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Growth and Biochemical Responses of Sea Lettuce (*Ulva rigida* C. Agardh) to Different Nitrogen Sources Under Controlled Culture Conditions

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Abstract

This study evaluated the effects of daily applications of sodium nitrate ($NaNO_3$) and ammonium nitrate (NH_4NO_3) at different concentrations on the growth, antioxidant activity, phenolic compounds, flavonoids, condensed tannins and pigment contents of the green macroalga *Ulva rigida*. Under the batch regime, the control received sodium nitrate only at the beginning of each five-day culture cycle, whereas treatment groups received daily nitrogen additions. The highest specific growth rate was recorded in the NO_3-3 group (3.84±0.32% day^{-1}), whereas the lowest value was observed in NH_4-5 (−0.70±0.14% day^{-1}) ($P<0.05$). The DPPH inhibition activity ranged from 17.72±2.87% (NO_3-4) to 41.10±1.73% (NO_3-3). The lowest IC_{50} value was also determined in NO_3-3 (8.65±0.48 mg g^{-1} ext) ($P<0.05$). Total phenolic, flavonoid and condensed tannin contents ranged from 0.80±0.10 to 2.35±0.08 mg GAE g^{-1} ext, 1.64±0.06 to 3.05±0.04 mg RE g^{-1} ext and 66.72±2.22 to 125.61±0.74 µg CE g^{-1} ext, respectively ($P<0.05$). Chlorophyll *a* and total carotenoid contents were highest in the control group, whereas chlorophyll *b* was highest in the NO_3-5. Correlation analysis and PCA supported differences among nitrogen treatments. These results showed that nitrogen source and application regime affected the growth, antioxidant activity and biochemical characteristics of *U. rigida*.

Introduction

Macroalgae are used in many areas, such as food, agriculture, pharmaceuticals and cosmetics, because they are rich in amino acids, fatty acids, minerals, polysaccharides, polyphenolic compounds and vitamins (Dominguez & Loret, 2019; Warnasooriya et al., 2024; Heriansah et al., 2025). Red and brown algae constitute most cultivated algal species and are important sources of carrageenan, alginate and agar. Green algae represent a smaller share of global cultivation and include edible taxa used as food (Buschmann et al., 2017). Among the green macroalgae, *Ulva* species are commonly found in coastal ecosystems and exhibit rapid growth (Sousa et al., 2025). These species are considered suitable biomass sources for food, feed and biotechnological applications due to their high growth rates and rapid nutrient uptake (Dominguez & Loret, 2019; Warnasooriya et al., 2024). They also contain vitamin A, B-complex vitamins, vitamin C, vitamin E and α-tocopherol and they are used as edible seaweeds and food ingredients (Ortiz et al., 2006; Pathan et al., 2023). Due to these properties, *Ulva* species have been used in many cultivation and biomass production studies in recent years (Simon et al., 2022; Zollmann et al., 2023; Duarte et al., 2025; Liu et al., 2025; Sousa et al., 2025).

The biochemical composition of macroalgae can vary with nutrient availability (Kang & Chung, 2018; Toth et al., 2020; Miguel et al., 2023). Nitrogen is one of the main limiting factors for macroalgal growth. Macroalgae

can use both nitrate and ammonium as nitrogen sources and these forms differ in their assimilation pathways. Ammonium is assimilated without prior reduction, while nitrate is reduced before cellular use (Ale et al., 2011). However, nitrogen requirements and nitrogen utilization may differ among species (Hurd et al., 2014). Ammonium nitrate provides both NH_4^+ and NO_3^- and therefore may influence growth and metabolism through multiple nitrogen assimilation pathways. Increased growth and physiological activity under ammonium enrichment have been reported in several macroalgal species (Luo et al., 2012; Kang et al., 2017; Kang & Chung, 2018). Reidenbach et al. (2017) found higher relative growth rate and total chlorophyll content in *Ulva australis* under low NH_4^+ enrichment. Growth in *Ulva* species depends on dissolved inorganic nitrogen in the culture medium (Viaroli et al., 1996; Wang et al., 2019). Ale et al. (2011) reported a higher maximum specific growth rate in NH_4Cl-treated *Ulva lactuca* than in nitrate-treated groups. Nitrogen source can also change photosynthetic activity and biochemical composition in *Ulva* species (Shahar et al., 2020).

Nitrogen is a structural element of amino acids, proteins and photosynthetic pigments (Piechulla & Heldt, 2024). Changes in nitrogen availability may therefore affect cellular metabolism and alter resource allocation among biochemical pathways (Shahar et al., 2020; Van Alstyne, 2018). These changes can alter phenolic, flavonoid and photosynthetic pigment levels (McCauley et al., 2018; Vega et al., 2020). Macroalgae contain phenolic compounds, pigments and polysaccharides related to antioxidant activity (Leandro et al., 2019). These compounds differ among environmental and cultivation conditions. McCauley et al. (2018) reported differences in pigment composition, phenolic contents and antioxidant activity in cultivated *Ulva* sp. under different nutrient conditions. Similarly, Neves et al. (2026) reported differences in antioxidant responses in *Ulva rigida* under different nutrient conditions, whereas phenolic contents remained unchanged. Sousa et al. (2025) also reported variation in phenolic contents in cultivated *Ulva lacinulata* under different cultivation periods. In *Ulva* species, total phenolics, flavonoids and tannins are often analyzed together with antioxidant activity (Ak & Türker, 2019). Changes in photosynthetic pigment content may reflect physiological responses to nutrient conditions and environmental stress (Figueroa et al., 2009). Nitrogen availability has been reported to affect pigment composition in *Ulva* species. Gómez Pinchetti et al. (1998) found higher chlorophyll *a* and chlorophyll *b* levels in nitrogen-enriched *Ulva rigida* cultures and lower pigment levels in nitrogen-free cultures. Similarly, McCauley et al. (2018) reported lower total chlorophyll content under low-nitrogen conditions than under high-nitrogen conditions.

Nitrogen availability and nitrogen form affect growth and biochemical composition in *Ulva* species. Previous studies have mostly focused on growth and pigment responses. Antioxidant responses under controlled culture conditions have been studied less. Data on the combined effects of nitrogen source and daily application regime on growth, pigments, antioxidant activity and phenolic compounds in *U. rigida* are still limited. This study compared daily applications of $NaNO_3$ and NH_4NO_3 with a batch nitrate treatment. Growth performance, pigment content, 1,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity, total phenolic content, total flavonoid content and condensed tannin content were measured in *U. rigida* under controlled culture conditions.

Materials and Methods

Algal Source and Stock Culture Conditions

The study was conducted at the Plankton Stock Unit and Processing Technology Laboratories of the Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye, between August and October 2019. *Ulva rigida* thalli were obtained from laboratory-maintained stock cultures. Before establishing stock cultures, the thalli were washed with seawater filtered through a 0.45 µm membrane and sterilized with ultraviolet light. The thalli were maintained as stock cultures for one month in a modified Artificial Seawater Medium based on the formulation provided by the Culture Collection of Algae at the University of Texas at Austin (UTEX) and adjusted to a salinity of 35‰ (UTEX Culture Collection of Algae, 2019). The medium contained 35 g L^{-1} NaCl, 2.6 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.6 g L^{-1} KCl, 0.3 g L^{-1} $CaCl_2 \cdot 2H_2O$, 0.05 g L^{-1} KH_2PO_4 and 0.22 g L^{-1} $NaHCO_3$. The P-II trace-metal stock solution contained 0.1 g $Na_2EDTA \cdot 2H_2O$, 0.114 g H_3BO_3, 4.9 mg $FeCl_3 \cdot 6H_2O$, 16.4 mg $MnSO_4 \cdot H_2O$, 2.2 mg $ZnSO_4 \cdot 7H_2O$ and 0.48 mg $CoCl_2 \cdot 6H_2O$ per 100 mL. The chelated iron stock solution was prepared using 10 g $Na_2EDTA \cdot 2H_2O$, 0.81 g $FeCl_3 \cdot 6H_2O$ and approximately 450 mL of 0.1 M HCl, with the final volume adjusted to 500 mL. Stock cultures were maintained at 20°C under a light intensity of 100 µmol photons m^{-2} s^{-1} and a 16 h light:8 h dark photoperiod. Continuous aeration was supplied from the bottom of the culture vessels to circulate the thalli and provide uniform exposure to light. Evaporative losses were compensated daily with distilled water, while temperature, salinity and pH were monitored throughout the stock culture period.

Experimental Conditions

After the stock culture period, the thalli were transferred into cylindrical glass culture vessels approximately 16 cm in diameter and 24 cm in height. Each vessel contained 3 L of culture medium and 15 g of wet algal biomass, corresponding to an initial biomass density of 5 g L^{-1}. The experimental culture medium was prepared using the same modified UTEX formulation used for the stock cultures. Two separate culture

experiments were conducted using $NaNO_3$ and NH_4NO_3 as nitrogen sources. Each nitrogen-source experiment comprised five daily-dose treatment groups and a corresponding batch control group. Each treatment and control group was run in triplicate and the culture vessels were randomly positioned within the culture area. Each experiment lasted 21 days. The $NaNO_3$ treatment groups were designated as NO_3-1–NO_3-5. The daily $NaNO_3$ doses were 2.12 mM day^{-1} (NO_3-1), 2.82 mM day^{-1} (NO_3-2), 3.53 mM day^{-1} (NO_3-3), 4.24 mM day^{-1} (NO_3-4) and 4.94 mM day^{-1} (NO_3-5). The NH_4NO_3 treatment groups were designated as NH_4-1–NH_4-5. The daily NH_4NO_3 doses were 1.12 mM day^{-1} (NH_4-1), 1.50 mM day^{-1} (NH_4-2), 1.87 mM day^{-1} (NH_4-3), 2.25 mM day^{-1} (NH_4-4) and 2.50 mM day^{-1} (NH_4-5). The NH_4NO_3 doses were calculated on a nitrogen-equivalent basis to provide total nitrogen concentrations comparable to those of the corresponding $NaNO_3$ treatments. The nominal ammonium-N and nitrate-N concentrations of the treatments were calculated from the amounts of $NaNO_3$ and NH_4NO_3 added to the 3-L culture volume. In each experiment, the batch control received 17.65 mM $NaNO_3$ only at the beginning of each five-day culture cycle. The culture medium in all treatment and control groups was completely renewed every five days. Following each renewal, the corresponding nitrogen treatments were re-established. Cultures were maintained at 24.0±1.0°C under a light intensity of 100 µmol photons m^{-2} s^{-1} and a 16 h light:8 h dark photoperiod. Continuous aeration was supplied from the bottom of each vessel to mix the culture medium and circulate the thalli. Biomass collected before and after each experiment was dried at 35°C for 48 h until constant weight was reached and used for antioxidant and biochemical analyses.

Water Quality

Temperature and pH were monitored with a multi-parameter probe (Hanna Instruments HI9829) during the experiment. Salinity was recorded using a refractometer. The culture medium was replaced every five days.

Growth Measurements

Specific growth rate (SGR) followed Shpigel et al. (2019):

$$Specific\ Growth\ Rate(\%\ day-1) = \frac{(lnWt - lnWo)}{t}\ x\ 100$$

Where W_0 is the initial biomass and W_t is the biomass measured at time t.

Culture density was maintained at 5 g L^{-1} during the experiment. Growth was measured at five-day intervals throughout the experiment. After each measurement, excess biomass was removed as needed and the culture density was readjusted to 5 g L^{-1}. SGR was calculated separately for each cultivation interval and the average of the interval-specific SGR values was used to represent growth performance. After removing surface water, the thalli were weighed using a precision balance (Kern 440-49N) until constant weight was reached.

1,2-diphenyl-1-picrylhydrazyl (DPPH) Radical-Scavenging Assay

Antioxidant activity was measured with the DPPH method of Brand-Williams et al. (1995). Extracts were dissolved in methanol at 1 mg mL^{-1}. For each reaction, 0.1 mL extract solution was mixed with 3.9 mL methanolic DPPH solution (6×10^{-5} M). The mixture was kept in the dark at room temperature for 30 min and absorbance was read at 517 nm. Results were reported as DPPH inhibition (%).

DPPH scavenging activity (%) was calculated using the following equation:

$$Inhibition\ (\%) = \frac{Acontrol - Asample}{Acontrol}\ x\ 100$$

Where A_{sample} is the sample absorbance and $A_{control}$ is the control absorbance.

Extracts were assayed at different concentrations. IC_{50} values were obtained from inhibition–concentration curves by nonlinear regression. Vitamin C and butylated hydroxytoluene (BHT) were included only as positive controls in DPPH inhibition and IC_{50} analyses.

Total Phenolic Content

Total phenolic content was analyzed following Djeridane et al. (2006). Extract, distilled water and Folin–Ciocalteu reagent were placed in the reaction tube. Sodium carbonate was added to start the colour reaction. After 30 min at room temperature, absorbance was read at 750 nm. Gallic acid calibration was used and the values were given as mg GAE g^{-1} ext.

Total Flavonoid Content

Total flavonoid content was measured following Quettier-Deleu et al. (2000). The methanolic extract was combined with 2% $AlCl_3$ in methanol. The reaction mixture was held at room temperature for 1 h and absorbance was read at 430 nm. Rutin was used as the calibration standard and total flavonoid content was calculated from the rutin calibration curve. Results were expressed as mg rutin equivalents per g extract (mg RE g^{-1} ext).

Condensed Tannin Content

Condensed tannin content was measured following Price et al. (1978). A 0.5 g sample was extracted with methanol containing 1% HCl. The extract was kept at 30°C for 20 min and centrifuged. Vanillin

reagent was added to the samples. The blank samples, 4% HCl in methanol. After 20 min of incubation, absorbance was read at 500 nm. Results were given as µg catechin equivalent per gram of extract (µg CE g^{-1} ext).

Pigment Analysis

Samples were collected at the end of the experiment for pigment analysis. Samples were kept in 90% acetone at 4°C in the dark for 24 h. Thalli were homogenized with sea sand and filtered through a membrane filter. The filtrate was centrifuged at 1500 rpm for 5 min. Chlorophyll *a*, chlorophyll *b* and total carotenoid contents were determined according to Jeffrey and Humphrey (1975) and Lichtenthaler (1987). Absorbance values were measured at 750, 664, 630 and 450 nm.

Statistical Analysis

Data are presented as mean±standard deviation. Data distribution was checked with the Shapiro–Wilk test and variance homogeneity was checked with Levene's test. Group differences were tested by one-way analysis of variance (ANOVA) and Tukey's Multiple Comparison test. The significance level was set at $P<0.05$. Spearman correlation analysis was used to assess associations among antioxidant activity, growth, biochemical variables and pigment parameters. Principal Component Analysis (PCA) was applied to standardized data to evaluate the distribution of treatment groups and variables. Analyses were conducted in R version 4.5.3 (R Core Team, Vienna, Austria). Vitamin C and BHT were included only as positive controls in DPPH inhibition and IC_{50} analyses. They were excluded from the Spearman correlation and PCA analyses.

Results

Growth Performance

Temperature and pH remained relatively stable throughout the experiment, averaging 23.1±0.8°C and 8.4±0.2, respectively. SGR values differed significantly among groups ($P<0.05$) and ranged from -0.70±0.14% day^{-1} (NH_4-5) to 3.84±0.32% day^{-1} (NO_3-3) (Figure 1). In NO_3 applications, growth increased up to the NO_3-3 group, whereas in NH_4NO_3 applications, growth increased up to the NH_4-2 group (2.18±0.14% day^{-1}), followed by lower values at higher doses. Negative growth was recorded in NH_4-5.

DPPH Radical-scavenging Activity

The highest inhibition value was observed in the NO_3-3 group (41.10±1.73%), whereas the lowest value was determined in the NO_3-4 group (17.72±2.87%) (Table 1). Significant differences in inhibition values were found among the treatment groups ($P<0.05$). IC_{50} values ranged from 8.65±0.48 (NO_3-3) to 22.61±0.82 mg g^{-1} extract (NO_3-4) ($P<0.05$). Vitamin C and BHT showed higher inhibition values and lower IC_{50} values than the algal extract groups.

Total Phenolic, Flavonoid and Condensed Tannin Contents

Total phenolic, flavonoid and condensed tannin contents differed among nitrogen treatments (Table 2). Total phenolic contents ranged from 1.10 to 2.18 mg GAE g^{-1} ext in NO_3 applications and from 0.80 to 1.08 mg GAE g^{-1} ext in NH_4NO_3 applications. The highest value was determined in the control group (2.35±0.08 mg GAE g^{-1} ext), followed by NO_3-2 and NO_3-3. The lowest values were observed in NH_4-1, NH_4-2 and NH_4-5.

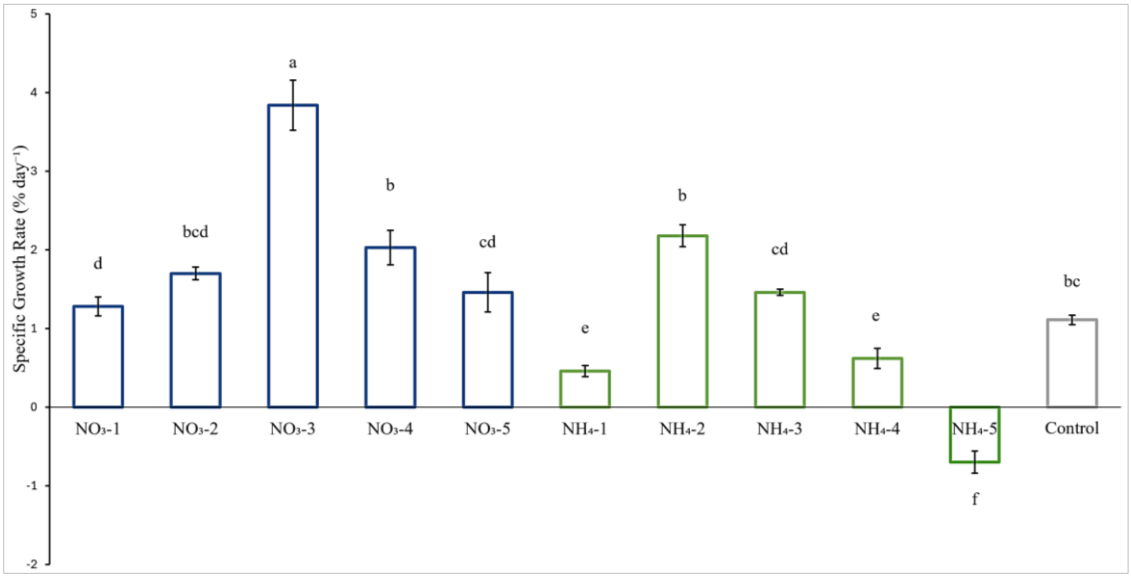

Figure 1. Specific growth rate of *U. rigida* under different nitrogen source and dose regimes. Values are presented as Mean±SD (n=3). Different letters indicate significant differences among groups ($P<0.05$).

Total flavonoid content ranged from 1.64±0.06 mg RE g^{-1} ext. (NH_4-2) to 3.05±0.04 mg RE g^{-1} ext. (NO_3-5). Flavonoid contents ranged from 2.35 to 3.05 mg RE g^{-1} ext. in NO_3 applications and from 1.64 to 2.13 mg RE g^{-1} ext. in NH_4NO_3 applications. Significant differences were observed among groups (P<0.05).

Condensed tannin contents differed among groups (P<0.05). The highest value was observed in the NO_3-4 group (125.61±0.74 µg CE g^{-1} ext), while the control group showed 114.77±9.99 µg CE g^{-1} ext. The lowest value was observed in the NH_4-2 group (66.72±2.22 µg CE g^{-1} ext).

Pigment Content

The highest chlorophyll *a* content was recorded in the Control group (5.14±0.23 mg g^{-1}), whereas the lowest value was observed in the NO_3-2 group (3.44±0.52 mg g^{-1}) (Table 3). Chlorophyll *b* content ranged from 1.93±0.23 mg g^{-1} (NO_3-2) to 3.06±0.39 mg g^{-1} (NO_3-5). Chlorophyll contents differed among groups (P<0.05). In NO_3 treatments, chlorophyll *b* reached the highest value in NO_3-5, whereas chlorophyll *a* was the highest in the control group. In NH_4NO_3 treatments, chlorophyll *a* and *b* decreased at high doses and the lowest values were observed in NH_4-5.

Total carotenoid content ranged between 1.45±0.12 mg g^{-1} (NH_4-5) and 2.49±0.06 mg g^{-1} (control). Differences among groups were significant (P<0.05). The highest value was observed in the control group, whereas lower values were observed in the high NH_4NO_3 treatments.

Correlation and Principal Component Analyses

According to Spearman correlation analysis (Table 4), DPPH inhibition was strongly negatively correlated with IC_{50} (r=-0.941, p<0.001). Positive correlations were observed between DPPH inhibition and total phenolic (r=0.628, p<0.001) and total flavonoid contents (r=0.505, p=0.003). DPPH inhibition also showed a moderate positive correlation with SGR (r=0.452, p=0.008). Significant correlations were also observed between DPPH inhibition and chlorophyll *b* (r=0.358, p=0.041) and total carotenoid content (r=0.388, p=0.026), although these correlations were weaker than those observed for phenolic and flavonoid contents. No significant correlation was observed between DPPH inhibition and condensed tannin content (r=0.015, p=0.932).

IC_{50} showed negative correlations with total phenolic (r=-0.561, p=0.001) and total flavonoid contents (r=-0.496, p=0.003). A weak negative correlation was also observed between IC_{50} and SGR (r=0.352, p=0.045). Total phenolic and total flavonoid contents showed a strong positive correlation (r=0.779,

Table 1. DPPH inhibition (%) and IC_{50} values of *U. rigida* under different nitrogen source and dose regimes

Group	Inhibition (%)	IC_{50} (mg g^{-1}ext)
NO_3-1	29.07±0.72^{de}	13.65±0.89^{d}
NO_3-2	29.88±2.45^{d}	14.22±0.30^{d}
NO_3-3	41.10±1.73^{b}	8.65±0.48^{e}
NO_3-4	17.72±2.87^{g}	22.61±0.82^{a}
NO_3-5	35.50±2.56^{c}	9.72±0.76^{e}
NH_4-1	23.37±0.69^{f}	20.06±0.28^{b}
NH_4-2	26.32±0.34^{def}	18.39±0.52^{b}
NH_4-3	24.69±0.67^{def}	19.86±0.48^{b}
NH_4-4	21.80±0.83^{fg}	22.34±0.41^{a}
NH_4-5	24.37±0.57^{ef}	19.60±0.64^{b}
Control	27.01±3.37^{de}	17.74±1.76^{c}
Vitamin C	98.92±0.18^{a}	1.35±0.85^{f}
BHT	98.95±0.07^{a}	1.35±0.35^{f}

Values are presented as Mean±SD (n=3). Different superscript letters within the same column indicate significant differences among groups according to Tukey's multiple comparison test (P<0.05). Vitamin C and BHT were used as positive controls.

Table 2. Total phenolic, total flavonoid, and condensed tannin content of *U. rigida* under different nitrogen source and dose regimes

Group	Total Phenolics (mg GAE g^{-1} ext)	Total Flavonoids (mg RE g^{-1} ext)	Condensed Tannins (µg CE g^{-1} ext)
NO_3-1	1.67±0.03^{d}	2.89±0.03^{b}	118.99±5.19^{ab}
NO_3-2	2.18±0.02^{ab}	2.35±0.03^{c}	93.33±4.54^{d}
NO_3-3	2.04±0.04^{bc}	2.92±0.04^{b}	101.31±0.42^{cd}
NO_3-4	1.10±0.07^{e}	2.88±0.04^{b}	125.61±0.74^{a}
NO_3-5	1.89±0.04^{c}	3.05±0.04^{a}	109.76±1.03^{bc}
NH_4-1	0.80±0.10^{f}	2.12±0.01^{d}	102.91±2.49^{cd}
NH_4-2	0.80±0.07^{f}	1.64±0.06^{f}	66.72±2.22^{e}
NH_4-3	1.05±0.06^{e}	1.90±0.08^{e}	115.90±18.10^{ab}
NH_4-4	1.08±0.10^{e}	2.13±0.04^{d}	76.22±2.98^{e}
NH_4-5	0.82±0.06^{f}	2.01±0.04^{de}	91.19±1.54^{d}
Control	2.35±0.08^{a}	2.96±0.04^{ab}	114.77±9.99^{ab}

Values are expressed as Mean±SD (n=3). Different letters within the same column indicate significant differences among groups (P<0.05).

p<0.001). Strong correlations were also observed among pigment parameters (r=0.889–0.929, p<0.001).

Principal component analysis showed that the first two components explained 75.4% of the total variance (PC1: 53.5%, PC2: 21.9%) (Figure 2). Samples tended to separate according to nitrogen treatment along PC1. NH_4NO_3-treated samples were primarily clustered on the negative side of PC1, whereas NO_3-treated samples were clustered on the positive side. The control group clustered more closely with the NO_3 treatments. The loading plot indicated that chlorophyll *a*, chlorophyll *b*, total carotenoids, total flavonoids, total phenolics, DPPH inhibition activity, condensed tannins and SGR were positively associated with PC1, whereas IC_{50} was negatively associated with this axis (Figure 3).

Discussion

Growth Response to Nitrogen Treatments

Growth differed among nitrogen treatments in *U. rigida*. The highest SGR was recorded in NO_3-3, while negative growth was observed in NH_4-5. This pattern shows that growth increased at intermediate NO_3 levels and decreased at high NH_4NO_3 doses under the applied culture conditions. Previous studies reported different growth responses of *Ulva* species to nitrate and ammonium sources. Wang et al. (2019) reported that *Ulva prolifera* biomass decreased at nitrate concentrations above 284 µmol L^{-1} and associated this decrease with growth inhibition. The study also showed that increases in nitrate uptake rate had a limited effect on relative growth rate, with growth increasing up to a certain uptake rate and then decreasing. Li et al. (2016) observed mean relative growth rates of 2.8±0.03% day^{-1} for NO_3^- and 4.0±0.11% day^{-1} for NH_4^+ at a nitrogen concentration of 40 µM. Maximum relative growth rates were recorded at 7.6±0.14% day^{-1} and 8.1±0.31% day^{-1} for NO_3^- and NH_4^+, respectively. Under NH_4-based nutrition, growth rates reached 14.3±1.5% day^{-1} compared with 7.5±3.0% day^{-1} under NO_3-based nutrition, which was attributed to higher uptake rates and lower energy requirements of NH_4 assimilation (Shahar et al., 2020). Maximum relative growth rates of 13.1% day^{-1} with NO_3– and 16.9% day^{-1} with $NH4^+$ were calculated for *U. prolifera*, while corresponding values for *Ulva linza* were 9.8% day^{-1} and 11.6% day^{-1} (Luo et al., 2012). The same study identified a higher affinity for NH_4^+ than for NO_3^- and linked this response to the lower energy requirement of ammonium assimilation.

Table 3. Chlorophyll *a*, chlorophyll *b*, and total carotenoid contents of *U. rigida* under different nitrogen source and dose regimes

Group	Chl *a* (mg g^{-1})	Chl *b* (mg g^{-1})	Total carotenoid (mg g^{-1})
NO_3-1	4.42±0.39^{abc}	2.67±0.11^{ab}	2.07±0.21^{abc}
NO_3-2	3.44±0.52^{c}	1.93±0.23^{c}	1.66±0.17^{abc}
NO_3-3	4.43±0.61^{abc}	2.54±0.36^{abc}	2.15±0.32^{abc}
NO_3-4	4.40±0.39^{abc}	2.57±0.40^{abc}	2.08±0.20^{abc}
NO_3-5	4.84±0.57^{a}	3.06±0.39^{a}	2.32±0.43^{a}
NH_4-1	4.42±0.07^{abc}	2.53±0.03^{abc}	2.03±0.04^{abc}
NH_4-2	4.43±0.56^{abc}	2.50±0.42^{abc}	2.03±0.37^{abc}
NH_4-3	4.69±0.09^{ab}	2.47±0.01^{abc}	1.96±0.01^{abc}
NH_4-4	4.15±0.54^{abc}	2.20±0.25^{bc}	1.56±0.61^{bc}
NH_4-5	3.58±0.26^{bc}	2.01±0.14^{bc}	1.45±0.12^{c}
Control	5.14±0.23^{a}	2.67±0.17^{a}	2.49±0.06^{ab}

Values are expressed as Mean±SD (n=3). Different letters within the same column indicate significant differences among groups (P<0.05).

Table 4. Spearman correlation coefficients among growth, antioxidant, biochemical, and pigment parameters in *U. rigida*

	Inhibition	IC_{50}	SGR	TPC	TFC	CT	Chl *a*	Chl *b*	TCar
Inhibition	1.000	-0.941* (0.000)	0.452* (0.008)	0.628* (0.000)	0.505* (0.003)	0.015 (0.932)	0.257 (0.148)	0.358* (0.041)	0.388* (0.026)
IC_{50}		1.000	-0.352* (0.045)	-0.561* (0.001)	-0.496* (0.003)	0.014 (0.940)	-0.061 (0.736)	-0.251 (0.159)	-0.250 (0.160)
SGR			1.000	0.480* (0.005)	0.318 (0.071)	0.156 (0.386)	0.403* (0.020)	0.388* (0.026)	0.541* (0.001)
TPC				1.000	0.779* (0.000)	0.330 (0.060)	0.286 (0.106)	0.375* (0.032)	0.349* (0.047)
TFC					1.000	0.527* (0.002)	0.405* (0.019)	0.626* (0.000)	0.550* (0.001)
CT						1.000	0.467* (0.006)	0.545* (0.001)	0.539* (0.001)
Chl *a*							1.000	0.895* (0.000)	0.889* (0.000)
Chl *b*								1.000	0.929* (0.000)
TCar									1.000

TPC: total phenolic content; TFC: total flavonoid content; CT: condensed tannins; TCar: total carotenoids. Note. Values are Spearman correlation coefficients (r), p-values are given in parentheses. * P<0.05.

In the present study, growth increased at lower NH_4NO_3 doses, whereas reduced growth and negative growth were observed at higher doses. Differences among groups may be related to differences in nitrogen utilization pathways. Ammonium directly participates in assimilation, whereas nitrate must be transported into the cell and reduced before assimilation (Glibert et al., 2016). Nitrogen compounds were added daily to the culture medium at relatively high concentrations (1.12–2.50 mM day^{-1} in NH_4NO_3 groups). In the present study, growth decreased at high NH_4NO_3 doses. Ji et al. (2019) also reported reduced growth under high $NH4^+$ enrichment, with species-specific responses.

Antioxidant and Biochemical Responses

DPPH inhibition and IC_{50} values differed among treatments. NO_3-3 showed the highest DPPH inhibition and the lowest IC_{50} value. NO_3-4 showed the lowest DPPH inhibition and the highest IC_{50} value. These results indicate that antioxidant activity varied among nitrogen sources, concentration and application regime under controlled culture conditions. Similar variation in antioxidant responses has been reported in previous studies. Vega et al. (2020) observed that the half-maximal effective concentration increased in *U. rigida* cultivated under ammonium-rich IMTA conditions. DPPH radical-scavenging activity may vary depending on collection site, season and culture conditions. Ak and Türker (2019) found an IC_{50} value of 3.76 mg g^{-1} extract and approximately 41% inhibition in *U. rigida* collected from the Çanakkale Strait. IC_{50} values ranging from 0.18 to 0.41 µg mL^{-1} were obtained from different extracts of the same species collected from the Ras-Djebel coast of Tunisia (Mezghani et al., 2016).

Higher phenolic contents were generally observed in the control and NO_3 groups than in the NH_4NO_3 groups. Neves et al. (2026) reported no significant change in the phenolic content of *U. rigida* under different nutrient conditions. In the present study, phenolic contents differed among nitrogen treatments. Other studies reported variable phenolic contents in *Ulva* species under different environmental and cultivation conditions (Yildiz et al., 2012; Trigui et al., 2013; Ak & Türker, 2019; Casado et al., 2023; Duarte et al., 2025; Sousa et al., 2025). These differences may be

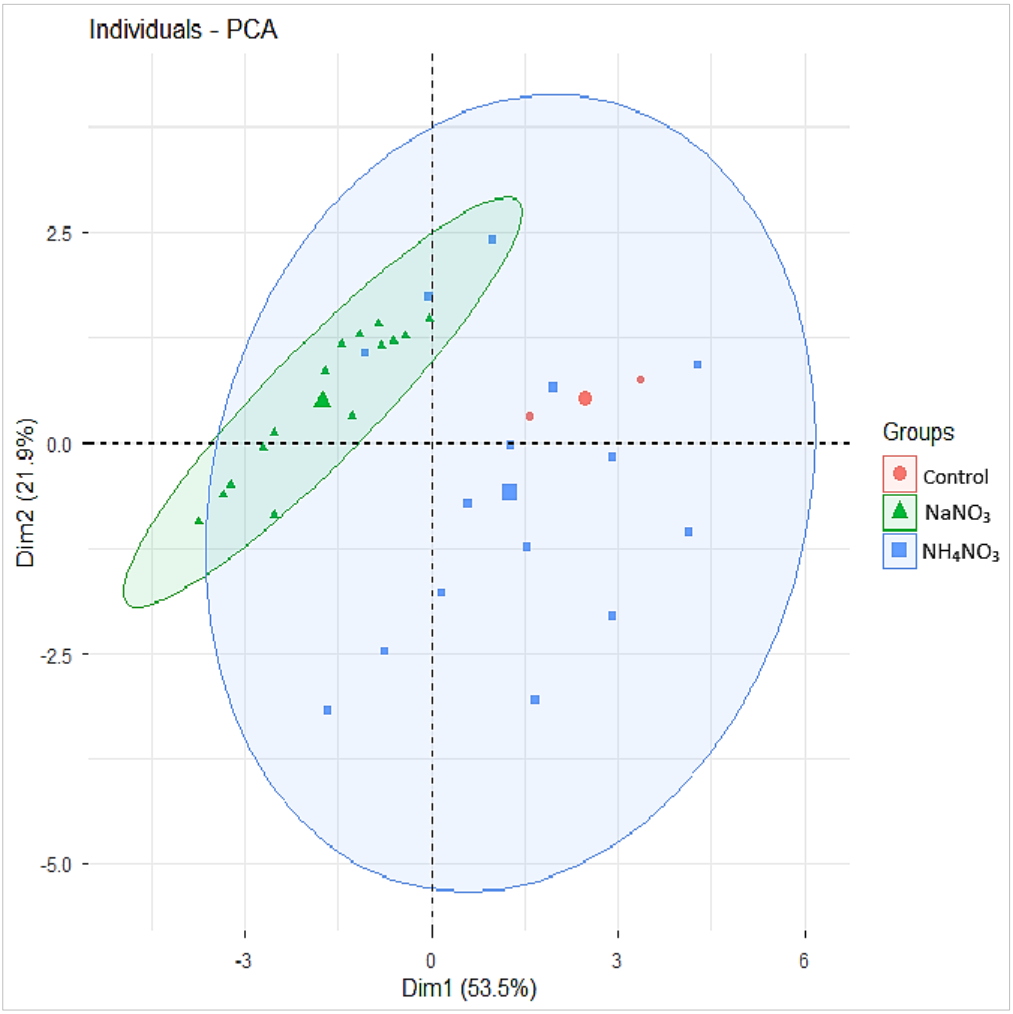

Figure 2. Principal component analysis (PCA) score plot based on growth, antioxidant, biochemical and pigment parameters of *U. rigida* cultivated under different nitrogen source and dose regimes. PC1 and PC2 explained 53.5% and 21.9% of the total variance, respectively.

related to species, cultivation systems, sampling periods, nutrient conditions and environmental characteristics.

Flavonoid contents were higher in NO_3 applications than in NH_4NO_3 applications. Similar differences in flavonoid contents have been reported under different environmental and cultivation conditions. Marinho et al. (2019) reported seasonal variations in flavonoid contents, whereas Schiavon et al. (2012) observed increased flavonoid levels in *Ulva* sp. under selenium-induced oxidative stress.

Condensed tannin contents also differed among treatments. Reports on condensed tannin contents in marine macroalgae remain limited. Previous studies reported condensed tannin contents of 3.76 mg CE g^{-1}ext. in *U. rigida* (Ak & Türker, 2019), while Petchidurai et al. (2019) reported variable tannin contents among drifted coastal macroalgae. Direct comparison among studies is difficult due to differences in analytical methods, standards, units of expression and sample bases.

Pigment Responses

Pigment contents differed among nitrogen treatments. Chlorophyll *a* and total carotenoid contents were highest in the control group, whereas chlorophyll *b* was highest in NO_3-5. In NH_4NO_3 treatments, chlorophyll *a*, chlorophyll *b* and total carotenoid contents were lower at high doses.

Nitrogen availability has been reported to affect pigment composition in *Ulva* species. Xu et al. (2014) observed increased chlorophyll contents under NH_4^+ enrichment, whereas Van Alstyne (2018) reported that nitrate availability and light affected growth and resource allocation in Ulva species. Zheng et al. (2019) determined changes in chlorophyll content at different nitrogen levels under varying salinity conditions. In contrast, Neves et al. (2026) found no significant changes in photosynthetic pigment composition under different nutrient ratios in *U. rigida*. Chlorophyll contents also vary among cultivation systems and experimental conditions. Total chlorophyll content ranged from 5.8 to 10.6 mg g^{-1} in *U. lacinulata* cultivated in an integrated multi-trophic system (Sousa et al., 2025), whereas chlorophyll *a* content reached 2.51 mg g^{-1} in *Ulva* sp. cultivated in an RAS-associated system (Duarte et al., 2025). Reduced total chlorophyll contents were observed under nutrient-limited conditions (McCauley et al., 2018).

Carotenoid contents also varied among treatments. Zheng et al. (2019) showed that nitrogen level significantly affected carotenoid content in *U. prolifera*. Xu et al. (2014) reported that NH_4^+ enrichment increased carotenoid content, although this response varied with light intensity. McCauley et al. (2018) observed strong pigment loss under nutrient-limited conditions. Ak and Turker (2018) reported a carotenoid content of 414.2 µg g^{-1} in *U. rigida* collected from the Çanakkale Strait. Carotenoid values ranged from 0.9 to

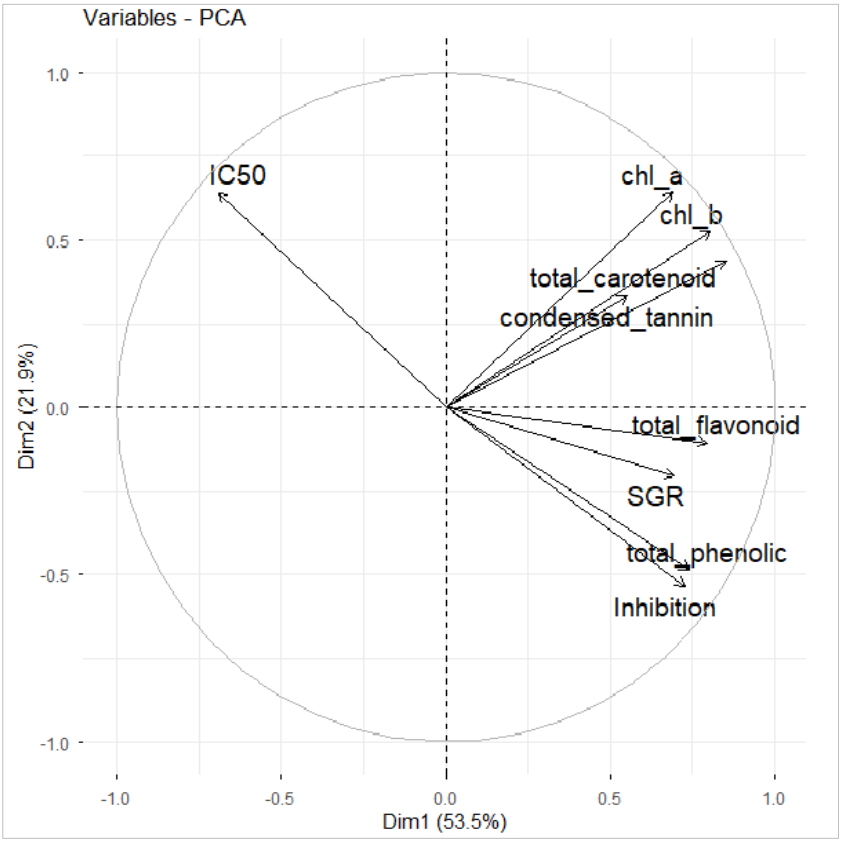

Figure 3. Principal Component Analysis (PCA) loading plot showing the contribution of growth, antioxidant, biochemical and pigment parameters to PC1 and PC2 in *U. rigida* under different nitrogen source and dose regimes.

1.8 mg g^{-1} in *U. lacinulata* cultivated in an integrated multi-trophic system (Sousa et al., 2025). The carotenoid content reached 0.58 mg g^{-1} in *Ulva* sp. cultivated under RAS conditions (Duarte et al., 2025).

Relationships Among Antioxidant, Biochemical and Pigment Parameters

Correlation analysis showed positive associations of DPPH inhibition with total phenolic and total flavonoid contents. IC_{50} showed the opposite pattern. In this study, phenolic and flavonoid contents were more closely linked to antioxidant activity than condensed tannin content. Similar associations between antioxidant activity and phenolic compounds were reported for *U. rigida* by Mezghani et al. (2016), Trigui et al. (2013) and for other *Ulva* species by Yao et al. (2022). Ak and Türker (2019) also reported significant relationships between antioxidant activity parameters and phenolic and flavonoid contents. In *Gongolaria barbata*, Ak et al. (2022) and Yılmaz et al. (2021) reported lower IC_{50} values in relation to higher phenolic and flavonoid contents. These reports agree with the correlation pattern found here. However, antioxidant activity does not always follow phenolic content. Neves et al. (2026) reported that phenolic content remained stable in *U. rigida* under different nutrient conditions. Their PCA showed that antioxidant activity was related to low nutrient supply. Ak et al. (2022) found high DPPH activity in some *G. barbata* groups with lower phenolic contents. Sousa et al. (2025) reported parallel changes in pigments and phenolic compounds in *U. lacinulata*. In the present study, pigment parameters showed weaker correlations with DPPH inhibition than phenolic and flavonoid contents.

The PCA supported the differences among nitrogen treatments. The first two components explained 75.4% of the total variance. NH_4NO_3-treated samples were mainly on the negative side of PC1, whereas NO_3-treated samples were mainly on the positive side. The control group was closer to the NO_3 treatments. The loading plot showed that growth, pigment content, DPPH inhibition and phenolic-related variables were positively associated with PC1, whereas IC_{50} was negatively associated with PC1. These results indicate that nitrogen source and application regime influenced growth, pigment composition, antioxidant activity and biochemical characteristics in *U. rigida*. A similar effect of nitrogen availability on the biochemical composition of *Ulva* species was reported by Queirós et al. (2021).

Conclusions

This study examined the effects of different nitrogen sources and application regimes on the growth and biochemical characteristics of *U. rigida* under controlled culture conditions. Growth performance, antioxidant activity, pigment composition and biochemical characteristics differed among treatments. The highest specific growth rate and DPPH inhibition activity were observed in the NO_3-3 treatment, whereas the lowest growth rate was recorded in NH_4-5. Total phenolic, flavonoid and condensed tannin contents differed among treatments, with higher phenolic contents generally observed in the control and NO_3 treatments than in NH_4NO_3 treatments. Correlation and principal component analyses supported the differences observed among nitrogen treatments. The results showed that nitrogen source and application regime influenced not only growth performance but also pigment composition, antioxidant activity and biochemical characteristics of *U. rigida*. Future studies should investigate how nitrogen sources and application regimes can be optimized to meet specific production targets, such as biomass production, pigment enrichment, or antioxidant-rich biomass.

Ethical Statement

This study did not involve any vertebrates or cephalopods. Therefore, ethical approval was not required, as per national and institutional regulations.

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Author Contribution

Conceptualization: ÖÖ, İA; Methodology: ÖÖ, İA; Investigation and experimental work: ÖÖ; Data analysis: ÖÖ, İA; Writing – original draft: ÖÖ, İA; Writing – review and editing: İA; Supervision: İA.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal interests that could have influenced the work reported in this paper.

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References

Ak, I., & Turker, G. (2018). Antioxidant activity of five seaweed extracts. *New Knowledge Journal of Science*, 7(2), 149–155.

Ak, İ., Çankırılıgil, E. C., Türker, G., Sever, O., & Abomohra, A. (2022). Enhancement of antioxidant properties of *Gongolaria barbata* (Phaeophyceae) by optimization of combined light intensity and salinity stress. *Phycologia, 61*(6), 584–594.

https://doi.org/10.1080/00318884.2022.2099136

Ak, İ., & Türker, G. (2019). Free radical scavenging activity and biochemical characteristics of *Ulva rigida* (Ulvophyceae) and *Arthrospira platensis* (Cyanophyceae). *Turkish Journal of Agriculture - Food Science and Technology, 7*(sp1), 145–149. https://doi.org/10.24925/turjaf.v7isp1.145-149.2789

Ale, M. T., Mikkelsen, J. D., & Meyer, A. S. (2011). Differential growth response of *Ulva lactuca* to ammonium and nitrate assimilation. *Journal of Applied Phycology, 23*(3), 345–351. https://doi.org/10.1007/s10811-010-9546-2

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology, 28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Buschmann, A. H., Camus, C., Infante, J., Neori, A., Israel, Á., Hernández-González, M. C., Pereda, S. V., Gómez-Pinchetti, J. L., Golberg, A., Tadmor-Shalev, N., Critchley, A. T. (2017). Seaweed production: overview of the global state of exploitation, farming and emerging research activity. *European Journal of Phycology*, *52*(4), 391–406. https://doi.org/10.1080/09670262.2017.1365175

Casado, R., Pinto, F. R., Barosso, S., & Gil, M. M. (2023). Phenolic compounds content and antioxidant activity of wild macroalgae *Ulva* sp. vs cultivated *Ulva rigida*. In the *24th International Seaweed Symposium*, 19–27 February 2023, Hobart, Tasmania, Australia. https://iss2023.net-/2022/08/19/phenolic-compounds-content-and antioxi-dant-activity-of-wild-macroalgae-ulva-sp-vs-cultivated-ulva-rigida/

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., & Vidal, N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry, 97*(4), 654–660. https://doi.org/10.1016/j.foodchem.2005.04.028

Dominguez, H., & Loret, E. P. (2019). *Ulva lactuca*, a source of troubles and potential riches. *Marine Drugs, 17*(6), 357. https://doi.org/10.3390/md17060357

Duarte, A. M., Carvalhal, G., Assunção, J., Guedes, A. C., Pinto, I. S., & Costa, I. (2025). Cultivation of *Ulva* sp. and *Gracilaria* sp. associated with a recirculating aquaculture system. *Journal of Applied Phycology, 37*(3), 1525–1539. https://doi.org/10.1007/s10811-024-03424-6

Figueroa, F. L., Israel, A., Neori, A., Martínez, B., Malta, E., Ang, P. O., Jr., Inken, S., Marquardt, R., & Korbee, N. (2009). Effects of nutrient supply on photosynthesis and pigmentation in *Ulva lactuca* (Chlorophyta): Responses to short-term stress. *Aquatic Biology, 7*, 173–183. https://doi.org/10.3354/ab00187

Glibert, P. M., Wilkerson, F. P., Dugdale, R. C., Raven, J. A., Dupont, C. L., Leavitt, P. R., Parker, A. E., Burkholder, J. M., & Kana, T. M. (2016). Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions. *Limnology and Oceanography, 61*(1), 165–197. https://doi.org/10.1002/lno.10203

Gómez Pinchetti, J. L., del Campo Fernández, E., Moreno Díez, P., & Reina, G. G. (1998). Nitrogen availability influences the biochemical composition and photosynthesis of tank-cultivated *Ulva rigida* (Chlorophyta). *Journal of Applied Phycology, 10*(4), 383–389. https://doi.org/10.1023/A:1008008912991

Heriansah, H., Syamsuddin, R., Najamuddin, N., Syafiuddin, S., & Hidayani, M. T. (2025). Studying how to minimize nutrient waste while maximizing macroalgae biomass: A vertical method study on an integrated aquaculture recirculating system. *Aquaculture Studies, 25*(3), 128–138. https://doi.org/10.4194/AQUAST1874

Hurd, C. L., Harrison, P. J., Bischof, K., & Lobban, C. S. (2014). *Seaweed ecology and physiology* (2nd ed.). Cambridge University Press. https://doi.org/10.1017/CBO9781139192637

Jeffrey, S. W., & Humphrey, G. F. (1975). New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae and natural phytoplankton. *Biochemie und Physiologie der Pflanzen, 167*(2), 191–194. https://doi.org/10.1016/S0015-3796(17)30778-3

Ji, Z., Zou, D., Gong, J., Liu, C., Ye, C., & Chen, Y. (2019). The different responses of growth and photosynthesis to NH_4^+ enrichments between *Gracilariopsis lemaneiformis* and its epiphytic alga *Ulva lactuca* grown at elevated atmospheric CO_2. *Marine Pollution Bulletin, 144*, 173–180. https://doi.org/10.1016/j.marpolbul.2019.04.049

Kang, J. W., & Chung, I. K. (2018). The interactive effects of elevated CO_2 and ammonium enrichment on the physiological performances of *Saccharina japonica* (Laminariales, Phaeophyta). *Ocean Science Journal, 53*(3), 487–497. https://doi.org/10.1007/s12601-018-0014-2

Kang, J. W., Kambey, C., Shen, Z., Yang, Y., & Chung, I. K. (2017). The short-term effects of elevated CO_2 and ammonium concentrations on physiological responses in *Gracilariopsis lemaneiformis* (Rhodophyta). *Fisheries and Aquatic Sciences, 20*, 18. https://doi.org/10.1186/s41240-017-0063-y

Leandro, A., Pereira, L., & Gonçalves, A. M. M. (2020). Diverse applications of marine macroalgae. *Marine Drugs, 18*(1), 17. https://doi.org/10.3390/md18010017

Li, H., Zhang, Y., Han, X., Shi, X., Rivkin, R. B., & Legendre, L. (2016). Growth responses of *Ulva prolifera* to inorganic and organic nutrients: Implications for macroalgal blooms in the southern Yellow Sea, China. *Scientific Reports, 6*, 26498. https://doi.org/10.1038/srep26498

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In *Methods in Enzymology* (Vol. 148, pp. 350–382). Academic Press. https://doi.org/10.1016/0076-6879(87)48036-1

Luo, M., Liu, F., & Xu, Z. (2012). Growth and nutrient uptake capacity of two co-occurring species, *Ulva prolifera* and *Ulva linza*. *Aquatic Botany, 100*, 18–24. https://doi.org/10.1016/j.aquabot.2012.03.006

Marinho, G. S., Sørensen, A.-D. M., Safafar, H., Pedersen, A. H., & Holdt, S. L. (2019). Antioxidant content and activity of the seaweed *Saccharina latissima*: A seasonal perspective. *Journal of Applied Phycology, 31*(2), 1343–1354. https://doi.org/10.1007/s10811-018-1650-8

McCauley, J. I., Winberg, P. C., Meyer, B. J., & Skropeta, D. (2018). Effects of nutrients and processing on the nutritionally important metabolites of *Ulva* sp. (Chlorophyta). *Algal Research, 35*, 586–594. https://doi.org/10.1016/j.algal.2018.09.016

Mezghani, S., Csupor, D., Bourguiba, I., Hohmann, J., Amri, M., & Bouaziz, M. (2016). Characterization of phenolic compounds of *Ulva rigida* (Chlorophycae) and its antioxidant activity. *European Journal of Medicinal Plants, 12*(1), 1–9.

Miguel, S. P., D'Angelo, C., Ribeiro, M. P., Simões, R., & Coutinho, P. (2023). Chemical composition of

macroalgae polysaccharides from Galician and Portugal coasts: Seasonal variations and biological properties. *Marine Drugs, 21*(11), 589. https://doi.org/10.3390/md21110589

Neves, F. F., Robles-Carnero, V., Dal Grande, I. G., Benitez, A. A., Bonomi-Barufi, J., Celis-Plá, P. S. M., & Figueroa, F. L. (2026). Biomass productivity, photosynthesis and bioactive compounds in land-based cultivated *Ulva rigida*: Stocking density and nutrient availability. *Aquaculture, 614*, 743544. https://doi.org/10.1016/j.aquaculture.2025.743544

Ortiz, J., Romero, N., Robert, P., Araya, J., Lopez-Hernández, J., Bozzo, C., Navarrete, E., Osorio, A., & Rios, A. (2006). Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. *Food Chemistry, 99*(1), 98–104. https://doi.org/10.1016/j.foodchem.2005.07.027

Pathan, A., Mahajan, M. M., Jain, P. G., Zambad, S. P., Bhandari, G. S., Darak, A. D., Ahire, E. D., Rajora, A. K., & Surana, K. R. (2023). Nutraceutical properties of seaweed vitamins. In E. D. Ahire, R. K. Keservani, K. R. Surana, S. Singh, & R. K. Kesharwani (Eds.), Vitamins as nutraceuticals (pp. 167–184). Wiley. https://doi.org/10.1002/9781394175543.ch7

Petchidurai, G., Nagoth, J. A., John, M. S., Sahayaraj, K., Murugesan, N., & Pucciarelli, S. (2019). Standardization and quantification of total tannins, condensed tannin and soluble phlorotannins extracted from thirty-two drifted coastal macroalgae using high performance liquid chromatography. *Bioresource Technology Reports, 7*, 100273. https://doi.org/10.1016/j.biteb.2019.100273

Piechulla, B., & Heldt, H. W. (2024). *Plant biochemistry*. Academic Press.

Price, M. L., Van Scoyoc, S., & Butler, L. G. (1978). A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry, 26*(5), 1214–1218. https://doi.org/10.1021/jf60219a031

Queirós, A. S., Circuncisão, A. R., Pereira, E., Válega, M., Abreu, M. H., Silva, A. M. S., & Cardoso, S. M. (2021). Valuable nutrients from *Ulva rigida*: Modulation by seasonal and cultivation factors. *Applied Sciences, 11*(13), 6137.

Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., Cazin, M., Cazin, J.-C., Bailleul, F., & Trotin, F. (2000). Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *Journal of Ethnopharmacology, 72*(1–2), 35–42. https://doi.org/10.1016/S0378-8741(00)00196-3

Reidenbach, L. B., Fernandez, P. A., Leal, P. P., Noisette, F., McGraw, C. M., Revill, A. T., Hurd, C. L., & Kübler, J. E. (2017). Growth, ammonium metabolism, and photosynthetic properties of *Ulva australis* (Chlorophyta) under decreasing pH and ammonium enrichment. *PLOS ONE, 12*(11), e0188389. https://doi.org/10.1371/journal.pone.0188389

Schiavon, M., Moro, I., Pilon-Smits, E. A. H., Matozzo, V., Malagoli, M., & Dalla Vecchia, F. (2012). Accumulation of selenium in *Ulva* sp. and effects on morphology, ultrastructure and antioxidant enzymes and metabolites. *Aquatic Toxicology, 122–123*, 222–231. https://doi.org/10.1016/j.aquatox.2012.06.014

Shahar, B., Shpigel, M., Barkan, R., Masasa, M., Neori, A., Chernov, H., Salomon, E., Kiflawi, M., & Guttman, L. (2020). Changes in metabolism, growth and nutrient uptake of *Ulva fasciata* (Chlorophyta) in response to nitrogen source. *Algal Research, 46*, 101781. https://doi.org/10.1016/j.algal.2019.101781

Shpigel, M., Guttman, L., Ben-Ezra, D., Yu, J., & Chen, S. (2019). Is *Ulva* sp. able to be an efficient biofilter for mariculture effluents? *Journal of Applied Phycology, 31*(4), 2449–2459. https://doi.org/10.1007/s10811-019-1748-7

Simon, C., McHale, M., & Sulpice, R. (2022). Applications of *Ulva* biomass and strategies to improve its yield and composition: A perspective for *Ulva* aquaculture. *Biology, 11*(11), 1593. https://doi.org/10.3390/biology11111593

Sousa, C., Sousa-Pinto, I., Oliveira, I., & Marinho, G. S. (2025). Seasonal variation in the composition and antioxidant potential of *Codium tomentosum* and *Ulva lacinulata* produced in a land-based integrated multi-trophic aquaculture system. *Journal of Applied Phycology, 37*(3), 1557–1572. https://doi.org/10.1007/s10811-025-03489-x

Toth, G. B., Harrysson, H., Wahlström, N., Olsson, J., Oerbekke, A., Steinhagen, S., Kinnby, A., White, J., Albers, E., Edlund, U., Undeland, I., & Pavia, H. (2020). Effects of irradiance, temperature, nutrients, and pCO_2 on the growth and biochemical composition of cultivated *Ulva fenestrata*. *Journal of Applied Phycology, 32*(5), 3243–3254. https://doi.org/10.1007/s10811-020-02155-8

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. https://doi.org/10.1007/s10811-012-9866-5

UTEX Culture Collection of Algae. (2019). Artificial seawater medium. UTEX Culture Collection of Algae. Retrieved June 4, 2026, from https://utex.org/products/artificial-seawater-medium?variant=30991787884634

Van Alstyne, K. L. (2018). Seawater nitrogen concentration and light independently alter performance, growth, and resource allocation in the bloom-forming seaweeds *Ulva lactuca* and *Ulvaria obscura* (Chlorophyta). *Harmful Algae, 78*, 27–35. https://doi.org/10.1016/j.hal.2018.07.005

Vega, J., Álvarez-Gómez, F., Güenaga, L., Figueroa, F. L., & Gómez-Pinchetti, J. L. (2020). Antioxidant activity of extracts from marine macroalgae, wild-collected and cultivated, in an integrated multi-trophic aquaculture system. *Aquaculture, 522*, 735088. https://doi.org/10.1016/j.aquaculture.2020.735088

Viaroli, P., Naldi, M., Bondavalli, C., & Bencivelli, S. (1996). Growth of the seaweed *Ulva rigida* C. Agardh in relation to biomass densities, internal nutrient pools and external nutrient supply in the Sacca di Goro lagoon (Northern Italy). *Hydrobiologia, 329*, 93–103. https://doi.org/10.1007/BF00034550

Wang, C., Su, R., Guo, L., Yang, B., Zhang, Y., Zhang, L., Xu, H., Shi, W., & Wei, L. (2019). Nutrient absorption by *Ulva prolifera* and the growth mechanism leading to green-tides. *Estuarine, Coastal and Shelf Science, 227*, 106329. https://doi.org/10.1016/j.ecss.2019.106329

Warnasooriya, V., Gunawardena, S., Weththasinghe, P., Jayawardana, B. C., Qader, M., & Liyanage, R. (2024). Nutritional properties, antioxidant activity, and heavy metal accumulation in selected marine macro-algae species of Sri Lanka. *Nutraceuticals, 4*(1), 50–64. https://doi.org/10.3390/nutraceuticals4010004

Xu, Z., Wu, H., Zhan, D., Sun, F., Sun, J., & Wang, G. (2014). Combined effects of light intensity and NH_4^+-enrichment on growth, pigmentation, and photosynthetic performance of *Ulva prolifera* (Chlorophyta). *Chinese Journal of Oceanology and Limnology, 32*(5), 1016–1023. https://doi.org/10.1007/s00343-014-3332-y

Yao, L., Liang, Y., Sun, M., Song, S., Wang, H., Dong, Z., Feng, T., & Yue, H. (2022). Characteristic volatile fingerprints of three edible marine green algae (*Ulva* spp.) in China by HS-GC-IMS and evaluation of the antioxidant bioactivities. *Food Research International, 162*, 112109. https://doi.org/10.1016/j.foodres.2022.112109

Yildiz, G., Celikler, S., Vatan, O., & Dere, S. (2012). Determination of the anti-oxidative capacity and bioactive compounds in green seaweed *Ulva rigida* C. Agardh. *International Journal of Food Properties, 15*(6), 1182–1189. https://doi.org/10.1080/10942912.2010.517341

Yılmaz, M., Türker, G., & Ak, İ. (2021). The effect of different solvents on antioxidant properties of *Gongolaria barbata* (Phaeophyceae). *Çanakkale Onsekiz Mart University Journal of Marine Sciences and Fisheries, 4*(2), 197–201. https://doi.org/10.46384/jmsf.1021387

Zheng, M., Lin, J., Zhou, S., Zhong, J., Li, Y., & Xu, N. (2019). Salinity mediates the effects of nitrogen enrichment on the growth, photosynthesis, and biochemical composition of *Ulva prolifera*. *Environmental Science and Pollution Research, 26*(19), 19982–19990. https://doi.org/10.1007/s11356-019-05364-y

Zollmann, M., Liberzon, A., & Golberg, A. (2023). Modeling of growth of the macroalga *Ulva* sp. in a controlled photobioreactor based on nitrogen accumulation dynamics. *Applied Phycology*, *4*(1), 121–140. https://doi.org/10.1080/26388081.2023.2224423