



## Physiological Effects of Light Intensity on the Opportunistic Algae *Ulva rigida* C. Agardh (Chlorophyta)

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### Abstract

Intertidal seaweeds exposed to strong fluctuation in light quantity and spectral light quality. Light plays an important role controlling seaweed growth and physiology. Therefore, we conducted a culture experiment to determine the physiological effects of light intensity on the opportunistic algae *Ulva rigida* C. Agardh. For this reason, samples were exposed to two different light intensities ( $55 \mu\text{mol photons m}^{-2}\text{s}^{-1}$  and  $100 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) and their some physiological features including photosynthetic performance, growth rate, pigment content, total protein and nitrate reductase activity were measured. According to our results, the maximum relative electron transport rate and saturation irradiance point were not significantly different between the treatments. On the other hand, relative growth rate, nitrate reductase activity and chlorophyll-*a* concentrations of *U. rigida* were significantly decreased under low light condition. Consequently, our data indicated that photosynthetic performance of *Ulva rigida* was not affected to treated light intensity, while growth and nitrogen metabolism were primarily controlled by light.

**Keywords** — Growth Rate, Light Intensity, Nitrate Reductase, Photosynthesis, *Ulva rigida*

### 1 Introduction

Macroalgal blooms occur worldwide and have potential to cause severe ecological and economic damage. Macroalgal blooms in coastal areas are largely composed of fast growing species that rapidly utilize resources, including many species in the genus *Ulva* L. [1]. *Ulva* species are considered opportunistic because of their simple thallus, fast growth rate, high surface area to volume ratio and capacity to rapidly metabolize inorganic nitrogen [2].

*Ulva* blooms are a common occurrence at Bursa coast and these blooms have increased in frequency and duration over the last years. Blooms frequently occurred in the late spring and summer, rarely observed in the late fall and winter at the temperate regions [3]. This blooms caused to decrease dissolved oxygen at the water column, which can result in fish kills, suffocation of benthic fauna and reduced shellfish recruitment [4]. Moreover, blooms interfere with coastal commercial and recreational activities [5].

Intertidal seaweeds exposed to strong fluctuation in light quantity and spectral light quality [6]. Light plays an important role on controlling seaweed growth and physiology.

Many researchers reported that *Ulva* species have been saturated photosynthesis at the current inorganic carbon pool of seawater under saturated light intensity [7]. However, growth rate of *Ulva* varies with environmental parameters such as light. Therefore, the aim of this study was to assess how light intensity affects the physiology and abundance of common bloom forming alga *Ulva rigida* C. Agardh.

### 2 Materials and methods

#### 2.1 Algal material and Experimental set-up

*U rigida* cultures at the Uludag University (Turkey) were used for this study. The algae were originally collected from the south shore of the Sea of Marmara in the Bursa-Zeytinbagi region of Turkey ( $40^{\circ} 22.8' \text{N}$ ,  $28^{\circ} 48.0' \text{E}$ ). The algal blades were preincubated for 2 days at  $20^{\circ}\text{C}$ , a salinity of 23 which is the natural salinity level for Sea of Marmara,  $55 \mu\text{mol photons m}^{-2}\text{s}^{-1}$  light intensity and a 12:12h light:dark cycle in filtered artificial seawater containing  $\frac{1}{4}$  strength Provasoli enrichment medium [8]. Calcium chloride ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) was added to the enrichment medium to a final concentration of 10 mM to ensure the presence of calcium. Thalli used in the experiment were carefully selected from cultures and cleaned of epiphytes. For cultivation, thalli were cut into discs of 2 cm in diameter. Discs of



the *U. rigida* were acclimated for 3 days in a light and temperature controlled growth chamber before the experiment. Culture conditions were prepared with two different irradiation conditions ( $55 \mu\text{mol photons m}^{-2}\text{s}^{-1}$  and  $100 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ ). Each treatment was repeated four times in four independent experiments where each treatment was replicated once. Samples were incubated in glass tanks containing 15 l of filtered artificial seawater. Each tank initially contained 20 discs of *U. rigida*. The seawater was filtered through a Whatman polycap capsule filter ( $0.45 \mu\text{m}$  pore size) and circulated in each tank using magnetic stir bars. To avoid nutrient depletion culture medium was renewed every 3 days. During the light period, desired PAR (400-700nm) levels were provided by one or three Osram daylight fluorescence tube (Osram Luminix Plus Daylight L18W/11-860, Munich, Germany). Light measurements were made with a LI-COR 250A underwater quantum sensor 192A.

## 2.2 Photochemical measurements

Optimum quantum yield of photosystem II was determined by measuring variable chlorophyll fluorescence of PSII with a PAM 2500 device (Walz, Germany) following the methods by Bischof et al. [9]. maximum quantum yield ( $F_v/F_m$ ) was calculated as the ratio of variable to maximum fluorescence of dark acclimated algae for 10 min. Additionally, samples were irradiated with increasing levels of actinic light. Every 30 s, a saturating pulse was applied to measure effective quantum yield of photosynthesis before actinic irradiation was increased again. The parameters initial linear slope ( $\alpha$ ), maximum relative electron transport rate ( $rETR_{\text{max}}$ ) and saturation irradiance ( $I_k$ ) were calculated from the photosynthesis and light data using the Eilers and Peeters [10] model. All photochemical measurements were performed in the same medium used for the treatment.

## 2.3 Growth and Biochemical Measurements

Relative growth rates (RGR) were determined by measuring the fresh weight of one marked algal thallus in each treatment tank over time after blotting the thalli dry with paper towels. RGR expressed as percent of fresh weight per day ( $\% \text{FW d}^{-1}$ ) were calculated as  $\ln(W_t/W_0)/t \times 100$ , where  $W_t$  and  $W_0$  are the fresh weights at time  $t$  (days) and the initial fresh weight, respectively.

For the determination of chlorophyll-*a* and *b* contents, samples were extracted with N,N-dimethylformamide for 12 h at  $4^\circ\text{C}$  in the dark, after which, the absorbance of extracts was measured spectrophotometrically and concentrations were calculated according to Inskeep and Bloom [11]. Total protein content was determined spectrophotometrically at

595 nm and concentrations were calculated by comparing with a calibration curve of bovine serum albumin [12].

Nitrate reductase activity of *U. rigida* was measured using fresh material directly from the cultures according to the in situ method improved for *U. rigida* by Corzo and Niell [13]. Fresh algal discs were placed into 5 ml incubation medium (0.1 M phosphate buffer, 0.5 mM EDTA, 0.1% 1-propanol, 30 mM  $\text{KNO}_3$  and  $10 \mu\text{M}$  glucose; pH 8.0), which is bubbled with  $\text{N}_2$  gas before and after introducing the algal discs for 2 min. After the incubation in the dark at  $20^\circ\text{C}$  for 30 min, 1 ml of the assay buffer was removed and the nitrite concentrations were determined colorimetrically [14] after the addition of  $200 \mu\text{l}$  of 4% sulphanilimide and  $300 \mu\text{l}$  of 0.1% *n*-(1-naphthyl ethylenediamine dihydrochloride).

## 2.4 Statistics

Data are presented as the mean  $\pm$  standard error. Data were tested for normality (Kolmogorov-Smirnov test) and subjected to Levene's test to verify the homogeneity of variances among groups. Two-way anova or t-test was used to establish differences among treatments at a level of  $p < 0.05$ . The analyses were carried out with the commercial software program SPSS 17.0 (IBM Corporation).

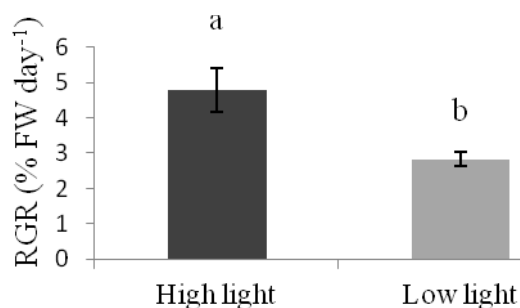
## 3 Results

Table 1 presents the mean of maximum quantum yield ( $F_v/F_m$ ), initial linear slope ( $\alpha$ ), saturation irradiance point ( $I_k$ ) and  $rETR_{\text{max}}$  of *U. rigida* exposed to the different light conditions. In this study, the mean  $F_v/F_m$  ratios of *Ulva rigida* exposed to low and high light conditions were significantly different between the treatments ( $F_{5,30} = 27.943$ ,  $p = 0.000$ , Table 1). Unlike the light intensity, the incubation time was not impact the  $F_v/F_m$  ratio ( $F_{5,30} = 3.020$ ,  $p = 0.064$ ). Similarly, there was not interaction between the light intensity and incubation time ( $F_{5,30} = 2.890$ ,  $p = 0.071$ ). The  $\alpha$  represents the ETR efficiency of PSII at low irradiance.  $\alpha$  values in this study significantly decreased with low light. The  $rETR_{\text{max}}$  as a function of increasing actinic light intensity and saturation irradiance point were not significantly different between the treatments ( $F_{5,30} = 0.525$ ,  $p = 0.474$ ;  $F_{5,30} = 2.677$ ,  $p = 0.112$ , respectively).

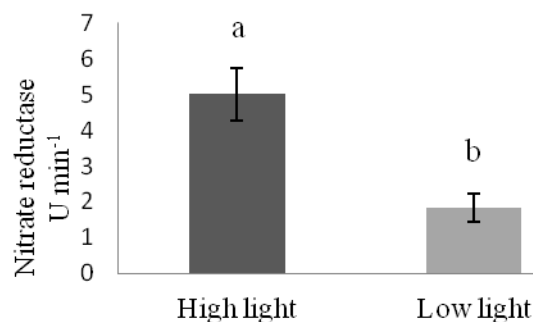
Total protein content of *U. rigida* was not significantly affected by light intensity ( $p = 0.780$ ) after 21 days (Table 2). On the other hand, RGR based on fresh weight and nitrate reductase activity were significantly decreased under low light condition ( $p = 0.037$ ;  $p = 0.014$ , respectively). The average RGR was  $4.79\% \text{ day}^{-1}$  in the high light treatment, but decreased to  $2.83\% \text{ day}^{-1}$  in low light treatment (Figure 1). Similarly, the mean nitrate reductase activity was  $5.01 \text{ U min}^{-1}$  in the high light treatment, but decreased to  $1.82 \text{ U min}^{-1}$  in the low light treatment (Figure 2).

**Table 1.** Means ( $\pm$ SD) of the maximum quantum yields (Fv/Fm), maximum relative electron transport rate (rETRmax), initial linear slope (alpha) and light saturation points (Ik) of *U rigida* exposed to different light intensity.

		Fv/Fm	rETRmax	alpha	Ik
Week-1	High light	0.744 $\pm$ 0.004	39.18 $\pm$ 1.16	0.363 $\pm$ 0.006	108.07 $\pm$ 4.11
	Low light	0.767 $\pm$ 0.003	39.03 $\pm$ 1.13	0.345 $\pm$ 0.006	113.23 $\pm$ 3.20
Week-2	High light	0.753 $\pm$ 0.004	36.23 $\pm$ 1.72	0.345 $\pm$ 0.007	105.10 $\pm$ 4.74
	Low light	0.772 $\pm$ 0.005	38.12 $\pm$ 2.61	0.339 $\pm$ 0.009	113.03 $\pm$ 8.68
Week-3	High light	0.761 $\pm$ 0.002	35.60 $\pm$ 1.45	0.353 $\pm$ 0.005	100.94 $\pm$ 4.22
	Low light	0.767 $\pm$ 0.003	37.00 $\pm$ 2.05	0.337 $\pm$ 0.006	109.63 $\pm$ 5.89



**Figure 1.** Means ( $\pm$ SD) of the relative growth rate of *U rigida* exposed to different light intensity after 21 days.



**Figure 2.** Means ( $\pm$ SD) of the nitrate reductase activity of *U rigida* exposed to different light intensity after 21 days.

The mean photosynthetic pigment concentrations of *U. rigida* exposed to different light intensity are displayed in Table 2. Content of pigments give fundamental information on the light harvesting. Chlorophyll-*b* concentrations were not significantly affected by light intensity ( $p = 0.418$ ). However, chlorophyll-*a* contents of samples ranged from 2.16 to 2.88 mg g<sup>-1</sup> and chlorophyll-*a* concentrations of *U.*

*rigida* were significantly decreased by exposure to low light ( $p = 0.023$ ).

**Table 2.** Means ( $\pm$ SD) of the total protein, chlorophyll-*a* and chlorophyll-*b* of *U rigida* exposed to different light intensity.

	Total protein (mg g <sup>-1</sup> )	Chlorophyll- <i>a</i> (mg g <sup>-1</sup> )	Chlorophyll- <i>b</i> (mg g <sup>-1</sup> )
High light	6.94 $\pm$ 0.45	2.73 $\pm$ 0.05	2.25 $\pm$ 0.04
Low light	7.11 $\pm$ 0.38	2.40 $\pm$ 0.10	2.20 $\pm$ 0.05

#### 4 Discussion and Conclusion

Pulse Amplitude Modulation technique has been widely used for ecophysiological studies [15]. Among the fluorescence parameters Fv/Fm ratio, which is the ratio of the variable to the maximum fluorescence after dark adaptation, indicates the efficiency of energy transferred from the antenna systems to the reaction centers of PSII and it has been used as an stress indicator.

In this study, decreasing Fv/Fm ratio indicated that samples were photosynthetically stressed under high light condition. Krause and Weis [16] indicated that decreased Fv/Fm reflects absorbed energy dissipated as heat. These data indicate that decrease of Fv/Fm at the high light here may related to change in the antenna composition. Higher alpha and chlorophyll-*a* contents at the high light condition supported to this idea. The alpha represents the ETR efficiency of PSII at low irradiance levels. Alpha values in this study significantly increased at high light treatment. But, increased antenna size at the high light treatment due to higher pigment contents was not effected rETRmax. We expected that photosynthetic efficiency would increase with increasing light, because more light is available for photosynthesis. The



unexpected rETR<sub>max</sub> values at the different light intensities showed that photosynthesis of *U. rigida* was saturated at the both treatments. Growth rate is the most important parameters to determine the ecological success for seaweeds. In this study, growth rate was stimulated with higher light intensity. Rautenberger et. al. [17] also reported growth is limited by light. Similarly, nitrate reductase activity of samples exposed to high light intensity was drastically increased to support higher growth. Nitrate reductase catalyzes the reduction of nitrate to nitrite, which is usually identified as the rate-limiting step in nitrate assimilation by algae [18]. Many studies also reported that nitrate reductase activity can vary with light regimes [19, 20, 21]. Moreover, the growth rate of *U. rigida* was entirely governed by N-assimilation.

In conclusion, physiological features of *U. rigida* was sensitive to light intensity. Photosynthetic performance was not affected to treated light intensity, while growth and nitrogen metabolism were primarily controlled by light. *U. rigida* act as a opportunistic and invasive species in coastal areas of Turkey. According to our results, blooms of *U. rigida* could be especially occur at intertidal zone due to stimulated growth, because more light is available at intertidal zone than subtidal.

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