in the macroalgal genus *Ulva*. *Food Chemistry, 209*, 332–340.
Ménesguen, A., Desmit, X., Dulière, V., Geneviève, L., Thouvenin, B., Thieu, V., & Dussauze, M. (2018). How to avoid eutrophication in coastal seas? A new approach to derive river-specific combined nitrate and phosphate maximum concentrations. *Science of The Total Environment, 628*, 400–414.
Mohsen, A. F., Nasr, A. H., & Metwalli, A. M. (1973). Effect of temperature variations on growth, reproduction, amino acid synthesis, fat and sugar content in *Ulva fasciata* delile plants. *Hydrobiologia, 42*(4), 451–460.
Msuya, F. E., & Neori, A. (2008). Effect of water aeration and nutrient load level on biomass yield, N uptake and protein content of the seaweed *Ulva lactuca* cultured in seawater tanks. *Journal of Applied Phycology, 20*(6), 1021–1031.
Naldi, M., & Wheeler, P. A. (1999). Changes in nitrogen pools in *Ulva fenestrata* (Chlorophyta) and *Gracilaria pacifica* (Rhodophyta) under nitrate and ammonium enrichment. *Journal of Phycology, 35*(1), 70–77.
Ortiz, J., Romero, N., Robert, P., Araya, J., Lopez-Hernandez, J., Bozzo, C., et al. (2006). Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. *Food Chemistry, 99*(1), 98–104.
Peña-Rodríguez, A., Mawhinney, T. P., Ricque-Marie, D., & Cruz-Suárez, L. E. (2011). Chemical composition of cultivated seaweed *Ulva clathrata* (Roth) C. Agardh. *Food Chemistry, 129*(2), 491–498.
Reed, D. C., Brzezinski, M. A., Coury, D. A., Graham, W. M., & Petty, R. L. (1999). Neutral lipids in macroalgal spores and their role in swimming. *Marine Biology, 133*(4), 737–744.
Robertson, J. A., & Eastwood, M. A. (1981). An examination of factors which may affect the water holding capacity of dietary fibre. *British Journal of Nutrition, 45*(01), 83–88.
Rodolfi, L., Chini Zittelli, G., Bassi, N., Padovani, G., Biondi, N., Bonini, G., & Tredici, M. R. (2009). Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering, 102*(1), 100–112.
Smith, S. V., Swaney, D. P., Talaue-Mcmanus, L., Bartley, J. D., Sandhei, P. T., McLaughlin, C. J., et al. (2003). Humans, hydrology, and the distribution of inorganic nutrient loading to the ocean. *Bioscience, 53*(3), 235–245.
Steinhoff, F. S., Graeve, M., Wiencke, C., Wulff, A., & Bischof, K. (2011). Lipid content and fatty acid consumption in zoospores/developing gametophytes of *Saccharina latissima* (Laminariales, Phaeophyceae) as potential precursors for secondary metabolites as phlorotannins. *Polar Biology, 34*(7), 1011–1018.
Tang, D., Han, W., Li, P., Miao, X., & Zhong, J. (2011). CO_2 biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO_2 levels. *Bioresource Technology, 102*(3), 3071–3076.
Trigui, M., Gasmi, L., Zouari, I., & Tounsi, S. (2013). Seasonal variation in phenolic composition, antibacterial and antioxidant activities of *Ulva rigida* (Chlorophyta) and assessment of antiacetylcholinesterase potential. *Journal of Applied Phycology, 25*(1), 319–328.
Tsubaki, S., Oono, K., Hiraoka, M., Ondab, A., & Mitanid, T. (2016). Microwave-assisted hydrothermal extraction of sulfated polysaccharides from *Ulva* spp. and *Monostroma latissimum*. *Food Chemistry, 210*, 311–316.
Tsuzuki, M., Ohnuma, E., Sato, N., Takaku, T., & Kawaguchi, A. (1990). Effects of CO_2 concentration during growth on fatty acid composition in microalgae. *Plant Physiology, 93*(3), 851–856.
Voutsinas, L. P., & Nakai, S. (1983). A simple turbidimetric method for determining the fat binding capacity of proteins. *Journal of Agricultural and Food Chemistry, 31*(1), 58–63.
Willett, W., Manson, J., & Liu, S. (2002). Glycemic index, glycemic load, and risk of type 2 diabetes. *The American Journal of Clinical Nutrition, 76*(1), 274S–280S.
Wong, K. H., & Cheung, P. C. K. (2000). Nutritional evaluation of some subtropical red and green seaweeds: Part I—proximate composition, amino acid profiles and some physico-chemical properties. *Food Chemistry, 71*(4), 475–482.
Yaich, H., Garna, H., Besbes, S., Paquot, M., Blecker, C., & Attia, H. (2011). Chemical composition and functional properties of *Ulva lactuca* seaweed collected in Tunisia. *Food Chemistry, 128*(4), 895–901.
Zhu, C. J., Lee, Y. K., & Chao, T. M. (1997). Effects of temperature and growth phase on lipid and biochemical composition of *Isochrysis galbana* TK1. *Journal of Applied Phycology, 9*(5), 451–457.