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The effects of elevated-CO_2 and UVR on photosynthetic performance and nitrate reductase activity of *Ulva flexuosa* Wulfen 1803

G Yildiz

Bursa Uludag University, Department of Biology, 16059, Bursa, Turkey

[E-mail: gamze@uludag.edu.tr]

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After the industrial revolution, increasing anthropogenic CO_2 emission causes a number of changes in seawater. These changes are known as ocean acidification and affect the seaweeds in various ways. Therefore, this study is aimed to determine the ecological succession of *Ulva flexuosa* Wulfen 1803 in future predicted CO_2-induced low pH conditions alone and in combination with naturally relevant ultraviolet radiation (UVR). For this purpose, acidification experiments with and without UVR were conducted on *U. flexuosa* from the Mediterranean coast, and important physiological features of algae was investigated. In this study, the Fv/Fm ratios of *U. flexuosa* ranged from 0.718±0.01 to 0.754±0.009. While rETRmax values of samples exposed to elevated-CO_2 were measured between 112.13 – 151.93 µmol $e^-m^{-2}s^{-1}$, it was determined between 111.7 – 158.4 µmol $e^-m^{-2}s^{-1}$ in samples exposed to ambient sea water. According to our results, increased CO_2 concentration in seawater did not improve the photosynthetic efficiency of *U. flexuosa*. However, when the specimens were exposed to elevated-CO_2, nitrate reductase activity of *U. flexuosa* was declined drastically. According to the results, it is suggested that the elevated CO_2 may regulate the nitrogen preference of *U. flexuosa*. Besides, the data also show that *U. flexuosa* was not sensitive to UVR.

[**Keywords**: Carbonic anhydrase, Chlorophyll fluorescence, Nitrate reductase, Ocean acidification]

Introduction

Macroalgae are important organisms for maintaining the stability of marine ecosystems. Their communities constitute a reproduction, sheltering, and feeding area for other marine organisms. However, after the industrial revolution increasing anthropogenic CO_2 emissions[1] causes several changes in their habitat. These changes, including the increased pCO_2 and decreased pH of seawater[1], are known as ocean acidification and affect the reproduction, diversity, and dominance of seaweeds in various ways[2]. In many studies, the effects of increasing CO_2 concentration on photosynthesis, growth, cellular structure and nutrient metabolism of macroalgae were investigated[3,4]. The results show that macroalgae exhibit species-specific responses against elevated CO_2. For example, under elevated CO_2 photosynthetic performance was enhanced in the green algae *U. lactuca*[5] while it was declined in *U. linza*[3]. Also, although the net photosynthetic rate of *U. prolifera* remained unchanged, its growth rate was increased under future predicted CO_2 conditions[6]. The increased growth rates were also reported for *U. lactuca*[7] and *Caulerpa taxifolia*[8] after exposure to elevated CO_2. On the contrary, elevated CO_2 had no detectable effects on growth rates of *U. rigida*[9] but it induced a significant reduction in the growth rate of *U. linza*[3].

It is known that photosynthetic responses of macroalgae to increasing CO_2 are largely related to their carbon uptake strategies. HCO_3^- is the most abundant inorganic carbon form in natural seawater. However, some seaweeds are only dependent on CO_2 for the carbon fixation of photosynthesis, which is not saturated at natural carbon concentrations. The general opinion is that these species will be the winners of future inorganic carbon alterations[10]. On the other hand, most of the seaweeds have saturated or near-saturated photosynthesis in the present inorganic carbon pools[11]. These species have many strategies to provide the CO_2 required for their photosynthesis from HCO_3^-. Among these strategies, called carbon-concentrating mechanisms (CCMs), dehydration of HCO_3^- to CO_2 by extracellular carbonic anhydrase activity and direct transport of HCO_3^- via anion exchange proteins into the cell are the best known[12]. The ability and strategies of seaweed species to use HCO_3^- ions are different among species. In addition, studies have shown that in enriched CO_2 environments, these species regulate

their carbon concentrating mechanisms by reducing their use of HCO_3^- and displaying the preference of CO_2 in their photosynthesis[13]. Therefore, different species will be affected to different degrees by increasing CO_2, and this will determine their species-specific responses against future predicted ocean acidification.

Nitrate and ammonium are important inorganic nitrogen sources in seawater for seaweeds[14]. Among them, ammonium is taken into the cell by facilitated diffusion that does not require energy, being directly incorporated into amino acids. Nitrate is taken up by active transport, which requires ATP, and then it is reduced to nitrite and ammonium, respectively, for the incorporation into amino acids[14]. On parity with carbon metabolism, nitrogen metabolism of seaweeds is also affected by ocean acidification in different ways, including the preference and uptake of inorganic nitrogen and enzymatic activity involved in nitrogen assimilation such as nitrate reductase[15].

In addition to ocean acidification, defined in IPCC's CO_2 emission-representative concentration pathway (RCP) 8.5, ultraviolet radiation (UVR) reaching the earth has increased due to damage to the stratospheric ozone layer by increased greenhouse gases since the industrial revolution[1]. Seaweeds, especially those of intertidal habitats, are predominantly exposed to high UVR. Therefore, extensive studies have been performed to determine the effects of UVR on seaweeds[16]. Species such as *Ulva olivascens*[17], *Ulva linza*[18] and *Cladophora sp.*[19] have a broad physiological tolerance to UVR, whereas species living in deeper waters such as *Ulva rotundata* are more sensitive to UVR[17]. Studies show that intertidal and subtidal species have different sensitivity and tolerance to UVR[20]. Tevini[21] has stated that the harmful effect of UVR varies depending on wavelength, intensity, duration of exposure, and the genetic structure, morphological structure and protective mechanisms of an organism. In addition to independent effects, combined effects of ocean acidification and UVR on seaweeds have also been investigated, particularly in calcareous species[22]. Studies stated that UVR might act synergistically, antagonistically or independently with ocean acidification[23]. However, very little is known about the combined effect of UVR and ocean acidification on non-calcareous seaweeds.

As a result, it is clearly shown that some species may be losers, while some species may be the winners in the future predicted ecological conditions. Therefore, our study is aimed to determine the ecological succession of Mediterranean alga *Ulva flexuosa* Wulfen 1803 in future predicted CO_2-induced low pH conditions alone and in combination with UVR, which reaches the Earth. For this purpose, acidification experiments in CO_2-enriched cultures with and without UVR were conducted on *U. flexuosa* for three weeks and important physiological features of the alga such as photosynthetic performance, pigment content and nitrogen metabolism were investigated.

Material and Methods

Sampling and experimental set-up

Ulva flexuosa samples (~120-150 g) were collected from the upper parts of the rocks in the intertidal zone (0-1 m) on the shores of Mersin (Mediterranean Sea, Turkey; 36°34' 29" N, 34°15' 51" E) in September, 2014. Samples were transferred to the laboratory in seawater as soon as possible. Epiphytes and other particles were removed by gently brushed with filtered synthetic seawater (Red Sea coral pro salt). Before the experiment, selected healthy samples were acclimated for three days at 27 ºC (measured in the field) and 80 µmol photon m^{-2} s^{-1} (to reduce epiphyte growth and prevent photosynthetic inhibition) and 12:12 L:D regimes. After that, four combinations of two different pH (present 8.2 and future 7.7) and two different radiation regimes (photosynthetically active radiation (PAR) alone and in combination with ultraviolet radiation (UVA+UVB) were prepared for culture experiment. The pH and radiation treatments consisted of pH: 8.2-PAR; pH: 7.7-PAR; pH: 8.2-PAR+UVA+UVB; and pH: 7.7-PAR+UVA+UVB. Each treatment tank was duplicated and each measurement was replicated 3 times from each tank.

The desired pH in the application tanks was adjusted by the addition of CO_2 gas. The pH values that change during the culture period due to metabolic activity of the samples were manipulated using the Mettler Toledo Transmitter (M800 model multichannel analytical transmitter connected with Mettler Toledo pH sensor InPro3100i/SG/225), which consisted of controlled gas valves that were set to open and close when the recorded pH is above and below the desired pH. Seawater in the tanks was changed three times a week. Filtered seawater via Whatman polycap GW filter with 0.45 µm pore size

was enriched with Provasoli[24], vitamins, and $CaCl_2.2H_2O$ to prevent nutrient deficiency. At the beginning of the experiment, each tank contained a 5 g algal sample in 10 L filtered seawater. In order to mimic conditions measured in the field, temperature, salinity and light:dark photoperiod was maintained at 27 ºC, 30 ‰ and 12:12 L:D respectively, during culture experiment for three weeks. PAR was applied below the values measured in the field (to prevent epiphyte growth and photoinhibition) (Table 1). UVR was applied as the average value measured in the field at noon on a sunny day (Table 1). Since the United Nations Environment Programme and Scientific Assessment of Ozone Depletion by the World Meteorological Organization have shown that the ozone-depleting gases were decreasing and the ozone layer is continued to recover[25], high UVR intensities were not applied in the study. Desired light intensity was provided with enough fluorescent lamps (Philips master TLD 90 deluxe 36W/950; Philips TL-K 40W/10-R UV-A; Philips TL 20W/01 RS). PAR was measured with LI-COR, 250A light meter with LI-192 underwater light quantum sensor. UVR was measured with Trios spectroradiometer with a SAM ACC UV model sensor. Inorganic carbon concentrations of seawater were estimated by the CO2SYS program using temperature, salinity, pH and alkalinity values. Total alkalinity was determined using Hach Lange test kit, In the culture tanks, seawater inorganic carbon calculations were conducted twice a week and before/after every water changes.

The determination of the photosynthetic performance of samples was conducted using pulse amplitude modulated chlorophyll fluorometer (Walz PAM 2500) by measuring variable chlorophyll-*a* fluorescence of photosystem II (PSII). The maximum quantum yield of PSII (Fv/Fm) was estimated as the ratio of the variable to the maximum chlorophyll-*a* fluorescence in samples pre-incubated in darkness for 10 min. For the rapid light curve, samples were irradiated with increasing levels of actinic light (40 – 2063 µmol photon m^{-2} s^{-1}). Every 30 s, a saturating pulse (10,000 µmol photon m^{-2} s^{-1}) was applied to measure the effective quantum yield of PSII, then actinic irradiation was increased again. Photosynthetic parameters including saturation irradiance (Ik), initial linear slopes (alpha) and relative maximum electron transfer rate (rETRmax) were calculated from the effective quantum yield and light data using the model proposed by Eilers and Peeters[26]. All photochemical measurements were performed in the same medium used for the treatments.

Table 1 — Underwater photosynthetically active radiation (PAR) and ultraviolet radiation (UVR) measured in the field at noon and in culture conditions

	Field	Culture
PAR (µmol photon m^{-2} s^{-1})	165.80	80
UVA (W m^{-2})	2.950	2.5
UVB (W m^{-2})	0.342	0.320

For the chlorophyll analyses, approximately 0.2 g samples were homogenized with N, N-Dimethylformamide and incubated in darkness for 24 h. Then, absorbance of the extracts was measured and chlorophyll *a* and *b* contents of sample were calculated from the formula given below using the extinction coefficient proposed by Inskeep & Bloom[27]; Chl-*a* = 12.70 x $A_{664.5}$ – 2.79 x A647; and Chl-*b* = 20.70 x A_{647} – 4.62 x $A_{664.5}$. Pigment measurements were conducted every week.

For UV absorbing contents (rUVACs), samples of *U. flexuosa* were extracted with 5 ml 25 % methanol and incubated 2 h at 45 ºC. Extracts were centrifuged at 5000 rpm for 5 min. The absorbance of supernatants was measured at between 250-400 nm and corrected for 0.01 g fresh weights. The relative amount of UVACs was expressed as absorption spectra. The relative concentration of UVACs was estimated on the basis of the absorption peak at 336 nm according to Dunlap *et al*.[28]. UVACs measurements were conducted after three weeks of culture period.

Total carbonic anhydrase activity (extracellular and intracellular) was determined by measuring the algal homogenate obtained by grinding ~ 30 mg of alga in the buffer used for the assay of activity. Activity was measured based on the method Haglund *et al*.[29]. Relative enzyme activity was calculated as (T_b / T_s) – 1, where T_b is time required for non-enzymatic reaction (assay buffer, blank); and T_s is time required for enzymatic reaction (algal homogenate). Nitrate reductase activity was performed according to the method reported by Corzo & Niell[30]. Nitrate reductase activity was calculated as µmol NO_2^- g FW^{-1} min^{-1}.

Data are presented as the mean ± standard deviation (SD). Prior to all statistical analyses, all data were tested for normality and homogeneity of variances using the Kolmogorov-Smirnov' test and Levene's test, respectively. The effects of the factors on the physiological responses of *U. flexuosa* were assessed

using analysis of variance. Three factors were considered as independent factors: pH (8.2 and 7.7), irradiance (UVR- and UVR+) and time (week-1, week-2, and week-3). Seawater inorganic carbon parameters and UVACs were tested using two-way analysis of variance. A post-hoc test for multiple comparisons (Tukey's HSD) was performed when the data revealed significant differences at a level of $p < 0.05$. The analyses were performed using the commercial software program SPSS 23 (IBM Corporation).

Results

During the culture experiment, recorded seawater inorganic carbon parameters for each of the four treatments are presented in Table 2. The alkalinity of culture medium was maintained at values close to each other among all treatments. As expected, the inorganic carbon species varied in different pH treatments (Table 3). In the CO_2 induced lower pH treatments, HCO_3^- and CO_2 concentrations were increased while CO_3^{2-} concentration was decreased.

Table 4 presents the photosynthetic performance measurements including the Fv/Fm ratio, Ik and alpha values of *Ulva flexuosa* exposed to different pH and irradiance treatments for three weeks. Fv/Fm ratios which are used as stress indicators of the sample did not significantly differ in either pH or irradiance treatment (Table 5). The alpha represents the ETR efficiency of PSII at low light intensities. In this study, alpha did not differ significantly among the treatments. However, incubation time had a significant effect on alpha values. Recorded high

Table 2 — Means (±sd) of the inorganic carbon parameters for each treatment

pH	8.2	7.7	8.2	7.7
Irradiance	PAR	PAR	PAR+UVA+UVB	PAR+UVA+UVB
Alkalinity (µmol kg/L)	2760 ± 145	2822 ± 63	2717 ± 69	2800 ± 35
pCO_2 (µatm)	314.5 ± 17.2	1260.5 ± 28.5	309.5 ± 8.3	1250.9 ± 15.8
HCO_3 (µmol/kg SW)	1981.8± 108.7	2511.9 ± 56.8	1949.9 ± 52.2	2492.7 ± 31.4
CO_3 (µmol/kg SW)	330.5 ± 18.1	132.5 ± 3.0	325.2 ± 8.7	131.5 ± 1.7
CO_2 (µmol/kg SW)	8.69 ± 0.48	34.83 ± 0.78	8.55 ± 0.23	34.57 ± 0.44

Table 3 — Results of two-way ANOVA for seawater inorganic carbon variables in culture tanks at different pH (two levels: 8.2 and 7.7) and irradiance (two levels: PAR and PAR+UVR). (df means degree of freedom, F means the value of F statistic, MS means the mean square and Sig. means the *p*-value)

	df	CO_3^2			HCO_3^-			CO_2		
		MS	*F*	*P-value*	MS	*F*	*P-value*	MS	*F*	*P-value*
pH	1	153501.363	368.949	0.0	1150926.660	61.388	0.0	2720.144	2510.487	0.0
Irradiance	1	40.037	0.096	0.762	2608.911	0.139	0.716	0.164	0.151	0.704
pH*Irradiance	1	18.512	0.044	0.836	159.580	0.009	0.928	0.014	0.013	0.910
Error	12									

Table 4 — Means (±sd) of the maximum quantum yields (Fv/Fm), initial linear slope (alpha), light saturation points (Ik), chlorophyll-*a* and chlorophyll-*b* contents of *Ulva flexuosa* exposed to different pH and radiation conditions

pH	Irradiance	Time	Photosynthetic parameters				
			Fv/Fm relative units	Alpha relative units	Ik µmol photon s^{-1} m^{-2}	Chlorophyll-*a* mg g^{-1}	Chlorophyll-*b* mg g^{-1}
8.2	-PAR	*week-1*	0.754 ± 0.009	0.30 ± 0.01	381.43 ± 51.82	1.155 ± 0.06	0.651 ± 0.029
		week-2	0.728 ± 0.012	0.274 ± 0.004	471.03 ± 6.84	1.201 ± 0.03	0.683 ± 0.024
		week-3	0.731 ± 0.019	0.278 ± 0.005	392.83 ± 123.54	1.255 ± 0.1	0.677 ± 0.055
7.7	PAR	*week-1*	0.729 ± 0.032	0.291 ± 0.006	494.15 ± 31.65	1.168 ± 0.02	0.652 ± 0.006
		week-2	0.736 ± 0.003	0.284 ± 0.00	502.47 ± 25.06	1.198 ± 0.11	0.642 ± 0.059
		week-3	0.739 ± 0.004	0.271 ± 0.011	520.30 ± 34.45	1.269 ± 0.14	0.715 ± 0.067
8.2	PAR+UV A+UVB	*week-1*	0.746 ± 0.004	0.296 ± 0.012	358.83 ± 43.12	1.303 ± 0.11	0.723 ± 0.053
		week-2	0.745 ± 0.005	0.283 ± 0.00	421.35 ± 24.19	1.356 ± 0.19	0.740 ± 0.093
		week-3	0.737 ± 0.015	0.276 ± 0.018	438.53 ± 58.17	1.299 ± 0.07	0.716 ± 0.05
7.7	PAR+UV A+UVB	*week-1*	0.739 ± 0.013	0.290 ± 0.011	387.15 ± 10.51	1.276 ± 0.14	0.767 ± 0.087
		week-2	0.728 ± 0.009	0.290 ± 0.004	527.13 ± 10.98	1.25 ± 0.18	0.706 ± 0.106
		week-3	0.718 ± 0.010	0.285 ± 0.013	508.23 ± 32.82	1.188 ± 0.06	0.679 ± 0.04

Table 5 — Results of three-way ANOVA for maximum quantum yields (Fv/Fm), initial linear slope (alpha) and light saturation point (Ik) in *Ulva flexuosa* cultured at different pH (two levels: 8.2 and 7.7) and irradiance (two levels: PAR and PAR+UVR), and examined at different times (three levels: 1, 2 and 3 week). (df means degree of freedom, F means the value of F statistic, MS means the mean square and Sig. means the *p*-value)

Source	df	Fv/Fm			Alpha			Ik		
		MS	*F*	*P*-value	MS	*F*	*P-value*	MS	*F*	*P*-value
pH	1	0.001	4.841	0.159	2.083e-6	0.022	0.882	75350.901	32.355	0.0
Irradiance	1	4.688e-6	0.029	0.881	0.0	1.654	0.207	4880.333	2.096	0.156
Time	2	0.0	2.698	0.081	0.001	12.174	0.0	25151.733	10.800	0.0
pH*irradiance	1	0.0	0.633	0.510	0.0	1.225	0.276	1534.541	0.659	0.422
pH*Time	2	0.0	0.287	0.777	0.0	2.749	0.077	1126.441	0.484	0.620
Trradiance*Time	2	0.0	0.264	0.791	0.0	1.357	0.270	6836.359	2.935	0.066
pH*Irradiance*Time	2	0.001	3.338	0.047	9.702e-5	1.042	0.363	7225.488	3.103	0.057
Error	36									

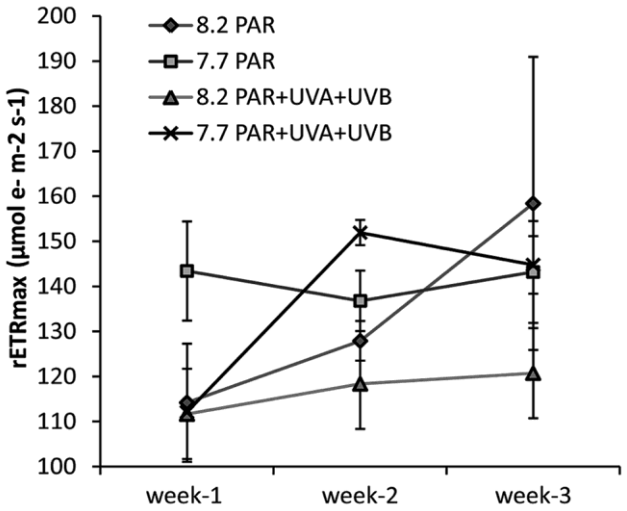

Fig. 1 — Means (±sd) of the maximum relative electron transfer rates of *Ulva flexuosa* exposed to different pH and radiation

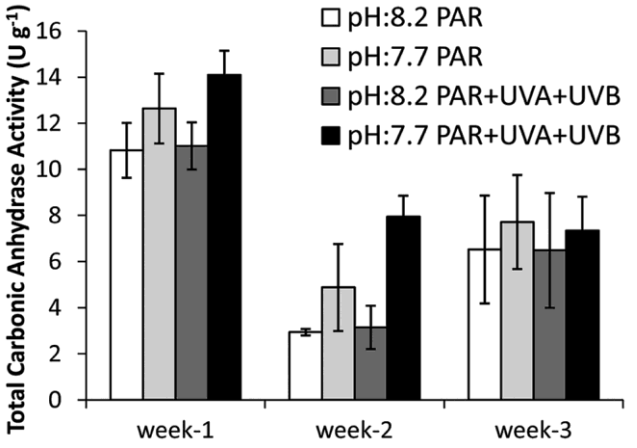

Fig. 2 — Means (±sd) of the carbonic anhydrase activity of *Ulva flexuosa* exposed to different pH and radiation

alpha values in the first week were reduced at week-2 and 3 in all treatments. Ik values were significantly higher at CO_2 induced lower pH treatments. In addition, incubation time at the lower pH increased the Ik values. However, different irradiance conditions did not affect the saturation irradiance point of photosynthesis of *U. flexuosa*. Similar to Fv/Fm and alpha, rETRmax was not affected by low pH and UVR after three weeks (Fig. 1). The lowest rETRmax values were observed in the pH: 8.2-PAR+UVA+UVB treatment, whereas the highest values were observed in the pH: 8.2-PAR treatment at the end of the three weeks.

Figure 2 shows the carbonic anhydrase activity of *U. flexuosa*. Carbonic anhydrase activity of samples exposed to different pH and irradiance was not statistically different from one another, but all of them significantly declined from the initial enzyme activity (Table 6). The nitrate reductase activity of *U. flexuosa* is depicted in Figure 3. No statistical differences were found depending on UVR. However, statistical analysis indicated that pH had a significant effect on the nitrate reductase activity in *U. flexuosa*. Nitrate reductase activity was drastically reduced when samples were exposed to CO_2-induced lower pH, especially for more than two weeks of incubation (Table 6).

The mean chlorophyll-*a* and chlorophyll-*b* concentration of *U. flexuosa* exposed to different pH and light conditions are presented in Table 4. Neither chlorophyll-*a* nor chlorophyll-*b* concentration was significantly affected by pH or UVR. But, the algae exposed to low pH had significantly higher relative-UVACs content than the algae exposed to ambient pH (Fig. 4). However, relative-UVACs were not significantly affected by UVR (Table 7).

Table 6 — Results of three-way ANOVA for carbonic anhydrase and nitrate reductase activity of *Ulva flexuosa* cultured at different pH (two levels: 8.2 and 7.7) and irradiance (two levels: PAR and PAR+UVR), and examined at different times (three levels: 1, 2 and 3 week). (df means degree of freedom, F means the value of F statistic, MS means the mean square and Sig. means the *p*-value)

Source	df	Carbonic anhydrase			Nitrate reductase		
		MS	*F*	*P*-value	MS	*F*	*P*-value
pH	1	60.645	11.313	0.078	76.007	100.333	0.0
Irradiance	1	6.572	2.006	0.292	0.673	0.888	0.352
Time	2	229.968	37.156	0.040	18.675	24.652	0.0
pH*Irradiance	1	4.673	1.907	0.301	0.081	0.107	0.745
pH*Time	2	5.363	2.189	0.314	13.664	18.037	0.0
Irradiance*Time	2	3.277	1.337	0.428	0.953	1.257	0.297
pH*Irradiance*Time	2	2.450	1.055	0.359	1.886	2.489	0.097
Error	36						

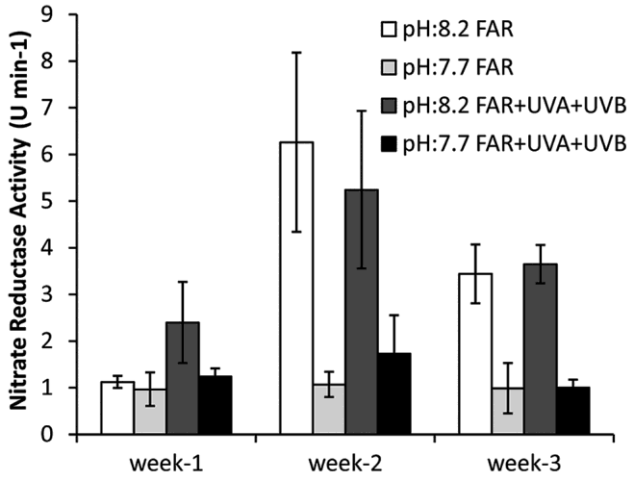

Fig. 3 — Means (±sd) of the nitrate reductase activity of *Ulva flexuosa* exposed to different pH and radiation

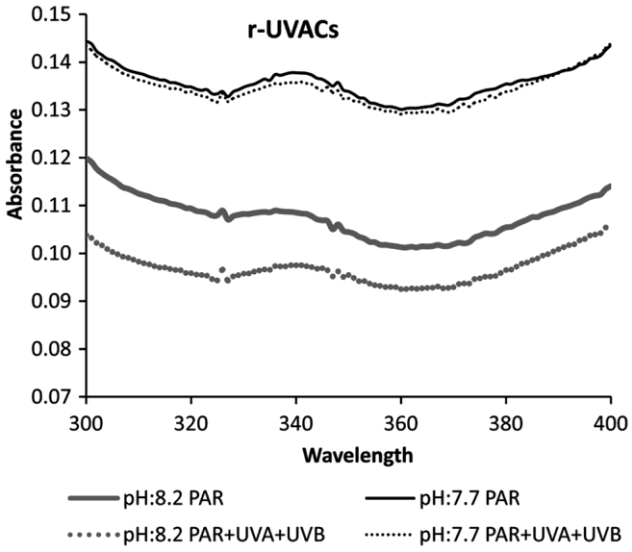

Fig. 4 — Absorbance spectra of the methanolic extracts of *Ulva flexuosa* after 3 week exposure at different pH and radiation

Table 7 — Results of three-way ANOVA for rUVACs of *Ulva flexuosa* cultured at different pH (two levels: 8.2 and 7.7) and irradiance (two levels: PAR and PAR+UVR). (df means degree of freedom, F means the value of F statistic, MS means the mean square and Sig. means the *p*-value)

	df	UVACs		
		MS	*F*	*P-value*
pH	1	0.004	14.217	0.003
Irradiance	1	0.0	0.586	0.459
pH*Irradiance	1	9.458e-5	0.303	0.592
Error	12			

Discussion

The results of this study indicate that CO_2-induced lower pH and UVR does not affect the physiology of *Ulva flexuosa*. Among the fluorescence parameters, the Fv/Fm represents the maximum quantum efficiency of PSII and it is widely used as an algal healthy indicator in ecophysiological studies. The Fv/Fm values obtained in this study showed that *U. flexuosa* was not photosynthetically stressed under the CO_2-induced lower pH. In accordance with the Fv/Fm, alpha values and chlorophyll contents of *U. flexuosa* showed that the light-harvesting efficiency of samples was not affected by the pH and UVR. Compared to ambient pH, more CO_2 is available for RuBisCO in the lower pH treatments. Therefore, an increase in photosynthetic activity at lower pH is predicted. However, rETRmax values did not differ among treatments in this study. This result suggests that photosynthesis of *U. flexuosa* is saturated at the present level of inorganic carbon. The increased CO_2 concentration in seawater did not improve the photosynthetic efficiency of *U. flexuosa*.

Recent studies have shown that *Ulva* species have CCMs that provides CO_2 required for RuBisCO[9,31]. Among these CCMs, extracellular carbonic anhydrase

activity, which participates in the conversion of HCO_3^- ions in seawater into CO_2, and anion exchange proteins, which provide direct transport of HCO_3^- into the cell, are the best-known mechanisms[32]. In the present study, the fact that the rETRmax values did not differ significantly between the normal pH and the lower pH treatments indicates that *U. flexuosa* favours HCO_3^- as a carbon source, in other words, *U. flexuosa* has CCMs. Similarly, many studies have reported that *Ulva* species prefer HCO_3^- as a primary carbon source for their photosynthesis[31].

Despite the saturated photosynthetic performance of *U. flexuosa* in the ambient carbon treatment, the fact that the carbonic anhydrase activity did not differ between ambient and low pH treatments suggests that *U. flexuosa* depends not only on carbonic anhydrase for provision of CO_2 from HCO_3^-. Gao *et al.*[31] reported that ocean acidification completely inhibits the extracellular carbonic anhydrase activity in *Ulva linza* and down-regulates intracellular carbonic anhydrase activity. In addition, researchers determined that *Ulva* species possess the acidic compartments as one of CCMs pathways. Rautenberger *et al.*[9] also mentioned that the known HCO_3^- use mechanisms that are not sufficient to maintain the internal inorganic carbon pool required for a high growth rate of *Ulva* species and suggested the presence of an active HCO_3^- carrier system that works in conjunction with light along with other known mechanisms for *Ulva rigida*. Similar mechanisms have been demonstrated in *Ulva prolifera*[33] and *Ulva linza*[34].

Nitrate reductase catalyzes the reduction of NO_3^- to NO_2^-, the first step in nitrate assimilation, and thus the activity of this enzyme may provide important information about nitrogen metabolism. CO_2- induced ocean acidification also affects the nitrogen metabolism of marine algae in different ways[35]. Elevated-CO_2 increased NO_3^- uptake in *Ulva rigida*[36] as well as in the red and brown algae[37] while it decreased NO_3^- uptake in *Ulva lactuca*[38]. In addition, Gordillo *et al.*[36] have indicated an enhanced nitrate reductase activity in *Ulva rigida* cultured at high CO_2. Similarly, high CO_2 stimulated nitrate reductase activity in *Ulva linza* is reported by Gao *et al.*[3]. On the contrary, in this study nitrate reductase activity of *U. flexuosa* decreased by elevated CO_2.

Pritchard *et al.*[15] have shown that all macroalgal species uptake and utilize nitrate to somewhat. It is known that this utilization is catalyzed by nitrate reductase. However, in this study nitrate reductase activity of *U. flexuosa* was reduced at elevated CO_2. Falkowski & Raven[39] have demonstrated that *Ulva* sp. prefers to take ammonium because it requires less energy for assimilation. But, there is no information about what type of inorganic nitrogen is specifically preferred by *U. flexuosa*. According to results of present study, it is suggested that elevated CO_2 may regulate the nitrogen preference of *U. flexuosa*. When the specimens are exposed to elevated-CO_2, their nitrate uptake, which is energetically expensive, maybe down-regulated and it may have a high affinity for ammonium. Similar results were also reported by Kang *et al.*[40] for the red alga *Gracilaria lemaneiformis*, which exhibited a rapid ammonium uptake rate under elevated CO_2 compared to ambient levels of CO_2.

Decreased nitrate reductase activity was also observed for *Sargassum muticum* at higher CO_2[(ref. 41)]. Researchers suggested that there may be an increase in the synthesis of H^+ transport proteins that counteract with acid-base perturbation, which may have reduced nitrate reductase synthesis. Recent studies indicated that low pH might disrupt the acid-base balance of the algal cell surface and reduce the membrane permeability, thereby damaging the transport of nitrate through the membrane[42]. Although, all of the studies mentioned above support the suggestion given based on present study that CO_2 may regulate the nitrogen preference of *U. flexuosa*, further investigations are necessary to provide more evidence.

Saved energy due to uptake of ammonium instead of nitrate in the low pH may be used either for growth or UVACs synthesis. In the present study, the growth rate could not be determined because of the branched filamentous thallus structure of *U. flexuosa*, but it was found that there was an increase in the absorbance of UVACs in enriched-CO_2 (Higher absorbance values indicates relatively higher amount UVACs). Among the UVACs, the most known substances are mycosporine-like amino acids (MAAs). The primary function of MAAs is that they are protective against excess light, both PAR and UVR[43]. Recently, it has been noted that these compounds are good antioxidants, and environmental variables such as osmotic stress, drying and thermal stress[44] may also induce their synthesis. But, synthesis of secondary metabolites such as UVACs requires additional energy input. Thus there is an antagonistic

relationship between growth rate and their synthesis. As mentioned before, the growth rate of *U. flexuosa* could not be determined in this study

The intensity of UVR used in this study affected neither photosynthetic performance of *U. flexuosa* nor its nitrate reductase and carbonic anhydrase activities, which give information about nitrogen and carbon metabolism. Also, the relative concentration of UVACs that have a protective role against UVR, did not differ between two different radiation regimes. The obtained data showed that *U. flexuosa* was not sensitive to used UVR-doses. The physiological effects caused by UVR in seaweeds have been studied by many researchers. Similar to findings of present study, many studies have reported that macroalgae, which are distributed in intertidal and supralittoral zones, such as *Ulva olivascens*[17], *Ulva linza*[18] and *Cladophora sp.*[19] have a wide physiological tolerance against UVR. However, it is known that species that are distributed in deeper waters are more sensitive to UVR[20].

Conclusion

In comparison to other species, *Ulva* spp. have rapid growth rates due to high ammonium and nitrate uptake capabilities. They can exhibit overgrowth, especially in eutrophic waters. Excessive growth of *Ulva* species can cause harmful environmental conditions (such as anoxia) for marine life, especially for benthic organisms. These algae have been reported to cause mortality of some calcareous marine organisms, such as bivalve and crab larvae[45]. However, it is known that the growth rate of *Ulva* species was limited by trophic level and light rather than CO_2 concentration in seawater. Current study shows that high CO_2 concentration on seawater does not alter the photosynthetic performance of *Ulva flexuosa*. Therefore, it may be stated that *Ulva* species, known as opportunistic and/or invasive species, may not show excessive growth due to increased CO_2 in future seawater carbon concentrations. Moreover, their growth can also be suppressed by the seaweed species that will be positively affected by the increased CO_2 levels.

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Conflict of Interest

Author declares no conflict of intrests.

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