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Performance of a Potentially Invasive Species of Ornamental Seaweed *Caulerpa sertularioides* in Acidifying and Warming Oceans

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Abstract: *Caulerpa*, a (sub) tropical seaweed, is a notorious taxonomic group and an invasive seaweed worldwide. Similar to several species that have been introduced to benthic habitats through aquariums, *Caulerpa sertularioides* has also been introduced into Korean aquariums, although it is not native to the region. Thus, it is necessary to evaluate the potential of this species for invading domestic macroalgal habitats. Therefore, an indoor mesocosm experiment was conducted to examine the ecophysiological invasion risk of non-native seaweed *C. sertularioides* under various climate conditions and exposure to three future climate scenarios: acidification (doubled CO_2), warming (5 °C increase from ambient temperature), and greenhouse (GR: combination of acidification and warming); additionally, we compared the invasion risk between future and present climates (control: 20 °C and 470 µatm CO_2). High CO_2 concentrations and increased temperatures positively affected the photosynthesis and growth of *C. sertularioides*. Photosynthesis and growth were more synergistically increased under GR conditions than under acidification and warming. Consequently, the performance of this potentially invasive species in the native macroalgal Korean habitat will be higher in the future in coastal environments. Therefore, proper management is required to prevent the geographic expansion of *C. sertularioides* in the Korean coastal ocean.

Keywords: *Caulerpa sertularioides*; climate change; CO_2; growth; introduced species; temperature; photosynthesis

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1. Introduction

One of the most serious environmental issues worldwide is the loss of species diversity, with international policies also focusing on maintaining species diversity [1]. Species diversity loss is accelerating due to the rapid increase in environmental stress, such as warming, acidification, hypoxia, introduction of invasive species, and habitat destruction caused by anthropogenic activities [2]. Presently, these issues are the subject of active research in marine ecosystems, along with other topics such as habitat restoration and ecosystem engineering. Among the mentioned factors, climate change affects the distribution range of marine organisms, along with their ecophysiological characteristics [3]. The ecophysiological characteristics of benthic macroalgae are significantly affected by ocean acidification and warming; directly inducing loss of species diversity in benthic ecosystems (e.g., [4,5]).

The increase in greenhouse gas emissions caused by anthropogenic activities has accelerated ocean acidification and warming. If global greenhouse gas emissions continue according to the RCP 8.5 scenario, by 2100 the surface seawater temperature is expected to increase by 2.6–4.8 °C and pH is expected to decrease by 0.31 [6]. As studies on climate change are being actively designed, research themes on the adaptation of marine organisms to climate change factors, from the physiological response to community structure, are equally enhanced. Previous studies have reported that seawater warming has a positive effect on the behavior of coastal benthic fauna and coralline algae, but negatively affects photosynthesis and growth of primary producers, including macrophytes [7]. Ocean acidification has a positive effect on the growth, photosynthesis, and primary production of marine autotrophs, but negatively affects several benthic mobile and sessile invertebrate taxa [8]. Specifically, ocean acidification can affect the survival, calcification, growth, and development of marine mollusks, echinoderms, crustaceans, and fishes [8]. These species- and taxon-specific adaptation strategies for future climate change accelerate the migration of marine organisms [3]; thus, it is possible that the responses of various marine organisms in future benthic communities may be substantially different than expected.

As anthropogenic activities increase, the movement of living organisms occurs through various pathways of introduction, and the resulting establishment and invasion can cause disturbances in native ecosystems [9]. Recently, ecological collapse from large-scale seaweed invasion has been reported in several benthic ecosystems (e.g., [10–12]). For example, massive *Sargassum* patches were carried to the beaches of Caribbean coasts, East China Sea, and Yellow Sea, causing enormous economic damage to the surrounding coastal ecosystems and industries [13,14]. These floating seaweed patches have the potential to become a pathway for transportation of various hitchhiker organisms [15]. Furthermore, invasive (or alien) seaweeds are introduced through various vectors, such as ballast water, hull fouling, imports, and aquariums [16]. *Caulerpa taxifolia* (nicknamed "killer algae") is a notorious invasive alga that spreads and invades coasts of the Mediterranean, Sydney (Australia), and San Diego (USA), causing the benthic ecosystem to collapse [17]. *Caulerpa taxifolia* was released off the Mediterranean coast at the Oceanographic Museum of Monaco in 1984, covering 30,000 ha of surrounding coasts after 10 years and inhibited the growth of benthic macrophyte habitats [18]. Fortunately, species of the invasive *Caulerpa* genus have not been observed in South Korea, and two native species inhabit natural habitats: *C. okamurae* and *C. geminata* [19,20]. However, two ornamental *Caulerpa* species, *C. sertularioides* and *C. racemose*, are traded in Korea and have been recognized as invasive seaweeds from the coast of Costa Rica and the Mediterranean coast, respectively [21,22]. Since *Caulerpa* species contain similar biological characteristics (e.g., siphonous green algae), they will be able to spread rapidly in future coastal environments; thus, it is important to carefully monitor the introduction and spread of these organisms.

Caulerpa sertularioides attaches to a sandy substrate or reef flat at a depth of 10 m in tropical and subtropical waters through stolons and rhizoids and has a feather-like frond. There is very little ecophysiological information about this species, and its invasion risk is still unknown. This species is traded as an aquarium plant in various regions, and hence, is worth paying close attention. In particular, ecophysiological studies on *Caulerpa*, a potential invasive species in the northern seaweed community, are necessary because climate change can increase opportunities for the introduction and settlement of tropical/subtropical organisms in temperate regions. Therefore, this study investigated the photosynthesis and growth of *C. sertularioides* according to climate change scenarios, as this species is morphologically very similar to killer algae.

2. Materials and Methods

2.1. Sample Preparation and Species Identification

The *C. sertularioides* utilized in the experiment was purchased from an aquarium in Seoul and transferred to a laboratory (November 2017). Post-purchase, the species was confirmed as *C. sertularioides* by DNA analysis after removing epiphytes from the algal tissue (DNA was extracted from the frond part and the *tuf*A gene was analyzed) [23]. The samples were cultured in a laboratory environment similar to aquarium conditions. In 10 L of artificial seawater, incubation conditions were maintained at a salinity of 33 (NO_3^- free and PO_4^{3-} free; Instant Ocean, Sarrebourg, France), light intensity of 80 μmol photons m^{-2} s^{-1} (L:D = 18:6 h), and temperature of 25 °C, and the water temperature was gradually lowered to 20 °C. For stable substrate attachment of rhizoids, coral sand with a thickness of approximately 5 cm was used as a substrate during the pre-adaptation period. Each sample was attached to the coral sand within 5 days. The stably growing thallus was fixed to a plastic mesh (1 × 1 cm mesh size) during the climate change experiment.

2.2. Experimental Conditions and Indoor Mesocosm System

An indoor mesocosm experiment was performed to investigate the individual and combined effects of acidification and warming. We designed ocean acidification (OA: doubled CO_2 with 20 °C) and ocean warming (OW: present CO_2 with 25 °C) conditions and compared them to the present conditions (control: present (470 µatm CO_2) CO_2 with 20 °C) based on the 2100 prediction results of IPCC Scenario WG GCP 8.5 [6]. The combined effect of the two future conditions was designed to represent a greenhouse effect (GR: doubled CO_2 with 25 °C).

The mesocosm system consisted of a water tank for sample incubation and exposure to experimental treatments as well as a gas mixing and control (M & C) unit. This part consisted of a mass flow controller (MFC, MR-300, MJ Technics, Incheon, Korea) and ball-type flow meter. We fixed the flow rate of ambient air (from the outside building) using the ball-type flow meter and mixed a small volume of pure CO_2 gas into ambient air using the mass flow controller. Ambient air was used for control and OW treatments. Elevated CO_2 conditions (OA and GR) were set using the MFC, and the flow rate of the pure CO_2 gas was finely adjusted to achieve a doubled pCO_2 (compared to the control and OW, respectively). The water tank consisted of a two-layer structure, four upper tanks (10 L; n = 4) for sample incubation, and a lower tank (80 L) pertaining to gas control for the experimental treatment. In the lower tank, the target CO_2 gas was adjusted to satisfy the experimental conditions, and the sample was then exposed to these conditions by pumping the seawater into the upper tank using a 65 W Universal-2400 pump (Eheim, Deizisau, Germany). To create the high-CO_2 treatment (i.e., OA and GR), the lower tank was aerated strongly with a mixture of ambient and pure CO_2 gases produced by the gas M & C unit. To create a warming treatment (i.e., OW and GR), a heating stick with a capacity of 500 W was connected to the temperature control system of the chiller, and temperatures were kept constant during the experiment (±0.1 °C). Our experiment is methodologically incomplete in that CO_2 gas was aerated into one large (lower) tank and distributed to four small upper tanks, resulting in so-called pseudo-replication. To overcome this methodological limitation, we conducted our experiment with a sufficiently large-scale lower tank (the lower seawater storage tank was eight times larger than the culture tank) and strong aeration using the targeted CO_2 gases. Experimental conditions were maintained with a venturi microbubble generator which suppressed biological responses. Detailed descriptions of the mesocosm system have been published by Kim et al. [4] and Kang et al. [5].

2.3. Determination of Carbonate Chemistry

Seawater samples were collected to determine seawater pH and total alkalinity (A_T). Samples were quickly transferred to 500-mL Pyrex bottles from the four upper tanks, without introducing air bubbles, and further poisoned immediately by the addition of saturated $HgCl_2$ [24]. All measurements were completed within 24 h of sampling. Seawater pH was determined by spectrophotometric pH measurements [25]. Meta-cresol purple (mCP) is commonly used for the measurement of seawater pH over a range of 7.2–8.2. The pH was calculated based on absorbance at wavelengths of 434, 578, and 730 nm before and after the addition of mCP. A_T was determined in a laboratory using a potentiometric titration system [26]. The titration system utilized in this study consisted of a Metrohm 765 Dosimat titrator and an Orion 920A pH meter [27]. The accuracy and precision of the pH and A_T measurements were checked daily against seawater reference materials (CRMs) with known pH and A_T values (certified by A. Dickson, Scripps Institution of Oceanography, San Diego, CA, USA). The precision of the measurement was approximately ±0.004 units for pH and ±1.5 µmol kg^{-1} for A_T. All carbonate chemistry parameters were calculated from the measured pH and A_T values using the CO2SYS software. In addition, pH, temperature, and salinity were monitored daily with a multi-meter, to ensure that the experimental conditions were maintained during the experiment period.

2.4. Oxygenic Photosynthesis and In Vivo Fluorescence

The oxygenic photosynthetic rate of the *C. sertularioides* thallus (one frond and 1 cm stolon) was measured after 15 days of acclimation to the experimental conditions. We randomly selected thallus from three tanks among the four exposure tanks and used them for photosynthesis experiments (n = 3). We used only three of the four tanks considering the complex procedure for maintaining the experiment and that three replicates are sufficient for statistical analysis. Measurement was conducted in the dark (0) and at four light levels (53, 197, 570, and 1010 μmol photons m^{-2} s^{-1}). The light source was provided by a 15 W LED lamp (light gradients were generated with a neutral density screen). After inserting the sample in a double-jacketed glass bottle (ca. 133 mL volume), the oxygen concentration was measured at 5 s intervals using an oxygen meter (ProODO Optical Dissolved Oxygen Instrument, YSI, OH, USA) and BOD probe (ProOBOD Optical BOD Probe) equipped with a self-stirring system. The seawater for bottle incubation was collected from the water tank of each environmental treatment.

In vivo chlorophyll-a fluorescence was measured using Mini PAM-II/R (Walz, Effeltrich, Germany) after 15 days of acclimation to each treatment. We conducted the PAM measurement using the thallus from four tanks (n = 4) of each experimental treatment. The relative electron transport rate (rETR) was calculated to construct rapid light curves (RLCs). The rETR was calculated using the effective quantum yield of PSII (Φ_{PSII}) at eight actinic light (AL) steps (24, 44, 63, 88, 122, 185, 277, and 409 μmol photons m^{-2} s^{-1}) after 10 min of quasi-dark acclimation. In addition, Φ_{PSII} was calculated as $\Phi_{PSII} = (F_m{}' - F)/F_m{}'$, where F and $F_m{}'$ represent the steady-state minimum fluorescence and maximum fluorescence after 10 s of acclimation to AL, respectively. The rETR was calculated as rETR = $0.5 \times 0.84 \times \text{AL} \times \Phi_{PSII}$. The mathematical model of photosynthesis-light curves (P-E curves) and RLCs were fitted using the exponential function of Platt, Gallegos, and Harrison [28] to obtain the photosynthetic parameters.

2.5. Growth Rate

The growth rate was calculated by measuring the length of the frond and stolon through image analysis (OptiView®, Jacksonville, FL, USA) captured with a digital camera every 3 days. We obtained sample images of thallus from four tanks (n = 4) of each experimental treatment. A coin was placed beside the sample in each image as a scale reference to allow the exact length to be determined. The relative growth rate (RGR) of frond and stolon was calculated as RGR (μ, d^{-1}) = $(\ln L_2 - \ln L_1)/(T_2 - T_1) \times 100$, where L_2 and L_1 represent the lengths of fronds or stolons at T_2 and T_1, respectively. Whole plant growth was calculated as the sum of frond and stolon length, using the RGR equation.

2.6. Statistical Analysis

All statistical analyses were performed using SPSS 23.0 (IBM, Armonk, NY, USA). When the Shapiro-Wilk normality test and Levene's homogeneity test of variance assumptions were fulfilled, separate two-way Model I analyses of variances (ANOVAs) were performed to identify differences in photosynthetic parameters of P-E curves and RLCs and RGR of *C. sertularioides* between CO_2 and temperature treatments. When significant differences were identified ($p < 0.05$), Tukey's HSD post-hoc comparison was performed.

3. Results

3.1. Carbonate Chemistry

In this study, the CO_2 concentrations of the control and OW conditions were 474.2 ± 3.2 μatm CO_2 and 476.6 ± 2.7 μatm CO_2, respectively (Table 1). The high CO_2 conditions (OA, GR) were 963.2 ± 10.6 μatm CO_2 and 879.5 ± 5.5 μatm CO_2, respectively

(Table 1). The pH values of OA and GR were lowered by approximately 0.25 units as compared to the control and OW, respectively. However, the pH difference between the control and GR was approximately 1.5 because the pH was increased by an increase in water temperature. HCO_3^- displayed the lowest concentration in the control (1911.0 ± 3.6 µmol kg^{-1}SW) and the highest concentration under the OA condition (2150.0 ± 7.6 µmol kg^{-1}SW). The dissolved inorganic carbon (DIC) concentration was 8.4- and 2.4-fold higher in OA and GR compared to the control and OW, respectively. The total alkalinity (A_T) was the highest in the OW condition (2632.0 ± 5.0 µmol kg^{-1}SW).

Table 1. Seawater carbonate chemistry under present and three simulated future ocean conditions.

Treatments	**Temp.**	**pH**	A_T **(µmol kg^{-1}SW)**	**DIC (µmol kg^{-1}SW)**	**pCO_2 (µatm CO_2)**	**HCO_3^- (µmol kg^{-1}SW)**	**CO_3^{2-} (µmol kg^{-1}SW)**
Control	20 °C	7.919 ± 0.002	2308.2 ± 2.9	2074.5 ± 3.3	474.2 ± 3.2	1911.0 ± 3.6	162.6 ± 0.3
Acidification (OA)	20 °C	7.647 ± 0.003	2390.3 ± 7.2	2249.0 ± 7.4	963.2 ± 10.6	2150.0 ± 7.6	98.7 ± 0.9
Warming (OW)	25 °C	8.020 ± 0.002	2632.0 ± 5.0	2291.8 ± 3.3	476.6 ± 2.7	2049.4 ± 2.0	242.3 ± 1.3
Greenhouse (GR)	25 °C	7.771 ± 0.004	2430.7 ± 3.5	2239.1 ± 3.1	879.5 ± 5.5	2104.0 ± 3.3	134.9 ± 1.1

Data are presented as the mean ± SE (n = 4).

3.2. Photosynthesis

Photoinhibition was observed at a light intensity of >200 µmol photons m^{-2} s^{-1} from the photosynthesis-light (P-E) curves, and the highest photosynthetic rate was exhibited under the GR condition (Figure 1). Contrarily, the highest respiration rate and lower photosynthesis rate were observed under the OW condition (Table 2). The maximum gross photosynthesis (GP_{max}) was the lowest under the OW condition (0.416 ± 0.045 mg O_2 g^{-1} DW h^{-1}), which was significant lower from the OA and GR conditions in which CO_2 was increased (Table 2). The increase in CO_2 increased GP_{max} ($F_{1,8}$ = 29.226, p = 0.001), but the effect of increased temperature was not statistically significant ($F_{1,8}$ = 0.510, $p > 0.05$; Table 3). The maximum net photosynthesis (NP_{max}) displayed higher values in the order of GR, OA, and OW conditions. Although there was no variation in NP_{max} due to increased temperature ($F_{1,8}$ = 0.089, $p > 0.05$), an increase in CO_2 had a positive effect on NP_{max} ($F_{1,8}$ = 66.747, $p < 0.001$; Table 3). In addition, there was a synergistic effect on NP_{max} when both CO_2 and temperature increased ($F_{1,8}$ = 6.044, $p < 0.05$; Table 3). The photosynthetic efficiency (α) was 0.0137 ± 0.0017 under the GR condition, which was higher than that of the control, while the corresponding values under the OA and OW conditions were lower than that of the control (Table 2). However, there was no significant difference between the experimental treatments ($p > 0.05$). Additionally, α was not significantly affected by individual and combination effects of high CO_2 concentrations and an increased temperature (Table 3). Saturation light (E_k) was the lowest under the control condition (43.9 ± 2.7 µmol photons m^{-2} s^{-1}), and the highest under the OW condition (81.5 ± 32.8 µmol photons m^{-2} s^{-1}; Table 2). For E_k, there was no significant difference between the experimental treatments. An increased CO_2 concentration and temperature did not affect E_k ($F_{1,8}$ = 0.050, p = 0.829 for CO_2, and $F_{1,8}$ = 0.587, p = 0.466 for temperature; Table 3). The dark respiration (R_d) was 0.262 ± 0.013 mg O_2 g^{-1} DW h^{-1} under the OW condition, which was significantly higher than that of the other conditions ($F_{3,12}$ = 7.104, p = 0.012; Table 2). R_d displayed a significantly decrease when CO_2 increased ($F_{1,8}$ = 10.157, p = 0.013), but displayed an increase when the temperature increased ($F_{1,8}$ = 7.079, p = 0.029; Table 3).

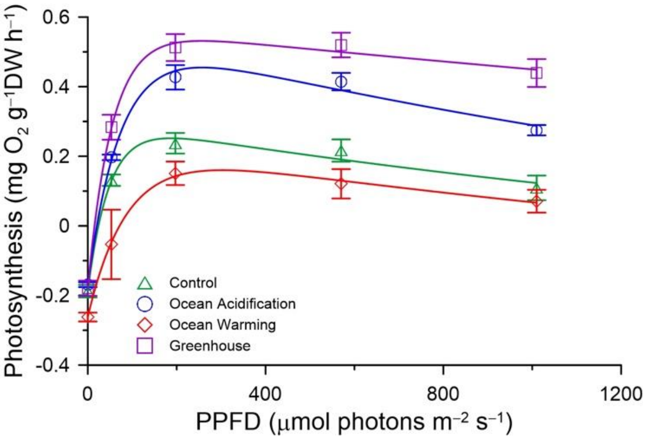

Figure 1. Net photosynthesis of *Caulerpa sertularioides* after exposure to present and three simulated future ocean conditions for 15 days. Data are represented as mean ± SE (n = 3).

Table 2. Photosynthetic parameters of *Caulerpa sertularioides* from oxygen measurement (P-E curves) and chlorophyll-a fluorescence measurement (RLCs: rapid light curves) after 15 days under present and three future ocean conditions.

Treatments		Control	Acidification	Warming	Greenhouse
P-E curves	GP_{max}	0.440 ± 0.033^{ab}	0.624 ± 0.044^{bc}	0.416 ± 0.045^{a}	0.711 ± 0.053^{c}
	NP_{max}	0.252 ± 0.031^{a}	0.455 ± 0.037^{b}	0.154 ± 0.037^{a}	0.532 ± 0.036^{b}
	α	0.0101 ± 0.0008	0.0096 ± 0.0002	0.0072 ± 0.0027	0.0137 ± 0.0017
	E_k	43.9 ± 2.7	65.0 ± 3.6	81.5 ± 32.8	52.9 ± 3.9
	R_d	0.188 ± 0.018	0.169 ± 0.007	0.262 ± 0.013 *	0.179 ± 0.022
RLCs	$rETR_{max}$	4.523 ± 0.226^{ab}	4.238 ± 0.191^{a}	5.719 ± 0.413^{b}	4.854 ± 0.485^{ab}
	α_{RLC}	0.3925 ± 0.0298	0.3737 ± 0.0335	0.3465 ± 0.0306	0.3910 ± 0.0274
	$E_{k,RLC}$	12.0 ± 01.2	12.0 ± 1.3	17.0 ± 2.3	12.9 ± 1.9

Maximum gross photosynthetic rate (GP_{max}; mg O_2 g^{-1} DW h^{-1}), maximum net photosynthetic rate (NP_{max}; mg O_2 g^{-1} DW h^{-1}), photosynthetic efficiency (α), minimum saturation light (E_k; μmol photons m^{-2} s^{-1}), dark respiration (R_d; mg O_2 g^{-1} DW h^{-1}), maximum relative electron transport rate ($rETR_{max}$), electron transport efficiency (α_{RLC}) and saturation light from RLCs ($E_{k,RLC}$; μmol photons m^{-2} s^{-1}). Different superscript letters and asterisk "*" indicate significant differences among the treatments (Tukey's post-hoc test, $p < 0.05$). Data are represented as mean ± SE (n = 3 for P-E curves and n = 4 for RLCs).

The RLCs obtained by in vivo chlorophyll-a fluorescence measurements exhibited a relatively higher rETR under the OW condition than under the other conditions (Figure 2). There was no significant difference in RLC parameters between the control, OA, and GR conditions except for $rETR_{max}$ (Tukey's test: $p > 0.05$). The $rETR_{max}$ was the highest under the OW condition at 5.719 ± 0.413, which displayed a significant difference from the OA condition (Tukey's test: $p < 0.05$; Table 2). The $\alpha_{,RLC}$ displayed the highest value under the control condition and the lowest value in OW, but there was no significant difference between treatments ($p > 0.05$; Table 2). The $E_{k,RLC}$ under the OW condition was 17.0 ± 2.3 μmol photons m^{-2} s^{-1}, which was higher than that under the control and OA conditions. Temperature favored the increase in $rETR_{max}$ ($F_{1,12} = 6.655$, $p = 0.024$; Table 3), but there was no significant effect resulting from an increased CO_2 or the combination of the two factors (increased CO_2: $F_{1,12} = 2.681$, $p = 0.128$; orthogonal effect: $F_{1,12} = 0.680$, $p = 0.426$; Table 3).

Table 3. Two-way ANOVAs to evaluate the individual and combined effects of CO_2 and temperature on photosynthetic parameters of *Caulerpa sertularioides*.

Parameter		Source	Type III SS	Df	MS	F	p
P-E curves	GP_{max}	CO_2	0.173	1	0.173	29.226	0.001
		Temp.	0.003	1	0.003	0.510	0.495
		CO_2 × Temp.	0.009	1	0.009	1.555	0.248
		Error	0.047	8	0.006		
	NP_{max}	CO_2	0.253	1	0.253	66.747	<0.001
		Temp.	0.000	1	0.000	0.089	0.773
		CO_2 × Temp.	0.023	1	0.023	6.044	0.039
		Error	0.030	8	0.004		
	α	CO_2	0.0000267	1	0.0000267	3.279	0.108
		Temp.	0.000001107	1	0.000001107	0.136	0.722
		CO_2 × Temp.	0.00003604	1	0.00003604	4.426	0.069
		Error	0.00006515	8	0.00000814		
	E_k	CO_2	41.642	1	41.642	0.050	0.829
		Temp.	489.028	1	489.028	0.587	0.466
		CO_2 × Temp.	1853.107	1	1853.107	2.224	0.174
		Error	6665.503	8	833.188		
	R_d	CO_2	0.008	1	0.008	10.157	0.013
		Temp.	0.005	1	0.005	7.079	0.029
		CO_2 × Temp.	0.003	1	0.003	4.074	0.078
		Error	0.006	8	0.001		
RLCs	$rETR_{max}$	CO_2	1.324	1	1.324	2.681	0.127
		Temp.	3.286	1	3.286	6.655	0.024
		CO_2 × Temp.	0.335	1	0.335	0.680	0.426
		Error	5.924	12	0.494		
	α_{RLC}	CO_2	0.001	1	0.001	0.178	0.681
		Temp.	0.001	1	0.001	0.222	0.646
		CO_2 × Temp.	0.004	1	0.004	1.081	0.319
		Error	0.044	12	0.004		
	$E_{k,RLC}$	CO_2	17.270	1	17.270	1.415	0.257
		Temp.	35.575	1	35.575	2.916	0.113
		CO_2 × Temp.	17.663	1	17.663	1.448	0.252
		Error	146.421	12	12.202		

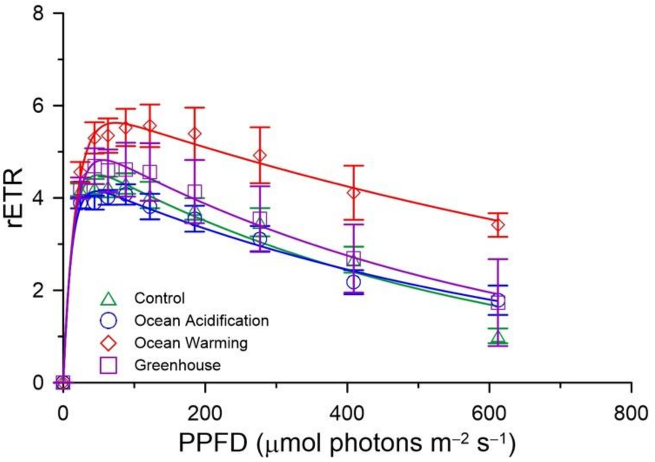

Figure 2. Relative electron transport rate (rETR) of *Caulerpa sertularioides* after exposure to present and three simulated future ocean conditions for 15 days. Data are represented as mean ± SE (n = 4).

3.3. Growth Rate

The frond, stolon, and whole plant growth of *C. sertularioides* varied among the future climate conditions (Figure 3; Table 4). The highest and lowest whole individual length was exhibited under GR and OA conditions, respectively (170.0 ± 22.53 mm for GR and 62.62 ± 8.03 mm for OA) (Figure 3A). The whole plant growth of *C. sertularioides* was 2.10 ± 0.92 d^{-1} in the control after 15 days of acclimation (Figure 3D). In the OA environment, the relative growth rate (RGR) was slightly higher than that of the control, but there was no significant difference (Tukey's test: $p > 0.05$). The RGR of *C. sertularioides* under the OW condition, where only the temperature increased, was 6.35 ± 1.71 d^{-1}. Under the GR condition, the whole individual growth rate was 9.16 ± 0.26 d^{-1}, which was significantly higher than the rate observed under the control and OA conditions (Tukey's: $p < 0.05$).

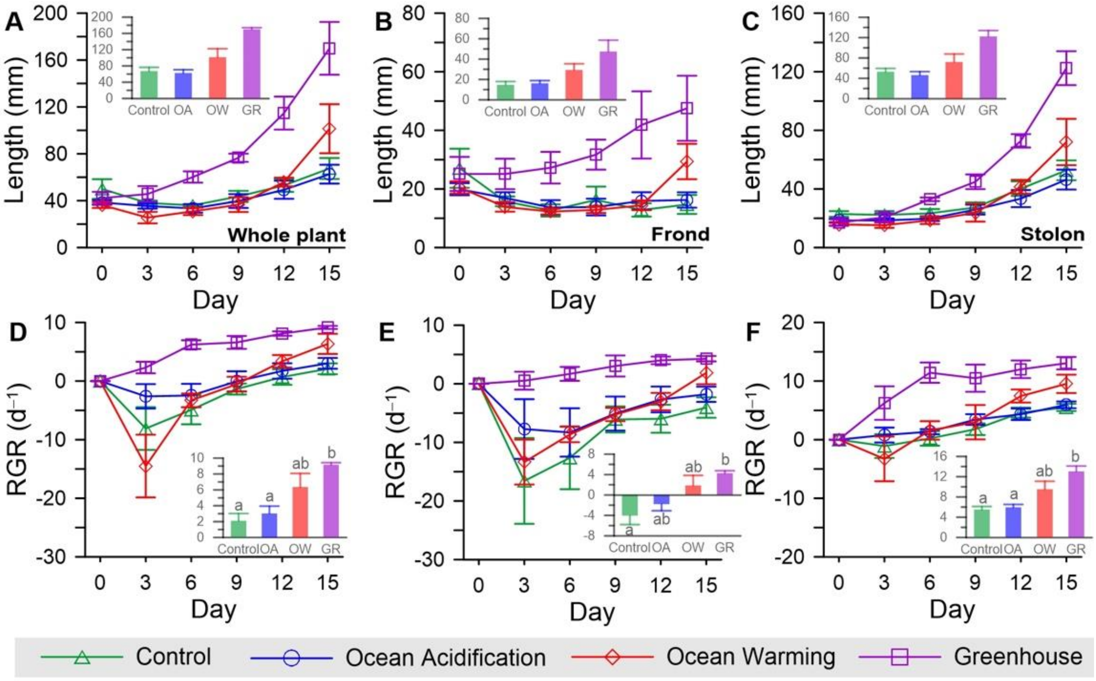

Figure 3. Thalli length (**A**–**C**) and relative growth rates (RGR, (**D**–**F**)) of whole plant (**A**,**D**), frond (**B**,**E**), and stolon (**C**,**F**) for *C. sertularioides* under present and three simulated future ocean conditions. Bar graphs represent the thalli lengths and RGR on the 15 days of the experiment. The different letters indicate significant differences between treatments based on Tukey's HSD post-hoc comparison ($p < 0.05$). Data are represented as mean ± SE (n = 4).

Table 4. Two-way ANOVAs to evaluate the individual and combined effects of CO_2 and temperature on relative growth rate (RGR) of *Caulerpa sertularioides*.

	Parameter	Source	Type III SS	Df	MS	F	*p*
Growth	RGRfrond	CO_2	21.798	1	21.798	2.478	0.141
		Temp.	143.972	1	143.972	16.368	0.002
		CO_2 × Temp.	0.018	1	0.018	0.002	0.965
		Error	105.551	12	8.796		
	RGRstolon	CO_2	15.654	1	15.654	3.655	0.080
		Temp.	123.672	1	123.672	28.875	<0.001
		CO_2 × Temp.	9.493	1	9.493	2.216	0.162
		Error	51.397	12	4.283		
	RGRwhole	CO_2	13.942	1	13.942	2.947	0.112
		Temp.	107.949	1	107.949	22.814	<0.001
		CO_2 × Temp.	3.550	1	3.550	0.750	0.403
		Error	56.779	12	4.732		

More specifically, the stolon grew more rapidly than the fronds during the experiment (Figure 3B,C,E,F). The fronds grew steadily for 15 days under the GR condition, approximately 1.8 times that of day 0 (reaching a length of 47.53 ± 11.10 mm) (Figure 3B). The highest RGR of the fronds was exhibited in the GR condition (4.28 ± 0.94 d^{-1}) (Figure 3E). Conversely, the fronds did not grow but declined under the control and OA conditions (-1.79 ± 1.30 d^{-1}). Under the GR condition, the RGR of the frond increased steadily until day 12, and then stagnated. The stolon showed elongation in all treatments, including control, and was elongated 2.4 (OA) to 7.2 (GR) times compared to day 0 on day 15 (Figure 3C). The stolon exhibited the highest RGR in GR, like the frond growth rate (13.05 ± 1.06 d^{-1} on day 15), and the samples also exhibited a higher RGR under OW conditions than under the control conditions, at 9.53 ± 1.157 d^{-1} (Figure 3F). The RGR of the stolon rapidly increased to the stagnation phase (after day 6) under the GR condition but continued to increase up to 15 days in the other treatments. There was a slight single effect of high CO_2 concentrations on the whole plant growth of *C. sertularioides*, but increased temperature had a strong positive effect on the growth rate of all individuals (CO_2: $F_{1,12} = 2.947$, $p = 0.112$; Temp.: $F_{1,12} = 22.814$, $p < 0.001$; Table 4). However, the combination of high CO_2 concentrations and an increased temperature did not exhibit a statistical difference (orthogonal effect: $F_{1,12} = 0.750$, $p = 0.403$), even if GR conditions exhibited the greatest effects on the RGR of *C. sertularioides*.

4. Discussion

This study investigated the photosynthesis and growth responses to future climate change scenarios of *C. sertularioides*, which is morphologically similar to the world-famous invasive 'killer algae'. Southern Korea is experiencing a rapid climatic transition from temperate to subtropical ecosystems, and a regime shift has been observed with the recent emergence of subtropical benthic plants (for example, *Ulva ohnoi* [5]; *Halophila nipponica* [29]). Most of the species belonging to the genus *Caulerpa* inhabit tropical and subtropical regions. Furthermore, since their habitats are expanding to high latitudes due to global warming, it is very important to evaluate the possibility of invasion into temperate regions due to future climate change. In particular, the performance of potentially invasive species of *Caulerpa* is very high because it overcomes geographical barriers very easily and is commercially traded in aquariums [30,31]. Therefore, studies evaluating the performance of a potentially invasive species based on ecophysiological (e.g., photosynthesis and growth) and biological (e.g., propagule density, dispersal, settlement capabilities, and interactions with native species) characteristics under elevated temperature and high CO_2 concentrations, which are predictable coastal conditions in the future, are very important for understanding the regime shift of future seaweed communities. In this study, ecophysiological acclimation strategies and the performance of a potentially invasive species in response to future climate conditions were compared between species examined in our previous indoor mesocosm studies (crustose coralline algae (CCA) and *U. ohnoi*) and *C. sertularioides* [4,5].

First, the photochemical properties (i.e., rETR) were up-regulated under warming conditions (i.e., OW), but this effect was negated by the increase in CO_2 (GR). In a study of a different subtropical algae (*U. ohnoi*) under the same experimental conditions, the rETR was up-regulated under the OA condition. However, the increased temperature displayed a negative effect, even under the GR condition [5]. Similarly, rETR of temperate CCA, *Chamberlainium* sp. was up-regulated under OA, but was reduced by increased temperature (OW and GR) [4]. Synergistically, photochemical properties vary along macroalgal species under future climate conditions. In addition, the combined effect of OA and OW (i.e., GR) could reduce the up-regulated photophysiology caused by specific climate factors (OW for *C. sertularioides* (in this study), OA for *U. ohnoi,* and temperate CCA) [4,5].

The oxygen evolution of *C. sertularioides* increased markedly under high CO_2 conditions (i.e., OA and GR). In our previous research on *U. ohnoi* and temperate CCA, oxygenic photosynthesis decreased under the OW and OA conditions, respectively, and was not higher than that of the control under all climate change conditions [4,5]. From these results, we concluded that high CO_2 concentrations and an increased temperature had various effects on photosynthetic characteristics among macroalgal species, and an energy mismatch between photochemical properties and O_2 production occurred. For *C. sertularioides*, rETR was expected to be very high under high CO_2 conditions (i.e., OA and GR), as O_2 evolution was significantly higher compared to the other treatments, but rETR was not different from the control (Figures 1 and 2). This suggests that processes such as oxygen-consuming electron transfer pathways (e.g., Mehler reaction) to reduce photo-stress may have been enhanced under warming conditions (OW) [32]. Moreover, we assumed that high O_2 production, even though the electron transfer efficiency is low under high CO_2 conditions (i.e., OA and GR), may be unable to down-regulate light utilization under high light conditions because most electrons are related to O_2 production. High CO_2 concentrations and an increased temperature greatly affect the electron transport chain and oxygen evolution/carbon dioxide fixation process during photosynthesis [33]. Increased CO_2 concentrations enhance photosynthesis via gas diffusion of the carbon source and induces down-regulation of carbon concentrating mechanisms, resulting in enhanced growth. In addition, as the light utilization efficiency is improved, there is no concomitant increase in the electron transfer efficiency (e.g., [34,35]). However, this physiological model does not appear in *C. sertularioides*, and it seems that elevated temperatures have a more positive effect than increased CO_2 concentrations. *C. sertularioides* grows optimally in (sub) tropical warm water, and high growth is expected under warming conditions (i.e., OW and GR) because an increase in electron transfer efficiency and an increase in oxygen evolution are observed. Growth is also expected to increase under the OA condition, as oxygen evolution is also increased.

Several species of *Caulerpa* exhibit optimal growth at 20–30 °C [20]. The increase in temperature has a positive effect on the growth of fronds and stolons in *C. sertularioides*. Based on our results, *C. sertularioides* exhibited better growth at 25 °C than at 20 °C. Although the temperature had a stronger effect than the increase in CO_2 concentrations, the combination of the two effects resulted in the highest growth with synergistic effects. Interestingly, there was a difference in the growth rates of fronds and stolons. The frond continued to grow during the experiment only under the GR condition, but in the other three conditions, the initial frond partially fell off and then gradually grew. Fragments of *Caulerpa* species can migrate and survive for several days and can re-grow under suitable conditions [36]. Under three conditions (control, OA, and OW), the fronds continued to grow after day 3, but only under the OW condition did they recover to the initial state after 15 days of incubation. When the *C. sertularioides* fragment was under suitable conditions, we observed a high possibility of recovery growth. In particular, the performance of this potentially invasive species also increased when *C. sertularioides* was exposed to the optimal temperature conditions. In addition, since stolons grow more actively than fronds, if stolons succeed in overwintering, there is a high possibility that *C. sertularioides* will survive, continuously grow, and spread. A representative invasive seaweed, *C. taxifolia*, exhibited high growth in the OA environment, suggesting that it may pose higher risks to invasive areas in the future [37].

Asexual reproduction is very important to the dispersal of *Caulerpa* species [38,39]. The proliferation of *Caulerpa* species is exhibited by growth patterns, fragmentation, and formation of propagules. In the invasive population of *C. taxifolia*, the density and biomass of stolons, fronds, and fragmented fronds were much higher than those of the native population [39]. Piazzi et al. [40] observed that the frond of *C. racemosa* decreased in winter in northern Italy, but the stolon persisted throughout the year. The rapid proliferation by stolon allows the stolon apex to continuously grow and disseminate from the substrate, enabling frond growth [22]. In our study, the growth of the stolon was higher than that of the frond during the experimental period, especially under the GR condition. This suggests that future climate change in the coastal environment will have a positive effect on the dispersal of *C. sertularioides*, and the rapid growth of stolons may accelerate its spread.

5. Conclusions

Based on our study, the invasive potential of *C. sertularioides* could be increased by acidification and warming seawater. Although this species has not yet been observed in Korea's natural seaweed habitat, these results indicate the possibility of successful early settlement under future coastal environments due to intensive anthropogenic activities. As the invasive potential increases in the future environment, it is necessary to propose a policy management plan to prevent proliferation and dispersion.

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