

Unlocking the Potential for Carbon Dioxide Removal (CDR) by *Ulva prolifera*: How Does the Addition of CO₂ Enhance Growth and Photosynthesis Rates?

Kaho Yamaha^{1*}, Eri Inomata², Hikari Nagoe², Yoichi Sato², and Gregory N. Nishihara³

¹Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Nagasaki, Japan

²Algal Research and Innovation Centre, Riken Food Co., Ltd., Miyagi, Japan

³Organization for Marine Science and Technology, Institute for East China Sea Research, Nagasaki University, Nagasaki, Japan

ABSTRACT

In Japan, climate change has led to decreasing yields of *Ulva prolifera* from aquaculture farms, and therefore land-based aquaculture techniques were developed for this species. We investigated how temperature and CO₂ concentration affected relative growth rates (RGR) and net photosynthesis rates (NPR), with the intent to optimize the *U. prolifera* production in land-based aquaculture tanks. RGR and NPR of *U. prolifera* were measured at three temperature levels (10, 20, and 30°C) and a range of CO₂ concentrations (0 to 200 mg L⁻¹). To assess RGR, the samples were cultured for 6 days in flasks and the wet weight was recorded. To assess NPR, the increase in dissolved oxygen concentrations in 100 mL bottles was measured over time. A generalized additive model was fit to the data to elucidate the CO₂ concentration that leads to maximum RGR and NPR.

RGR and NPR increased with increasing CO₂ concentrations and declined after reaching a peak. Our analysis suggests that the maximum RGR occurred at CO₂ concentrations of 4.8, 14.0, and 22.2 mg L⁻¹ at 10, 20, and 30°C, respectively. Whereas the maximum NPR occurred at CO₂ concentrations of 2.0, 13.8, and 3.5 mg L⁻¹ at 10, 20, and 30°C, respectively. These results reveal that the addition of CO₂ enhances growth and photosynthesis rates and provides us with valuable information regarding carbon dioxide removal (CDR) potential of *U. prolifera* aquaculture. Additional experiments are being done to verify these results, and to provide more insight regarding why a reduction in the CO₂ concentration maximized NPR at 30°C.

Keyword: Carbon dioxide/ Carbon dioxide removal/ Land-based aquaculture/ Macroalga/ Net photosynthetic rate/ Relative growth rate

1. INTRODUCTION

Ulva prolifera O.F. Müller is a species of filamentous green alga that can often be observed in brackish environments, such as estuaries. This intertidal species is well adapted to fluctuating environmental conditions, therefore it is resistant to environmental changes. The growth rate of *Ulva prolifera* is reported to be maximized at 20°C and salinity 32. However, the algal thalli have been shown to grow at 5°C, suggesting a wide tolerance for temperature and salinity fluctuations [1].

U. prolifera is consumed as food in Japan and is coveted for its distinctive aroma. One of the major production areas of this species was the Shimanto River in Kochi Prefecture, where the thriving season is divided into two periods, from December to January and late April. *U. prolifera* was also intensively cultivated in the Yoshino River estuary in Tokushima Prefecture since 1983 [2] However, more recently, its quality and yield have been unstable, which can be attributed to the effects of climate change on water temperature and weather conditions [3,4].

To stabilize quality and yield, land-based aquaculture techniques have been developed (i.e., the Germling cluster method [5]). Previous studies have reported that CO₂ aeration promoted the growth of this species [6,7]. However, our pilot research indicated that the growth rate decreased at CO₂ concentrations higher than 250 mg L⁻¹. Therefore, we hypothesize that the growth rate of *U. prolifera* has a maximal value when the concentration of CO₂ supplied to the *U. prolifera* clusters is below 250 mg L⁻¹ and that maximal growth rates occur at different CO₂ concentrations under different cultivation

*Corresponding Author: Gregory N. Nishihara
E-mail address: greg@nagasaki-u.ac.jp

temperatures (10, 20 and 30°C). Our group has investigated the response to CO₂ concentration on the relative growth rate and net photosynthesis rate of *U. proliferate*.

Currently, there is growing interest in the carbon dioxide removal (CDR) potential of various seaweed species, with ongoing research in this area. This study aims to enhance the efficiency of *U. prolifera* production in land-based aquaculture systems, which could contribute to increased CO₂ absorption. Such advancements may also provide opportunities for the application of gases emitted from sources such as thermal power plants [8,9].

2. METHODOLOGY

2.1 Growth experiments

The impact of CO₂ concentrations on the relative growth rate (RGR) of *Ulva prolifera*, were examined. The seawater used for the experiments were prepared using sterilized natural seawater. First the sterilized seawater was aerated with CO₂ gas using an air tube and an air stone. This high CO₂ concentration seawater was then diluted with sterilized seawater to create 8 to 9 different CO₂ concentrations of seawater. All growth conditions used the same seawater supply which was pumped from a distance of 160 m from the shoreline. Salinity was periodically measured and ranged from 28 to 34 psu. Samples were incubated in 1L flasks containing 1.1 medium at 10, 20 and 30°C (CN-40A, MITSUBISHI) under conditions of 150 μmol m⁻² s⁻¹ (12 h photoperiod) with stirring. CO₂ concentration was modified using a portable carbon dioxide concentration meter (CGP-31, DKK-TOA) and the light source was 3LH-64 (NK system). The relative growth rate was determined with equation (1). Briefly, the wet weight of the sample measured on day 0 and day *t* was recorded. The difference of the natural logarithm transformed wet weights were calculated and divided by the duration of the growth experiment [10].

$$\text{RGR} = \frac{(W_t) - (W_0)}{\text{days}} \quad (1)$$

Where; W_t represents the wet weight at day *t* and W_0 represents the wet weight of day 0.

2.2 Photosynthesis rates

To assess the impact of CO₂ concentration on photosynthesis rates, the net photosynthetic rate (NPR) was examined using samples pre-cultured for 1-4 days under conditions of 18 μmol m⁻² s⁻¹ (24 h photoperiod), and 20°C. The NPRs were measured in a 100 mL BOD bottle containing a single cluster and medium that was prepared similarly to the RGR experiments. The CO₂ concentrations were adjusted to 7 to 9 levels. Samples were secured within the bottles using a net to prevent movement. The medium was poured into the bottle and a BOD sensor (Xylem) connected to ProSolo (Xylem) was carefully inserted into the bottle to prevent the trapping of bubbles. Bottles were placed in a glass water bath and provided light. The temperature of the water bath was controlled with a chiller and heater as needed. Three water temperatures were selected for this experiment and were 10°C, 20°C, and 30°C. The light intensity of 200 μmol m⁻² s⁻¹ was maintained. Dissolved oxygen (DO) was measured every 5 minutes for 1 hour; the water in the BOD bottles were continuously stirred with a stirrer attached to the BOD sensor. The NPR was determined from the slope of the linear regression model fitted to the DO concentration time series. pH was measured with D-52S (HORIBA). To account for the influence of photosynthesis on CO₂ concentration, the adjusted mean CO₂ concentration was calculated and used as the representative value.

2.3 Statistical analysis

All statistical analyses were done using R version 4.4.1 [R core team 2024]. The linear regression was fit using a least-squares approach, with time as the explanatory variable and DO concentration as the response variable. The response of RGR and NPR to various temperature and CO₂ concentrations were examined using a generalized additive model (GAM), using a P spline with 5 basis functions for

the mean response and 7 basis functions for each temperature treatment. The response variable was either RGR or NPR and the explanatory variable was the natural logarithm of the CO₂ concentration. For the both models, a gamma distribution was assumed for the error distribution and the link-function was the natural logarithm. A Bayesian approach was used to fit the GAM and the priors for the model were defined with a gamma distribution (location 0 and shape of 2) for the model error. Model fit was assessed visually by inspecting the residuals and posterior distributions.

3. RESULTS AND DISCUSSION

In the relative growth experiment the RGR appeared to increase with increased CO₂ concentration, then decreased at higher concentrations; the responses were different with respect to temperature (Figure 1). Under the experimental conditions of 10°C, the maximum RGR (day⁻¹) reached 0.404 at the CO₂ concentration of 2.9 mg L⁻¹. Under the experimental conditions of 20°C, the maximum RGR reached 0.818 at the CO₂ concentration of 16.4 mg L⁻¹. Under the experimental conditions of 30°C, the maximum RGR reached 0.703 at the CO₂ concentration of 16.7 mg L⁻¹. In each experiment, the RGR was observed to have gradual reductions in areas with higher concentration of CO₂. However, the model predicted that the peak RGR would likely occur at 4.8 mg L⁻¹ at 10°C, 14.0 mg L⁻¹ at 20°C, and 22.2 mg L⁻¹ at 30°C.

As in the RGR experiment, the NPR responded to changing CO₂ concentrations and temperature similarly (Figure 2). Under the experimental conditions of 10°C, the maximum NPR (µg O₂ g_{ww}⁻¹ min⁻¹) reached 48.9 at the CO₂ concentration of 5.77 mg L⁻¹. Under the experimental conditions of 20°C, the maximum NPR reached 160 at the CO₂ concentration of 6.69 mg L⁻¹. Under the experimental conditions of 30°C, the maximum NPR reached 143 at the CO₂ concentration of 7.41 mg L⁻¹. However, the model prediction predicts that the peak NPR will likely occur at 1.95 mg L⁻¹ at 10°C, 13.8 mg L⁻¹ at 20°C, and 2.52 mg L⁻¹ at 30°C.

The photosynthesis and growth of macroalgae requires an adequate supply of CO₂ and optimal temperature conditions. When CO₂ concentrations are too low or too high, photosynthesis rates and growth rates of macroalgae should be suboptimal. Additionally, when temperatures are the extremes of the tolerable temperature range of a species, suboptimal photosynthesis and growth can occur. The dome-shaped response of RGR and NPR observed in *U. prolifera* suggests that there is a combination of CO₂ concentration and water temperature that leads to maximal rates. In our study, RGR and NPR generally peaked at CO₂ concentrations between 2.0 to 22.2 mg L⁻¹ (Fig. 1 and 2) and that the occurrence of these peaks were different with respect to temperature.

While optimal temperatures for plant growth are well-established [6], seaweeds also exhibit species-specific optimal temperature ranges that often reflect their natural habitats. For instance, *Undaria pinnatifida* and gametophytes of *Saccharina japonica* have reported maximum photosynthetic rates at 22.2°C and 23.3°C, respectively [11,12]. Xiao et al. observed high specific growth rates in *U. prolifera* between 14°C and 32°C, indicating its adaptability to a wide temperature range [1]. Our results align with these findings, as we observed no significant differences in relative growth rates between 20°C and 30°C.

Several studies have examined the effects of elevated CO₂ on seaweed growth, revealing species-specific responses. Similar to our findings, *Gracilaria* sp. and *G. chilensis* exhibited enhanced growth under elevated CO₂ conditions [13]. *Porphyra yezoensis* also demonstrated larger thalli in higher CO₂ concentrations [14]. Additionally, Wang et al. reported increased nitrate transporter activity in *U. prolifera* under elevated CO₂ conditions. *Hizikia fusiformis* not only showed accelerated growth but also increased average nitrate uptake rates and nitrate reductase activity, indicating enhanced nitrogen assimilation [15].

Studies investigating the combined effects of elevated CO₂ and temperature have yielded varied results. For instance, *Chlorella vulgaris* exhibited increased growth rates at 30°C under elevated CO₂ conditions [16]. In contrast, *Synechococcus* showed increased phycobilin and chlorophyll a content but no difference in growth rate [17]. *Kappaphycus alvarezii* cultured in vitro with CO₂ supplementation

exhibited cellular changes such as cell wall thickening, starch granule formation, and the appearance of organelles associated with intense cellular activity, suggesting improved stress tolerance and productivity when transported to offshore aquaculture farms [18].

While previous studies examined a wider range of temperatures, they generally showed a trend of increasing RGR in *U. prolifera* at temperatures around 10, 20, and 30°C, respectively [1,19]. Our results are consistent with this general pattern. We hypothesize that at low temperature, respiration and enzymatic reactions are down regulated, while at high temperatures, enzyme denaturation and respiration increased respiration rate reduce photosynthesis efficiency. The dome-shaped response of the growth to temperature is well-documented in various microalgae studies and can also be expected in *U. prolifera* [20,7]. As a result, CO₂ demand decreases compared to 20°C. In other words, temperature is likely a more significant limiting factor for the growth compared to CO₂ concentration.

A slight increase in CO₂ supply is expected to enhance the RGR of *U. prolifera*. Several factors may contribute to this, the primary one being energy conservation. Short-term low pH conditions can reduce the energy required for proton transport across membranes, potentially leading to increased energy available for growth. Additionally, restrictions on the carbon concentrating mechanisms (CCMs) may also lead to energy conservation that can be allocated to growth. CCMs widely found in seaweeds convert HCO₃⁻ to CO₂, enhancing the CO₂ environment to RUBISCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase), the central photosynthetic enzyme [21]. CCM requires more substance transport and energy consumption than general photosynthesis. Therefore, the suppression of CCM might have promoted growth. In addition, a previous study reported that the NRT gene expression increased significantly under elevated CO₂ [22]. As a result, nutrient uptake was promoted and the growth rate increased.

The net photosynthesis rates in our study exhibited significant variability. One possible explanation for this is the displacement of the sample from the light during the experiment. Additionally, when comparing samples with the same CO₂ concentration, we observed a positive correlation between NPR and sample weight. It is possible that larger samples experienced oxygen saturation.

In aquaculture systems where production must be carried out within limited land areas, technologies to improve productivity are indispensable. Our study found that the growth of *U. prolifera* is promoted when supplied with 10-30 mg L⁻¹ of CO₂ at 20°C. Therefore, we can expect that productivity will improve if similar land-based aquaculture tanks are placed in similar environments. Furthermore, since the optimal temperature and CO₂ concentration vary depending on the species being cultured, it is important to establish the optimal culture environment for each species. Improving the production efficiency of *U. prolifera* in land-based aquaculture systems could further enhance CDR. However, when cultivated in open-air tanks, an excessive supply of CO₂ not only fails to increase production but also results in the release of excess CO₂ back into the atmosphere. Therefore, when utilizing this species as a carbon sink, it is crucial to maintain an optimal CO₂ concentration to ensure efficient carbon absorption.

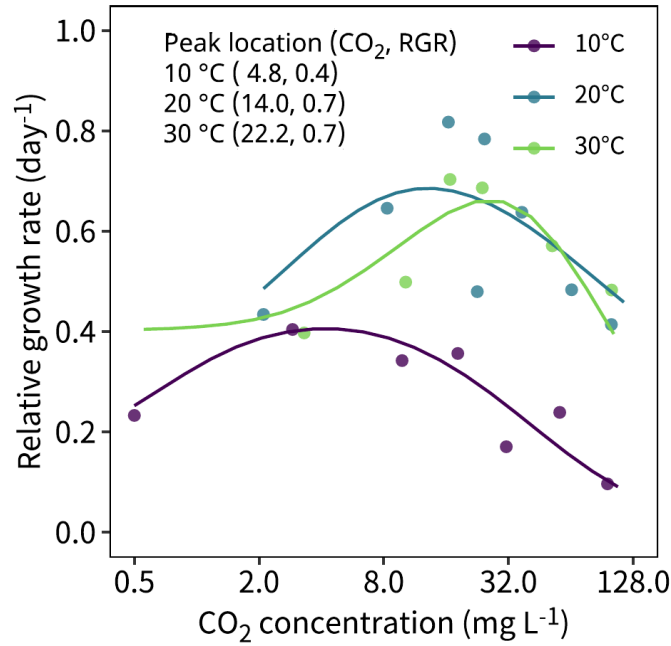


Figure 1. The RGR (day⁻¹) of *U. prolifera* in several CO₂ concentrations for 6 days' cultivation.

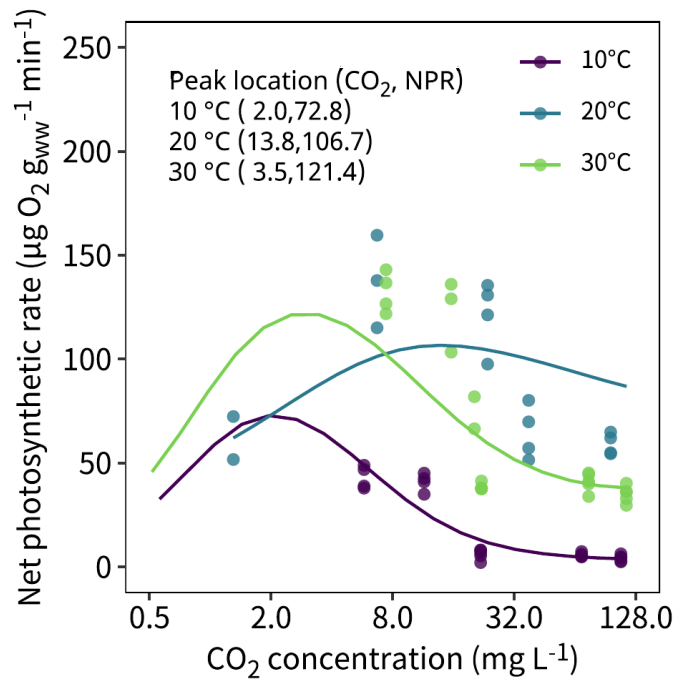


Figure 2. The NPR (μg O₂ g_{mass}⁻¹ min⁻¹) of *U. prolifera* in several CO₂ concentrations (n = 5)

4. CONCLUSIONS

This study aimed to determine the optimal CO₂ concentration for the production of *Ulva prolifera*. Our results indicated that the optimal concentration varied with temperature. However, these values should be considered as guidelines rather than absolute limits for CO₂ supply. Furthermore, the results of net photosynthesis rates exhibited significant variability, reducing their reliability. The reasons why growth is promoted by increasing CO₂ concentrations remain unclear, necessitating further investigation into the underlying mechanisms. A clearer understanding of these mechanisms would enable us to more effectively supply optimal amounts of CO₂ in response to environmental changes.

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