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Research Paper

Characterization of photosynthesis and growth of *Monostroma latissimum* (*Ulvophyceae*) collected from the intertidal area in Kochi, Japan

Jayvee Ablaña Saco[1,2], Satoko Sekida[1], and Ichiro Mine[1*]

[1] Laboratory of Cell Structure and Function, Graduate School of Kuroshio Science, Kochi University, 2-5-1 Akebono-cho, Kochi, 780-8520, Japan

[2] Present Address: Verde Island Passage Center for Oceanographic Research and Aguatic Life Sciences and college of Arts and Sciences, Batangas State University, Rizal Avenue, Batangas City, Batangas 4200, Philippines

Abstract

The photosynthesis and growth of the commercially important and edible green seaweed, *Monostroma latissimum* (Ulvophyceae), from a naturally occurring population in the intertidal area were examined in the laboratory. The natural population inhabits the middle to upper intertidal area and is usually attached to rocks. Cultivation occurs mostly in the shallow and calm waters under full light. The species was subjected to 10, 15, 20, or 25° C temperatures, and irradiances ranging from 0 to 1200 μmol photon m^{-2} s^{-1} in order to evaluate the effect of photosynthesis on growth. Photosynthesis was determined and characterized using photosynthesis-irradiance curves from oxygen production and PAM fluorometry. The maximum net photosynthetic rate (per thallus area and total chlorophyll content) did not differ significantly at the different temperatures, which were chosen to reflect the autumn to mid-spring temperatures that the seaweed would be expected to experience. The growth rates over 5 and 10 days were the same for all temperatures, but it decreased at 25° C after 15 days of culturing, which suggested that prolonged exposure to higher temperatures might have an adverse effect on growth. Similarly, the maximum quantum yield decreased as the temperature increased, which indicated that photosystem II (PSII) was under physiological stress at higher temperatures. In contrast, the light compensation point, saturating irradiance, initial slope, and PSII light absorption efficiency increased as the temperature rose. This suggested that the species optimized photosynthesis to accommodate the low and high light conditions that it may experience through its growing season. These conditions are characterized by irradiance limitation and low temperatures in winter, and higher irradiance and temperatures in spring. There were no photoinhibitory responses, which suggested that this alga was tolerant to higher irradiance. The growth rate significantly increased as irradiance rose, which may indicate higher growth rate responses at higher irradiances. Overall, the photosynthetic responses were in parallel with the growth rate response by *M. latissimum*. Therefore, the results from this study on *M. latissimum*, photosynthetic characteristics can be used to improve its cultivation.

Key words: cultivation, PAM fluorometry, *P-I* curve, percentage growth rate

INTRODUCTION

Monostroma latissimum Wittrock is a green benthic seaweed that has a single-layered blade-like thallus with a single parietal chloroplast per cell. It mostly inhabits the middle to the upper intertidal area where it usually grows on rocks or larger seaweeds. This species typically grows from late autumn to mid-spring and matures around March to May

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*Correspondence. Email address: mine@kochi-u.ac.jp

(Segi and Kida 1968, Ohno 1993, Bast 2011). In Japan, this species has been successfully cultivated on a commercial scale and has been a staple food for many centuries (Nisizawa et al. 1987, Bast 2011). The cultivation of *M. nitidum* and *M. latissimum* Wittrock mostly occurs in shallow and calm areas of inner bays or river mouths that are fully irradiated (Segi and Kida 1968, Ohno 1993). These species are cultivated by collecting the spores using culture nets on which the sporelings have settled. Two "seed collection" methods are used. One is a natural method where the zoospores that are naturally liberated in the cultivation sites are used, and the other is an artificial method that utilizes the zygotes produced by the *in vitro* fertilization of isolated gametes in the laboratory. These are then subsequently installed at the cultivation site (Ohno 1993, Bast 2011).

Earlier studies by Maegawa (1980) and Maegawa and Aruga (1974, 1983) provide detailed information on the photosynthetic and growth characteristics of the cultivated *M. latissimum* population under natural, ambient conditions. Population photosynthesis was more highly correlated with cultivation period and frond age than water temperature variations (5-35℃) and weather types. The highest photosynthetic activity was observed during the earlier cultivation period (November-December) and during fine days (~1600 µmol photons m^{-2} s^{-1} and ~13 ± 2°C). The lowest activity was recorded during the later cultivation period (January-April) and during rainy days (~300 µmol photons m^{-2} s^{-1} and ~13 ± 2°C). However, respiration is less affected by these factors (Maegawa and Aruga 1974, 1983, Maegawa 1980). The species was shown to have relatively high saturating irradiance levels (about 370-555 µmol photons m^{-2} s^{-1}) and no photoinhibitory response beyond 1850 µmol photons m^{-2} s^{-1}, which reflects its adaptation to high light levels (Maegawa and Aruga 1974, 1983). In contrast, the population growth rate increased during the earlier cultivation period (November-December) and gradually declined during the later period (January-April), but when the fronds were harvested or cut off by wave action, there was a slight increase in growth (Maegawa and Aruga 1974). In addition, no significant effect on the population growth rate when different weather types i. e., rainy days and fine days (~300 and ~1600 µmol photons m^{-2} s^{-1}, respectively, and ~13 ± 2°C) were investigated (Maegawa 1980).

Previous studies showed that photosynthesis and growth were affected by cultivation period and weather types (Maegawa and Aruga 1974, 1983, Maegawa 1980). However, the effect of environmental conditions on photosynthesis and growth have not been fully understood due to the use of natural, ambient conditions that cannot be controlled artificially. In addition, previous reports did not mention the relationship between photosynthesis and growth. Understanding the basic mechanisms controlling primary production is important if efficient cultivation is to be achieved, particularly as photosynthesis is one of the most important factors affecting algal growth. Although photosynthesis in seaweeds is affected by different environmental conditions, e. g., salinity and nutrients, irradiance and temperature are also two important parameters that drive photosynthesis and affect molecular activities and metabolism, respectively, and subsequently their growth (Lobban et al. 1985, Lobban and Harrison 1994). In this study, we measured photosynthesis by recording oxygen evolution and PAM chlorophyll fluorometry of *M. latissimum* collected from the natural habitat. While the growth was measured using unialgal culture strains of *M. latissimum* where source materials were collected from natural habitat. These were measured under laboratory conditions at various controlled temperatures and light levels. We also discussed the dependency of *M. latissimum* growth on photosynthetic activities and the physiological background of the natural population including relation to the growing season.

MATERIALS AND METHODS

Algal material

Monostroma latissimum was collected from the intertidal area of Usa, Tosa City, Kochi, Japan (33° 26′21.8″N, 133°26′ 30.5″E) between March and May 2016 during low tide (Fig. 1). The samples were placed inside a plastic bag with a minimal amount of ambient seawater and kept inside a styrofoam container filled with ice packs. The samples were transported to the laboratory within 40 min where they were immediately cleaned of epiphytes, epizoans, and debris using filtered seawater. The samples were then maintained inside a growth chamber at 15℃, about 15 µmol photons m^{-2} s^{-1}, and with a

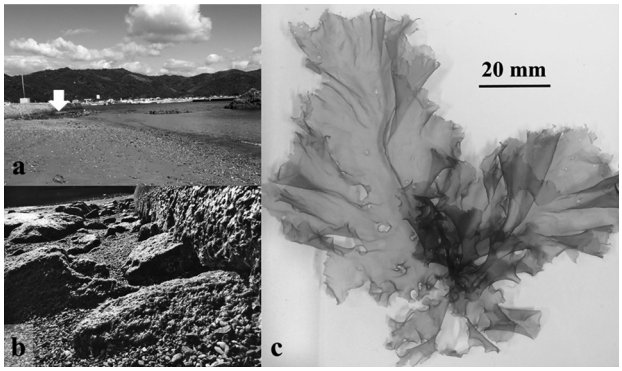

Fig. 1. Photographs of the collection site (a, b) and a freshly collected *M. latissimum* sample (c). (a) The intertidal area where the sample was growing (filled arrow). (b) Closer image of the species on rocks. (c) *M. latissimum* showing the light green colored thallus with a fan-shaped blade and the plane elliptical margin.

12L:12D photoperiod until needed for further processing. A different set of individual thalli was used in each experiment.

Measurement of photosynthetic oxygen evolution

The photosynthetic measurements using oxygen evolution technique were based on the procedures used by Saco et al. (2017). The photosynthesis of an 8-mm diameter thallus disc (n = 5) was measured using the oxygen evolution technique under unilateral illumination at eight light intensities (3, 26, 80, 120, 210, 300, 610, or 1,200 µmol photons m^{-2} s^{-1}) and four different temperatures (10 ± 0.1, 15 ± 0.1, 20 ± 0.1, or 25 ± 0.1°C) without pre-acclimation to the experimental temperatures. The oxygen evolved was measured using a Clark-type oxygen electrode (9002SP, Toko Chemical Laboratories Co., Ltd., Tokyo, Japan) fitted in a glass specimen chamber (OC-100, Toko Chemical Laboratories Co., Ltd). The temperature was controlled by recirculating water jackets connected to a water bath (RTE-9, Neslab Instruments, Inc., Portsmouth, USA). These were placed inside a sealed black box to prevent outside light intrusion and to measure respiration rates when the light was turned off. The discs were punched near the margins of five individual thalli about 12 h in advance and kept inside a growth chamber under the conditions mentioned above to allow the wounds to heal prior to their use in experiments the following day.

A halogen lamp with a reflecting mirror (JDR110V60W/K5S, Toshiba Lighting and Technology Corp., Yokosuka, Japan) provided photosynthetically active radiation. Two heat absorbing filters (HA-50, Hoya Corporation USA, Milpitas, USA) were placed at the bottom of the chamber and were kept perpendicular to the disc using a light guide. Neutral density filters (AND-25S-01, 05, 13, 25, 50, 70, Sigma Koki Co., Ltd., Tokyo, Japan) were used to produce the different light intensities, which were measured with a phototransistor (NJL7502L, New Japan Radio Co., Ltd., Tokyo, Japan) housed in a black painted aluminum tube calibrated with a quantum meter (BQM, Apogee Instrument, Inc., Logan, USA).

The glass specimen chamber was filled with 0.6 ml of filtered seawater. The disc was placed flat on the raised bottom and then a round stainless-steel mesh (PMY40X-K-50-50, Misumi Corporation, Tokyo, Japan) was placed on top to immobilize the disc during continuous stirring by a cylindrical magnetic stirrer, which was placed on top. The bottom was raised about 9 mm using a transparent acrylic column that had an outer diameter that exactly fitted the inner diameter of the cylindrical chamber. This minimized the inner capacity of the chamber. Photosynthetic oxygen concentration was measured for 3 min. Then the light was turned off for 3 min to measure the respiration rate. The filtered seawater was replaced before each cycle.

A Hansatech oxygen control box (CB1-D3, Hansatech Instrument, Ltd., King's Lynn, England) was used to amplify the current signal of the oxygen electrode and convert it to a voltage signal. The oxygen electrode was calibrated using an oxygen solubility table (YSI Incorporated, 2009) at a salinity of 36.1 ppt and atmospheric pressure of 760 mm Hg. The saturated oxygen concentration was obtained using filtered seawater and the zero oxygen concentration was obtained using 2% Na_2SO_3 in 10 mM $Na_2B_4O_7$ solution. The photosynthetic and respiration rates were calculated from the slope of the linear regression for oxygen evolved within the 3 min incubation time.

Chlorophyll determination

The total chlorophyll content (Chls *a* and *b*) was extracted immediately from each disc (ø = 8-mm) after oxygen production measurement that lasted approximately 50 min per disc for each temperature treatment. The excess seawater was removed from the disc using tissue paper and then the disc was soaked in 1.0 ml 100% N, N-dimethylformamide (DMF) solvent for 1 h at room temperature in the dark. The light absorbance of the extract was measured spectrophotometrically (JASCO UV-VIS V-550, Jasco Corporation, Tokyo, Japan) using the following formula (Porra et al. 1989):

[Chl *a* + Chl *b* (µg mL^{-1}) = 17.67 $A_{646.8}$ + 7.12 $A_{663.8}$].

PAM fluorometry

The PAM fluorometry measurements were based on Saco et al. (2018). A PAM fluorometer (JUNIOR-PAM, Walz, Effeltrich, Germany) was used to determine the chlorophyll fluorescence parameters of an 8-mm diameter disc thallus (n = 5). The discs were punched near the margins of five individual thalli about 12 h in advance and kept inside a growth chamber under the conditions mentioned above to allow the wounds to heal prior to their use in experiments the following day. The measurements were taken in the dark and with saturating actinic light under stable fluorescence conditions and at different temperatures (10 ± 0.1, 15 ± 0.1, 20 ± 0.1, or 25 ± 0.1°C). The thallus disc was held together in a specimen holder that maintained a distance of about 1.0 mm between the light fiber optic and the surface of the disc. The specimen holder was placed inside a tin container filled with filtered seawater under continuous stirring. This was then placed in a transparent water bath with recirculating water jackets (RTE-9, Neslab Instruments, Inc., Portsmouth, USA) that controlled the temperature when the measurements were being taken.

Measurements were performed using 0.6 µmol photons

m^{-2} s^{-1} measuring light and a 6000 µmol photons m^{-2} s^{-1} saturating pulse. The maximum quantum yield of PSII [F_V/F_M = (F_M − F_O) / F_M] (Genty et al. 1989) was determined from dark-adapted samples over 10 min. The effective quantum yield of PSII [ϕ_{PSII} = (F_M' − F') / F_M'] (Genty et al. 1989) was determined from saturating actinic light (625 µmol photons m^{-2} s^{-1}) that was stable for about 16 min. At the end of the light-adapted measurement, far-red light was used to determine the non-photochemical quenching coefficient [q_N = 1 − [(F_M' − F_O') / (F_M − F_O)]] (van Kooten and Snel 1990). The stable fluorescence showed that after a sufficiently long actinic light illumination period, the fluorescence reading reached an almost constant level within 2-5 min, after which a total of 3-5 saturating pulses were given every 5-10 sec, which produced 3-5 ϕ_{PSII} values that were averaged to represent ϕ_{PSII} in saturating actinic light.

Growth measurement

Whole thalli were induced to mature so that they could be used to produce unialgal cultures from isolated biflagellate zoids. The thalli were washed several times with fresh water and placed inside culture dishes containing filtered seawater. These dishes were then placed inside a growth chamber under continuous light of about 15 µmol photons m^{-2} s^{-1} and at 15℃ for about 3-4 days until a brownish-yellow color appeared at the thallus margin. The washing of freshwater and incubation under continuous light of the thalli provide physiological stress in order to induce and facilitate faster maturity. The zoids were collected using the positive phototactic response in a sterilized petri dish containing autoclaved Provasoli Enriched Seawater (PES). The collected zoids were allowed to grow into foliose thalli under the culture conditions mentioned above with a 12L: 12D photoperiod and once a week PES replacement for about 30-40 days prior to experimentation. The filtered natural seawater used for the PES medium was collected from the Usa Marine Biological Institute, Kochi University, which was near the collection site.

The laboratory growth rate experimental set up followed that of Mine et al. (2015). Each foliose unialgal culture (n = 6) was transferred to a tissue culture plate (Product No. 3506, 6 wells with a flat bottom and a low evaporation lid, Corning Inc., Corning, USA) with about 10 ml of PES in each well. Three plates containing the culture were stacked together and placed directly under illumination provided by four white light emitting diodes (OSW4XME1C1S-100, OptoSupply Ltd., Hong Kong) from above. Two layers of black cheese cloths were placed on top of the middle and bottom plates to reduce the intensity of the incident lights. This mean that there were different light intensities at the top, middle, and bottom of the plates. These were 400, 80, and 20 µmol photons m^{-2} s^{-1}, respectively. The photoperiod was 12L:12D. The plates were incubated at 10 ± 2, 15 ± 2, 20 ± 2, or 25 ± 2℃ for 15 days, and the wet weight (mg) was measured every 5 days after gently removing excess seawater with a sterile tissue. The PES was also replaced at this point. The plates were shaken about 2-3 times every day. The percentage growth rate (% d^{-1}) was measured following the formula used by Yong et al. (2013): Daily Growth Rate = [(W_t / Wo)$^{1/t}$ − 1] × 100 where W_t is the final wet weight, W_o is the initial wet weight and t is days of culture. The initial wet weight used was approximately 0.8-1.9 mg and the average length was about 6 mm.

Data analysis

Photosynthetic rates were plotted against their respective light level and a curve was fitted using the non-linear least square method, which compared differences between actual and estimated data using the Solver Module in Excel (Microsoft Corp., Redmond, USA) (Roleda et al. 2006). The method followed a model that used an exponential equation (Webb et al. 1974, Jassby and Platt 1976, Platt et al. 1980, Henley 1993) as cited by Borlongan et al. (2017). This was [P^n = $Pmax$(($1 - exp(-\alpha / Pmax)(I)) - Rd$] where P^n is the net photosynthesis, $Pmax$ is the maximum photosynthesis, α is the initial slope of the curve, I is the incident irradiance, and Rd is the dark respiration. The maximum net photosynthesis was computed as $P^n max$ = ($Pmax$ − Rd), while the saturation and compensation irradiances were Ik = ($Pmax$ / α) and Ic = [$Pmax$(ln(($Pmax$) / (Rd − $Pmax$)) / α], respectively.

The procedure used by Dawes (1992) was applied to allow statistical comparisons among photosynthesis-irradiance (*P-I*) curve parameters. The photosynthetic rate at each light level from a disc taken from five different individuals was curve-fitted using the above procedure. This generated five data sets for each parameter.

The data were tested for normality using the Shapiro-Wilk test prior to further analysis and were log transformed if the data were not uniformly distributed i.e., *Ik* and *Ic*. One-way analysis of variance (ANOVA) was used to determine significant differences among the *P-I* curves, PAM fluorescence parameters, and the extracted chlorophyll content to temperature values ($p < 0.05$). Two-way ANOVA was used to determine significant differences between the growth rate to temperature and irradiance ($p < 0.05$). Tukey's HSD was used for post-hoc analysis ($p < 0.05$). Regression analysis was used to determine the relationships among the *P-I* curves and PAM fluorescence parameters, the extracted chlorophyll content to temperature, and the growth rate relationship with temperature and irradiance ($p < 0.05$). These were undertaken using R software (version 3.2.0, R Core Team, Vienna, Austria).

RESULTS

Photosynthesis-irradiance (*P-I*) curves

The *M. latissimum* photosynthetic response to increasing irradiance per thallus area (μmol O_2 m^{-2} s^{-1}), and the total chlorophyll content (μmol O_2 g Chl *a* + Chl b^{-1} s^{-1}) response to the various temperatures were compared using *P-I* curves and the respective extracted chlorophyll contents (Figs. 2 and 3; Tables 1 and 2).

Temperature did not significantly affect $P^n max$ (per thallus area and total chlorophyll content), but *Rd* (per thallus area and total chlorophyll content) increased as the temperature rose. However, a shift in the *P-I* curve under 25°C was observed when the per thallus area values were compared to the total chlorophyll content values. This might be due to the slightly higher total chlorophyll content extracted from the thalli used during the experiment, but it was not significantly different from the others. The α (per thallus area and total chlorophyll content) decreased steepness as the temperature increased. Both *Ic* and *Ik* increased as the temperature rose between 8 and 74 μmol photons m^{-2} s^{-1} and 102 and 234 μmol photons m^{-2} s^{-1} , respectively. There was no decrease in $P^n max$ beyond the saturating irradiance point for all temperatures, even at the highest irradiance level of 1200 μmol photons m^{-2} s^{-1} .

PAM fluorometry

The *M. latissimum* PAM fluorescence parameters, i.e., F_V/F_M, ϕ_{PSII}, and q_N responses to various temperatures were compared (Fig. 4; Table 2). In the dark-adapted state, F_V/F_M decreased as the temperature rose, and showed a significantly higher response (0.77 ± 0.01) at 10°C, but a significantly lower response (0.71 ± 0.02) at 25°C. In the light-adapted

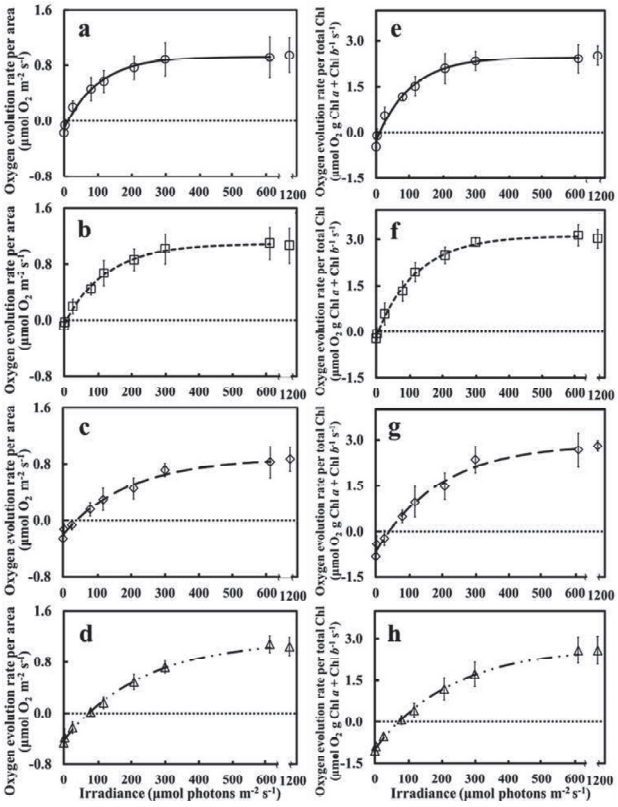

Fig. 2. Photosynthesis-irradiance (*P-I*) curves for *M. latissimum* (a-d) per thallus area and (e-h) for total chlorophyll content at (a, e) 10, (b, f) 15, (c, g) 20, and (d, h) 25°C. The photosynthetic rate was curve-fitted up to the highest irradiance level, but the line regression after 600 μmol photons m^{-2} s^{-1} was omitted so that the photosynthetic rates at lower irradiance levels could be shown. The markers and vertical lines are the means and standard deviations for the discs of five different individuals, respectively.

Table 1. *P-I* curve parameters and chlorophyll content of *M. latissimum* to various temperatures.

Temperature (°C)	Parameters of photosynthesis-irradiance (*P-I*) curve								Chlorophyll content[4]
	per thallus area[1]			per total chlorophyll content[2]			*Ik*[3]	*Ic*[3]	
	$P^n max$	*Rd*	α	$P^n max$	*Rd*	α			
10	0.93±0.25	0.11±0.07[a]	0.010±0.003[a]	2.40±0.39	0.27±0.09[a]	0.027±0.003[ac]	102±13[a]	11±4[a]	1.93±0.60
15	1.08±0.25	0.07±0.05[a]	0.010±0.002[ab]	3.11±0.36	0.21±0.11[a]	0.029±0.004[a]	117±29[a]	8±4[a]	1.73±0.29
20	0.89±0.18	0.20±0.06[b]	0.006±0.002[b]	2.87±0.32	0.67±0.28[b]	0.019±0.007[bc]	204±57[b]	39±8[b]	1.57±0.25
25	1.14±0.14	0.42±0.12[b]	0.007±0.001[b]	2.72±0.51	0.98±0.07[c]	0.016±0.003[b]	234±34[b]	74±16[b]	2.22±0.66

[1]μmol O_2 m^{-2} s^{-1}; [2]μmol O_2 g Chl *a*+b^{-1} s^{-1}; [3]μmol photons m^{-2} s^{-1}; [4]μg Chl *a* + Chl *b*/cm^2 thallus area

ANOVA and Tukey's HSD at $p < 0.05$; mean±standard deviation with the same letter is not significantly different.

Data from discs of five different individuals are shown.

Table 2. Regression coefficient (r^2) of the *P-I* curve and PAM fluorescence parameters and chlorophyll content of *M. latissimum* to various temperatures.

Parameters of photosynthesis-irradiance (*P-I*) curve						Parameters of PAM fluorescence					Chlorophyll content[4]
per thallus area[1]			per total chlorophyll content[2]			*Ik*[3]	*Ic*[3]	F_V/F_M	ϕ_{PSII}	q_N	
$P^n max$	*Rd*	α	$P^n max$	*Rd*	α						
0.19	0.61*	0.31*	0.21*	0.61*	0.48*	0.65*	0.60*	0.68*	0.65*	0.09	0.02

[1]μmol O_2 m^{-2} s^{-1}; [2]μmol O_2 g Chl *a*+*b*$^{-1}$ s^{-1}; [3]μmol photons m^{-2} s^{-1}; [4]μg Chl *a* + Chl *b*/cm^2 thallus area
*p < 0.05; Data from discs of five different individuals are shown.

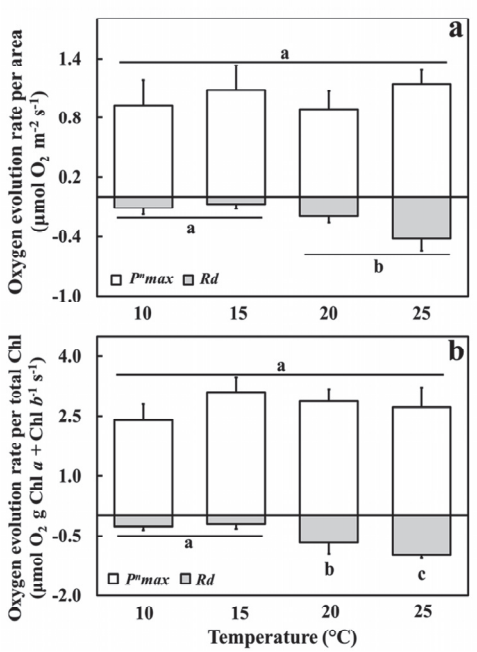

Fig. 3. Maximum net photosynthetic rate (white part) and respiration in the dark (gray part) for *M. latissimum* (a) per thallus area and (b) for total chlorophyll content at different temperatures. Vertical bars with the same letter are not significantly different. The vertical bars and lines are the means and standard deviations for discs from five different individuals, respectively.

state, ϕ_{PSII} increased as the temperature rose, and showed significantly lower responses (0.12 ± 0.02 and 0.13 ± 0.03) at 10 and 15℃, respectively, but significantly higher responses (0.23 ± 0.02 and 0.2 1 ± 0.02) at 20 and 25℃, respectively. However, q_N ranged from 0.85 ± 0.06 to 0.092 ± 0.02 and there were no significant differences between temperatures.

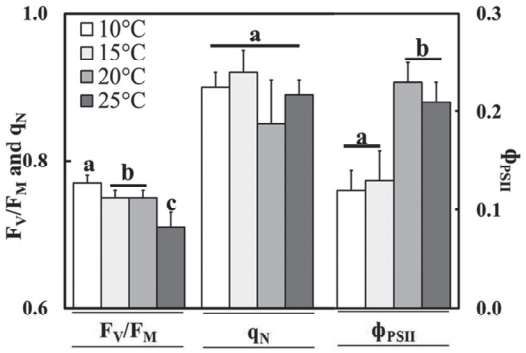

Fig. 4. PAM fluorescence parameters of *M. latissimum* at different temperatures. Vertical bars with the same letter are not significantly different. The vertical bars and lines are the means and standard deviations for the discs from five different individuals, respectively.

Growth rate

The growth rate (% d^{-1}) did not differ significantly between 10-25℃ for all irradiance levels when *M. latissimum* was cultured for 5 days (Fig. 5; Table 3). The rates ranged from 6 to 13% d^{-1} at 20 μmol photons m^{-2} s^{-1}; from 11 to 19% d^{-1} at 80 μmol photons m^{-2} s^{-1}, and from 17 to 23% d^{-1} at 400 photons m^{-2} s^{-1}. The growth rate was generally similar between 10-25℃ at all irradiance levels when the thalli were cultured for 10 days. The rates ranged from 6 to 7% d^{-1} at 20 μmol photons m^{-2} s^{-1}; from 8 to 20% d^{-1} at 80 photons m^{-2} s^{-1}, and from 13 to 19% d^{-1} at 400 photons m^{-2} s^{-1}. However, the growth rate decreased significantly at 25℃ when the thalli were cultured for 15 days. The rates ranged from 2% d^{-1} at 20 μmol photons m^{-2} s^{-1}, 6% d^{-1} at 80 photons m^{-2} s^{-1}, and 10% d^{-1} at 400 photons m^{-2} s^{-1}. The growth rate increased as the irradiance level rose for all temperature levels when the samples were cultured for 5, 10, and 15 days. The light levels

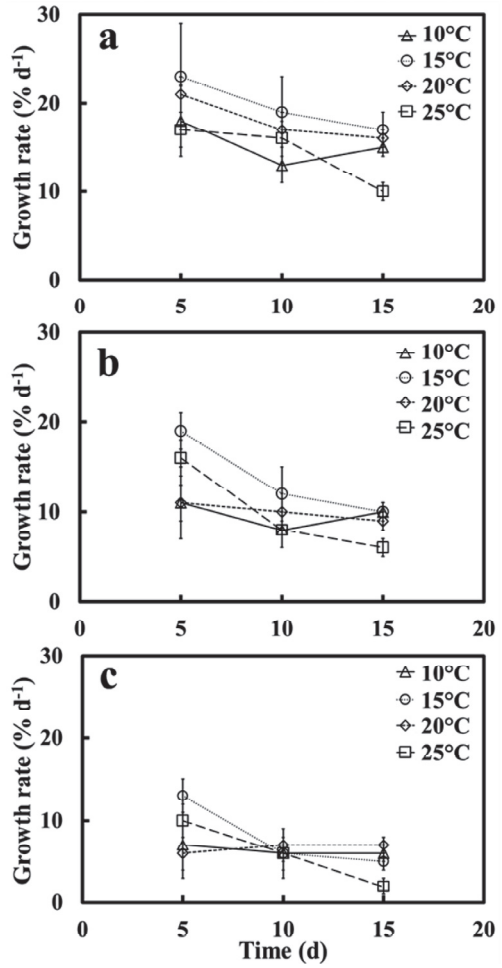

Fig. 5. Percentage growth rates of *M. latissimum* cultured over 5, 10, and 15 days at different temperatures (10, 15, 20, and 25°C) and irradiance levels: (a) 400, (b) 80, and (c) 20 μmol photons m^{-2} s^{-1}. Markers and vertical lines are the means and standard deviations from six different individuals, respectively.

Table 3. Regression coefficient (r^2) of the percent growth rate of *M. latissimum* to various (a) temperatures at each irradiance level and (b) irradiances at each temperature level, cultured in 5, 10 and 15 days.

		Growth Rate (% day^{-1})		
		5 days	10 days	15 days
(a)[1]	20	0.00	0.00	0.31*
	80	0.02	0.00	0.40*
	400	0.01	0.05	0.27*
(b)[2]	10	0.50*	0.65*	0.77*
	15	0.29*	0.67*	0.82*
	20	0.87*	0.82*	0.78*
	25	0.23*	0.70*	0.68*

[1]μmol photons m^{-2} s^{-1}; [2]°C; *$p < 0.05$

Data from six different individuals are shown.

used in the growth rate experiment were within the light compensation point, and saturating irradiance ranges obtained from their *P-I* curve. Overall, both temperature and irradiance significantly affected growth, but no interaction effect from both the temperature and irradiance was detected on any of the monitoring days while the samples were being cultured.

DISCUSSION

This study showed the strong influence of irradiance and temperature on the photosynthesis and growth rate responses by *M. latissimum*. The $P^n max$ value trend was similar to the growth rate response when the temperatures reflected the temperatures experienced in the late autumn to mid-spring growing season. However, the growth rate decreased at a higher temperatures, which indicated that prolonged exposure to higher temperatures might have a negative effect. The maximum quantum yield revealed the sensitivity of the PSII complexes to higher temperatures. In contrast, the steeper α, the lower *Ic, Ik*, and ϕ_{PSII} values at 10-15°C, and the gradual change in α and the higher *Ic, Ik*, and ϕ_{PSII} values at 20-25°C might indicate that the species was able to optimize its photosynthetic responses to low and high light conditions during its growing season, which was characterized by irradiance limitation and low temperatures in winter, and higher irradiance and temperatures in spring. The lack of a photoinhibitory response might indicate that *M. latissimum* can tolerate higher irradiance levels. Furthermore, the growth rates of the species increased as irradiance rose, which indicated a higher growth rate response as the irradiance levels increased.

Photosynthetic characteristics revealed by the *P-I* curves and PAM fluorometry

The similar light-saturated photosynthesis ($P^n max$) levels for *M. latissimum* at the temperatures used in this study clearly reflected the growing season, which was late autumn to mid-spring when the temperature is between ~11 and 23°C (Bast 2011). Similarly, Xiao et al. (2016) suggested that the significantly higher photosynthetic response of *Ulva prolifera* Müller at moderate temperature levels of 14-27°C indicated that the local environmental conditions in the southwestern Yellow Sea in China between April and May could lead to *U. prolifera* blooming. In addition, Mattieson and Dawes (1986) observed similar photosynthetic responses at a number of different temperatures (12-27°C) by the tropical green benthic alga, *M. oxyspermum* (Kützing) Doty, collected in Florida, USA, which indicated that most cosmopolitan species have a broad tolerance to temperature. Furthermore, Yokohama (1973) observed that the *M. nitidum* collected during the

winter season from Izu Peninsula in Tokyo, Japan had considerable resistance to temperatures up to 35℃. The results suggest that there was no decrease in photosynthesis at 25℃. This may indicate adaptation to the warm water of the Kuroshio Current circulating within the collection site (Qiu 2001, Tanaka et al. 2012). Therefore, future investigations should be conducted to confirm whether this is a general characteristic of all species in this area.

The F_V/F_M values for *M. latissimum* clearly decreased as the temperature increased, which suggests that there was a negative effect on the PSII complexes at higher temperatures. Roháček and Barták (1999), and Beer et al. (2014) showed that F_V/F_M has been widely used as a stress indicator for PSII complexes. Similarly, Xu et al. (2013) showed that a decreasing F_V/F_M in *Ulva linza* Linnaeus at higher temperatures indicated that the PSII complexes were under stress.

The *Rd* values for *M. latissimum* per thallus area and for total chlorophyll content increased as the temperature rose, which might indicate a metabolic adjustment to increasing temperature. Terrados and Ros (1992) and Xiao et al. (2016) also reported that respiration increased when the temperature rose in the intertidal green benthic algae *Caulerpa prolifera* (Forsskal) Lamouroux and *U. prolifera*, respectively. *Rd* is dependent on temperature. Therefore, it is probable that the $P^n max$ is dependent on temperature as well. Yokohama (1973) observed that *M. nitidum* is dependent on both photosynthesis and respiration when temperatures increase. However, due to the wide variations in $P^n max$ recorded in this study, the responses were not significantly different.

The α, *Ic*, *Ik*, and ϕ_{PSII} values for *M. latissimum* seems to be dependent on temperature, which could reflect an adaptation that optimizes photosynthesis to low light conditions during winter, when there is a shorter photoperiod and lower solar irradiation, and to high light conditions during spring, when the photoperiod is longer and solar irradiation is higher. Similarly, O'Neal and Prince (1988), and Terrados and Ros (1992) reported steeper α, and low *Ic* and *Ik* values for the green benthic algae, *Caulerpa paspaloides* (Bory) Greville and *C. prolifera*, respectively, which had been collected during the winter season when light availability and temperatures are low. O'Neal and Prince (1988) also reported lower α, and higher *Ic* and *Ik* values for the green benthic alga *C. paspaloides*, collected during the summer season, which is characterized by higher light conditions and temperatures. In addition, Xiao et al. (2016) observed an increasing light compensation point and saturating irradiance level for the detached green alga *U. prolifera* when the temperature rose. Furthermore, Fan et al. (2014) reported increasing ϕ_{PSII} values in the green-tide macroalga *U. prolifera* as the temperature increased, which indicated that there was an adjustment to match its light requirement. In contrast, Maegawa and Aruga (1974, 1983) showed that *M. latissimum* was able also to saturate at higher irradiance levels, but they were not affected by increasing temperature.

Monostroma latissimum showed no decrease in the photosynthetic rate beyond the saturating irradiance point at all temperatures and there was no photoinhibitory response, which confirmed the results reported by Maegawa and Aruga (1974) and Xiao et al. (2016) for the green benthic algae *M. latissimum* and *U. prolifera*, respectively. This indicated that it had a high irradiance tolerance. Similarly, no differences were observed in the q_N values for *M. latissimum*, which suggests that it has a photoprotective mechanism that enables it to tolerate a wide range of temperatures when exposed to fluctuating temperatures in the intertidal area, especially during tidal emersion and immersion where the temperatures are relatively high and low, respectively. Kang et al. (2013) showed that the higher non-photochemical quenching coefficient for the supralittoral green alga, *Prasiola stipitata* Suhr ex Jessen could be an ecological strategy to avoid damage to the photochemical pathway caused by excess photon energy.

Most published studies on seaweeds pre-acclimatized their algae to the experimental temperatures prior to photosynthetic measurement so that they could provide stable responses and avoid possible transient responses due to sudden temperature shifts (Terrados and Ros 1992, O'Neal and Prince 1988, Xiao et al. 2016). However, the photosynthetic responses in *M. latissimum* might not have been affected by sudden temperature changes for the following reasons: the experimental temperatures used in this study were within the normal ranges found during the *M. latissimum* growing season (~11-23° C) (Bast 2011), and the results of this study confirmed those reported by most of the studies cited above. It also verified their growth rate response results. Similarly, Serisawa et al. (2004) investigated the photosynthetic responses of the brown seaweed samples of *Ecklonia cava* Kjellman taken from warmer (Kochi Pref., southern) and colder (Shizuoka Pref., central) localities in Japan and subjected them to different temperatures (10-29° C). They found that the responses reflected the growing season and locality of the species. This was achieved without pre-acclimation to experimental temperatures prior to photosynthetic measurements.

Growth rate and its dependency on photosynthesis

Kalita and Titlyanov (2013) reported a periodic change with a cycle of 2 to 3 days in the growth rates of *Ulva lactuca.* If a similar phenomenon occurs also in *M. latissimum*, the growth measurement in the present study with 5-day intervals would have failed to measure the highest growth rate that may

possibly be achieved during the long interval. On the other hand, the results of the present study, as shown in Fig. 5, would still be able to show the significant dependency of the growth on the light intensity and temperature in the long-term experiments. The growth rates of *M. latissimum* at temperatures that reflected the growing season of the species from late autumn to mid-spring (~11-23℃) (Bast 2011) were similar. However, when cultured for a longer period of time, the higher temperatures had a negative effect on the growth rate of the species. Similarly, Xiao et al. (2016) showed that the local environmental conditions in the southwestern Yellow Sea in China from April-May increased the growth rate of *U. prolifera*. However, lower (< 14℃) and higher temperatures (> 27℃) had adverse effects on the growth rate. In addition, Bast et al. (2011) showed that the maximum thallus length of a naturally occurring population of *M. latissimum* collected in Kochi, Japan was observed during the colder season from November to July.

The irradiance effect on the growth rate of *M. latissimum* seems to suggest that it has a high light requirement. Similarly, Fu et al. (2008) observed that the growth rate of unialgal *Enteromorpha prolifera* (Müller) J. Agardh cultured over 7 days was significantly dependent on increasing irradiance and temperature up to 40 μmol photons m^{-2} s^{-1} and 25° C, respectively, which suggested a tolerance to relatively higher irradiance and temperature levels. No negative growth effects were detected the highest light level (400 μmol photons m^{-2} s^{-1}) used in the growth rate experiment in this study, even though it was beyond the saturating irradiance (Ik = ~100-200 μmol photons m^{-2} s^{-1} measured from their *P-I* curves) for *M. latissimum*. Therefore, future studies should determine the light levels at which the species produces a positive growth rate response. This could indicate the adaptability of this commercially important green seaweed to climate change i.e., variations on the solar radiation cause about by changes on the weather patterns.

The similar photosynthetic responses paralleled the growth rate responses of *M. latissimum* at the different temperatures and the growing season of the species from late autumn to mid-spring (Bast 2011). Similarly, Xiao et al. (2016) showed that both the net photosynthetic rate and the growth rate of the detached green alga *Ulva prolifera* Müller were significantly higher at moderate temperature levels (14-27℃), which indicated that the local environmental conditions in the southwestern Yellow Sea in China between April and May favored *U. prolifera* blooms. However, the decrease in photosynthesis and growth at lower and higher temperatures might indicate adverse effects on the species. The ability of *M. latissimum* to optimize photosynthesis under low and high light conditions, and its tolerance to higher irradiance affected its growth rate, which was positively correlated with increasing irradiance. This suggested that it had a higher light requirement for growth under these conditions. Similarly, Segi and Kida (1968) mentioned that cultivated *M. latissimum* generally grows better when the light levels are high.

In summary, the study clearly showed that photosynthetic rates were related to the growth rate responses of *M. latissimum* at different temperatures and light intensities. The photosynthetic and growth rate responses of the species indicated the growing season of the species from late autumn to mid-spring, but prolonged exposure to higher temperatures had an adverse effect. Furthermore, the species was able to optimize its photosynthetic and growth responses to low and high light conditions during the growing season, which is characterized by irradiance limitation and low temperatures in the winter and by high irradiance and temperatures in the spring. Furthermore, the species was able to tolerate higher irradiance levels. The results generated by this study provide science-based information that can be used to improve the cultivation of this species or any related taxa. We recommend that the temperature should be kept within 10-20℃ as higher temperatures have a detrimental effect, and irradiance should not exceed 400 μmol photons m^{-2} s^{-1}. However, field-testing and further studies should be conducted to validate our suggestions. Overall, understanding the mechanisms controlling photosynthesis is necessary if *M. latissimum* growth requirements are to be determined.

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