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Article

Sustainable Large-Scale Aquaculture of the Northern Hemisphere Sea Lettuce, *Ulva fenestrata*, in an Off-Shore Seafarm

Sophie Steinhagen [1,*], Swantje Enge [1], Karin Larsson [2], Joakim Olsson [3], Göran M. Nylund [1], Eva Albers [3], Henrik Pavia [1], Ingrid Undeland [2] and Gunilla B. Toth [1]

[1] Department of Marine Sciences-Tjärnö, University of Gothenburg, SE-452 96 Strömstad, Sweden; swantje.enge@marine.gu.se (S.E.); goran.nylund@marine.gu.se (G.M.N.); henrik.pavia@marine.gu.se (H.P.); gunilla.toth@marine.gu.se (G.B.T.)
[2] Department of Biology and Biological Engineering-Food and Nutrition Science, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden; karin.larsson@chalmers.se (K.L.); undeland@chalmers.se (I.U.)
[3] Department of Biology and Biological Engineering-Industrial Biotechnology, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden; joakim.olsson@chalmers.se (J.O.); eva.albers@chalmers.se (E.A.)
* Correspondence: sophie.steinhagen@gu.se

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Abstract: The growing world population demands an increase in sustainable resources for biorefining. The opening of new farm grounds and the cultivation of extractive species, such as marine seaweeds, increases worldwide, aiming to provide renewable biomass for food and non-food applications. The potential for European large-scale open ocean farming of the commercial green seaweed crop *Ulva* is not yet fully realized. Here we conducted manipulative cultivation experiments in order to investigate the effects of hatchery temperature (10 and 15 °C), nutrient addition (PES and 3xPES) and swarmer density (500 and 10,000 swarmers ml^{-1}) on the biomass yield and biochemical composition (fatty acid, protein, carbohydrate, pigment and phenolic content) of off-shore cultivated *Ulva fenestrata* in a Swedish seafarm. High seedling densities were optimal for the growth of this northern hemisphere crop strain and significantly increased the mean biomass yield by ~84% compared to low seedling densities. Variations of nutrients or changes in temperature levels during the hatchery phase were not necessary to increase the subsequent growth in an open-water seafarm, however effects of the factors on the thallus habitus (thallus length/width) were observed. We found no significant effect of the environmental factors applied in the hatchery on the total fatty acid or crude protein content in the off-shore cultivated *Ulva*. However, low seedling density and low temperature increased the total carbohydrate content and furthermore, high temperature in combination with high nutrient levels decreased the pigment content (chlorophyll a, b, carotenoids). Low temperature in combination with high nutrient levels increased the phenolic content. Our study confirms the successful and sustainable potential for large-scale off-shore cultivation of the Scandinavian crop *U. fenestrata*. We conclude that high seedling density in the hatchery is most important for increasing the total biomass yield of sea-farmed *U. fenestrata*, and that changing temperature or addition of nutrients overall does not have a large effect on the biochemical composition. To summarize, our study contributes novel insights into the large-scale off-shore cultivation potential of northern hemisphere *U. fenestrata* and underpins suitable pre-treatments during the hatchery phase of seedlings to facilitate a successful and cost-efficient large-scale rope cultivation.

Keywords: *U. fenestrata*; *U. lactuca*; aquaculture; biochemical composition; protein; carbohydrate; fatty acids

1. Introduction

As the world population continues to grow, the urgent need for sustainable biomasses that can be converted to nutritious food, renewable materials and novel biomolecules was emphasized by the sustainability goals of the United Nations (UN General Assembly, 2015). A central point of reaching these important goals is a sustainable increase of agricultural production, which is concomitant with the development, successful establishment and subsequent usage of new, sustainable resources and farm grounds. According to present-day research, oceans remain the only environment capable of extensive but yet sustainable agricultural expansion, e.g., [1]. Aquaculture, which is defined as the husbandry and farming of aquatic animals and plants [2], is recently among the fastest expanding economies and achieved a 7.5% annual growth rate between 1990 and 2009 [3]. Seaweed aquaculture in particular is worth more than 6 billion USD (US Dollar) per year and is a continuously growing industry worldwide [2].

Seaweeds contain a large number of high-value compounds which make it suitable for a wide range of applications [2]. Besides being commercially exploited by traditional markets of food and phycocolloids (e.g., alginates, agars, carrageenans) seaweeds are, for example, used as animal feed to improve health and productivity [4,5] and to reduce green-house gas emissions of cattle [6,7]. Furthermore, seaweed-derived products function as plant fertilizers and soil conditioners [8,9]. The cell components of seaweeds are additionally used in the biomaterials sector [10–13] and can provide alternative replacements for fossil fuels [14,15].

Even though less than 0.1% of the total seaweed production is accounted for by green seaweeds [2,16], the green seaweed *Ulva*—generally known as Sea Lettuce—has received a lot of attention by the aquaculture sector due to its compelling traits [17–19]. Combining the characteristics of being ubiquitously distributed [20], having a high environmental tolerance and being resistant towards changing abiotic factors [21–23], *Ulva* spp. exhibit high and fast growth rates [18,24] and are capable of thriving under high stocking densities [25,26], which makes them excellent aspirants for large-scale aquacultures.

Several important economic sectors are already profiting off the multipurpose usage of *Ulva* biomass. *Ulva* biomass can be rich in protein (4–44% dw) [27], essential amino acids [28], fatty acids (0.3–6.1%) [27], minerals, antioxidants, vitamins and dietary fibers [29,30] and thus exhibits great nutritional properties and benefits from direct consumption as food and feed [31–33]. Additionally, value-added products such as functional foods, cosmeceuticals, nutraceuticals and pharmaceuticals can be produced from their many bioactive compounds [34,35]. *Ulva* biomass can exhibit high total carbohydrate contents (15–65% dw) [27,36,37] and comprises the soluble sulphated polysaccharide ulvan. Ulvan can be used in water-conditioning hydrogels [11] and can be processed into heparin-like oligosaccharides as well as into rare monosaccharides, such as rhamnose and iduronic acid [10]. Recent studies have shown that environmental growth conditions have significant effects on the relative growth rate as well as on the biochemical composition of the abovenamed high-value compounds, e.g., [36–40] which underlines the importance of the optimization of cultivation conditions in aquaculture settings [40].

To date, cultivation of *Ulva* spp. in Europe has mainly been limited to coastal near-shore areas (cages, nets) and on-shore tanks, basins or (paddle wheel) pond-based (in- and outdoor) cultivation methods [17,24,41,42]. Land-based cultivation systems are especially challenged by their dependence on the massive intake of seawater [43,44] and the distinctive fixed and variable costs for construction, operation and maintenance [24]. Consequently, tank cultivation requires high power inputs and the use of expensive materials and equipment and is, if not operated effectively, in most cases too costly and inappropriate for commercial-scale production of seaweeds [45]. However, it has been shown that on-land-based tank cultivation produces the highest yields of biomass (per m^2 of water surface) in comparison to comparable cultivation methods [45]. Furthermore, it offers several additional advantages such as full control over the cultivation parameters which allows for manipulative cultivation as well as simple operation during harvest periods. Nevertheless, to be able to compete with terrestrial crops, cost-efficient methods for a sustainable large-scale production of *Ulva* biomass, as well as evaluations and breeding of best performing crop strains, are urgently needed. This is especially true for northern hemisphere cultivations where irradiance and temperature regimes strongly fluctuate.

The overall aim of this study was to assess the potential for large-scale aquaculture of Scandinavian *Ulva fenestrata* Postels and Ruprecht in a Swedish offshore seaweed farm. We investigated how changes in hatchery cultivation conditions (single or interactive effects of temperature, level of growth medium addition and gamete density levels) affect the growth and biochemical composition (total fatty acid, crude protein, carbohydrate, pigment and phenolic content) of the cultivated biomass.

2. Materials and Methods

2.1. Algal Source Material and Fertility Induction

Clonal, gametophytic algal material for this study was taken from a long-term indoor tank cultivation located at the Tjärnö Marine Laboratory, University of Gothenburg, Sweden (TML, 58°52′36.4′′ N 11°6′42.84′′ E). Because the genus *Ulva* exhibits several species with extraordinary phenotypic plasticity [20–23], adequate identification of the used biomass can, in most of the cases, only be obtained by applying modern molecular identification techniques such as DNA barcoding. Detailed information on applied cultivation conditions as well as molecular identification of the parental biomass of *U. fenestrata* can be found in [40].

To induce fertility and thus obtain gametes of the gametophytic strain of *U. fenestrata*, round discs with a radius of 4 cm were punched out from the vegetative thallus tissue and subsequently transferred into seawater-filled 14 L aquaria at 10 °C. Permanent aeration was applied. After 4–5 days a darkening of the thalli was observed and the formation of gametangia was validated by light microscopy. The fertile material was washed under sterile filtrated seawater and transferred to a beaker filled with approximately 80–100 mL of sterile seawater. After transferring the discs, the gametangia immediately started to release the motile gametes. To concentrate the gamete solution, a centrifugation step in a chilled centrifuge (10 °C) at 4000 rpm for 5 min was carried out. To induce immobilization of the motile, phototactic gametes, the concentrated solution was kept in the dark for 24 h at 10 °C. The density of swarmers was calculated by the help of a hemocytometer.

2.2. Experimental Setup

The following experiment was conducted to examine if manipulated hatchery conditions affect the later growth performance and biochemical composition of off-shore cultivated *U. fenestrata* in a large-scale seafarm. An orthogonal design with two levels of gamete density (low and high), temperature (10 and 15 °C) and nutrient supply (two concentrations of growth medium) was used to manipulate the hatchery conditions. The concentrated solution of immobilized gametes was diluted into different stock solutions containing 500 (low density, LD) and 10,000 (high density, HD) gametes mL^{-1}. The solutions were applied to spools which were coiled with 10 m (±50 cm) of nylon cord (ø = 2–3 mm) and had an absorbance of 7 AU. The spools were submersed in 1 L aquaria supplied with sterile filtered (0.2 µm + UV, 9 L h^{-1}) seawater at an average irradiance of 80–100 µmol m^{-2} s^{-1} under a 12:12 h L:D light regime (light source: OSRAM Lumilux Cool daylight L 58W/865). The settled gametes were allowed to grow in the hatchery for six weeks between September to October 2019 in a temperature-controlled room (10 °C). Elevated water temperature of 15 °C (±1 °C) was achieved using submersed heaters (EHEIM Aquarium Heaters, 600–1000 L. 230 V, 300 W). Growth medium (1× PES or 3× PES, respectively) was added once per week following the concentration specifications of [46] and was connected with a weekly performed water change. To prevent diatom growth, 1 mg L^{-1} GeO_2 was added to all treatments.

After growing for six weeks under hatchery conditions, the juvenile thalli were acclimatized to the prevailing Swedish late-fall conditions by decreasing the hatchery temperature to 8 °C with steps of 0.5–1 °C per day over one week. After one more week of acclimatization, the seaweeds were deployed in an off-shore seafarm (2 ha á 100 × 200 m)

(Figure 1), located in the sheltered waters of the Koster archipelago (Skagerrak), Sweden (N58.859901, E11.068660). The Kosterhavet national park is characterized by rocky shores which are typical for the Scandinavian west coast of Sweden and Norway and it is one of the most species-rich marine areas in Sweden. The mean salinity of the surface water at the cultivation site (during cultivation periods, i.e., October–May) is 27.6 ± 3.3 PSU (±SD) with a mean temperature of 7 ± 4.2 °C (±SD). For a detailed schematic representation of the seafarm see Figure 1. The nylon cords with the juvenile thalli from the different treatments ($n = 5$) were applied in a random order to the ropes of the seaweed farm. In April 2020, after a growth period of six months, the seaweeds were harvested and all biomass from different hatchery treatments was stored separately in plastic bags within a chilled container until further processing in the laboratory on the same day of harvest ($n = 5$; i.e., a total of 40 spools). Subsequent analyses of the below-described biochemical composition were performed on each of the five replicates per treatment; furthermore, two technical replicates per sample were included.

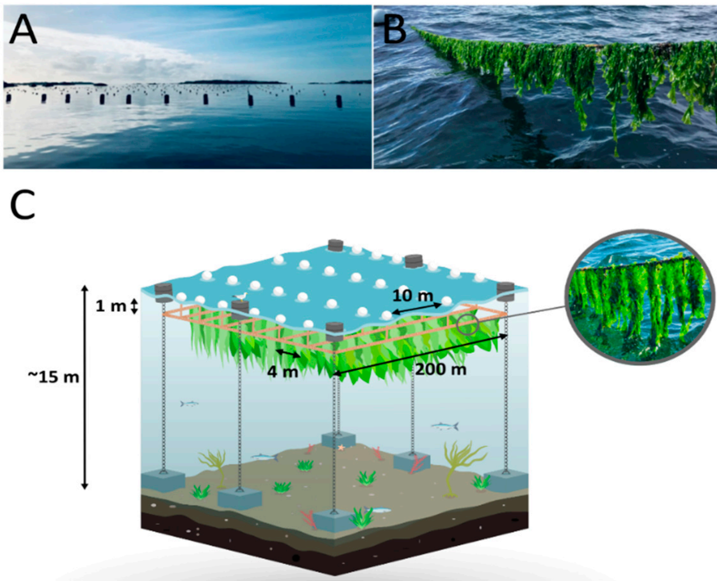

Figure 1. Overview about the off-shore cultivation site located in the Swedish Koster archipelago, Skagerrak. (**A**) Picture of the seafarm with (**B**) *Ulva fenestrata* individuals before their harvest in April. (**C**) Schematic overview about the experimental setup. The nylon chord with the attached juvenile seaweed thalli was coiled and fixed around fabric long ropes (each 200 m). The long ropes were attached by buoys at each site, which were anchored to the bottom. To keep the ropes suspended at 1–1.50 m below the water surface, buoys were added every 10 m to the rope. The ropes were arranged in parallel rows at intervals of 4 m.

2.3. Growth Measurements

The biomass yield was determined immediately after harvest and was expressed as fresh weight (fw) and dry weight (dw) (after lyophilization) per m $[rope]^{-1}$. Photographs were taken of ten randomly chosen individuals per replicate spool and the average length and width of the thalli was quantified using ImageJ [47]. Since no fouling organisms were detected, no measurements on the amount of epibionts were carried out. The biomass of each sample was frozen, lyophilized, homogenized and stored at −80 °C before further analysis of the biochemical composition. Dw was determined on a lab-scale (Sartorius TE1502S) after lyophilization.

2.4. Protein Content and Fatty Acid Content and Composition

Total nitrogen content of seaweed was determined by the combustion method (Dumas) using a LECO Trumac nitrogen analyzer. Protein content was calculated using a nitrogen-to-protein conversion factor of 5 [48].

Fatty acid content and composition in ~25 mg lyophilized seaweed were determined by a direct transesterification method described in detail by [49] using C17:0 as the internal standard for quantification. Identification of fatty acids was done using GLC-463 Reference standards (Nu-Check Prep, Inc., Elysian, MN, USA). In addition, C16:1n9, C16:2n6, C16:3n3, C16:4n3, C18:4n3 and C20:4n3 were identified using the MS library.

2.5. Carbohydrate Content and Composition

The total carbohydrate content and composition were calculated as previously described by Olsson et al. [37]. In short, freeze-dried biomass samples were hydrolyzed in two steps with sulphuric acid and released monosaccharides were analyzed by high-performance anion exchange chromatography; A Thermo ScientificTM DionexTM, ICS-3000 system (Dionex, Sunnyvale, CA, USA) with a pulsed amperometric detector was used with a Dionex CarbopacTM PA1 4 mm × 250 mm column and a 4 mm × 50 mm guard.

2.6. Total Phenolic Content

Total phenolic content in off-shore cultivated *U. fenestrata* was extracted using 60 mg lyophilized and homogenized algal material in 1.5 mL of 70% ethanol for 1.5 h in 20 °C. After extraction, the samples were centrifuged (1 min at 14,000 rpm and 20 °C) and the supernatant was collected. Total phenolic content was estimated colorimetrically using the Folin-Ciocalteu phenol reagent (Merck) with gallic acid (Sigma-Aldrich) as a standard. One mL supernatant and 0.5 mL Folin-Ciocalteu's reagent were mixed with 7 mL distilled H_2O, after which 1.5 mL Na_2CO_3 (200 gL^{-1}, Merck) was added. The samples were incubated for 2 h at 20 °C, after which the absorbance was measured spectrophotometrically at 765 nm (Lambda XLS+, Perkin Elmer). Total phenolic content was calculated as % of dw.

2.7. Pigment (Chlorophyll a, b, Carotenoids) Analysis

Total content of chlorophyll a, b, and carotenoids of *U. fenestrata* were extracted using 60 mg of lyophilized and homogenized algal material in 10 mL of 90% acetone. The samples were ultrasonicated for 10 min and subsequently placed on an orbital shaker for 1 h in 20 °C in darkness. After extraction, the samples were centrifuged (5 min at 4000 rpm and 20 °C) and the supernatant was collected. Absorbance was measured on a spectrophotometer at four different wavelengths: 647 nm, 664 nm, 510 nm and 480 nm. The total content of chlorophyll a and b was calculated using the spectrophotometric equations for higher plants and green algae [50] and total carotenoids [51].

2.8. Statistical Analysis

Data on the total amount of biomass yield (dw, fw), thallus length and width, and the biochemical composition of *U. fenestrata* from the experiments in the present study were statistically analyzed in JMP (JMP®, Version 15, SAS Institute Inc., Cary, NC, USA) using orthogonal 3-way analysis of variance (ANOVA, Tables 1 and 2) with temperature, nutrients and density as fixed 2-level factors.

Significant differences among means were compared using the Student's *t*-test in JMP. Before statistical analysis, data were tested for normality using Shapiro-Wilk test and for homogeneity of variances using Cochran's test [52]. To meet the assumption of normality and homogeneity of variances the data on biomass, thallus length and thallus width were square root transformed prior to statistical analyses.

Table 1. ANOVAs of (**a**) dry weight (g [m rope]$^{-1}$), (**b**) thallus length (cm), (**c**) thallus width (cm) (**d**) fatty acid content (% dw), (**e**) protein content (% dw), (**f**) carbohydrate content (% dw), (**g**) chlorophyll a (µg mg^{-1}), (**h**) chlorophyll b (µg mg^{-1}), and (**i**) carotenoids (µg mg^{-1}) in *Ulva fenestrata* cultivated under different levels of temperature, nutrients and densities. Data on mean values and SEM are presented in Figures 2 and 3. Significant *p*-values are indicated with italics and red color.

Source of Variance		**(a) Dry Weight**			**(b) Thallus Length**			**(c) Thallus Width**			**(d) Total Fatty Acids**			**(e) Crude Proteins**			**(f) Carbohydrates**		
	Df	MS	F ratio	*p*	MS	F ratio	*p*	MS	F ratio	*p*	MS	F ratio	*p*	MS	F ratio	*p*	MS	F ratio	*p*
Temperature	1	1.21	1.67	0.20	1.63	40.28	*<0.01*	2.06	60.05	*<0.01*	0.094	2.42	0.881	0.17	0.022	0.918	16.32	4.90	*0.034*
Nutrients	1	3.17	4.40	*0.04*	<1.01	0.12	0.73	0.07	2.23	0.13	0.077	1.99	0.151	16.45	2.16	0.151	1.31	0.39	0.535
Density	1	114.2	158.4	*<0.01*	0.15	3.81	0.052	0.01	0.51	0.47	>0.001	0.001	0.179	14.34	1.88	0.169	19.15	5.75	*0.022*
Temperature × Nutrients	1	3.87	5.36	0.02	0.69	17.23	*<0.01*	0.47	13.73	*<0.01*	0.032	0.83	0.840	0.32	0.041	0.838	0.083	0.025	0.875
Temperature × Density	1	0.63	0.87	0.35	<0.01	0.001	0.97	<0.01	0.001	0.97	0.118	3.06	0.097	22.14	2.91	0.096	0.54	0.162	0.690
Nutrients × Density	1	0.19	0.26	0.60	0.52	13.01	*<0.01*	0.39	11.38	*<0.01*	0.04	1.05	0.926	0.06	0.008	0.907	13.03	3.914	0.057
Temp. × Nut. × Dens.	1	4.21	5.84	0.02	0.05	1.37	0.242	0.03	0.91	0.33	0.022	0.56	0.838	0.32	0.042	0.847	9.67	2.903	0.098
Residual	32	0.721			0.041			0.034			0.0388			7.59			3.331		

Source of variance		**(g) Chlorophyll a**			**(h) Chlorophyll b**			**(i) Carotenoids**			**(j) Phenolic**		
	Df	MS	F ratio	*p*	MS	F ratio	*p*	MS	F ratio	*p*	MS	F ratio	*p*
Temperature	1	0.167	3.648	0.065	0.318	3.661	0.065	0.053	5.391	*0.026*	<0.01	0.118	0.733
Nutrients	1	0.088	1.925	0.175	0.042	0.483	0.492	0.276	2.797	0.104	<0.01	0.197	0.660
Density	1	0.053	1.168	0.288	0.095	1.101	0.302	0.004	0.042	0.838	<0.01	2.930	0.097
Temperature × Nutrients	1	0.224	4.892	*0.034*	0.426	4.896	*0.034*	0.645	6.520	*0.015*	0.012	5.411	*0.027*
Temperature × Density	1	0.028	0.620	0.437	0.010	0.118	0.734	0.126	1.277	0.266	0.016	7.391	*0.011*
Nutrients × Density	1	0.034	0.747	0.394	0.120	1.386	0.248	0.011	1.178	0.286	<0.01	0.092	0.763
Temp. × Nut. × Dens.	1	0.631	1.376	0.249	0.187	0.215	0.646	0.008	0.886	0.353	<0.01	0.399	0.531
Residual	32	0.045			0.087			0.009			<0.01		

Table 2. Student's test results of significant 2-way interactions (see also ANOVA Table 1) with respective values of mean.

Interactions		(a) Thallus Length (cm)		(b) Thallus Width [cm]		(c) Chla ($mg \cdot g^{-1}$)		(d) Chlb ($mg \cdot g^{-1}$)		(e) Carotenoids ($mg \cdot g^{-1}$)		(f) Phenolic (%)	
Temp.	**Nut.**	**Student's Test**	**Mean**	**Student's Test**	**Mean**	**Student's Test**	**Mean**	**Student's Test**	**Mean**	**Student's Test**	**Mean**	**Student's Test**	**Mean**
10 °C	PES	B	43.03	B	13.31	A	1.64	AB	1.11	A	0.69	A	0.24
10 °C	PESx3	A	57.04	A	18.30	A	1.69	A	1.25	A	0.72	A	0.29
15 °C	PES	BC	36.94	C	10.46	A	1.66	A	1.14	A	0.70	A	0.27
15 °C	PESx3	C	31.41	C	9.31	B	1.42	B	0.87	B	0.57	A	0.25
Temp.	**Dens.**	**Student's Test**	**Mean**	**Student's Test**	**Mean**								
10 °C	HD	-	-	-	-	-	-	-	-	-	-	A	0.30
10 °C	LD	-	-	-	-	-	-	-	-	-	-	B	0.23
15 °C	HD	-	-	-	-	-	-	-	-	-	-	B	0.25
15 °C	LD	-	-	-	-	-	-	-	-	-	-	AB	0.27
Nut.	**Dens.**	**Student's Test**	**Mean**	**Student's Test**	**Mean**								
PES	HD	B	38.10	C	15.50	-	-	-	-	-	-	-	-
PES	LD	B	41.86	B	12.86	-	-	-	-	-	-	-	-
PESx3	HD	A	52.20	A	15.00	-	-	-	-	-	-	-	-
PESx3	LD	B	36.25	BC	12.11	-	-	-	-	-	-	-	-

3. Results

3.1. Biomass Yield, Growth and Performance

We found a significant interaction between temperature, nutrient addition and swarmer density treatments on the dw of off-shore cultivated *U. fenestrata* in the hatchery phase of cultivation (Table 1a). When means were compared with the Student's *t*-test, we found that the high swarmer density treatment resulted in a significant ($p < 0.05$) 84.1% higher dw yield in the off-shore seafarm compared to low swarmer density (Figure 2A). Furthermore, nutrient addition in the high swarmer density treatments had a significant ($p < 0.05$) opposite effect on dw yield in different temperatures. When grown in 10 °C in the hatchery, a simultaneous high nutrient addition resulted in 27.4% higher dw yield, while in 15 °C a high nutrient addition resulted in 45.5% lower dw yield compared to a low nutrient addition (Figure 2A). In the low swarmer density treatment, nutrient addition and temperature did not affect subsequent dw yield in the off-shore seafarm (Figure 2A).

Significant interactions between temperature and nutrients as well as between nutrients and density were observed when data on mean thallus habitus/size (measured as total length and width) of off-shore cultivated *U. fenestrata* was analyzed (Table 1b,c). Thallus length and width increased on average with 51.83% and 65.09% at 10 °C/PESx3 and with 28.81% and 24.46% at PESx3/HD compared to the means of the other treatment combinations (Figure 2B,C, Table 2a,b). In general, a positive effect of low temperature in combination with high nutrients and density on the average thallus width and length was observed (Figure 2B,C, Table 2a,b).

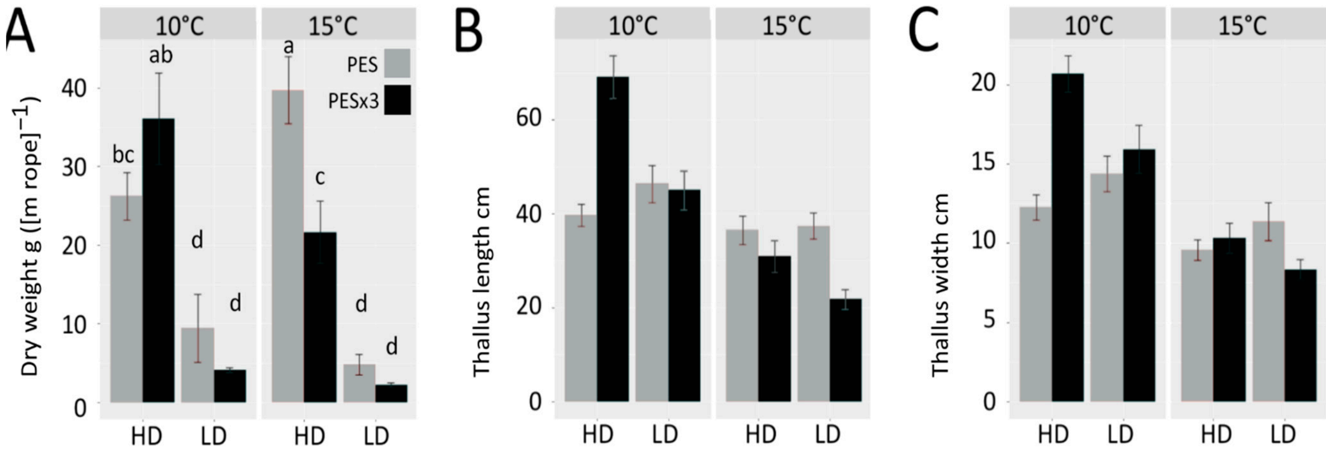

Figure 2. Mean total dry weight (g [m rope]$^{-1}$) (**A**), mean thallus length (cm) (**B**) and mean thallus width (cm) (**C**) of off-shore cultivated *Ulva fenestrata* after exposure to different pre-treatments (temperature, nutrient addition and density) during an indoor hatchery phase ($n = 5$). Error bars show SEM.

3.2. Fatty Acid Content and Relative Composition

The mean total fatty acid content of off-shore cultivated *U. fenestrata* was in a range of 3.2–3.55% on a dry weight (dw) basis (Figure 3A). We found no statistically significant effects of different hatchery conditions on the total fatty acid content in off-shore cultivated *U. fenestrata* (Table 1d).

The relative content of the 22 most prevalent fatty acids was analyzed (Figure 4, Table S1). Our results show that alpha-linolenic acid (C18:3n3) (~22% ± SEM) and palmitic acid (C16:0) (~21% ± SEM) occurred in highest percentages, followed by stearidonic-acid (C18:4n3) (~15% ± SEM), hexadecatetraenoic acid (C16:4n3) (~14% ± SEM), vaccenic acid (C18:1n7) (~11% ± SEM) and linoleic acid (C18:2n6) (~4% ± SEM). Besides the abovenamed fatty acids, some of the health-beneficial long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) were detected, including *docosapentaenoic* acid (C22:5n3) (~3% ± SEM) and eicosapentaenoic acid (C20:5n3) (~0,8–1% ± SEM) (for detailed FA composition see Figure 4 and Supplementary Table S1).

Among the abovenamed six most common fatty acids, the relative proportion only varied for C18:3n3, C16:0, C16:4n3 and C18:1n7 and no statistically significant effect was observed for variations in proportions of C18:4n3 and C18:2n6 among the treatments (Table 3).

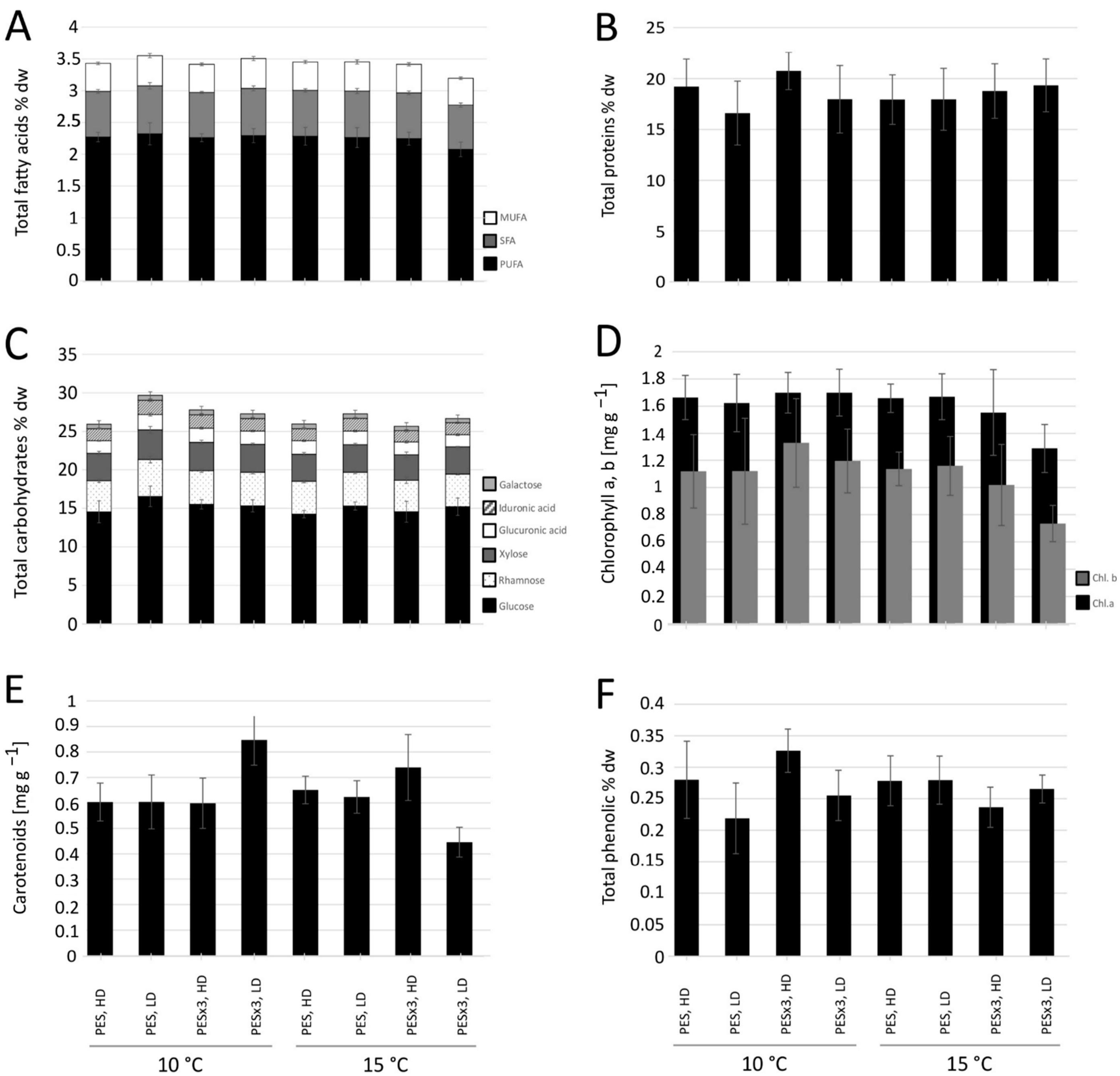

Figure 3. Mean (**A**) total fatty acid (% dw), (**B**) protein (% dw), (**C**) total carbohydrate (% dw), (**D**) chlorophyll a and b (mg g^{-1}), (**E**) carotenoids (mg g^{-1}) and (**F**) phenolic (% dw) content in off-shore cultivated *Ulva fenestrata* after exposure to different pre-treatments (temperature, nutrient addition and density) during an indoor hatchery phase (n = 5). Error bars show SD.

Table 3. ANOVAs of (a) C18:3n3 % of total fatty acids, (b) C16:0 % of total fatty acids, (c) C18:4n3 % of total fatty acids (d) C16:4n3 % of total fatty acids, (e) C18:1n7 % of total fatty acids, and (f) C18:2n6 % of total fatty acids in *Ulva fenestrata* cultivated under different levels of temperature, nutrients and densities. Data on mean values and SEM are presented in Figures 2 and 3. Significant *p*-values are indicated with italics and red color.

		(a) C18:3n3			**(b) C16:0**			**(c) C18:4n3**			**(d) C16:4n3**			**(e) C18:1n7**			**(f) C18:2n6**		
Source of variance	Df	MS	F ratio	*p*	MS	F ratio	*p*	MS	F ratio	*p*	MS	F ratio	*p*	MS	F ratio	*p*	MS	F ratio	*p*
Temperature	1	0.323	1.525	0.226	0.339	2.210	0.147	0.067	0.620	0.437	0.340	1.602	0.215	0.047	0.242	0.626	0.011	0.299	0.588
Nutrients	1	0.006	0.032	0.859	0.222	1.451	0.237	0.010	0.098	0.757	0.291	1.368	0.251	0.016	0.083	0.775	0.008	0.216	0.645
Density	1	2.699	12.743	*0.001*	1.542	10.042	*0.003*	0.038	0.352	0.557	2.089	9.828	*0.004*	1.111	5.668	*0.023*	0.030	0.797	0.379
T × N	1	0.333	1.574	0.219	0.416	2.710	0.110	0.006	0.061	0.807	0.415	1.956	0.172	0.109	0.556	0.461	0.022	0.601	0.444
T × D	1	0.288	1.361	0.252	0.060	0.394	0.535	0.001	0.012	0.912	0.017	0.083	0.775	0.084	0.432	0.516	0.013	0.358	0.554
N × D	1	0.021	0.101	0.753	0.081	0.528	0.473	0.004	0.044	0.835	0.0004	0.002	0.965	0.213	1.089	0.305	0.022	0.602	0.444
T × N × D	1	0.023	0.110	0.743	0.076	0.498	0.486	0.005	0.053	0.819	0.1025	0.482	0.493	0.025	0.128	0.723	0.017	0.458	0.504
Residual	32	0.211			0.153			0.109			0.212			0.196			0.037		

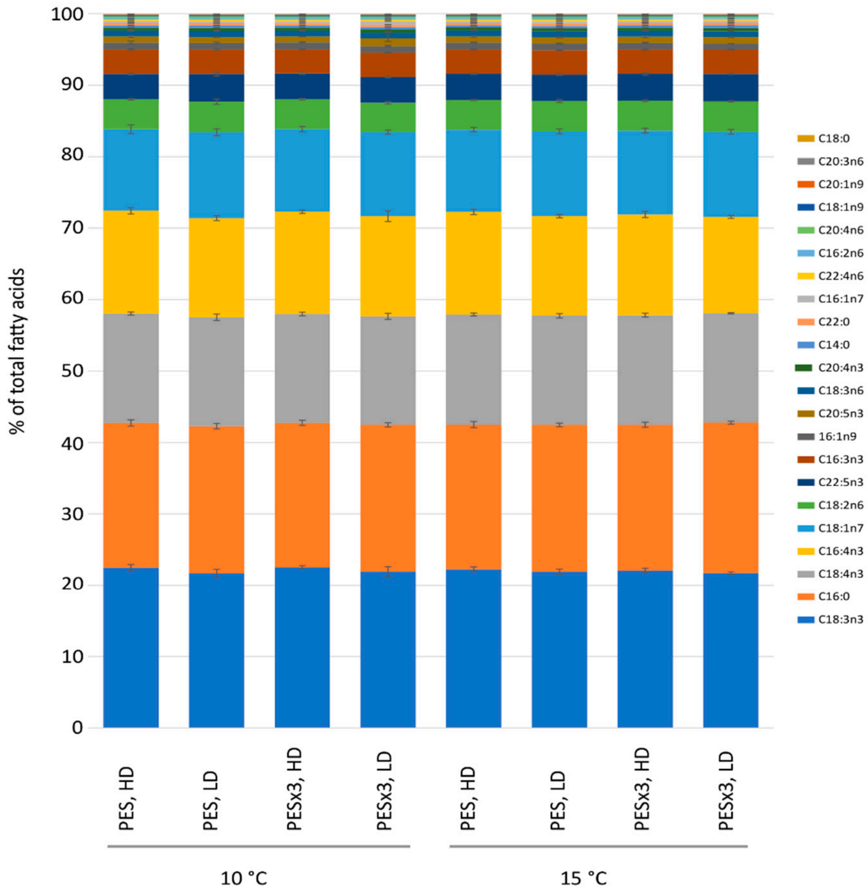

Figure 4. Fatty acid composition (% of total fatty acids) in off-shore cultivated *Ulva fenestrata* after exposure to different treatment combinations during seedling hatcheries (temperature = 10 °C, 15 °C, nutrient addition = PES, PESx3, swarmer density = 10,000 swarmer mL^{-1} (HD), 500 swarmer mL^{-1} (LD)). Error bars show SD. Detailed information on the fatty acid content can be found in supplementary Table S1.

Even though the effect sizes were relatively small (Figure 4), we found that density treatments changed the proportion of some fatty acids in off-shore cultivated *U. fenestrata* (Table 3). However, nutrient addition and temperature had no significant effect (Table 3). The proportion of C18:3n3 and C16:4n3 significantly ($p < 0.04$) increased with higher seedling densities, while both C18:1n7 and C16:0 increased significantly ($p < 0.03$) with decreasing densities (Table 3).

3.3. Crude Protein Content

The mean crude protein content for off-shore cultivated biomass of *U. fenestrata* was in a range of 16.60–20.75% dw (Figure 3B). Similar to the total fatty acid content, there was no statistically significant effect of the factors applied during the hatchery to affect the protein content of the cultivated biomass (Table 1e).

3.4. Carbohydrate Content and Composition

The mean total monosaccharide content and composition of off-shore cultivated *U. fenestrata* was determined after full hydrolysis of the polysaccharides of the respective samples (Figure 3C). There was a statistically significant effect of the factor temperature and density on the total carbohydrate content of the off-shore cultivated *U. fenestrata* (Table 1f). Varying nutrient regimes during hatchery had no significant effect on the total carbohydrate content of *U. fenestrata* in the present study (Table 1f, Figure 3C). Low densities and lowered temperature in the hatchery increased carbohydrate content by 5.25% and 4.846%, respectively, compared to the other treatment combinations. Similar to the fatty acid composition, the measured effect sizes were relatively small (Figure 3C).

When the composition profile was analyzed, we found that glucose was the dominating monosaccharide, with contents in the range 14.2–16.5% dw, whereas rhamnose was the second largest monosaccharide at 4.0–4.7% dw, followed by xylose at 3.5–3.9% dw. Glucoronic acid was detected in the range 1.6–1.9% dw, whereas the potentially high-value monosaccharide iduronic acid was detected at 1.4–1.8% dw. Galactose was found at fairly low values of 0.5–0.6% dw (Figure 3C). The chosen treatments had significant effects on the relative proportion of the monosaccharides (supplementary Table S1), however, as described for the composition of fatty acids, the effect size was relatively small (Figure 3).

3.5. Pigment Content

The mean total chlorophyll a, b and carotenoid content for off-shore cultivated biomass of *U. fenestrata* is displayed in Figure 3D,E.

There was a significant interaction between temperature and nutrient addition on the mean total content of all measured pigments (Table 1g–i). Higher temperature in combination with 3x PES-enriched nutrients during the hatchery reduced the pigment content in the biomass of off-shore cultivated *U. fenestrata* by 14.9% for chl a, 25.58% for chl b and 19.38% for carotenoids compared to the other temperature/nutrient combinations (Figure 3D,E, Table 2c–e).

3.6. Phenolic Content

The mean total phenolic content of off-shore cultivated biomass of *U. fenestrata* can be found in Figure 3F. There was a significant interaction between temperature and nutrient addition as well as of temperature and density on the phenolic content (Table 1j). Lower temperature in combination with high seedling density increased the phenolic content by 13.29% compared to the overall mean. Post-hoc testing did not find significant differences between the four temperature/nutrient combinations.

4. Discussion

Ulva biomass has recently gained attention in several economic sectors due to its multipurpose use in commodity products. Thus, the production of sustainable biomass feedstock beyond experimental or pilot scale cultivation is a crucial target. Our study confirmed the commercial scale production potential of Scandinavian *U. fenestrata* in a classical and sustainable rope-cultivation approach within a Swedish off-shore seafarm. This study further demonstrates the importance of applied hatchery conditions on the total biomass yield and on certain biochemical traits of the cultivated biomass.

As described by Toth et al. [40], an initial hurdle is the selection of adequate reference studies to place results in a broader, more global picture, since *Ulva* spp. are notoriously hard to identify, e.g., [20]. The exact taxonomic identity of *Ulva* spp. applied in mariculture literature is mostly ambiguous [16] due to the lack of molecular identification of the source material and morphological misidentifications caused by the extreme phenotypic plasticity of *Ulva* spp. [20]. We strongly emphasize the importance of molecular species identification in future aquaculture studies to not only enable disentangling the effect of genetic or environmental factors on biomass yield and biochemical composition, but to also support the growing Blue Economy with geographic and site-specific selections of suitable *Ulva* spp. and strains. This enables the generation of solid databases of potential *Ulva* crop strains and subsequent breeding approaches.

4.1. Biomass Yield, Growth and Performance

Even though most studies on European *Ulva* aquaculture are focusing on tank- or pond-based cultivation approaches, e.g., [24,42,53], our study confirms that a sustainable large-scale cultivation of *U. fenestrata* is benefited by sea-based rope cultivation. Whereas on-shore cultivation of *Ulva* spp. indeed has several advantages—such as permanent control of abiotic factors and overall cultivation conditions, easy accessibility of the biomass and less laborious harvests—a prevailing main problem is that, for now, the profitability of seaweed cultivation in many parts of Europe is questionable, especially due to the high costs of producing biomass [45,54]. A way to maximize the value of on-shore produced seaweed biomass is to apply cultivation conditions that elevate the content of high-value compounds. However, off-shore cultivation could also contribute immensely to the economic profitability by keeping maintenance costs low and biomass yields high.

An initial hurdle to facilitate large-scale, off-shore aquaculture of *Ulva* spp. is to obtain and concentrate viable swarmers (gametes or spores) to be seeded on suitable growth substrates. Using an established protocol for *U. mutabilis* [55], we could successfully manipulate gametangia induction and swarmer release in Swedish *U. fenestrata* which guarantees the independence of natural, seasonal reproduction patterns and further supports a profitable large-scale cultivation. Our results further showed that a high seeding density is favored in order to achieve a commercially viable large-scale cultivation of *U. fenestrata* and growth at scale. High seeding density resulted in an increased biomass yield (g [m rope]$^{-1}$), which is in agreement with previous findings on tubular *Ulva* spp. [56]. However, the comparison of optimal seeding densities of tubular, e.g., [56], and foliose *Ulva* spp.—like the *U. fenestrata* strain used in this study—is only possible on a relative scale because their habitus differs enormously, and adult tubular specimens generally take up less space than foliose individuals. There was no evidence for intraspecific competition or shading observed in high density treatments; furthermore, the fouling of epiphytes was in general very low in April.

As discussed by Carl et al. [56], a key factor crucial for biomass yield of *Ulva* spp. is the nursery or hatchery period prior to grow out. Increased contact time and thus longer nursery periods were found to minimize detachment and seedling loss caused by hydrodynamic forces [56,57], but concomitantly also lead to a more cost-intensive hatchery phase since on-shore facilities are needed, and resource limitations have to be counteracted. The relatively long hatchery periods of this study (six weeks) were chosen to allow the seedlings to develop a vigorous rhizoidal zone before their application in a Scandinavian off-shore seafarm during prevailing winter conditions. Evaluating if a shorter seedling nursery is viable was not part of the present study but should be considered in future studies to minimize the economic detriment during the hatchery phase.

The species *U. fenestrata* has proven to be an ideal candidate for biomass application in northern Europe even during relatively harsh winter and early spring conditions. The large-scale off-shore cultivation potential of northern hemisphere *U. fenestrata* opens up new abilities to integrate it with well-established mariculture branches such as fish- or mussel farms—which are important aquaculture industries in Scandinavia [58,59]—to concomitantly extrapolate bioremediation benefiting effects of *Ulva* biomass [18]. The feasible commercial usage of off-shore cultivated *U. fenestrata* biomass in different economic sectors is further supported by its many biochemical compounds with high-value applications.

4.2. Fatty Acids and Proteins

The total fatty acid content of off-shore cultivated *U. fenestrata* investigated in the present study (3.2–3.55% dw) was above the upper range (~1.6% dw) of what was reported for *Ulva* spp. in previous literature [27,40]. This suggests that off-shore cultivation of *U. fenestrata* mainly leads to a fatty acid composition similar to that from tank cultivation systems, however there is a significant increase of the total amount of fatty acids [40]. Whereas previous literature states that varying abiotic factors, such as temperature, nutrients or pCO_2, e.g., [38–40,60–62], have an influence on the fatty acid content, we found no statistically significant effect of different temperatures and nutrient supplies during the hatchery phase on the total fatty acid content in the harvested biomass. However, potential differences after the six weeks in hatchery were not evaluated in this study and could have been equalized during the six months in off-shore culture.

It is widely accepted that proteins of plant origin have a significantly lower carbon footprint than animal protein. Consumers' awareness of this, and also of the documented negative health effects from red meat consumption, has largely increased the demand for vegetarian proteins, which is often named a dietary protein shift. Indeed, this shift could benefit from sustainable off-shore cultivated extractive seaweed crops. The total protein content is a crucial factor for the application of *Ulva* biomass as food or feed and strongly influences the overall nutritional value [63]. The mean total protein content of the biomass investigated in this study (16.6–20.7% dw) was significantly higher than in previous lab-based experiments with *U. fenestrata* (8.09–12.36% dw) [40] and in an average range compared with previous studies on *Ulva* spp. (4–44% dw) [27]. A lab-based study on the same strain of *U. fenestrata* showed that increased temperature and irradiance negatively affect the total protein content [40], whereas raising nitrate levels had a strong positive effect [40]. Our analyses of the present study revealed that the factors applied during seedling hatcheries (temperature, nutrient addition, seedling density) had no significant effect on the crude protein content of the off-shore cultivated biomass. Our study thus confirms that off-shore cultivated *U. fenestrata* has several desirable traits considering its growing application for food, e.g., [49,64] and feed purposes [65,66]. Presumably, the observed high protein and fatty acid contents of off-shore cultivated *U. fenestrata* are benefited by the prevailing Scandinavian weather conditions. Low water temperatures and relatively short day length and thus favorable irradiance for protein and fatty acid rich biomass could be achieved by off-shore farming [27,40]. However, from a perspective of increased biomass growth as well as enrichment of total and desired bioactive compounds, different seasons, extended growth and shifted harvest periods need to be further investigated.

4.3. Carbohydrates

Seaweeds contain large amounts of polysaccharides, which mainly function as cell wall structures and storage polysaccharides [67]. Our data on total carbohydrates (25.95–29.69% dw) were distributed in the lower to middle range of what has previously been reported for *Ulva* spp. (15–65% dw) [27,36,37,68]. Notably, our data showed a tendency of increased carbohydrates when lower temperatures and low densities were applied during the hatchery phase. Even though the measured effect size was relatively low, our data contradict what has been reported previously; there was a positive effect of elevated temperature on the total carbohydrate composition [37,69], whereas raised nutrient levels resulted in deceased total carbohydrate contents [37,70].

The high-value iduronic acid can be used in commercially attractive biosynthesis pathways, such as in the synthesis of heparin fragment analogues with anti-thrombotic activities [27]. The present synthetic procedure is generally lengthy and cost-intensive and, at the moment, there is no large-scale source of commercial iduronic acid [71]. Thus, to obtain iduronic acid from a natural source, like cultivated *Ulva* biomass, could be beneficial [27]. Notably, the iduronic acid content of off-shore cultivated *U. fenestrata* was significantly higher than reported in a previous lab study (<1%) of a similar strain [37]. Additionally, rhamnose is a high-value monosaccharide that could be used in production of aroma compounds and rhamnolipids [27,72]. However, absolute differences in monosaccharide profiles of the here investigated biomass were small. In previous studies, it was shown that the total content of rhamnose and iduronic acid is positively correlated with increasing temperature and irradiance [37]. Furthermore, higher values of total rhamnose (11.74–17.39% dw) and iduronic acid (1.77–3.51% dw) contents have been found in Swedish wild-collected *Ulva* spp. during the summer months [36]. To conclude, if aiming for an increase of the total carbohydrate content and to especially enrich the high value bioactive compounds of rhamnose and iduronic acid in off-shore cultivated biomass of *U. fenestrata*, a harvest of the biomass during summer when water temperature and irradiance are significantly higher than during the harvest period (April) of the present study should be considered.

4.4. Pigments and Phenols

Phytochemicals such as phenolics, chlorophyll and carotenoids are known to be efficient scavengers of malign free radicals that can cause oxidative stress [73]. Besides significantly benefiting human health by their antioxidant [74] and anti-inflammatory [73] properties, additionally, chlorophylls show anti-tumoral activities by forming molecular complexes with carcinogens and thereby blocking their bioavailability [75], whereas carotenoids in particular help to prevent the free radical damage associated with the aging process [76]. This makes the pigment profile of *Ulva* species, which mainly consists of chlorophyll a and b, β-carotenes, lutein and different xanthophylls [77], highly interesting for several economic markets.

Our study showed that the average total chlorophyll a (1.29–1.69 mg g^{-1}), chlorophyll b (0.73–1.32 mg g^{-1}) and carotenoids (0.44–0.85 mg g^{-1}) content of off-shore cultivated *U. fenestrata* was in the average to upper range of what has previously been reported for *Ulva* spp. [53,78]. Previous studies have confirmed an increase of chlorophylls and carotenoids in *Ulva* spp. under raising temperatures and nitrogen enrichment [78]. However, our study showed that a high temperature in combination with high nutrients (PESx3) during hatchery decreased the mean total amount of chlorophyll a by 14.96%, chlorophyll b by 25.58% and carotenoids by 19.38% in the biomass of off-shore cultivated *U. fenestrata*. Thus, in-depth investigations of the physiological responses regarding biochemical profiles of species and northern hemisphere *Ulva* strains are required.

In comparison to brown seaweeds, red and green seaweeds have low concentrations of phenols [27]. The phenol content of dry seaweed biomass varies from <1 to 14% dw. Depending on the structure of the phenols, small amounts of bioactive secondary metabolites may increase the nutritional value of the biomass because the phenolic content in green algae shows a positive correlation with antibiotic and antioxidant activity [79]. Due to their anti-oxidative effect, phenols are relevant candidates for the development of functional foods, novel drugs and in general the nutraceutical and pharmaceutical industry. The total phenolic content of off-shore cultivated *U. fenestrata* of this study was in the average range (0.22–0.33% dw) of what has previously been reported for *Ulva* [79,80] and was higher than the total phenolic content observed during lab-cultivation of the same strain (0.122–0.202% dw) [40]. We observed higher phenolic contents at high seedling densities in combination with elevated nutrients. It has been shown that seasonality and consequently changing abiotic factors strongly influence the total phenol content and their free radical scavenging activity [76]. To further enrich off-shore cultivated *Ulva* biomass with desired phytochemicals, responses to seasonality, varying length of nursing periods and thallus age should be investigated in depth to support a productive future Blue Economy.

5. Conclusions

We conclude that Scandinavian *U. fenestrata* is a suitable crop for large-scale off-shore cultivation in the northern European hemisphere and that it copes very well with the prevailing, often harsh (storms, heavy precipitation, strong wave action) winter conditions. The ability to manipulatively induce gametogenesis and thus concentrate high swarmer quantities enables high seedling densities and makes this species a promising future crop. The off-shore cultivated biomass was found to be enriched by several high-value macro- and micronutrients that could find their application in several economic branches such as the food and feed industry and the neuro- and pharmaceutical sector, as well as in the biomaterial branch.

We were able to show that pre-treatments during the hatchery phase of the seedlings affect the biomass yield. However, even though some hatchery treatments had significant effects on the biochemical composition of off-shore cultivated *U. fenestrata*, their effect sizes in relation to the total amount of the respective compound were relatively small. However, as mentioned before, shorter cultivation periods could result in more significant effects of pre-treatments on the biochemical composition. Altogether, our study aims to provide first insights on the commercially attractive off-shore cultivation potential of *U. fenestrata* in the

European NE Atlantic to not only support the growing Blue Economy but to also tackle a shift towards more sustainable future resources.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jmse9060615/s1, Table S1: Fatty acid composition (% of total fatty acids) in *Ulva fenestrata* after exposure to different treatment combinations.

Author Contributions: S.S. conceptualization of the study, implementation of experiment, investigation, data analyses, visualization, original draft; S.E. data analyses, original draft; K.L. data analyses, refining of draft; J.O. data analyses, refining of draft; G.M.N. implementation of experiment; E.A. data analyses, refining of draft; H.P. funding acquisition, refining of draft; I.U. data analyses, refining of draft; G.B.T. data analyses, refining of draft. All authors have read and agreed to the published version of the manuscript.

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References

1. Duarte, C.M.; Holmer, M.; Olsen, Y.; Soto, D.; Marbà, N.; Guiu, J.; Black, K.; Karakassis, I. Will the Oceans Help Feed Humanity? *BioScience* **2009**, *59*, 967–976. [CrossRef]
2. FAO. *The State of World Fisheries and Aquaculture 2018—Meeting the Sustainable Development Goals*; FAO: Rome, Italy, 2018; 120p.
3. Troell, M.; Naylor, R.L.; Metian, M.; Beveridge, M.; Tyedmers, P.H.; Folke, C.; Arrow, K.J.; Barrett, S.; Crépin, A.S.; Ehrlich, P.R.; et al. Does aquaculture add resilience to the global food system? *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 13257–13263. [CrossRef]
4. Hanzal, H.; Divisova, M.; Murawska, D.; Janiszewski, P. The effect of dietary bio-alginate supplementation of the growth rate and body weights of common pheasant (*Phasianus cochicus*) chicks. *Pol. J. Nat. Sci.* **2016**, *31*, 363–371.
5. Morais, T.; Inácio, A.; Coutinho, T.; Ministro, M.; Cotas, J.; Pereira, L.; Bahcevandziev, K. Seaweed Potential in the Animal Feed: A Review. *J. Mar. Sci. Eng.* **2020**, *8*, 559. [CrossRef]
6. Kinley, R.D.; de Nys, R.; Vucko, M.J.; Machado, L.; Tomkins, N.W. The red macroalgae *Asparagopsis taxiformis* is a potent natural antimethanogenic that reduces methane production during in vitro fermentation with rumen fluid. *Anim. Prod. Sci.* **2016**, *56*, 282–289. [CrossRef]
7. Xixi, L.; Norman, H.C.; Kinley, R.D.; Laurence, M.; Wilmot, M.; Bender, H.; de Nys, R.; Tomkins, N. *Asparagopsis taxiformis* decreases enteric methane production from sheep. *Anim. Prod. Sci.* **2016**, *58*, 681–688.
8. Craigie, J. Seaweed extract stimuli in plant science and agriculture. *J Appl. Phycol.* **2011**, *23*, 371–393. [CrossRef]
9. Bird, M.I.; Wurster, C.M.; de Paula Silva, P.H.; Bass, A.M.; de Nys, R. Algal biochar—Production and properties. *Biores Technol.* **2011**, *102*, 1886–1891. [CrossRef] [PubMed]
10. Wahlström, N.; Nylander, F.; Malmhäll-Bah, E.; Sjövold, K.; Edlund, U.; Westman, G.; Albers, E. Composition and structure of cell wall ulvans recovered from *Ulva* spp. along the Swedish West coast. *Carbohydr. Polym.* **2020**, *233*, 115852. [CrossRef]
11. Wahlström, N.; Steinhagen, S.; Toth, G.; Pavia, H.; Edlund, U. Ulvan dialdehyde-gelatine hydrogels for removal of heavy metals and methylene blue from aqueous solution. *Carbohydr. Polym.* **2020**, 249. [CrossRef]
12. Wahlström, N.; Edlund, U.; Pavia, H.; Toth, G.; Jaworski, A.; Pell, A.J.; Choong, F.X.; Shirani, H.; Nilsson, K.P.R.; Richter-Dahlfors, A. Cellulose from the green macroalgae *Ulva lactuca*: Isolation, characterization, optotracing, and production of cellulose nanofibrils. *Cellulose* **2020**, *27*, 3707–3725. [CrossRef]
13. Chiellini, E.; Cinelli, P.; Ilieva, V.I.; Martera, M. Biodegradable thermoplastic composites based on polyvinyl alcohol and algae. *Biomacromolecules* **2008**, *9*, 1007–1013. [CrossRef]
14. Rowbotham, J.S.; Dyer, P.W.; Greenwell, H.C.; Theodorou, M.K. Thermochemical processing of macroalgae: A late bloomer in the development of third-generation biofuels? *Biofuels* **2012**, *3*, 441–461. [CrossRef]
15. Bruhn, A.; Dahl, J.; Nielsen, H.B.; Nikolaisen, L.; Rasmussen, M.B.; Markager, S.; Olesen, B.; Arias, C.; Jensen, P.D. Bioenergy potential of *Ulva lactuca*: Biomass yield, methane production and combustion. *Bioresour Technol.* **2011**, *102*, 2595–2604. [CrossRef]
16. Bolton, J.J.; Cyrus, M.D.; Brand, M.J.; Joubert, M.; Macey, B.M. Why grow *Ulva*? Its potential role in the future of aquaculture. *Perspect. Phycol.* **2016**, *3*, 113–120. [CrossRef]

17. Buchholz, C.M.; Krause, G.; Buck, B.H. "Seaweed and Man". In *Seaweed Biology: Ecological Studies 219*; Wiencke, C., Bischof, K., Eds.; Springer: Berlin/Heidelberg, Germany, 2012; pp. 471–493.
18. Lawton, R.J.; Mata, L.; de Nys, R.; Paul, N.A. Algal Bioremediation of Waste Waters from Land-Based Aquaculture Using *Ulva*: Selecting Target Species and Strains. *PLoS ONE* **2013**, *15*, e77344. [CrossRef] [PubMed]
19. Favot, G.; Cunha, M.E.; Quental-Ferreira, H.; Serrão, M. Production of *Ulva* sp. in multitrophic aquaculture in earth ponds. *Aquac. Fish. Stud.* **2019**, *3*, 24–31.
20. Hayden, H.S.; Blomster, J.; Maggs, C.A.; Silva, P.C.; Stanhope, M.J.; Waaland, J.R. Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. *Eur. J. Phycol.* **2003**, *38*, 277–294. [CrossRef]
21. Steinhagen, S.; Karez, R.; Weinberger, F. Cryptic, alien and lost species: Molecular diversity of *Ulva sensu lato* along the German coasts of the North and Baltic Seas. *Eur. J. Phycol.* **2019**, *54*, 466–483. [CrossRef]
22. Steinhagen, S.; Karez, R.; Weinberger, F. Surveying seaweeds from the Ulvales and Fucales in the world's most frequently used artificial waterway, the Kiel Canal. *Bot. Mar.* **2019**, *62*, 51–61. [CrossRef]
23. Steinhagen, S.; Weinberger, F.; Karez, R. Molecular analysis of *Ulva compressa* (Chlorophyta, Ulvales) reveals its morphological plasticity, distribution and potential invasiveness on German North Sea and Baltic Sea coasts. *Eur. J. Phycol.* **2019**, *54*, 102–114. [CrossRef]
24. Sebök, S.; Herppich, W.B.; Hanelt, D. Outdoor cultivation of *Ulva lactuca* in a recently developed ring-shaped photobioreactor: Effects of elevated CO_2 concentration on growth and photosynthetic performance. *Bot. Mar.* **2019**, *62*, 179–190. [CrossRef]
25. Mata, L.; Schuenhoff, A.; Santos, R. A direct comparison of the performance of the seaweed biofilters, *Asparagopsis armata* and *Ulva Rigida*. *J. Appl. Phycol.* **2010**, *22*, 639–644. [CrossRef]
26. Al-Hafedh, Y.S.; Alam, A.; Buschmann, A.H. Bioremediation potential, growth and biomass yield of the green seaweed, *Ulva lactuca* in an integrated marine aquaculture system at the Red Sea coast of Saudi Arabia at different stocking densities and effluent flow rates. *Rev. Aquacult.* **2015**, *7*, 161–171. [CrossRef]
27. Holdt, S.L.; Kraan, S. Bioactive compounds in seaweed: Functional food applications and legislation. *J. Appl. Phycol.* **2011**, *23*, 543–597. [CrossRef]
28. Shuuluka, D.; Bolton, J.J.; Anderson, R.J. Protein content, amino acid composition and nitrogen-to-protein conversion factors of *Ulva rigida* and *Ulva capensis* from natural populations and *Ulva lactuca* from an aquaculture system, in South Africa. *J. Appl. Phycol.* **2013**, *25*, 677–685. [CrossRef]
29. Carvalho, A.F.U.; Portela, M.C.C.; Sousa, M.B.; Martins, F.S.; Rocha, F.C.; Farias, D.F.; Feitosa, J.P.A. Physiological and physico-chemical characterization of dietary fibre from the green seaweed *Ulva fasciata* Delile. *Braz. J. Biol.* **2009**, *69*, 969–977. [CrossRef] [PubMed]
30. McDermid, K.J.; Stuercke, B. Nutritional composition of edible Hawaiian seaweeds. *J. Appl. Phycol.* **2003**, *15*, 513–524. [CrossRef]
31. Colombo, M.L.; Risè, P.; Giavarini, F.; Angelis, D.E.L.; Galli, C.; Bolis, C.L. Marine macroalgae as sources of polyunsaturated fatty acids. *Plant. Foods Hum. Nutr.* **2006**, *61*, 67–72. [CrossRef] [PubMed]
32. Abreu, M.H.; Pereira, R.; Sassi, J.F. "Marine algae and the global food industry". In *Marine Algae: Biodiversity, Taxonomy, Environmental Assessment, and Biotechnology*; Pereira, L., Magalhaes Neto, J., Eds.; CRC Press: Boca Raton, FL, USA, 2014; pp. 300–319. [CrossRef]
33. Santos, S.A.O.; Vilela, C.; Freire, C.S.R.; Abreu, M.H.; Rocha, S.M.; Silvestre, A.J.D. Chlorophyta and Rhodophyta macroalgae: A source of health promoting phytochemicals. *Food Chem.* **2015**, *183*, 122–128. [CrossRef]
34. Hafting, J.T.; Craigie, J.S.; Stengel, D.B.; Loureiro, R.R.; Buschmann, A.H.; Yarish, C.; Edwards, M.D.; Critchley, A.T. Prospects and challenges for industrial production of seaweed bioactivities. *J. Phycol.* **2015**, *51*, 821–837. [CrossRef]
35. Barzkar, N.; Tamadoni Jahromi, S.T.; Poorsaheli, H.B.; Vianello, F. Metabolites from marine microorganisms, micro, and macroalgae: Immense scope for pharmacology. *Mar. Drugs* **2019**, *17*, 464. [CrossRef]
36. Olsson, J.; Raikova, S.; Mayers, J.J.; Steinhagen, S.; Chuck, C.J.; Nylund, G.M.; Albers, E. Effects of geographical location on potentially valuable components in *Ulva intestinalis* sampled along the Swedish coast. *Appl. Phycol.* **2020**, *1*, 80–92. [CrossRef]
37. Olsson, J.; Toth, G.B.; Oerbekke, A.; Cvijetinovic, S.; Wahlström, N.; Harrysson, H.; Steinhagen, S.; Kinnby, A.; White, J.; Edlund, U.; et al. Cultivation conditions affect the monosaccharide composition in *Ulva fenestrata*. *J. Appl. Phycol.* **2020**, *32*, 3255–3263. [CrossRef]
38. Gao, G.; Clare, A.S.; Chatzidimitriou, E.; Rose, C.; Caldwell, G. Effects of ocean warming and acidification, combined with nutrient enrichment, on chemical composition and functional properties of *Ulva rigida*. *Food Chem.* **2018**, *258*, 71–78. [CrossRef] [PubMed]
39. Gao, G.; Clare, A.S.; Rose, C.; Caldwell, G.S. *Ulva rigida* in the future ocean: Potential for carbon capture, bioremediation and biomethane production. *Gbc Bioenergy* **2018**, *10*, 39–51.
40. Toth, G.B.; Harrysson, H.; Wahlström, N.; Olsson, J.; Oerbekke, A.; Steinhagen, S.; Kinnby, A.; White, J.; Albers, A.; Edlund, U.; et al. Effects of irradiance, temperature, nutrients, and pCO_2 on the growth and biochemical composition of cultivated *Ulva fenestrata*. *J. Appl. Phycol.* **2020**, *32*, 3243–3254. [CrossRef]
41. Lubsch, A.; Timmermans, K. Uptake kinetics and storage capacity of dissolved inorganic phosphorus and corresponding N:P dynamics in *Ulva lactuca* (Chlorophyta). *J. Phycol.* **2018**, *54*, 215–223. [CrossRef] [PubMed]
42. Califano, G.; Kwantes, M.; Abreu, M.H.; Costa, R.; Wichard, T. Cultivating the macroalgal holobiont: Effects of Integrated Multi-Trophic-Aquaculture on the microbiome of *Ulva rigida* (Chlorophyta). *Front. Mar. Sci.* **2020**, *7*, 52. [CrossRef]

43. Lapointe, B.E.; Williams, L.D.; Goldman, J.C.; Ryther, J.H. The mass outdoor culture of macroscopic marine algae. *Aquaculture* **1976**, *8*, 9–21. [CrossRef]
44. Lüning, K.; Pang, S.J. Mass Cultivation of Seaweed: Current Aspects and Approaches. *J. Appl. Phycol.* **2003**, *15*, 115–119. [CrossRef]
45. Titlyanov, E.A.; Titlyanova, T.V. Seaweed cultivation: Methods and problems. *Russ. J. Mar. Biol.* **2010**, *36*, 227–242. [CrossRef]
46. Provasoli, L. Media and prospects for the cultivation of marine algae. In *Cultures and Collections of Algae, Proceedings of the US-Japan Conference, Hakone, Japan, 12–15 September 1966*; Society of Plant Physiology: Tokyo, Japan, 1968.
47. Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **2012**, *9*, 671–675. [CrossRef]
48. Angell, A.R.; Mata, L.; de Nys, R.; Paul, N.A. The protein content of seaweeds: A universal nitrogen-to-protein conversion factor of five. *J. Appl. Phycol.* **2016**, *28*, 511–524. [CrossRef]
49. Harrysson, H.; Hayes, M.; Eimer, F.; Carlsson, N.G.; Toth, G.B.; Undeland, I. Production of protein extracts from Swedish red, green, and brown seaweeds, *Porphyra umbilicalis* Kützing, *Ulva lactuca* Linnaeus, and *Saccharina latissima* (Linnaeus) JV Lamouroux using three different methods. *J. Appl. Phycol.* **2018**, *30*, 3565–3580. [CrossRef]
50. Jeffrey, S.; Humphrey, G. New spectrophotometric equations for determining a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanz.* **1975**, *167*, 191–194. [CrossRef]
51. Parsons, T.R.; Maita, Y.; Lalli, C.M. *A manual for Chemical and Biological Methods for Seawater Analysis*; Pergamon Press: Oxford, UK, 1984.
52. Underwood, A.J. Techniques of analysis of variance in experimental marine biology and ecology. *Oceanogr. Mar. Biol. Annu. Rev.* **1981**, *19*, 513–603.
53. Martins, M.; Fernandes, A.; Torres-Acosta, M.A.; Collén, N.; Abreu, M.H.; Ventura, S.P.M. Extraction of chlorophyll from wild and farmed *Ulva* spp. using aqueous solutions of ionic liquids. *Sep. Purif. Technol.* **2021**, *254*, 117589. [CrossRef]
54. Palatnik, R.R.; Zilberman, D. Economics of natural resource utilization—The case of macroalgae. In *Modeling, Dynamics, Optimization and Bioeconomics II*; Pinto, A.A., Zilberman, D., Eds.; DGS 2014; Springer: Cham, Switzerland, 2017; pp. 1–21.
55. Kessler, R.W.; Taghreed, A.; Wichard, T. Purification of sporulation and swarming inhibitors from *Ulva*. In *Protocols for Macroalgae Research*; Charrier, B., Wichard, T., Reddy, C.R.K., Eds.; CRC Press: Boca Raton, FL, USA, 2018; pp. 139–158.
56. Carl, C.; de Nys, R.; Lawton, R.J.; Paul, N.A. Methods for the Induction of Reproduction in a Tropical Species of Filamentous *Ulva*. *PLoS ONE* **2014**, *9*, e97396. [CrossRef]
57. Zhang, C.; Li, X.; Kim, S. Application of marine biomaterials for nutraceuticals and functional foods. *Food Sci. Biotechnol.* **2012**, *21*, 625–631. [CrossRef]
58. Smaal, A.C. European mussel cultivation along the Atlantic coast: Production status, problems and perspectives. In *Sustainable Increase of Marine Harvesting: Fundamental Mechanisms and New Concepts*; Vadstein, O., Olsen, Y., Eds.; Developments in Hydrobiology; Springer: Dordrecht, The Netherlands, 2002; Volume 167. [CrossRef]
59. Taranger, G.L.; Karlsen, Ø.; Bannister, R.J.; Glover, K.A.; Husa, V.; Karlsbakk, E.; Kvamme, B.O.; Kroon Boxaspen, K.; Bjørn, P.A.; Finstad, B.; et al. Risk assessment of the environmental impact of Norwegian Atlantic salmon farming. *ICES J. Mar. Sci.* **2015**, *72*, 997–1021. [CrossRef]
60. Gao, G.; Clare, A.S.; Rose, C.; Caldwell, G.S. Eutrophication and warming-driven green tides (*Ulva rigida*) are predicted to increase under future climate change scenarios. *Mar. Pollut. Bull.* **2017**, *114*, 439–447. [CrossRef]
61. Chen, B.B.; Lin, L.D.; Ma, Z.L.; Zhang, T.T.; Chen, W.Z.; Zou, D.H. Carbon and nitrogen accumulation and interspecific competition in two algae species, *Pyropia haitanensis* and *Ulva lactuca*, under ocean acidification conditions. *Aquac. Int.* **2019**, *27*, 721–733. [CrossRef]
62. Mhatre, A.; Patil, S.; Agrawal, A.; Pandit, R.; Lali, A.M. Influence of nitrogen source on photochemistry and antenna size of the photosystems in marine green macroalgae, *Ulva lactuca*. *Photosynth Res.* **2019**, *139*, 539–551. [CrossRef]
63. Shpigel, M.; Ragg, N.L.; Lupatsch, I.; Neori, A. Protein content determines the nutritional value of the seaweed *Ulva lactuca* L for the abalone *Haliotis tuberculata* L. and *H. discus hannai* Ino. *J. Shellfish Res.* **1999**, *18*, 227–234.
64. Fleurence, J. Seaweed proteins: Biochemical, nutritional aspects and potential uses. *Trends Food Sci. Technol.* **1999**, *10*, 25–28. [CrossRef]
65. Angell, A.R.; Angell, S.F.; de Nys, R.; Paul, N.A. Seaweed as a protein source for mono-gastric livestock. *Trend Food Sci. Technol.* **2016**, *54*, 74–84. [CrossRef]
66. Øverland, M.; Mydland, L.T.; Skrede, A. Marine macroalgae as sources of protein and bioactive compounds in feed for monogastric animals. *J. Sci. Food Agric.* **2019**, *99*, 13–24. [CrossRef]
67. Kumar, C.S.; Ganesan, P.; Suresh, P.V.; Bhaskar, N. Seaweeds as a source of nutritionally beneficial compounds-a review. *J. Food Sci. Technol.* **2008**, *45*, 1.
68. Olsson, J.; Toth, G.; Albers, E. Biochemical composition of red, green and brown seaweeds on the Swedish west coast. *J. Appl. Phycol.* **2020**, *32*, 3305–3317. [CrossRef]
69. He, Y.; Hu, C.; Wang, Y.; Cui, D.; Sun, X.; Li, Y.; Xu, N. The metabolic survival strategy of marine macroalga *Ulva prolifera* under temperature stress. *J. Appl. Phycol.* **2018**, *30*, 3611–3621. [CrossRef]
70. Kumari, P.; Kumar, M.; Reddy, C.R.K.; Jha, B. Nitrate and phosphate regimes induced lipidomic and biochemical changes in the intertidal macroalga *Ulva lactuca* (Ulvophyceae, Chlorophyta). *Plant. Cell Physiol.* **2014**, *55*, 52–63. [CrossRef]

71. Mohamed, S.; Ferro, V. Synthetic approaches to l-Iduronic acid and l-Idose: Key building blocks for the preparation of glycosaminoglycan oligosaccharides. In *Advances in Carbohydrate Chemistry and Biochemistry*; Baker, D.C., Horton, D., Eds.; Academic Press: New York, NY, USA, 2015; Volume 72, pp. 21–61.
72. Muller, M.M.; Kugler, J.H.; Henkel, M.; Gerlitzki, M.; Hormann, B.; Pohnlein, M.; Syldatk, C.; Hausmann, R. Rhamnolipids-next generationsurfactants? *J. Biotech.* **2012**, *162*, 366–380. [CrossRef]
73. Abd El-Baky, H.H.; El-Baz, F.K.; El-Baroty, G.S. Natural preservative ingredient from marine alga *Ulva lactuca* L. *Int. J. Food Sci. Technol.* **2009**, *44*, 1688–1695. [CrossRef]
74. Lanfer-Marquez, U.M.; Barros, R.M.; Sinnecker, P. Antioxidant activity of chlorophylls and their derivatives. *Food Res. Int.* **2005**, *38*, 885–891. [CrossRef]
75. Egner, P.A.; Muñoz, A.; Kensler, T.W. Chemoprevention with chlorophyllin in individuals exposed to dietary aflatoxin. *Mutat. Res. Fundam. Mol. Mech. Mutagenesis* **2003**, *523*, 209–216. [CrossRef]
76. Khairy, H.M.; El-Sheikh, M.A. Antioxidant activity and mineral composition of three Mediterranean common seaweeds from Abu-Qir Bay, Egypt. *Saudi J. Biol. Sci.* **2015**, *5*, 623–630. [CrossRef]
77. Bocanegra, A.; Bastida, S.; Benedí, J.; Rodenas, S.; Sanchez-Muniz, F.J. Characteristics and nutritional and cardiovascular-health properties of seaweeds. *J. Med. Food* **2009**, *12*, 236–258. [CrossRef]
78. Eismann, A.I.; Reis, R.P.; da Silva, A.F.; Cavalcanti, D.N. *Ulva* spp. carotenoids: Responses to environmental conditions. *Algal Res.* **2020**, *48*, 101916. [CrossRef]
79. Sirbu, R.; Stanciu, G.; Tomescu, A.; Ionescu, A.M.; Cadar, E. Evaluation of Antioxidant and Antimicrobial Activity in Relation to Total Phenolic Content of Green Algae from Black Sea. *Rev. Chim.* **2019**, *70*, 1197–1203. [CrossRef]
80. Hashem, H.A.; Mansour, H.A.; El-Khawas, S.A.; Hassanein, R.A. The potentiality of marine macro-algae as bio-fertilizer to improve the productivity and salt stress tolerance of canola (*Brassica napus* L.) plants. *Agronomy* **2019**, *9*, 146.